THE MOLECULAR STRUCTURE OF GALACTOSE CONTAINING POLYSACCHARIDES
OF THE PLANT GUM AND HELICELLULOSE GROUPS

- by -

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Reprint
INTRODUCTION

SECTION I

THE GALACTOSE CONTAINING POLYSACCHARIDES OF THE PLANT GUMS

The plant gums have been defined (1) as "those substances of plant origin which are obtained as exudations from the fruit, trunks or branches of trees spontaneously or after mechanical injury of the plant by incision of the bark or by removal of a branch, or after invasion by bacteria or fungi". The exudates harden on drying, forming nodules of polysaccharide material, with various impurities such as lignin, protein, resinous material and bits of bark.

The gums show many similarities in properties and in molecular structure to the plant mucilages, and are normally reviewed together (1-6). They are usually distinguished, in that the mucilages exist inside the plant in the form of membrane thickening material, or intracellular substances, and are thought to act either as food stores, or as a means of retaining water. They have also been divided because of their different solubilities in water, the gums generally dissolving to give clear solutions and the mucilages forming colloids.

The plant gums are found on trees and shrubs of many species, but are most commonly produced by fruit trees, and by the small thorny trees which are widespread in hot, dry countries. Gum arabic is the best known and most important commercially of the gums. It has been in use for many centuries, the Egyptians employing it as a paint thickener, as far back as 2000 B.C. It is now harvested in considerable amounts in the Near East, often from trees specially grown in gardens for the production/
production of the gum.

Under the name of gum arabic go secretions from trees of the various species of the genus *Acacia*. This leads to confusion in structural work, and unless a gum has a known botanical origin, precise conclusions cannot be drawn about the molecular structure of the species. The commercially highest quality gum comes from *Acacia senegal* and the classical work of Smith (24,27,30,31,33,34) on the molecular composition of arabic acid, was carried out on gum of high quality known as Gum Kordofan.

The cause of formation of the gums is still in doubt. Although they seem to be exuded exclusively to seal off the attacked or invaded area, it is unknown whether they are produced by invading micro-organisms, or by the plant to protect itself against such attack, or if they are merely natural secretions of the tree. It has been found that some fungi will induce the formation of water soluble gums on *Acacia* trees (7), but, on the other hand, in the case of other gums, there is evidence against the secretions being bacterial products. For example, gum tragacanth is produced immediately the bark of the tree is cut. In addition the exudates from different trees of the same species, are remarkably similar in their properties, whereas, one might expect different bacteria to form different gums on trees of the same species. It is also interesting to note that gums are produced mainly by trees which are old, in poor health or short of water. Healthy trees with a plentiful supply of water, produce little gum.
PURIFICATION AND FRACTIONATION

The polysaccharide material normally exists in the secretions as the neutral or slightly acid salts with calcium, magnesium and potassium the usual cations, and is easily obtained from the crude gum. The nodules are dissolved in water, the solution filtered and the free gum acid precipitated from the filtrate by the addition of acidified ethanol.

After the isolation of the polysaccharide material from the crude gum, the next problem is to determine whether or not the material is homogeneous. If a mixture, it may be made up of polysaccharides, with different structural units, or polysaccharides with the same structural units linked in different ways. Further a mixture of polymers may have the same structural units linked in the same way, but in different proportions. Also a polymer may be heterogeneous in being made up of molecules containing the same repeating unit, but covering a range of molecular weights.

Fractionation has been attempted by a large number of methods, the main ones being fractional precipitation using an organic solvent as precipitant, and secondly the complexing of certain polysaccharides with reagents such as copper (8) and cetyltrimethylammonium bromide (9,10) to give precipitates while other components may be left in solution.

While most gums are homogeneous with respect to these chemical methods of separation, several have been separated into more than one constituent.
constituent. For example gum tragacanth (11) can be separated into three distinct components, a glycoside and two polysaccharides with different component monosaccharides. Other gums showing this type of heterogeneity are the gum component of Olibanum (12) and Khaya senegalensis gum (13).

Immunological fractionation depends on the fact that antipneumococcus sera give specific precipitates with certain polysaccharides. Gum arabic with Type II antipneumococcus serum yields a precipitate from which can be recovered a polysaccharide which has much less rhamnose than the original gum (14). On the other hand, electrophoresis has been applied to the plant gums and gum arabic appears to be electrophoretically homogeneous. It has also been separated from other gums including *Acacia cyanophylla* gum (15).

Ionophoresis on ordinary paper is not successful because of adsorption, and the method has been extended using glass fibre paper (16,17). With this, Lewis and Smith (18) have reported that more than one component has been detected in a number of the plant gums including *Acacia pycnantha*, *Acacia senegal*, *Acacia seyal*, gum ghatti and gum tragacanth, whereas Black Wattle gum from *Acacia decurrens* and Mesquite gum from *Prosopis juliflora* appeared to be homogeneous. In most of these was a major and one minor component, but, at the moment there is no evidence of the nature of the heterogeneity, so that it is not known whether the different fractions of the same gum have fundamental differences in structure or if they merely differ in some minor aspects.

**STRUCTURAL INVESTIGATION OF THE PLANT GUM POLYSACCHARIDES**

The /
The preliminary examination of the polysaccharide involves the determination of optical rotation, % ash, methoxyl, acetyl and uronic anhydride contents and the equivalent weight.

Next the nature of the component monosaccharides must be found. The polysaccharide can be broken down to aldobiouronic acids and neutral monosaccharides by heating at 100° in N-sulphuric or hydrochloric acids for about four hours. A rapid identification of the neutral sugars can be obtained by paper chromatography of the hydrolysate. This can be carried out on very small amounts of polysaccharide (10-20 mg.) but has the disadvantage that it will not differentiate D- and L-sugars. The method has been extended to give quantitative estimation of the sugars by Flood, Hirst, and Jones (19). The mixture is run, and the positions of the various monosaccharides determined by spraying side strips of the paper. The sugars are then eluted from the paper and estimated by some micro-method as that of Somogyi (20).

For final identification of the sugars, partition chromatography on cellulose powder is used. This enables the monosaccharides to be separated on a scale large enough for the sugars to be characterised by the formation of crystalline derivatives. The main monosaccharides occurring in the plant gums are D-galactose, L-arabinose, D-xylose, D-mannose, L-rhamnose, D-glucuronic acid and 4-methyl-D-glucuronic acid, and D-galacturonic acid while D-tagatose and L-fucose have also been detected.
Partial acid hydrolysis

Acid hydrolysis under various conditions is used to isolate simple fragments of the molecule whose structures may be completely determined thus providing information about the order and mode of linkages of the monosaccharides in the polymer.

If all the glycoside linkages were hydrolysed at the same rate, random partial hydrolysis would give the variety of possible products of incomplete hydrolysis. However the various types of linkages have different rates of hydrolysis so that, under given conditions, one type of linkage is hydrolysed and others are relatively untouched. Furanoside linkages are very easily hydrolysed being cleaved by heating in approximately 0.01 N-sulphuric acid at 100° for a few hours. Pyranoside bonds are less easily split requiring about 0.1 N-acid while uronic acid glycoside linkages are very resistant to acid and in the harsh conditions required for their hydrolysis, the sugars tend to decompose, particularly the uronic acid residues which undergo decarboxylation. Thus the hydrolysis, to a certain extent, can be made selective by changing acid strength and temperature, although, of course, the hydrolysis never occurs exclusively at one type of linkage. From the different hydrolysates, the various oligosaccharides can be isolated and examined.

It is known that oligosaccharides are synthesised from monosaccharides in acid conditions so that small amounts of oligosaccharides observed in acid hydrolysates of polysaccharides must be viewed with caution. For example, Jones and Nicholson (21) have found that L-arabinose /
L-arabinose in hydrochloric acid gives three reversion products and the presence of these in hydrolysates which contain arabinose indicates that reversion may be taking place. Certain samples of gum arabic in mild acid conditions (22) yields one of these disaccharides 3-\(\alpha\)-\(\beta\)-L-arabopyranosyl-L-arabinose. The absence of the other two however is evidence in favour of its being a true hydrolysis product and reversion products can generally be distinguished since their concentrations reach an equilibrium value, whereas actual fragments of the polysaccharides are hydrolysed on more prolonged heating.

When the polysaccharide is heated at 100\(^\circ\) in 0.01 N-sulphuric acid, the acid attack is mainly on the furanoside linkages. Heating an aqueous solution of the free acid polysaccharide (a process referred to as autohydrolysis) gives the same effect for gums with a high enough uronic acid content. Pentose containing disaccharides have been isolated from the hydrolysates of gums in these conditions and several have been characterised. Many gums seem to consist of an acid resistant nucleus, composed of monosaccharides linked by pyranoside or uronic acid glycosidic linkages, with the more acid labile sugars situated at the periphery of the molecule. In these cases mild acid hydrolysis strips off the outer sugars leaving behind this more acid stable nucleus, which is known as the degraded gum and which is often used in methylation studies.

Hydrolysis under the harsher conditions of around 0.1 N-sulphuric acid at 100\(^\circ\) attacks the degraded gum breaking it up into fragments, and disaccharides containing neutral monosaccharides have been isolated /
isolated from several gums. 3-O-β-D-Galactopyranosyl-D-galactose has been isolated from Acacia pycnantha gum (23), gum arabic (24) and golden apple gum (25) which has also yielded the 1:6 linked galactobiose.

N-Sulphuric acid at 100° is drastic enough to cleave all bonds in the polysaccharides except the uronic acid glycoside linkages so that under these conditions the molecule is hydrolysed to aldobiouronic acids and the neutral monosaccharides. A number of these acid disaccharides have been isolated and characterised.

**Methylation studies.**

The main method of determining the modes of linkage between the monosaccharides is methylation. All free hydroxyl groups are converted to methoxyl groupings and subsequent hydrolysis gives partially methylated sugars, the free hydroxyl groups of which indicate the positions of linkages in the molecule.

Disaccharides and linear polymers which contain only one component monosaccharide and one type of linkage, give unambiguous results, and the structure of these substances can be found from methylation studies alone. However, in all other cases methylation gives the mode of linkage of the constituents only, indicating whether they are end groups or situated at branch points etc.

Generally the mixture of component methylated sugars is separated /
separated on a column of cellulose powder and the sugars characterised by means of crystalline derivatives. The results of these hydrolysates are in the case of the gums extremely complex and very difficult to interpret. The degraded gums are methylated also and since their structures are simpler than those of the whole gums, the results of hydrolysis of the methylated, degraded gums are much easier to interpret. In addition, from a comparison of the methylation results on the whole and degraded gums, the points of attachment of the acid labile groupings can be approximately determined.

**Peridate oxidation studies.**

A further source of information is the study of the oxidation of the polysaccharide with sodium metaperiodate. Structures with hydroxyl groups on adjacent carbon atoms are oxidised by salts of metaperiodic acids as follows:

(a) \[
\begin{align*}
\text{CHOH} & + \text{I}_4^- \\
\text{CHOH} & \rightarrow \text{CHO} + \text{I}_5^- + \text{H}_2\text{O}
\end{align*}
\]

(b) \[
\begin{align*}
\text{CHOH} & + 2\text{I}_4^- \\
\text{CHOH} & \rightarrow \text{HCOOH} + 2\text{I}_3^- + \text{H}_2\text{O}
\end{align*}
\]

(c) \[
\begin{align*}
\text{CHOH} & + \text{I}_4^- \\
\text{CH}_2\text{OH} & \rightarrow \text{CHO} + \text{HOHO} + \text{I}_5^- + \text{H}_2\text{O}
\end{align*}
\]

Thus /
Thus the measurement of the uptake of periodate and the release of formaldehyde and formic acid give an indication of the mode of linkage and the extent of branching. An extension of this method has been developed by Barry (26). In this the oxidised polysaccharide is treated with phenylhydrazine and glacial acetic acid at 100°. This removes all the oxidised monosaccharides leaving behind a simpler degraded polysaccharide whose structure may be relatively easily determined. For example, take the following hypothetical polysaccharide -

\[
\text{Gal} \ 1 - 4 \ 	ext{Gal} \ 1 - 3 \ 	ext{Gal} \ 1 - [\text{Gal}]_n
\]

\[
\begin{align*}
\text{Gal} \ &\text{1} \ 4 \\
\text{Gal} \ &\text{1} \ 3 \\
\text{Gal} \ &\text{1} \\
\text{[Gal]}_n
\end{align*}
\]

\[
\begin{align*}
\text{CH} &= \text{N.NH.C}_6\text{H}_5 \\
\text{C} &= \text{N.NH.C}_6\text{H}_5 \\
\text{CH}_2\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{glycerosazone} \\
+ \\
\text{CH} &= \text{N.NH.C}_6\text{H}_5 \\
\text{CH} &= \text{N.NH.C}_6\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{CH} &= \text{N.NH.C}_6\text{H}_5 \\
\text{C} &= \text{N.NH.C}_6\text{H}_5 \\
\text{CHOH}
\end{align*}
\]

\[
\begin{align*}
\text{threosazone} \\
+ \\
\text{CH} &= \text{N.NH.C}_6\text{H}_5 \\
\text{CH} &= \text{N.NH.C}_6\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{glyoxalbisphenylhydrazone}
\end{align*}
\]
In cases of the more complex polysaccharides, the degradation procedure can be repeated until, for example, as in gum arabic after three such treatments, there is recovered a polysaccharide, which was resistant to further periodate oxidation and gave only galactose on hydrolysis, indicating a straight chain of 1:3 linked galactose residues.

The plant gums so far examined are extremely complex and from a comparison of all these results only the main structural features and possibly the main repeating unit can be determined. Gum arabic is the most extensively examined of the polysaccharides and a summary of the work carried out on it is given below.

THE STRUCTURE OF GUM ARABIC (Gum Kordofan)

The component monosaccharides of arabic acid, the free acid polysaccharide of gum arabic, have been established as D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid in the approximate molecular proportions of 3:3:1:1. Autohydrolysis or hydrolysis using 0.01 N-sulphuric acid (27) liberates arabinose, rhamnose and a disaccharide which was characterised as 3-O-α-D-galactopyranosyl-L-arabinose, leaving a degraded gum of galactose and glucuronic acid residues. More prolonged autohydrolysis frees a galactose containing disaccharide and this has been characterised as 3-O-β-D-galactopyranosyl-D-galactose (24). A fourth fragment of the molecule, the aldobiouronic acid 6-O-β-D-glucuronosyl-D-galactose (28,29), has been obtained by acid hydrolysis. The partial hydrolysis /
hydrolysis experiments are summarised below.

\begin{align*}
\text{(autohydrolysis or hydrolysis with 0.01 N-sulphuric acid at 100°)} & \rightarrow \text{L-arabinose} \\
\text{GUM (prolonged auto-ARABIC hydrolysis)} & \rightarrow 3-0-\beta-\text{D-galactopyranosyl-L-arabinose} \\
\text{(hydrolysis with N sulphuric acid)} & \rightarrow 6-0-\beta-\text{D-glucuronosyl-D-galactose}
\end{align*}

The degraded gum, which from the above experiments seems to be the more acid resistant nucleus of the molecule, was found by Smith (30) to contain 9 parts of galactose to 3 parts of glucuronic acid. On methylation and subsequent hydrolysis, it yielded the following sugars in the given proportions.

\begin{align*}
2:3:4:6-\text{tetra-0-methyl-D-galactose} & \quad (1) \\
2:3:4-\text{tri-0-methyl-D-galactose} & \quad (5) \\
2:4-\text{di-0-methyl-D-galactose} & \quad (3) \\
\text{and 2:3:4-tri-0-methyl-D-glucuronic acid} & \quad (3)
\end{align*}

This indicates that both sugars are in the pyranose form, that both 1:3 and 1:6 linkages occur and that the glucuronic acid residues occur exclusively as end group in the branched framework of the degraded arabic acid. Jackson and Smith (31) isolated, by the partial hydrolysis of the methylated degraded gum, the hexamethyl ether of 6-0-\beta-\text{D-glucuronosyl-D-galactose} showing that the terminal glucuronic acid residues are attached to /
to at least one galactose residue before the backbone in the degraded gum.

With the evidence so far, Smith proposed several possible formulae for the main repeating unit of the degraded arabic acid.

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Some of the other possibilities are:

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(III) (IV)

Further /
Further information about the structure of the backbone of the gum was obtained by periodate oxidation studies (32). The whole gum was oxidised with sodium metaperiodate and the oxidised sugar residues were removed by treatment with phenylhydrazine and glacial acetic acid at 100° according to Barry's method (26). The recovered polysaccharide was further twice degraded in the same way and yielded a polysaccharide which was resistant to periodate oxidation and which gave galactose only on hydrolysis, indicating a straight chain of 1:3 linked galactopyranose residues. This represents the backbone of the molecule so that the degraded arabic acid must be mainly made up of a main chain 1:3 linked galactose units with side chains of 1:6 linked galactose residues and with the glucuronic acid appearing as end group. Thus a working model for the degraded gum can be given by structure (III), the only one with a 1:3 linked backbone of galactopyranose residues.

A similar conclusion about the distribution of the 1:3 and 1:6 linkages in the galactan framework was reached by F. Smith and Spiessentbach (33). They oxidised the degraded gum with sodium metaperiodate and removed the cleaved aldobiouronic acid side chains by controlled acid hydrolysis. The remaining fragments were shown in methylation studies to contain only 1:3 linkages.

The whole gum on methylation and hydrolysis (34) gave the following sugars:
From these results it appears that the rhamnose exclusively occurs in the molecule as end group in the pyranose form, that the arabinose is always in the furanose form whether attached as end group or as non terminal units linked through carbon atoms 1 and 3, and that the glucuronic acid residues can be either end group or have an acid labile grouping attached to carbon atom 4. By a comparison of these results with those of the methylation studies on the degraded gum and with the results of partial hydrolysis, an approximate main repeating unit can be built up for gum arabic (V).
Andrews and Jones (22) have reported the presence of the disaccharide 3-\(\beta\)-L-arabopyranosyl-L-arabinose in the hydrolysis products of some samples of gum arabic so that the unit \(L\)-Araf \(\beta\)-3-\(L\)-Araf \(\beta\) may occur in the molecule although no 2:3:4-tri-\(O\)-methyl-L-arabinose was detected in the hydrolysis products of the methylated whole gum.

**MOLECULAR STRUCTURE OF OTHER PLANT GUMS**

A great deal of work has recently been carried out on the molecular structures of a large number of plant gums of known botanical origin. Structurally, these can be most conveniently divided into three main groups by the nature of their uronic residues.

1. Those containing D-glucuronic acid
2. Those containing 4-\(O\)-methyl-D-glucuronic acid
3. Those containing D-galacturonic acid.
Gums containing D-glucuronic acid (Tables I, p. 21 and II, p. 24)

Most members of this group of gums exhibit certain common general features although they differ in the more detailed aspects of their molecular structures. The main component sugars found are L-arabinose, D-galactose and D-glucuronic acid with L-rhamnose, D-xylose and D-mannose also occurring in certain cases. As in arabic acid, the arabinose is normally in the labile furanose form so that on mild hydrolysis, a degraded gum of galactose, glucuronic acid and sometimes mannose residues can often be recovered. In the gums which have been more thoroughly examined, the galactopyranose residues are mainly linked 1:3 and 1:6 and generally seem to make up the framework of the polysaccharides with the acid labile residues occurring on the periphery of the molecule. The glucuronic acid residues are usually linked to galactose or mannose units, with acid labile groupings often attached to carbon atom 4 of the acids.

The gums of the genus Acacia show similarities in their compositions. They all have the same component monosaccharides, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid in different proportions and the same aldobiouronic acid 6-O-β-D-glucuronosyl-D-galactose has been isolated in partial hydrolysis experiments from all members examined. This may not be the only acid disaccharide unit in all cases since 4-O-α-D-glucuronosyl-D-galactose has also been obtained from Acacia karroo gum (38).

Arabinose and sometimes rhamnose are liberated on autohydrolysis of the Acacia gums leaving degraded polysaccharides of galactose and glucuronic /
glucuronic acid units. The same disaccharides have been isolated from the autohydrolysates of different *Acacia* gums indicating further structural similarities between the gums. 5-0-α-D-Galactopyranosyl-L-arabinose has been isolated from the hydrolysates of gum arabic (*A. senegal*) (27) and *A. cyanophylla* gum (37), while *A. karroo* gum and some samples of gum arabic (22) give 3-0-β-L-arabopyranosyl-L-arabinose.

Gum arabic (24) and *A. pyronantha* gum (25) (the subject of much of the present work) yield on severer acid hydrolysis, 5-0-β-D-galactopyranosyl-D-galactose. Methylation and periodate oxidation studies (35) indicate further similarities between the two gums in that both have 1:3 and 1:6 linked frameworks of galactopyranosyl residues in which the backbone is predominately linked 1:3.

The gums of the genus *Prunus* show structural similarities to each other and to the *Acacia* gums although definite differences have been found.

Some contain D-mannose and D-xylose. The mannose free gums liberate, on acid hydrolysis, 6-0-D-glucuronosyl-D-galactose while the mannose containing gums give 2-0-D-glucuronosyl-D-mannose and the mannose seems to occur exclusively linked to glucuronic acid residues since there is no evidence of it elsewhere in the molecules.

The *Prunus* gums liberate arabinose, and sometimes rhamnose and xylose, on the autohydrolysis leaving degraded gums of galactose, glucuronic acid and sometimes mannose residues. Methylation studies on the whole and degraded gums show the galactose is branched 1:3 and 1:6 (as in gum arabic) and from the information so far gathered, the structure /
structure of these gums is a galactan backbone with glucuronic acid (with or without mannose) and pentose residues attached either directly on to the main chain or to side chains of further galactose residues. The arabinose is generally in the furanose form and may be attached terminally, linked 1:3 or 1:5, although 3-O-β-L-arabopyranosyl-L-arabinose has been isolated from peach and cherry gums (48). The xylose occurs in the pyranose form either as end group or linked 1:5.

Recent work on gum ghatti (70,71) shows that it contains some unusual structural features. The first three members of the series of oligosaccharides:

\[
\text{Gal 1}\left[\alpha 6\text{Gal 1}\right]_n\beta 6\text{Gal} \quad (i.e. \ n = 0,1,2)
\]

and the first four members of

\[
\text{Gal 1}\left[\beta 6\text{Gal 1}\right]_n\beta 5\text{Ara} \quad (i.e. \ n = 0,1,2,3)
\]

along with a small amount of 3-O-β-D-galactopyranosyl-D-galactose have been isolated after partial hydrolysis experiments and these results along with methylation studies on the whole and degraded gums indicate that the gum is made up of chains of 1:6 linked galactopyranose residues, joined by arabofuranose residues.

The two aldobiouronic acids of the Prunus gums have been isolated from the partial hydrolysis of gum ghatti (56) and the following structural features are thought to exist in the gums:

\[
R/
\]
R represents mainly terminal L-arabofuranose residues, but in some cases may represent L-rhamnopyranose residues or side chains composed of two or more arabinose units.

Chagual gum obtained from *Puya chilensis* (62) contains an unusually large amount of xylose and little arabinose. Graded hydrolysis yields the following sugars L-arabinose (7%), D-xylose (31%), D-galactose (36%) and 2-0-D-glucuronosyl-D-xylose (27%).

*Achras sapota* gum (63-66) shows marked differences from the other gums of this group. No hexoses have been detected, the component sugars being xylose, arabinose, glucuronic acid and possibly 4-0-methyl-D-glucuronic acid. Autohydrolysis does not liberate the arabinose preferentially and no disaccharides have been isolated although the aldobiouronic acid 2-O-D-glucuronosyl-D-xylose has been detected.
Methylation studies indicate a main chain of xylose residues with side chains containing glucuronic acid in both terminal and non-terminal positions and end groups of arabopyranose and xylose units.

Another gum differing in structure from the majority of this group is Sterculia caudata gum (72). Although all other Sterculia gums so far examined contain D-galacturonic acid, Sterculia caudata gum shows similarities in its structure to them. 2-O-α-D-Glucuronosyl-L-rhamnose has been obtained and thamnose occurs as branching points and linked through positions 1:2 but not terminally as in the other glucuronic acid gums. In addition galactose is linked 1:4 instead of 1:3 and 1:6.
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Key: -

\(\alpha\) = D-Glucuronic Acid
\(\beta\) = L-Galacturonic Acid
\(\gamma\)-me = 4-methyl-D-glucuronic acid
Gal A = D-galacturonic Acid
Gal  =  D-galactose
Ara =  L-arabinose
f  =  Pyranose

Xy = D-xylose
Fu = L-fucose
Ta = D-tagatose
Rh = L-rhamnose
Ma =  L-mannose
P  =  Pyranose
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(M) = Main Chain.
Gums containing 4-O-methyl-D-gluconic acid (Table III, p. 27)

The gums containing 4-methyl-gluconic acid tend to have higher uronic acid and lower arabinose contents than the gums of the preceding group. The arabinose, which is generally easily removed on auto-hydrolysis, occurs in several cases in the pyranose form, the disaccharide 3-O-β-L-arabopyranosyl-L-arabinose having been isolated from the hydrolysis of lemon gum (78) and the golden apple gum (80). Xylose, rhamnose and fucose also occur in some of the gums and xylose containing disaccharides have been isolated in a number of cases.

6-O-(4-O-Methyl-D-gluconosyl)-D-galactose and 4-O-(4-O-methyl-D-gluconosyl)-D-galactose are the main aldobiouronic acids isolated from the partial hydrolysis products of the gums so far examined and it is interesting to note that the corresponding unmethylated acid disaccharides are common in the gluconic acid containing gums. Also, in two cases, aldobiouronic acids containing arabinose, 4-O-(4-O-methyl-α-D-gluconosyl)-L-arabinose from lemon gum (77), and 3-O-(4-O-methyl-α-D-gluconosyl)-L-arabinose from golden apple gum (25) have been obtained on autohydrolysis indicating that uronic acid residues occur in the acid labile periphery of some of the gums.

Mesquite gum from Prosopis juliflora (83-90) which belongs to the same family as the Acacia trees, has been somewhat more extensively examined than the others of this group and methylation studies show that it resembles the Acacia and Prunus gums in having the galactose residues linked 1:3 and 1:6, and the arabinose, which is present in the furanose form, occurring as end groups and in non terminal/
terminal positions linked 1:3.

No information about the structures of the rest of this group of gums is, as yet, available from methylation studies so that the modes of linkages in the main framework of the molecules are unknown. However degraded golden apple gum (25) on partial hydrolysis liberates 3-β-D-galactopyranosyl-D-galactose and 6-β-D-galactopyranosyl-D-galactose showing that the galactose nucleus has, in this case at least, the 1:3 and 1:6 linkages predominating.
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>4-me-GA</th>
<th>Gal</th>
<th>Ara</th>
<th>Other Sugars</th>
<th>Disaccharides</th>
<th>Refs.</th>
</tr>
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<tr>
<td>Burseraceae</td>
<td>Commiphora</td>
<td>Myrrhha</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>-</td>
<td>4-me-GA 1 β 6 Gal</td>
<td>73, 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4-me-GA 1 α 4 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boswelliae</td>
<td>Carterii (Frankincense)</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>Fu</td>
<td>4-me-GA 1 β 6 Gal</td>
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<tr>
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<td>Grapefruit</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td></td>
<td>76</td>
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<tr>
<td></td>
<td></td>
<td>Lemon</td>
<td>12</td>
<td>34</td>
<td>17</td>
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<td>76, 77</td>
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<td>4-me-GA 1 α 4 Ara</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Fagara</td>
<td>Xanthoxyloidaes</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>79</td>
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<tr>
<td>Anacardiaceae</td>
<td>Spondias</td>
<td>Cytheria (Golden apple)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Xy</td>
<td>4-me-GA 1 β 3 Gal</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4-me-GA 1 α 3 Ara</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Arap 1 β 3 Ara</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Xyp 1 β 3 Ara</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Gal 1 β 3 Gal</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Gal 1 β 6 Gal</td>
<td></td>
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<td></td>
<td>Lannea</td>
<td>Grandis (Meadal)</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>4-me-GA 1 β 6 Gal</td>
<td>81-82</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Prosopis</td>
<td>Juliflora (Mosquite)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>4-me-GA 1 β 6 Gal</td>
<td>83-90</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4-me-GA 1 β 4 Gal</td>
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</tbody>
</table>
Gums containing D-galacturonic acid. (Table IV, p.30)

This group contains a number of gums which show marked differences from the glucuronic acid containing gums. These, the Karaya, Khaya and Sterculia gums, are partly acetylated, have high uronic acid and rhamnose contents, but no arabofuranose residues and so are resistant to autohydrolysis, and do not seem to contain a backbone of galactopyranose residues. The main aldobiouronic acids isolated so far are 4-O-D-galacturonosyl-D-galactose and 2-O-D-galacturonosyl-L-rhamnose. In addition, a D-galacturonosyl-D-galacturonic acid has been isolated from Sterculia setigera gum (91,92) and O-D-galacturonosyl-(1 → 2)-O-L-rhammopyranosyl-(1 → 4)-D-galactose has been obtained from the acid hydrolysis of Khaya grandifolia gum (94,95) which also yields 2-O-D-galacturonosyl-L-rhamnose and 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose.

Methylation studies (92,95,96) show that rhamnose can be present as branching points, end group, and linked 1:2, and that the galactose is linked 1:4 in contrast to the glucuronic acid gums in which rhamnose occurs exclusively as end group and the galactose residues are linked 1:5 and 1:6.

However one galacturonic acid containing gum which is more similar to the Acacia and Prunus gums is Cholla gum (Opuntia fulgida)(99). It has a lower uronic acid and higher pentose content than is usual and readily undergoes autohydrolysis liberating arabinose, xylose and 4-O- or 5-O-β-D-xylopyranosyl-L-arabinose leaving a degraded gum of D-galactose /
D-galactose and D-galacturonic acid residues. Methylation studies indicate that the galactose is linked 1:3 and 1:6 as in the *Acacia* and *Prunus* gums and the arabinose seems to occur in the furanose form terminally linked 1:5 and

\[
\begin{array}{c}
\text{2} \\
\text{5} \\
\text{1 Araf} \\
\end{array}
\]

Thus Cholla gum bears more resemblance to the *Acacia* and *Prunus* gums than to its own group, just as *Sterculia caudata* gum (72) is structurally more similar to the galacturonic acid gums than to its own group, the glucuronic acid gums.

Gum tragacanth (11,100,101) can be separated after methylation into three components, a methylated acid polysaccharide, a methylated neutral polysaccharide and a glycoside. The acid polysaccharide, tragacanthic acid, contains D-galacturonic acid, L-fucose and D-xylose and seems to be made up of 1:2 linked xylopyranose units, with end groups fucose and xylose, and both non terminal and branch point galacturonic acid residues. The neutral polysaccharide, which has as its monosaccharides L-arabinose and D-galactose gives, on methylation, products indicating that the arabinose residues occur terminally, linked 1:5 and thirdly

\[
\begin{array}{c}
\text{2} \\
\text{5} \\
\text{1 Araf} \\
\end{array}
\]

The galactose, isolated as dimethyl galactose, occurs as branch points.
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
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<th>GalA</th>
<th>Gal</th>
<th>Rh</th>
<th>Other Sugars</th>
<th>Disaccharides</th>
<th>Refs.</th>
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<td>5</td>
<td>Ta (1)</td>
<td>GalA 1 —— 2 Rh</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Xy ?</td>
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<td></td>
<td></td>
<td>Rh-</td>
<td>Ketose?</td>
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<td></td>
<td></td>
<td>Urens</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Grandifolia</td>
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<td>3</td>
<td>2</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>4-me-GA</td>
<td>GalA 1 —— 4 Gal</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Tormentosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<td>Senegalensis</td>
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<td>+</td>
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<td></td>
<td>13, 95.</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ara</td>
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<td>Bixaceae</td>
<td>Cochlosper-</td>
<td>Gossypium</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Ketc-</td>
<td>GalA 1 —— 2 Rh</td>
<td>96.</td>
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<tr>
<td></td>
<td>num</td>
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<td></td>
<td></td>
<td></td>
<td>hexose</td>
<td>GalA 1 —— 4 Gal</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(Joel)</td>
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<td></td>
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</tr>
<tr>
<td>Cactaceae</td>
<td>Opuntia</td>
<td>Fulgida</td>
<td>1</td>
<td>3</td>
<td>tr</td>
<td>Am (6)</td>
<td>Xyp 1 ^f (4 or) 5 Ara</td>
<td>99.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Cholla)</td>
<td></td>
<td></td>
<td></td>
<td>Xy (2)</td>
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<td>Leguminoseae</td>
<td>Astragalus</td>
<td>Tragacanth</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Xy</td>
<td></td>
<td>11, 101.</td>
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<td></td>
<td></td>
<td>A. Acid</td>
<td></td>
<td></td>
<td></td>
<td>Ara</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>B. Neutral</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Ara</td>
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<td>100.</td>
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SECTION II

THE GALACTOSE CONTAINING POLYSACCHARIDES OF THE HEMICELLULOSE GROUP.

The main constituents of plant cell walls are cellulose, other polysaccharides which are known as the hemicelluloses, and lignin.

The long threadlike macromolecules of the cellulose (102) are arranged in bundles which are, in certain areas, highly organised enough to be semi-crystalline due to hydrogen bonding between the free hydroxyl groups of the 1:4 linked glucopyranose residues of adjacent molecules. Other regions seem to be more amorphous in nature and are rich in lignin and the hemicelluloses which appear to be present to give the cell wall rigidity although their exact functions are not known.

The extraction and subsequent fractionation of the hemicellulose material into component polysaccharides must first be carried out. Generally the initial step is to remove lipids, fats, resins etc., with some organic solvent system such as hot benzene:ethanol. The material is next delignified by one of a number of methods. For example treatment with chlorous acid renders the lignin water soluble, and aqueous extraction leaves a residue of cellulose and hemicellulose known as holocellulose. The hemicellulose is then extracted from this with dilute alkali. Certain polysaccharides normally grouped with the hemicelluloses are themselves water soluble and they can be extracted directly after the original plant substance has been treated with benzene and ethanol.
The main constituent monosaccharides found in the hemicelluloses are D-xylose, D-glucose, D-mannose, D-galactose, L-arabinose and D-glucuronic acid, and the main polysaccharides are the xylans, the mannan polysaccharides which are frequently associated with glucose, and the galactans which are normally present as arabogalactans and are very often water soluble.

The fractionation of the hemicellulose mixture into its pure component polysaccharides is a very difficult problem. Several techniques are now available but the most successful separation method for each particular case must still be determined by trial and error.

Differences in solubilities of the various polysaccharides present in the mixture can be used in fractional precipitation and dissolution. Also solubility differences are sometimes more marked in the acetylated (105) or methylated polysaccharides than in the original material. Another method of fractionation is the graded precipitation of the polysaccharides from an aqueous solution by the addition of ammonium sulphate (104). As in the plant gums, complexing with various reagents is sometimes successful in precipitating the complex of one polysaccharide while others remain in solution.

Cetyltrimethylammoniumbromide (9,10) has been used to precipitate acid polysaccharides and the method has more recently been extended to the separation of neutral polysaccharides which form complexes with boric acid (105). Xylans form insoluble complexes with copper (106) so that they may be separated from the other polysaccharides present although the treatment generally has to be repeated several times.

Once /
Once the pure, or almost pure, polysaccharide has been isolated, analysis of its molecular structure can begin. The main methods used are the same as for the plant gums with methylation, partial acid hydrolysis and periodate oxidation being the most important.

The xylans are the most frequently occurring polysaccharides of the hemicellulose group, and from the investigations so far carried out they seem to have simpler structures than the plant gums generally being branched to a smaller extent. The xylose occurs exclusively in the pyranose form and is usually 1:4 linked in long backbones.

Wood xylans have been isolated in a number of cases, e.g. from Norway spruce (Picea excelsa) (107) and beech wood (Fagus sylvatica) (108) and these polysaccharides seem to be made up of xylose backbones with attached sidechains of single glucuronic acid residues. Arabinose, if present at all, is in very small amounts. The cereal xylans, however, generally contain around 5-10% arabinose, which occurs mainly as end group in the furanose form linked to C atom 3 of xylose residues.

Oat straw xylan (109) has most of the typical features of the cereal xylans and the main repeating unit of the polymer may be approximately described:

\[
\text{Xyl} \ 1...4 \text{ Xyl} \ 1...4 \text{ Xyl} \ 1...4 \text{ Xyl} \ 1...4 \text{ Xyl} \\
\ 3 \ 2 \\
\ 1 \ 1 \\
\text{Araf} \ 4\text{-me-Gl Acid}
\]

The /
The uronic acid residues are attached normally to C atom 2 of xylose units and partial hydrolysis experiments have yielded 2-O-D-glucuronosyl-D-xylose and 2-O-(4-O-methyl-D-glucuronosyl)-D-xylose from a number of xylans, one aldobiouronic acid generally being isolated from each polysaccharide. However in two cases, sea pine (Pinus pinaster) (110) and Monterey pine (Pinus radiata)(111), 3-O-(4-O-methyl-α-D-glucuronosyl)-D-xylose has been detected in addition to the normally occurring 1:2 linked aldobiouronic acid.

Esparto grass xylan (112) gives arabinose on hydrolysis but after repeated fractionation with copper a true xylan was obtained suggesting that these polymers may in fact be mixtures of related polysaccharides.

The mannans are frequently found in association with glucose, and sometimes with xylose and galactose, although a true mannan has been isolated. Since purification is so difficult, these more minor constituents may be due to impurities in certain cases.

Most glucomannans so far examined conform to the pattern of having a 1:4 linked main chain of D-mannopyranose and D-glucopyranose residues. Methylation studies have shown that end groups of mannose, glucose and also galactose residues may occur.

In addition to occurring in the plant gums, galactans are found as arabogalactans in the woods of certain trees and as what appear to be pure galactans in plant tissues in association with the pectic substances.
The seeds of *Lupinus albus* (113) yield a pure galactan which on methylation and hydrolysis gave 2:3:6-tri-O-methyl-D-galactose and a small amount of 2:3:4:6-tetra-O-methyl-D-galactose indicating a straight chain of 1:4 linked galactopyranosyl residues. A similar polysaccharide has been obtained from the seeds of *Strychnos nux-vomica* (114) although this galactan may be branched to a small extent through C atoms 1, 3 and 6.

Polysaccharides containing galactose and arabinose have been extracted from the various larch trees and from one or two other woods. The polysaccharides, which are called \( \alpha \)-galactans, have similar optical rotations and proportions of monosaccharides but seem to vary in their more detailed molecular structures. The galactans from European larch (*Larix decidua*) and from Western larch (*Larix occidentalis*) have been most extensively examined.

Campbell, Hirst and Jones (115) found that European larch \( \alpha \)-galactan gives on complete hydrolysis D-galactose and L-arabinose in the approximate ratio of 6:1, and stated that the methylated material could be separated into two components, one a methylated arabogalactan and the other a methylated pure galactan. The latter, on complete hydrolysis gave approximately 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 indicating a highly branched galactan with 1:3 and 1:6 linkages. In support of this evidence of heterogeneity Mosiman and Svedberg (116) found ultracentrifugal measurements indicating the presence of two distinct components in a sample of European /
European larch $\xi$-galactan.

However in more recent work Aspinall, Hirst and Ramstad (117) could find no evidence of heterogeneity and ultracentrifugal examination showed only one component in their sample. Heidelberg (118) also found no evidence of more than one molecular species in studies of precipitation reactions of the polysaccharide with various pneumococcal sera.

From partial hydrolysis of the European larch $\xi$-galactan in the mild conditions of 0.01 N-sulphuric acid at 100°, Jones (119) isolated a disaccharide which he characterised as being 5-0-\(\beta\)-D-arabopyranosyl-L-arabinose. Attempts, however, to remove all the arabinose residues (117) in mild acid conditions to give a degraded gum have been unsuccessful. Partial hydrolysis under severer conditions yielded two disaccharides 5-0- and 6-0-\(\beta\)-D-galactopyranosyl-D-galactoses and three trisaccharides which have the following probable structures:

\[
\text{Gal 1 - 3 Gal 1 - 5 Gal} \quad \text{Gal 1 - 6 Gal 1 - 6 Gal} \quad \text{Gal 1 - 5 Gal}
\]


Since the amounts of the 2:3:4-tri-0-methyl-L-arabinose and 2:5/
2:5-di-β-methyl-L-arabinose are almost equal and since the
disaccharide 3-β-β-L-arabopyranosyl-L-arabinose has been isolated the
main grouping of the arabinose residues in the molecule is Arap 1-3Araf 1.

Ignoring for the moment the arabinose grouping and the two
smaller amounts of methyl ethers of galactose, various possible structures
can be put forward, on the basis of methylation results, for the main
repeating unit of the polysaccharide. Some are shown below:

\[
\begin{align*}
3 \text{Gal} & - 3 \text{Gal} 1 \ldots & 6 \text{Gal} 1 & - 6 \text{Gal} 1 \ldots \\
6 & 6 & 3 & 3 \\
1 & 1 & 1 & 1 \\
\text{Gal} & \text{Gal} & \text{Gal} & \text{Gal} \\
6 & 6 & 6 & 6 \\
1 & 1 & 1 & 1 \\
\text{Gal} & \text{Gal} & \text{Gal} & \text{Gal}
\end{align*}
\]

(VI) (VII)

\[
\begin{align*}
3 \text{Gal} & - 6 \text{Gal} 1 - 3 \text{Gal} 1 \ldots & 6 \text{Gal} 1 & - 6 \text{Gal} 1 - 3 \text{Gal} 1 \ldots \\
6 & 6 & 6 \\
1 & 1 & 1 \\
\text{Gal} & \text{Gal} & \text{Gal}
\end{align*}
\]

(VIII)

\[
\begin{align*}
6 \text{Gal} 1 & - 6 \text{Gal} 1 - 6 \text{Gal} 1 & - 6 \text{Gal} 1 & - 6 \text{Gal} \\
3 & 3 & 3 \\
1 & 1 & 1 \\
\text{Gal} & \text{Gal} & \text{Gal}
\end{align*}
\]

(IX)

Evidence in favour of structure (VI) was obtained from periodate
oxidation studies and subsequent degradation by Barry's method (26).
The remaining polysaccharide on partial hydrolysis gave 3-β-β-D-
galactopyranosyl-D-galactose with only a small amount of the 1:6 linked
isomer.
isomer. Only a main repeating unit of structure (VI) conforms to these results. The groupings of:

\[
\text{Arap} \, 1 - 5 \text{ Araf} \, 1 -
\]

will be attached to some of the galactose residues while some galactose residues will be doubly linked through C atoms 1 and 3 and others will be branched through C atoms 1, 5, 4 and 6. On this evidence European larch \( \alpha \)-galactan is strikingly similar in structure to gum arabic (24, 27-54) and \textit{Acacia pycnantha} gum (23,35) in having a 1:3 linked backbone and 1:6 linked side chains of galactopyranose residues.

Western larch \( \alpha \)-galactan, which gives roughly the same optical rotation and 6:1 ratio of galactose and arabinose as European larch \( \alpha \)-galactan, has been extensively examined by White (120) and later by Bouveng and Lindberg (121,122).

White found that the arabogalactan on methylation and hydrolysis gave the following sugars:

- \(2:5:4:6\)-tetra-\(O\)-methyl-D-galactose (2 moles)
- \(2:5:4\)-tri-\(O\)-methyl-D-galactose (1 mole)
- \(2:4\)-di-\(O\)-methyl-D-galactose (3 moles)
- \(2:5:5\)-tri-\(O\)-methyl-L-arabinose (1 mole)

In addition, from the partial hydrolysis of the methylated gum he obtained two disaccharides octyl-methyl-(6-\(O\)-D-galactopyranosyl-D-galactose) and heptyl-methyl-(6-\(O\)-D-galactopyranosyl-D-galactose) the latter having the free hydroxyl group at C atom 3 of the reducing end group. On this evidence White stated that the following three groupings /
groupings occur in the molecule:

\[
\text{Gal}_1 - 6 \text{Gal}_1 - \text{Gal}_1 - 6 \text{Gal}_1 - \text{Araf}_1 - \frac{1}{3}
\]

Next the polysaccharide was partially hydrolysed and then methylated. From the hydrolysis products of this partly degraded methylated polysaccharide, White found evidence of the arabofuranose residues being linked to C atom 6 of galactose residues also linked through carbon atoms 1 and 3:

\[
\text{Araf}_1 - 6 \text{Gal}_5 - \frac{1}{3}
\]

and suggested that the arabogalactan had a 1:3 linked backbone of galactopyranose residues.

Later work has complicated the above picture. Bouveng and Lindberg (121) isolated two disaccharides which they characterised as 6-O-\(\beta\)-D-galactopyranosyl-D-galactose and 3-O-\(\beta\)-L-arabopyranosyl-L-arabinose. No evidence of end group arabopyranose units was found in White's methylation results.

In a later publication Bouveng and Lindberg (122) gave evidence of heterogeneity. Electrophoresis on glass fibre paper showed two components and separation on a larger scale using cetyltrimethylammonium hydroxide and boric acid gave two polysaccharides of similar compositions and optical rotations but of different molecular weights.

The higher molecular weight material was methylated and hydrolysed,
hydrolysed and the results show that the arabogalactan has a framework of 1:3 and 1:6 linked galactopyranose residues with terminal groupings of:

\[
\text{Arap} 1 - 3 \text{Araf} 1 -
\]

and \[
\text{Araf} 1 -
\]

White spruce (\textit{Picea glauca}) \(\xi\)-galactan has been examined by Adams (123) and no evidence of heterogeneity has been found from electrophoresis or chemical fractionation studies. The material, on complete hydrolysis, gave D-galactose and L-arabinose in the usual 6:1 ratio but the optical rotation \((\lbrack \alpha \rbrack_D = -7^\circ)\) was lower than normal. Traces of a xylan are thought to account for this. In addition there is present about 5\% uronic acid and this is probably present in association with the xylose as 2-O-(4-O-methyl-\(\alpha\)-D-glucuronosyl)-D-xylose and probably O-(4-O-methyl-\(\alpha\)-D-glucuronosyl)-(1 \(\rightarrow\) 2)-O-\(\beta\)-D-xylopyranosyl-(1 \(\rightarrow\) 4)-D-xylopyranose have been isolated.

Methylation results indicate that the arabinose is present as end group in the furanose form only, and that the galactan framework has 1:3 and 1:6 linkages predominating.

The same type of structure seems to occur in jack pine (\textit{Pinus banksiana Lamb}) according to methylation studies carried out by Bishop (124). The material has been shown to have a molar ratio of arabinose to galactose of 1:13 and again appears to be homogeneous.

Jeffrey pine (\textit{Pinus jeffreyi}) gives an arabogalactan (125) which contains more arabinose than normal, the ratio with galactose being /
being 4:5. Methylation studies of the material show that the arabinose can be doubly linked, as well as occurring as terminal arabofuranose units, and that the galactose can be linked

\[
\begin{array}{c|c|c|c}
\text{6} & \text{1 Gal} & \text{1 Gal} & \text{1 Gal} \\
\text{4} & \text{3} & \text{3} & \\
\end{array}
\]

Little end group galactose occurs.

Thus the hemicellulose arabogalactans so far examined have the same component monosaccharides D-galactose and L-arabinose usually in roughly the ratio of 6:1, and similar optical rotations. Their molecular structures are alike in that they have frameworks of galactopyranose residues in which 1:3 and 1:6 are the main linkages, with arabinose attached as end group in the furanose form or as \text{Arap} 1 - 3 \text{Araf} 1 -, and in these features they resemble the gums of the \text{Acacia} and \text{Prunus} trees.
SECTION I

THE MOLECULAR STRUCTURE OF ACACIA EYCHANTHA GUM

EXPERIMENTAL

GENERAL METHODS

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) Butan-1-ol : ethanol : water (4:1:5, upper layer).
(C) Ethyl acetate : acetic acid : water (3:1:3, upper layer).
(D) Butan-2-one, half saturated with water.

Unless otherwise stated, papers were sprayed with a saturated solution of aniline oxalate and developed at 120° for 2-3 minutes.

Also unless otherwise stated, chromatography of the methylated sugars was carried out in solvent (E), and Rf values refer to the rate of movement relative to 2:3:4:6-tetra-O-methyl-D-glucose in that solvent.

Thick paper sheets (Whatman 3MM), used for chromatographic separation of sugars, were first extracted with methanol in a Soxhlet.

Paper ionophoresis (126) was carried out using borate buffer at pH 10. After running for 4-5 hours at a potential of 500 volts, the papers were sprayed with aniline oxalate containing 5% glacial acetic acid.

Methoxyl determinations were carried out by means of the semi-micro Zeisel method (127,128).

Optical rotations were observed at 18 ± 2°.
Evaporation were carried out under reduced pressure at temperatures below 40°.

Small scale hydrolysis were carried out by heating at 100° samples of the material (ca. 25 mg.) with acid (1 ml.) of the given normality, in sealed glass tubes. Using sulphuric acid, neutralisation was effected with analar barium carbonate. The barium ions were removed with Amberlite resin IR-120(H) and the solution taken to dryness.

Hydrochloric acid hydrolysates were neutralised with silver carbonate and the silver ions removed by treatment with hydrogen sulphide. The filtrates were then taken to dryness, the organic material extracted from the residues with hot acetone and the extracts taken to dryness.

The hydrolysates were then ready for chromatographic examination.

The cellulose columns were packed dry, and washed with water and the solvents to be used for separation of the sugars. The solvents were purified as below.

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

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

Butan-2-one was distilled.

The solvent was allowed to soak in at the top of the column, and the sugar mixture added in the minimum amount of eluant. A thin layer of cellulose powder was added to the top of the column and solvent (5-25 ml.) added and allowed to soak in thoroughly.
The sugars were then eluted from the column using the given eluants and the eluate was collected on an automatic turntable in fractions of a suitable volume (5-20 ml.). A small sample (2 ml.) from every fifth tube was taken to dryness and examined chromatographically for sugars. Tubes containing the same sugars were combined, and the resulting solutions were evaporated to dryness. The residues were extracted with warm acetone: water, and filtered. The filtrates were taken to dryness, dried over phosphorus pentoxide and weighed.

**Demethylations** (129) were carried out by heating the sugar (ca. 5 mg.) with hydriodic acid (1 ml.) in a sealed glass tube for 5 minutes at 100°. The solution was then diluted with water and neutralised with silver carbonate. Silver ions were removed with hydrogen sulphide and the filtrates taken to dryness.

**Aniline derivatives** of sugars were prepared by refluxing the sugars (10-50 mg.) with equimolecular amounts of freshly distilled aniline in dry ethanol (5 ml.) for 20 minutes with the exclusion of light. The syrups obtained on the removal of the solvent crystallised and were re-crystallised from the given solvents.

**Preparation of Aldonolactones.** The sugar (10-50 mg.) was oxidised with an excess of bromine water for one day. Excess bromine was removed by aeration and the solution neutralised with silver carbonate, treated with hydrogen sulphide and evaporated to dryness. The organic material was extracted with hot acetone: water and recrystallised from the given solvents.
Preparation of Aldonamides. The lactone (5-50 mg.) was treated with dry methanolic ammonia (5 ml.) at 0° for 1 day. The solvent was then evaporated off and the resulting amide recrystallised from the given solvents.

Charcoal : celite columns were used to separate oligosaccharides from monosaccharides. The charcoal was washed with water and mixed with an equal weight of celite, which had previously been heated with concentrated hydrochloric acid : water (1:1) and washed free of acid. The mixture was packed as a slurry into columns and washed with water.

The mixture of mono- and oligosaccharides was allowed to soak into the charcoal : celite and the monosaccharides were removed by elution with water. Elution with the given concentration of aqueous ethanol removed the oligosaccharides. The eluates were found to be slightly acid and so were treated with Amberlite resin IR-4B(OH) or, in the case of acid oligosaccharides with a little barium carbonate, evaporated to small volume, filtered and the filtrates taken to dryness.

Periodate oxidation of methylated sugars (130). The methylated sugar (1-2 mg.) was treated with sodium metaperiodate (0.5N : 0.2 ml.) at 0° for one hour. The excess of periodate was destroyed by the addition of ethylene glycol (1 drop), and the solution allowed to come to room temperature. It was then reduced to small volume and examined chromatographically using solvent (B).

The following results were obtained:

Sugar /
<table>
<thead>
<tr>
<th>Sugar</th>
<th>Oxidation products</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:4-di-O-methyl-D-galactose</td>
<td>0.37 pink (unchanged 2:4-di-O-methyl-D-galactose)</td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-galactose</td>
<td>0.15 yellow (methoxymalondialdehyde)</td>
</tr>
<tr>
<td>2 methyl pentoses</td>
<td>0.15 yellow (methoxymalondialdehyde)</td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-galactose</td>
<td>0.67 grey</td>
</tr>
<tr>
<td></td>
<td>0.78 brown</td>
</tr>
<tr>
<td></td>
<td>0.86 grey</td>
</tr>
</tbody>
</table>
Preliminary

The gum exuded by *Acacia pycnantha* studied in this work, had previously been examined by Hirst and Perlin (23). They found that the gum is composed of residues of D-galactose (65%), L-arabinose (27%), L-rhamnose (12%) and D-glucuronic acid (5%). Partial hydrolysis afforded the aldobiouronic acid 6-O-β-D-glucuronosyl-D-galactose and 3-O-β-D-galactopyranosyl-D-galactose, both of which were characterised. Further a sample of the gum was methylated.

**Electrophoresis on glass fibre paper (16,17,18).**

The unmethylated *Acacia pycnantha* gum was examined electrophoretically on glass fibre paper, the runs being carried out using 2N-potassium hydroxide as electrolyte at 230 volts and 300 milliamperes for about 6 hours. The papers were dried at 100° and sprayed (131) with a solution of potassium permanganate in Na-sodium hydroxide (0.5% w./v.). The gum was found to be essentially homogeneous, the material travelling about 5 cm. A possible second spot (< 1% of total) remained on or near the starting line. However in another sample of *Acacia pycnantha* gum obtained from Dr. Frahn of the Commonwealth Scientific and Industrial Research Organisation a definite minor component (ca. 5% of total) was detected travelling about 0.5 cm. from the starting line.
PARTIAL ACID HYDROLYSIS OF AGACIA EYGMANTHA GUM

Trial Graded Hydrolysis

(a) At 100° The gum (200 mg.) was heated with 0.01N-sulphuric acid (25 ml.) at 100° and samples (5 ml.) were withdrawn at intervals. Each sample was cooled, neutralised with Amberlite resin IR-4B(OH), taken almost to dryness, and poured on to a small charcoal - celite column (1:1, 5g.).

<table>
<thead>
<tr>
<th>Time of Heating</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>Elution with water and subsequent chromatography of the evaporated eluate showed arabinose, a small amount of rhamnose and a trace of pink material which had $R_{Gal.} = 1.4$ in both solvents (C) and (D). Elution with 15% ethanol in water gave a small amount of the same unidentified sugar.</td>
</tr>
<tr>
<td>2 hours</td>
<td>The sugar was present in even smaller quantities in both the water and 15% ethanol eluates in addition to the arabinose and rhamnose.</td>
</tr>
<tr>
<td>4, 6 and 8 hours</td>
<td>The water and 15% ethanol eluates showed arabinose, rhamnose and a trace of galactose, but the unidentified sugar had now disappeared, suggesting that it was a pentose containing disaccharide.</td>
</tr>
</tbody>
</table>

(b) At 85° The above procedure was repeated with the gum (200 mg.) being heated at 85° with 0.01N-sulphuric acid (25 ml.). The samples were removed at $\frac{1}{2}$, 1, 4, 7$\frac{1}{2}$ and 14 hours.

Elution /
Elution with water showed arabinose and rhamnose increasing in amounts with time of heating, with a trace of galactose appearing after 7\(\frac{1}{2}\) hours.

Also present in all except the 14 hour eluate was a small amount of the above pink spot. It was present in maximum concentration at 4 hours. Elution with 15% ethanol showed the unknown sugar present at \(\frac{1}{2}\) hour, increasing to a maximum at 4 hours and fading away with further heating, the 7\(\frac{1}{2}\) hour eluate showing only a faint trace and the 14 hour none.

**Isolation of 3-O-L-arabofuranosyl-L-arabinose**

Whole gum (5g.) was dissolved in 0.01N-sulphuric acid (150 ml.) and the solution heated at 85° for 4 hours. The solution was then cooled, neutralised with Amberlite resin IR-4B(OH), concentrated to 50 ml. and alcohol (3 volumes) added. The precipitated degraded polysaccharide was centrifuged off and washed several times with alcohol.

The hydrolysis was repeated using a further 5 g. of whole gum, and the centrifugates and all washings combined and taken to dryness. **Yield = 2.2 g.**

Chromatography in solvents (C) and (D) showed galactose, arabinose, rhamnose and a quantity of the unidentified sugar. Small amounts of several other slower moving sugars were also detected.

The sugars of the hydrolysate were separated by partition chromatography on a cellulose column (2.8 x 80 cm.) using solvent (D) as eluant. Three fractions were collected.

*Fraction /*
**Fraction 1.** (1.7 g.) Chromatography in solvent (C) showed rhamnose, a large amount of arabinose and a trace of the suspected disaccharide.

**Fraction 2.** (0.105 g.) This contained the disaccharide with slight traces (ca. 1%) of galactose and arabinose.

**Fraction 3.** (0.070 g.) Chromatographic examination in solvent (C) showed a trace of the disaccharide and several slower moving sugars (including galactose) in small amounts. These were in too small quantities to be further examined.

**Examination of Fraction 2**

\[ [\alpha]_D^C = +89^\circ \quad (c = 1.0 \text{ in water}) \]

of 3-O-L-arabofuranosyl-L-arabinose (132)

\[ [\alpha]_D = +94^\circ \pm 4^\circ \quad (c = 0.5 \text{ in water}) \]

Hydrolysis gave arabinose alone.

**Estimation of Formaldehyde Release after Periodate Oxidation** (133, 134)

Fraction 2 syrup (10 mg.) was oxidised with sodium metaperiodate and the formaldehyde release estimated by complexing with dinitrophenyl. A further sample of the sugar (10 mg.) was reduced using potassium borohydride, periodate oxidised and the formaldehyde release determined. In the first case approximately 1 mole of formaldehyde per mole of sugar was liberated and in the second case 1.54 moles of formaldehyde per mole of sugar. This corresponds to an oligosaccharide with the reducing end group /
group arabinose linked through C atom 3.

The sugar (10 mg.) was dissolved in water (2.0 ml.) and N-sodium bicarbonate (2.0 ml.) and sodium metaperiodate solution (0.3M : 2.0 ml.) added. The solution was mixed and allowed to stand at room temperature for 1 hour. Next were added in turn, with thorough mixing, N-hydrochloric acid (3.0 ml.) and sodium arsenite solution (1.2 N : 2.0 ml.). When the precipitate and yellow colour were completely away sodium acetate (M : 2.0 ml.) and dimesdone reagent (1 ml. : 80 mg. 5.5 dimethyldihydroresorcinol per 1 ml. 95% ethanol) were added. The solution was heated on a boiling water bath for 10 minutes and stood at room temperature for 1 hour giving a crystalline precipitate. This was filtered off, dried at 85-90° for 20 minutes and weighed. The crystals melted sharply at 189-190° and mixed with dimesdone gave a depressed melting point. The formaldehyde release was calculated and came to 0.87 moles per mole of sugar.

A second sample of the sugar (10 mg.) was dissolved in water (2 ml.), potassium borohydride (20 mg.) added, and the solution left standing /
standing overnight. The excess of borohydride was destroyed with glacial acetic acid and the formaldehyde release after periodate oxidation estimated in situ giving 1.54 moles per mole of sugar.

Methylation and Hydrolysis of Fraction 2.

The sugar was finally characterised by methylation, hydrolysis and the isolation of 2:3:5-tri-O-methyl-L-arabinose, 2:5-di-O-methyl-L-arabinose and 2:4-di-O-methyl-L-arabinose. The two dimethyl arabinoses correspond to the reducing end of the disaccharide being in equilibrium between the pyranose and furanose forms in solution (X).

\[
\begin{align*}
\text{HO} & \\
\text{OH} & \\
\text{CH}_2\text{OH} & \\
\text{CH}_2\text{OH} & \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \\
\text{OH} & \\
\text{H} & \\
\end{align*}
\]

\[
(X)
\]

Fraction 2 syrup (80 mg.) was dissolved in water (8 ml.) and dimethyl sulphate (2 ml.) and sodium hydroxide (40% : 3 ml.) added dropwise at 0°C. The solution was stirred throughout the entire methylation. Next were added, again dropwise, dimethyl sulphate (12 ml.) and sodium hydroxide (40% : 22 ml.) over a period of 2 hours. The reaction mixture was allowed to come to room temperature (2 hours) and two further additions of dimethyl sulphate (6 ml.) and sodium hydroxide (40% : 9 ml.) /
(40% : 9 ml.) made, each over an hour. The mixture was then heated on a boiling water bath for 1 hour causing precipitation of sodium sulphate which was filtered off, dried and extracted with chloroform. The filtrate was extracted with chloroform in a continuous extractor for 16 hours and the combined extracts taken to dryness yielded the methylated disaccharide (80 mg.). Trial hydrolysis of the product (3 mg.) with N-sulphuric acid, on a boiling water bath for 4 hours showed, on chromatography of the hydrolysate in solvent (B) spots corresponding in Rₕ and colour to 2:3:5-tri-O-methyl-L-arabinose, 2:5-di-O-methyl-L-arabinose, 2:4-di-O-methyl-L-arabinose and traces of monomethyl arabinose.

The remainder of the methylated material was hydrolysed under the same conditions, and taken to dryness. The syrup (68 mg.) was separated on filter sheets using solvent (B) giving three fractions.

Fraction (a)      (0.020 g.)      Rₕ = 0.98

The sugar was chromatographically pure corresponding to 2:3:5-tri-O-methyl-L-arabinose and was characterised by conversion to the aldonic acid m.p. 132 - 133° undepressed in mixed m.p. with an authentic specimen of 2:3:5-tri-O-methyl-L-arabinose.

Fraction (b)      (0.010 g.)      Rₕ = 0.84

Chromatography in solvent (B) showed one spot corresponding to 2:5-di-O-methyl-L-arabinose. The sugar was characterised by conversion to the aldonic acid m.p. 123 - 124° and undepressed on admixture with authentic 2:5-di-O-methyl-L-arabinose.
Fraction (c) (0.014 g.) \( R_g = 0.65 \)

The fraction was chromatographically pure corresponding to 2:4-di-O-methyl-L-arabinose, and was characterised by conversion to the aniline derivative having m.p. = 131\(^\circ\)C undepressed in mixed m.p. with authentic 2:4-di-O-methyl-N-phenyl-L-arabinosylamine.

**PREPARATION OF DEGRADED ACACIA ECHINANTHA GUM**

**Graded hydrolysis with 0.01N-sulphuric acid**

Polysaccharide (1 g.) was heated with 0.01N-sulphuric acid (100 ml.) on a boiling water bath. Every hour a sample was withdrawn and the optical rotation taken.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\alpha]_D)</td>
<td>-2°</td>
<td>-6°</td>
<td>-2°</td>
<td>+6°</td>
<td>+10°</td>
<td>+14°</td>
<td>+17°</td>
<td>+20°</td>
<td>+23°</td>
<td>+29°</td>
<td>+35°</td>
<td>+39°</td>
</tr>
</tbody>
</table>

Every second hour, a sample (5 ml.) was withdrawn, neutralised with barium carbonate, filtered, deionised with Amberlite resin IR-120(H) and 3 volumes of ethanol added.

The partially hydrolysed polysaccharide precipitated from each sample was removed by centrifugation and washed thoroughly with ethanol to remove free sugars. It was then completely hydrolysed with N-sulphuric acid. The hydrolysates were neutralised and evaporated to syrups which were examined chromatographically using solvent (A).

The filtrate and ethanol washings from each sample were concentrated and examined on paper chromatograms again using solvent (A).
The arabinose was found to be almost completely removed from the molecule after 5-6 hours, and the rhamnose after 14 hours. Galactose, the least labile of the three component neutral sugars, appeared in the hydrolysate in small amounts after about 6 hours. No galactose containing oligosaccharides were detected in solution in these experiments.

**Large scale Hydrolysis**

Polysaccharide (29 g.) was heated with 0.01 N-sulphuric acid (1,500 ml.) on a boiling water bath. The optical rotation was followed until it reached the value corresponding to the complete removal of rhamnose and arabinose (14 hours).

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\alpha]_D$</td>
<td>-8°</td>
<td>-4°</td>
<td>+5°</td>
<td>+14°</td>
<td>+26°</td>
<td>+30°</td>
<td>+35°</td>
<td>+38°</td>
</tr>
</tbody>
</table>

The solution was then cooled, neutralised with Amberlite resin IR-4B(OH), concentrated to 500 ml., and poured into 4 volumes of ethanol to give a polysaccharide which was thoroughly washed with ethanol to remove free sugars. The precipitate was removed by centrifugation and dried in vacuo. Yield = 15 g.

This degraded *Acacia pycnantha* gum was, like the whole gum a white powder having $[\alpha]_D = +20°$ ($c = 1.2$ in water), and on complete hydrolysis with N-sulphuric acid gave galactose and a trace of arabinose.

**METATILATION STUDIES**
METHYLATION STUDIES

Methylation of Degraded Gum

Degraded polysaccharide (10 g.) was dissolved in water (100 ml.) and methylated with dimethyl sulphate (100 ml.) and sodium hydroxide (160 ml. : 40%). The reagents were added dropwise with vigorous stirring over a period of 6 hours. The reaction mixture was kept in ice under an atmosphere of nitrogen and left stirring overnight. Six further batches of reagents were added over a period of 6 days. At the sixth addition acetone was added to keep the partially methylated material in solution.

The solution was neutralised with sulphuric acid, and the methylated product extracted from the aqueous solution with chloroform, and dried over anhydrous sodium sulphate. Most of the chloroform was removed under reduced pressure and the methylated degraded gum precipitated as a white powder by the addition of 5 volumes petroleum ether.

Yield = 6.1 g.  OMe = 40.3%

Partially methylated polysaccharide (3 g.) was dissolved in refluxing methyl iodide (100 ml.) and silver oxide (4 g.) added over a period of 4 hours. The reaction mixture was kept under reflux for a further 4 hours. The solvent was then filtered off and the residue exhaustively extracted with boiling chloroform. All the filtrates were combined, concentrated, and the polysaccharide precipitated with petroleum ether. This procedure was repeated on the remaining partially methylated polysaccharide (3 g.).

Total Yield = 4.0 g.  OMe = 43.9%  \[\alpha\]_D = -38.2° (c = 1.0 in chloroform)
The methylation with methyl iodide and silver oxide was repeated and the final product was a white powder.

Yield = 3.1 g. OMe = 45.0%  [α]_D = -35° (c = 1.0 in chloroform)

Hydrolysis of Methylated Degraded Gum

The methylated degraded gum (2.9 g.) was suspended in N-hydrochloric acid (300 ml.) at 40° for 3 days. The solution was then heated at 100° until the rotation was constant (16 hours), cooled, neutralised with silver carbonate, and, after the removal of silver ions, as silver sulphide, treated with barium carbonate, filtered and concentrated to a syrup.  

Yield = 2.4 g.

Examination of Hydrolysis Products of Methylated Degraded Gum

The methylated sugars (2.4 g.) obtained from the hydrolysis of the methylated degraded gum were separated by chromatography on a cellulose column (90 x 3.5 cm.)

The sugars were eluted, first with light petroleum (b.p. 100 - 120°); butan-1-ol (1:1) saturated with water and then, after the dimethyl hexoses had come through, with butan-1-ol half saturated with water. When all the neutral sugars were removed, the column was washed with water (2 l.) to elute uronic acids.

The tubes were bulked, in the normal way, into fractions which were examined chromatographically in solvent (B). The probable sugar content of the fractions from this preliminary examination is tabulated below.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tubes</th>
<th>Weight</th>
<th>Colour</th>
<th>$R_g$</th>
<th>Sugars Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1-85</td>
<td>0.041</td>
<td>Grey green</td>
<td>1.01</td>
<td>2:3:4-tri-methyl rhamnose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grey</td>
<td>0.98</td>
<td>2:3:5-tri-methyl-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red/brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td>2.</td>
<td>86-105</td>
<td>0.525</td>
<td>Red/brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td>3.</td>
<td>106-139</td>
<td>0.056</td>
<td>Red/brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grey</td>
<td>0.84</td>
<td>dimethyl arabinose</td>
</tr>
<tr>
<td>4.</td>
<td>140-190</td>
<td>0.010</td>
<td>Red/brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red/brown</td>
<td>0.73</td>
<td>trimethyl galactose</td>
</tr>
<tr>
<td>5.</td>
<td>191-256</td>
<td>0.583</td>
<td>Red/brown</td>
<td>0.73</td>
<td>trimethyl galactose</td>
</tr>
<tr>
<td>6.</td>
<td>257-446</td>
<td>0.134</td>
<td>Red/brown</td>
<td>0.73</td>
<td>trimethyl galactose</td>
</tr>
<tr>
<td>7.</td>
<td>447-513</td>
<td>0.056</td>
<td>Red/brown</td>
<td>0.54</td>
<td>2:6-dimethyl galactose</td>
</tr>
<tr>
<td></td>
<td>514-533</td>
<td>0.223</td>
<td>Red/brown</td>
<td>0.54</td>
<td>2:6-dimethyl galactose</td>
</tr>
<tr>
<td></td>
<td>534-544</td>
<td>0.212</td>
<td>Red/brown</td>
<td>0.47</td>
<td>2:4-dimethyl galactose</td>
</tr>
<tr>
<td></td>
<td>545-555</td>
<td>0.047</td>
<td>Red/brown</td>
<td>0.47</td>
<td>2:4-dimethyl galactose</td>
</tr>
<tr>
<td></td>
<td>556-565</td>
<td>0.049</td>
<td>Brown</td>
<td>0.29</td>
<td>2-methyl galactose</td>
</tr>
<tr>
<td>11.</td>
<td>566-598</td>
<td>0.013</td>
<td>Brown</td>
<td>0.29</td>
<td>2-methyl galactose; arabinose and galactose</td>
</tr>
<tr>
<td>12.</td>
<td></td>
<td>0.165g</td>
<td></td>
<td></td>
<td>Hexamethyl ether of 6-2-β-D-glucuronosyl-2-galactose</td>
</tr>
<tr>
<td>13.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EXAMINATION OF FRACTIONS


0.041 g. \( R_G = 1.01, 0.98 \) and \( 0.89 \)
\[ [\alpha]_D = + 140^\circ (c = 0.40 \text{ in water}) \]

Paper chromatography in solvents (B) and (D) showed this fraction to be a mixture of the above three sugars with the trimethyl ether of arabinose predominating.

Demethylation gave rhamnose, arabinose and galactose.

Fraction 2. 2:3:4:6-tetra-O-methyl-D-galactose

0.525 g. \( R_G = 0.89 \)
\[ [\alpha]_D = + 117^\circ (c = 1.3 \text{ in water}) \]

The syrup gave, on paper chromatograms run in solvents (B) and (D), one spot corresponding in \( R_G \) and colour to 2:3:4:6-tetra-O-methyl-D-galactose. Seeded with a crystal of this sugar, the fraction crystallised out and, after recrystallisation from ether: light petroleum had m.p. 68\(^\circ\) undepressed on admixture with an authentic specimen of 2:3:4:6-tetra-O-methyl-D-galactose.

Recrystallised material had: \( [\alpha]_D = + 140^\circ \rightarrow + 117^\circ (c = 1.0 \text{ in water}) \)
\[
\text{CMe} = 52.2\% \text{ (calculated for } C_{10}H_{20}O_6, 52.5\%) \]

The material was characterised by conversion to the aniline derivative m.p. 194\(^\circ\), undepressed in mixed m.p. with authentic 2:3:4:6-tetra-
tetra-O-methyl-N-phenyl-D-galactosylamine.

Fraction 3. 2:3:4:6-tetra-O-methyl-D-galactose and dimethyl arabinose

0.056 g.  \( R_G = 0.89 \) and 0.84

\([\alpha]_D^o = +90.2^o \) (\( c = 1.1 \) in water)

Chromatography of the colourless syrup showed 2:3:4:6-tetra-O-methyl-D-galactose and a small amount of a slower moving material corresponding in colour (dark grey: pink in ultraviolet fluorescence) to 2:5-, and in \( R_G \) to 2:5- and 3:5-di-O-methyl-L-arabinoses.

Demethylation gave galactose and a small amount of arabinose.

The main component was characterised by conversion to 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine  m.p. 191°.

Fraction 4. 2:3:4:6-tetra-O-methyl-D-galactose and trimethyl galactose

0.010 g.  \( R_G = 0.89 \) and 0.73

\([\alpha]_D^o = +110^o \) (\( c = 0.2 \) in water)

Paper chromatography showed that this fraction was a mixture of the above sugars.

Demethylation gave only galactose.

Fraction 5. 2:4:6- (and 2:3:4-)tri-O-methyl-D-galactoses

0.583 g.  \( R_G = 0.73 \)

\([\alpha]_D^o = +128^o \rightarrow +94^o \)

Paper /
Paper chromatography gave one spot corresponding to 2:3:4 and/or 2:4:6-tri-O-methyl-D-galactoses. The fraction crystallised out and the crude crystals were recrystallised from acetone : ether : light petroleum (1:1:1) giving crystals m.p. 101° undepressed on admixture with authentic 2:4:6-tri-O-methyl-D-galactose and depressed to 71°-80° with 2:3:4-tri-O-methyl-D-galactose.

Recrystallised material had: \([\alpha]_D = +120° \rightarrow +90°\) (c = 0.71 in water)  
\(\text{OMe} = 41.8\%\) (calculated for C\(_9\)H\(_{18}\)O\(_6\), 41.9\%)

The aniline derivative was prepared in the normal manner and gave needle shaped crystals. Recrystallised from acetone : ether : light petroleum (1:1:1) these gave m.p. 164°-165° undepressed on admixture with authentic 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine and depressed in mixed m.p. with 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine.

The mother liquor was left for several weeks but no crystals of the 2:3:4-trimethyl ether aniline derivative (characteristically plate shaped) formed.

The formaldehyde release (p. 83) on periodate attack was estimated, and corresponded to the presence of 8\% of 2:3:4-tri-O-methyl-D-galactose and 92\% of 2:4:6-tri-O-methyl-D-galactose in the fraction.

Fraction 6. 2:4:6- and 2:5:4-tri-O-methyl-D-galactoses and (probably) 2:3-di-O-methyl-L-arabinose

0.134 g.  \(R_G = 0.75\) and 0.67  
\([\alpha]_D = +103.4°\) (c = 1.2 in water)
This fraction was obtained as a colourless syrup, which failed to crystallise. Paper chromatography showed tri-\text{-}O\text{-}methyl-D-galactose and a slower moving pink spot (pink also under ultraviolet) corresponding in colour and $R_f$ to 2:3-di-\text{-}O\text{-}methyl-L-arabinose in the ratio of about 10:1.

Demethylation gave galactose and a little arabinose.

The syrup was converted to the aniline derivative giving needle shaped crystals. These were recrystallised from ethanol and had m.p. 162$^\circ$ - 163$^\circ$ not depressed on admixture with authentic 2:4:6-tri-\text{-}O\text{-}methyl-N-phenyl-D-galactosylamine and depressed in mixed m.p. with 2:3:4-tri-\text{-}O\text{-}methyl-N-phenyl-D-galactosylamine. The mother liquor, after a week, yielded a second small crop of crystals, which, under the microscope, showed up to be a mixture of plates and needles. Attempts to separate these by recrystallisation were unsuccessful. The melting point was found to be depressed to 146$^\circ$.

The high value for the rotation and the mixed crop of crystals in the aniline derivative suggests that the fraction contained both the 2:3:4- and 2:4:6-tri-\text{-}O\text{-}methyl-D-galactoses in addition to a small amount of what is probably 2:3-di-\text{-}O\text{-}methyl-L-arabinose.

The formaldehyde release (p.83) on periodate oxidation was estimated, and, allowing for 10\% of dimethyl arabinose, corresponded to the presence of 35\% of 2:3:4-tri-\text{-}O\text{-}methyl-D-galactose and 55\% of 2:4:6-tri-\text{-}O\text{-}methyl-D-galactose.

Fraction /
Fraction 7. 2:6-di-O-methyl-D-galactose

0.056 g. \( R_G = 0.54 \)

The fraction corresponded to pure 2:6-di-O-methyl-D-galactose in solvents (B) and (D) using both aniline oxalate and p-anisidine hydrochloride as sprays. The sugar crystallised out and, recrystallised from chloroform: light petroleum gave crystals 98°- 99°, undepressed on admixture with authentic 2:6-di-O-methyl-D-galactose monohydrate and depressed to 84°C with 2:4-di-O-methyl-D-galactose.

Recrystallised material had: \([\alpha]_D = +52^\circ \rightarrow +82^\circ \ (c = 0.55 \text{ in water}) \)

\( \text{Ome} = 27.0\% \) (calculated for \( C_{6}H_{18}O_{6}, \text{H}_{2}O, 27.2\% \))

The aniline derivative was prepared and had m.p. = 119° after recrystallisation from ethanol mixed m.p. with 2:6-di-O-methyl-\( \bar{N} \)-phenyl-D-galactosylamine gave no depression.

Fraction 8. 2:4- and 2:6-di-O-methyl-D-galactoses

0.223 g. \( R_G = 0.54 \text{ and } 0.47 \)

\([\alpha]_D = +105^\circ \rightarrow +88^\circ \ (c = 0.88 \text{ in water}) \)

Chromatography in solvents (B) and (D), using aniline oxalate and p-anisidine hydrochloride as sprays showed that this fraction contained the above two sugars in ratio of about 5:1.

Demethylation gave only galactose.

The fraction partially crystallised on keeping in vacuo over \( \text{P}_{2}O_{5} \) for several weeks. The crude crystals were recrystallised from acetone /
acetone containing 1% water giving needles m.p. 101° undepressed on admixture with 2:4-di-O-methyl-D-galactose monohydrate. The main sugar was further characterised by the formation of the aniline derivative m.p. 216° - 217° undepressed on admixture with 2:4-di-O-methyl-N-phenyl-D-galactosylamine.

The remaining mother liquor was periodate oxidised and chromatography gave methoxylmalondialdehyde (R_F = 0.15 : from the 2:6-dimethyl ether), unchanged 2:4-di-O-methyl-D-galactose but no sign of the products characteristic of 2:3-di-O-methyl-D-galactose.

**Fraction 9. 2:4-di-O-methyl-D-galactose**

0.212 g. \[\text{R_g} = 0.47\]

\[\alpha_D^\circ = +116^\circ \rightarrow +35^\circ \quad (c = 1.0 \text{ in water})\]

The sugar was chromatographically pure and identical to 2:4-di-O-methyl-D-galactose in solvents (B) and (D).

Demethylation gave galactose only.

The fraction crystallised out almost immediately. The crude crystals were recrystallised as in fraction 8 giving m.p. 108° undepressed on admixture with 2:4-di-O-methyl-D-galactose monohydrate.

Recrystallised material had: \[\alpha_D^\circ = +123^\circ \rightarrow +35^\circ \quad (c = 0.66 \text{ in water})\]

\[\text{Ode} = 27.1\% \text{ (calculated for C_{18}H_{26}O_3, H_2O, 27.2\%)}\]

The sugar was finally characterised by conversion to the aniline derivative m.p. 218° (after recrystallisation from methanol) undepressed on admixture.
admixture with 2:4-di-Q-methyl-N-phenyl-D-galactosylamine.

**Fraction 10.** 0.047 g.  
\[ R_G = 0.47 \text{ and } 0.29 \]

\[ [\alpha]_D = +85^\circ \quad (c = 0.47 \text{ in water}) \]

Run in solvents (B) and (D), this syrup showed up as 2:4-di-Q-methyl-D-galactose with a small amount of material corresponding to 2-Q-methyl-D-galactose.

Demethylation gave galactose only.

**Fraction 11.** 2-Q-methyl-D-galactose  
0.049 g.  
\[ R_G = 0.29 \]

The fraction was chromatographically pure and corresponded to 2-Q-methyl-D-galactose, in solvents (B) and (D). The material crystallised on standing.

\[ [\alpha]_D = +65^\circ \rightarrow +82^\circ \quad (c = 0.49) \]

Recrystallisation from glacial acetic acid yielded a few crystals m.p. 148° - 149°, not depressed on admixture with an authentic specimen of 2-Q-methyl-D-galactose.

**Fraction 12.** 0.015 g.  

Paper chromatograms run in solvents (A), (B) and (D) showed small amounts of 2-Q-methyl-D-galactose, galactose and arabinose.
Fraction 13. Hexamethyl ether of 6-O-β-D-glucuronosyl-D-galactose 0.166 g.

Chromatography in solvent (B) showed only barium salts on the starting line. The fraction was dissolved in water, shaken up with Amberlite resin IR-120(H) to remove barium ions, filtered and taken to dryness.

Reduction and Hydrolysis of Methylated Aldobiouronic Acid

The dry syrup (108 mg.) was converted to the methyl ester methyl glycoside by refluxing with methanolic hydrogen chloride (3%: 40 ml.) until constant rotation was reached (6 hours). The cooled solution was neutralised with silver carbonate and taken to dryness.

The dried syrup was dissolved in purified tetrahydrofuran (50 ml.), lithium aluminium hydride in tetrahydrofuran (250 mg. in 10 ml.) added dropwise and the resulting solution refluxed for 2 hours. The excess hydride was destroyed by adding water, the organic layer separated and the aqueous layer taken to dryness. The residue was extracted several times with acetone and chloroform, and the extracts combined and taken to dryness under reduced pressure. Yield = 85 mg.

This syrup was hydrolysed with N-hydrochloric acid by refluxing at 100° for 4 hours. The cooled solution was neutralised with silver carbonate and taken to dryness (69.6 mg.). Run in solvent (B) this showed two spots only, corresponding to 2:3:4-tri-O-methyl-D-glucose and 2:5:4-tri-O-methyl-D-galactose.

Separation /


Separation of methylated sugars on cellulose column

The methylated sugars were fractionated on a cellulose column (50 x 2 cm.) using light petroleum (b.p. 100°-120°): ethanol (1:1) saturated with water as eluant, to give two fractions.

Fraction (a) 0.023 g.

This was chromatographically pure corresponding to 2:3:4-tri-O-methyl-D-glucose and characterised by conversion to the aniline derivative m.p. 134°. Mixed m.p. with authentic 2:3:4-tri-O-methyl-N-phenyl-D-glucosylamine gave no depression.

Fraction (b) 0.028 g.

\[[\alpha]_D^D = +116^\circ \quad (c = 0.28 \text{ in water})\]

This fraction was again chromatographically pure corresponding to 2:3:4-tri-O-methyl-D-galactose. It was characterised as the aniline derivative m.p. 169° after recrystallisation from acetone. Mixed m.p. with authentic 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine gave no depression.

HYDROLYSIS OF METHYLATED WHOLE GUM

The whole gum was methylated by Hirst and Perlin (23) giving a product which had: OMe = 40.5% and \[[\alpha]_D^D = -48^\circ \quad (c = 1.0 \text{ in chloroform}).\]

This methylated whole gum (6 g.) was refluxed with methanolic hydrogen chloride (4% : 300 ml.) until the optical rotation was constant (18 hours).
(18 hours). The methanol was removed under reduced pressure and the residue was heated at 100° with 0.5N-hydrochloric acid (300 ml.) to constation rotation (14 hours). The cooled solution was neutralised with silver carbonate, concentrated to a small volume, treated with barium carbonate, filtered and the filtrate evaporated to a syrup. Yield = 5.1 g.

**Separation of Methylated Sugars on a Cellulose Column**

The syrupy mixture of sugars (5.1 g.) was separated on cellulose (90 x 3.5 cm.) which had previously been washed with water, butan-1-ol half saturated with water and light petroleum (b.p. 100°-120°) - butan-1-ol (7:3) saturated with water. The sugars were eluted first with light petroleum - butan-1-ol (7:3) saturated with water and then, after the trimethyl hexoses had passed through the column, with light petroleum - butan-1-ol (1:1) saturated with water. When the dimethyl hexoses had come through, the solvent was changed to butanol half saturated with water. With this the monomethyl sugars and small amounts of unmethylated sugars were eluted and, finally, the cellulose was washed with water (2 l.).

The tubes were bulked in the normal way and the fractions dried, weighed and examined chromatographically in solvent (B). The results are tabulated below.

```
UNDEGRADED POLYSACCHARIDE /
```
### UNDEGRADED FOLYOSACCHARIDE

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tubes</th>
<th>Weight</th>
<th>Colour</th>
<th>( R_g )</th>
<th>Sugars present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1-100</td>
<td>0.641</td>
<td>Grey-green</td>
<td>1.91</td>
<td>2:3:4-trimethyl rhamnose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grey</td>
<td>0.98</td>
<td>2:3:5-trimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red-brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td>2.</td>
<td>101-170</td>
<td>0.717</td>
<td>Grey</td>
<td>0.98</td>
<td>2:3:5-trimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red-brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td>3.</td>
<td>171-205</td>
<td>0.042</td>
<td>Grey</td>
<td>0.98</td>
<td>2:3:5-trimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red-brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.84</td>
<td>dimethyl arabinose</td>
</tr>
<tr>
<td>4.</td>
<td>206-390</td>
<td>0.276</td>
<td>Grey</td>
<td>0.84</td>
<td>2:5-dimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td>0.84</td>
<td>3:5-dimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Red-brown</td>
<td>0.89</td>
<td>2:3:4:6-tetramethyl galactose</td>
</tr>
<tr>
<td>5.</td>
<td>391-442</td>
<td>0.217</td>
<td>Red-brown</td>
<td>0.73</td>
<td>2:3:4-(+2:4:6)-trimethyl galactose</td>
</tr>
<tr>
<td></td>
<td>511-595</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>443-510</td>
<td>0.351</td>
<td>Red-brown</td>
<td>0.73</td>
<td>2:3:4- and 2:4:6-trimethyl galactoses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pink</td>
<td>0.67</td>
<td>2:3-dimethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tri- and dimethyl galactoses</td>
</tr>
<tr>
<td>7.</td>
<td>596-685</td>
<td>0.053</td>
<td>Red-brown</td>
<td>0.47</td>
<td>2:4-dimethyl galactose</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>686-760</td>
<td>1.885</td>
<td>Red-brown</td>
<td>0.47</td>
<td>2:4-dimethyl galactose + traces X and Y.</td>
</tr>
<tr>
<td></td>
<td>801-1030</td>
<td></td>
<td>Red-brown</td>
<td>0.54</td>
<td>2:6-dimethyl galactose + tr. X and Y.</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>9.</td>
<td>761-800</td>
<td>0.067</td>
<td>Red-brown</td>
<td>0.47</td>
<td>2:4-dimethyl galactose + X, + tr. Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>1031-1070</td>
<td>0.031</td>
<td></td>
<td></td>
<td>2:4- + tr. 2:4:6-dimethyl galactoses + Y + tr. galactose</td>
</tr>
<tr>
<td>11.</td>
<td>1071-1100</td>
<td>0.010</td>
<td>Red-brown</td>
<td>2:4-dimethyl galactose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td>2-methyl galactose + tr. galactose</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>1101-1240</td>
<td>0.224</td>
<td>Brown</td>
<td>0.29</td>
<td>2-methyl galactose</td>
</tr>
<tr>
<td>13.</td>
<td>1241-1388</td>
<td>0.042</td>
<td></td>
<td></td>
<td>2-methyl galactose + arabinose + galactose</td>
</tr>
<tr>
<td>14.</td>
<td>Water wash</td>
<td>0.252</td>
<td></td>
<td></td>
<td>Hexamethyl ether of 6-2-3-glucuronosyl-2-galactose</td>
</tr>
</tbody>
</table>

\( X = 2:3\)-dimethyl galactose \( ? \) \( R_g \) 0.50 : \( Y = 2\)-methyl arabinose \( ? \) \( R_g \) 0.38
EXAMINATION OF FRACTIONS

Fraction 1. 2:3:4-tri-O-methyl-L-rhamnose, 2:3:5-tri-O-methyl-L-arabinose and a trace of 2:3:4:6-tetra-O-methyl-D-galactose

0.641 g.  \( R_g = 1.01, 0.98 \) and 0.89

\( [\alpha]_D = -23^\circ \)  (\( c = 0.64 \) in water)

This fraction was obtained as a very mobile syrup and paper chromatography showed two spots corresponding in \( R_g \) and colour to 2:3:4-tri-O-methyl-L-rhamnose (greenish grey in ultraviolet fluorescence) and 2:3:5-tri-O-methyl-L-arabinose (grey : pink in ultraviolet) in the approximate ratio of 1:4.

Demethylation gave arabinose and rhamnose.

The optical rotation \( [\alpha]_D = -23^\circ \) corresponded to a mixture of the two sugars in the proportions of 23:77.

\( (2:3:4\text{-tri-O-methyl-L-rhamnose} \ [\alpha]_D = +24^\circ \)  
and 2:3:5-tri-O-methyl-L-arabinose \( [\alpha]_D = -37^\circ \)

Separation of Methylated Sugars on a Cellulose Column

The methylated sugars (360 mg.) were fractionated on a cellulose column (50 x 2 cm.) using light petroleum (b.p. 100° - 120°) : butan-i-ol (7:3) saturated with water as eluant to give four fractions.

Subfraction 1a  0.047 g.  \( R_g = 1.01 \)

\( [\alpha]_D = +24^\circ \)  (\( c = 0.4 \) in water)

Chromatography gave a spot corresponding to 2:3:4-tri-O-methyl-L-rhamnose and the sugar was characterised by conversion to the aniline derivative. Recrystallised from light petroleum, this gave m.p. /
m.p. 111° undepressed on admixture with authentic 2:3:4-tri-O-methyl-L-phenyl-D-rhamnosylamine.

Demethylation gave rhamnose only.

Subfraction 1b 0.232 g.  \( R_G = 1.01 \) and 0.98

\[ [\alpha]_D^{20} = -28^\circ \quad (c = 1.0 \text{ in water}) \]

Chromatography in solvent (B) gave two spots corresponding to 2:3:5-tri-O-methyl-L-arabinose and 2:3:4-tri-O-methyl-L-rhamnose in the ratio of approximately 10:1.

Subfraction 1c 0.047 g.  \( R_G = 0.98 \)

\[ [\alpha]_D^{20} = -37^\circ \quad (c = 0.47 \text{ in water}) \]

The sugar had the chromatographic properties of pure 2:3:5-tri-O-methyl-L-arabinose and was characterised by conversion to the amide of the aldonic acid m.p. 135°-136° after recrystallisation from acetone. This was undepressed on mixed m.p. with an authentic specimen of 2:3:5-tri-O-methyl-L-arabinamide.

Subfraction 1d 0.007 g.  \( R_G = 0.89 \)

\[ [\alpha]_D^{20} = +100^\circ \quad (c = 0.14 \text{ in water}) \]

Chromatography in solvents (B) and (D) gave a spot corresponding to 2:3:4:6-tetra-O-methyl-D-galactose.

Fraction 2  2:3:4:6-tetra-O-methyl-D-galactose + 2:3:5-tri-O-methyl-L-arabinose

0.717 g.  \( R_G = 0.98 \) and 0.89

\[ [\alpha]_D^{20} = +92^\circ \quad (c = 0.72 \text{ in water}) \]

The optical rotation $\left[\alpha\right]_D = +92^\circ$ corresponded to a mixture of the two sugars in the proportion of 16:84.

(2:3:5-tri-O-methyl-L-arabinose has $\left[\alpha\right]_D = -37^\circ$ and 2:3:4:6-tetra-O-methyl-D-galactose $\left[\alpha\right]_D = +117^\circ$)

The aniline derivative was prepared and gave crystals, which on recrystallisation from ethanol had m.p. 191° undepressed on admixture with 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine.

Fraction 3. 2:3:5-tri-O-methyl-L-arabinose + 2:3:4:6-tetra-O-methyl-D-galactose and dimethyl arabinose

0.042 g. $R_g = 0.93, 0.89$ and 0.84

$\left[\alpha\right]_D = +62^\circ$ ($c = 0.41$ in water)

Chromatography in solvents (B) and (D) gave the tetramethyl galactose as the main component with some trimethyl arabinose and a slower moving spot corresponding in colour (dark grey and pink in ultraviolet) to 2:5- and $R_g$ values to 2:5- and 3:5-di-O-methyl-L-arabinose.

The main component was characterised by conversion to the aniline derivative m.p. 189° undepressed in mixed m.p. with 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine.

Fraction 4. /
Fraction 4. 2:5- and 3:5-di-0-methyl-L-arabinoses and 2:3:4:6-tetra-0-
methyl-D-galactose.

0.276 g. \[R_G = 0.84 \text{ and } 0.89\]

\[\left[\alpha\right]_D^\circ = +45^\circ \quad (c = 1.1 \text{ in water})\]

\[\text{Ome} = 36.3\% \text{ (calculated for } C_{7111405}, 54.8\%)\]

Paper chromatography in solvents (B) and (D) gave a main spot corresponding in \(R_G\) values to 2:5- and 3:5-di-0-methyl-L-arabinoses, and in colour (grey, pink under ultraviolet) to the former. In addition there was a small amount of material chromatographically identical to 2:3:4:6-tetra-0-methyl-D-galactose.

Demethylation gave arabinose and a little galactose.

Separation on a Cellulose Column

The syrup (160 mg.) was fractionated on a small cellulose column (50 x 2 cm.) using light petroleum (b.p. 100°-120°)-butan-1-ol (7:3), saturated with water as eluant. Two fractions were collected.

Subfraction 4a 0.018 g. \[R_G = 0.89\]

Chromatography of the syrup showed a single component corresponding in \(R_G\) and colour to 2:3:4:6-tetra-0-methyl-D-galactose.

Demethylation gave galactose alone.

The identity of the sugar was confirmed by conversion to the aniline derivative m.p. 190° undepressed on admixture with 2:3:4:6-tetra-0-methyl-N-phenyl-D-galactosylamine.

Subfraction 4b /
Subfraction 4b  0.122 g.  \( R_g = 0.34 \)

Paper chromatography in solvents (B) and (D) showed the one spot corresponding to the dimethyl arabinoses. Paper ionophoresis however, showed 2 spots, the main one corresponding in colour and distance travelled to 2:5-di-O-methyl-L-arabinose, and the smaller faster moving component to the 3:5-isomer, which is brown and shows up yellow under ultraviolet fluorescence. The ratio of the sugars was approximately 10:1.

Demethylation gave arabinose alone.

The main sugar was characterised by conversion to the crystalline amide of the aldonic acid which after recrystallisation from ethyl acetate, had m.p. 125°-126° undepressed on admixture with authentic 2:5-di-O-methyl-L-arabonamide.

Fraction 5. 2:3:4- (and 2:4:6-)tri-O-methyl-D-galactoses

0.217 g.  \( R_g = 0.75 \)

\([\alpha]_D^\infty = +113^\circ \quad (c = 1.0 \text{ in water})\)

\( \text{O-Me} = 41.2\% \) (calculated for \( C_9H_{18}O_6 \), 41.9%)

This fraction was obtained as a clear syrup and did not crystallise even after keeping in vacuo over \( P_2O_5 \) for several months.

Demethylation showed only galactose.

The syrup (100 mg.) was converted to the aniline derivative. The bulk of the solvent was removed and the remaining syrup, kept at 0° overnight, gave plate shaped crystals. These were recrystallised from acetone /
acetone and identified as 2:3:4-tri-\(\beta\)-methyl-\(\text{\textBeta}\)-phenyl-\(\text{\textBeta}\)-galactosylamine having m.p. 168° undepressed in mixed m.p. with an authentic specimen. Mixed m.p. with 2:4:6-tri-\(\beta\)-methyl-\(\text{\textBeta}\)-phenyl-\(\text{\textBeta}\)-galactosylamine, gave 145° - 149°.

The mother liquor kept at 0° gave no further crystals.

The formaldehyde release on periodate attack was estimated, and corresponded to the presence of 91% of 2:3:4-tri-\(\beta\)-methyl-\(\text{\textBeta}\)-galactose and 9% of 2:4:6-tri-\(\beta\)-methyl-\(\text{\textBeta}\)-galactose (p. 83).

**Fraction 6.** 2:3:4- and 2:4:6-tri-\(\beta\)-methyl-\(\text{\textBeta}\)-galactoses plus (probably) 2:3-di-\(\beta\)-methyl-L-arabinose.

0.351 g. \( R_G = 0.73 \) and 0.67

\[
[a]_D = +108.5^\circ \quad (c = 1.1 \text{ in water})
\]

OMe = 40.7% (calculated for \(C_{9}H_{18}O_{6}\), 41.9%)

On chromatograms run in solvents (B) and (D), this fraction gave a main spot corresponding to trimethyl galactose and a slower moving pink spot (pink under ultraviolet) chromatographically identical to 2:3-di-\(\beta\)-methyl-L-arabinose (cf. fraction 6 of methylated degraded gum). The ratio of the sugars was estimated as 10:1.

Demethylation gave galactose and a smaller amount of arabinose.

A sample (100 mg.) was treated with aniline in the normal way to give the aniline derivative. After removal of the solvent, the syrup was kept at 0° overnight. Plate shaped crystals formed and these, on recrystallisation from acetone, gave m.p. 167° and were characterised by /
by mixed m.p. as 2:3:4-tri-\(\text{O}\)-methyl-\(\text{N}\)-phenyl-\(\text{D}\)-galactosylamine. Again admixture with 2:4:6-tri-\(\text{O}\)-methyl-\(\text{N}\)-phenyl-\(\text{D}\)-galactosylamine gave a depression in m.p.. The mother liquor was left at 0° for several days and a second crop of crystals formed. These were a mixture of plates and needles with the latter predominating. After several recrystallisations a few needles were obtained m.p. 178°-180°. In admixture with 2:4:6-tri-\(\text{O}\)-methyl-\(\text{N}\)-phenyl-\(\text{D}\)-galactosylamine the melting point was undepressed. Melting point was depressed to 148° on mixing with 2:3:4-tri-\(\text{O}\)-methyl-\(\text{N}\)-phenyl-\(\text{D}\)-galactosylamine.

The formaldehyde release (p.83) on periodate attack was estimated, and, allowing for 10% of dimethyl arabinose, corresponded to the presence of 54% 2:3:4-tri-\(\text{O}\)-methyl-\(\text{D}\)-galactose and 36% 2:4:6-tri-\(\text{O}\)-methyl-\(\text{D}\)-galactose in the fraction.

Fraction 7. 0.053 g. \(R_G = 0.73\) and 0.54

\([\alpha]_D^0 = +109°\) \((c = 0.53\) in water\)

Examination by paper chromatography showed that this fraction contained trimethyl galactose and a slower moving sugar corresponding to 2:6-di-\(\text{O}\)-methyl-\(\text{D}\)-galactose.

Fraction 8. 2:4- and 2:6-di-\(\text{O}\)-methyl-\(\text{D}\)-galactoses (+ traces)

1.885 g. \(R_G = 0.47\) and 0.54

\([\alpha]_D^0 = +112° \rightarrow +35°\) \((c = 0.91\) in water\)

Chromatography showed a sugar corresponding in \(R_G\) and colour to 2:4-di-\(\text{O}\)-methyl-\(\text{D}\)-galactose. The material crystallised almost immediately /
immediately on drying and, on recrystallisation from acetone containing 1% water, gave needle shaped crystals m.p. 103°. The crystals and mother liquor were examined.

Examination of crystals: \([\alpha]_D = +122° \rightarrow +85° \) (\(c = 1.0\) in water)

\(\alpha Me = 27\%\) (calculated for C\(_{8}\)H\(_{16}\)O\(_{6}\), H\(_{2}\)O, 27.2%)

Mixed m.p. with authentic 2:4-di-\(\beta\)-methyl-D-galactose monohydrate gave 102°. The aniline derivative was prepared and the resulting crystals, recrystallised from methanol, were identified as 2:4-di-\(\beta\)-methyl-\(N\)-phenyl-D-galactosylamine m.p. 217°-218° by mixed m.p. with an authentic sample.

Examination of mother liquor. This was taken to dryness (240 mg.) and chromatography of the syrup in solvent (B) showed two spots corresponding to 2:4-di-\(\beta\)-methyl-D-galactose with a small amount of a faster moving sugar. Solvent (D) gave the smaller spot corresponding to 2:6-di-\(\beta\)-methyl-D-galactose (\(R_2:4\)-di-\(\beta\)-methyl-D-galactose = 1.38). Sprayed with p-anisidine hydrochloride the 2:4- showed up light brown and the 2:6-pink brown (129). Standard 2:3-di-\(\beta\)-methyl-D-galactose was grey brown and in solvent (D) had (\(R_2:4\) gal = 1.50).

Paper ionophoresis showed two components, the smaller travelling at the same rate as 2:6-di-\(\beta\)-methyl-D-galactose and the main one stationary (cf. 2:4-di-\(\beta\)-methyl-D-galactose).

Separation on a Cellulose Column.

The syrup from the mother liquor (228 mg.) was fractionated on a small cellulose column (50 x 2 cm.) using solvent (D) as eluant. Three fractions were collected.

Subfraction 8a /
Subfraction 8a 0.039 g.  \( R_g = 0.54 \) and 0.38

Chromatography in solvents (B) and (D) gave 2:6-di-Q-methyl-D-galactose with a small amount of a slower moving material. This was pink (pink also in ultraviolet fluorescence) and had \( R_{2:4 \text{ gal}} = 1.15 \) in solvent (D). Sprayed with p-anisidine hydrochloride, the main spot showed up pink brown and the smaller pink.

Demethylation gave galactose and a trace of arabinose.

Chromatography of the products of periodate oxidation showed a bright yellow spot \( (R_F 0.15) \) due to 2:6-di-Q-methyl-D-galactose and/or 2-Q-methylaldehyde and showed no trace of the pattern obtained on oxidation of 2:3-di-Q-methyl-D-galactose.

The fraction crystallised on keeping and recrystallised from chloroform : light petroleum had m.p. 99° - 100° undepressed on admixture with 2:6-di-Q-methyl-D-galactose monohydrate and depressed to 85° - 87° on admixture with the 2:4-dimethyl ether.

Examination of crystals: \([\alpha]_D = + 52° \rightarrow + 84° \) \((\alpha = 0.56 \text{ in water})\)

\( \text{OMe} = 27.0\% \) (calculated for \( C_{16}H_{16}O_6, H_2O, 27.2\% \))

The crystals were characterised by conversion to 2:6-di-Q-methyl-N-phenyl-D-galactosylamine m.p. and mixed m.p. 121° - 122°.

The second spot was probably a trace of 2-Q-methyl-L-arabinose.

Subfraction 8b. 0.027 g.  \( R_g = 0.47, 0.50 \) and 0.54

\([\alpha]_D = + 85° \) \((\alpha = 0.27 \text{ in water})\)

Demethylation /
Demethylation gave galactose only.

Chromatography in solvents (B) and (D) gave 2:4- and 2:6-di-O-methyl-D-galactoses in the ratio of about 2:1 with a trace of material corresponding in R_G and colour (with aniline oxalate and p-anisidine hydrochloride as sprays) to the 2:3-di-O-methyl ether.

Periodate oxidation confirmed this, giving the bright yellow spot R_P 0.15, a heavy pink spot corresponding to unattacked 2:4-di-O-methyl-D-galactose and small amounts of the three spots from 2:5-di-O-methyl-D-galactose (R_P's 0.67, 0.78, 0.86).

Subfraction 8c 0.152 g.  \( R_G = 0.47 \)

\[ [\alpha]_D = +115^\circ \rightarrow +85^\circ \qquad (\alpha = 1.0 \text{ in water}) \]

Chromatography in solvents (B) and (D) showed pure 2:4-di-O-methyl-D-galactose.

Chromatography of the products of periodate oxidation gave the sugar unaltered and demethylation yielded only galactose.

The fraction crystallised out immediately on drying and recrystallised from acetone containing 1% water, it yielded crystals of 2:4-di-O-methyl-D-galactose monohydrate m.p. and mixed m.p. 102° and was characterised by conversion to 2:4-di-O-methyl-N-phenyl-D-galactosylamine, m.p. and mixed m.p. 216°- 218°.

Fraction 9. 2:4- and 2:5-di-O-methyl-D-galactose (trace probably 2-O-methyl-L-arabinose)

0.067 g.  \( R_G = 0.47, 0.50 \text{ and } 0.38 \)

On /
On chromatograms run in solvents (B) and (D) and using both aniline oxalate and p-anisidine hydrochloride as sprays, this fraction showed the two dimethyl galactoses and a small amount of the material thought to be 2-0-methyl-L-arabinose.

Demethylation gave galactose and a small amount of arabinose.

Periodate attack followed by chromatography gave unattacked 2:4-di-O-methyl-D-galactose, the three spots characteristic of oxidised 2:3-di-O-methyl-D-galactose, and the bright yellow spot (R$_F$ 0.15) due probably to 2-0-methyl-L-arabinose in the absence of any 2:6-di-O-methyl-D-galactose.

The fraction did not crystallise and attempts to obtain the aniline derivatives of the main sugars were unsuccessful.

**Fraction 10.** 0.031 g.

Chromatography of this fraction showed 2:4-di-O-methyl-D-galactose, the spot corresponding to 2-0-methyl-L-arabinose and traces of unmethylated galactose and 2:6-di-O-methyl-D-galactose.

**Fraction 11.** 0.0100 g.

Chromatograms run in solvents (A), (B) and (D) showed this fraction to be a mixture of 2:4-di-O-methyl-D-galactose, a sugar corresponding to 2-0-methyl-L-galactose, a trace of galactose and a small unidentified spot R$_G$ = 0.90.

**Fraction 12.** 2-0-methyl-D-galactose.

0.244 g.  

\[ [\alpha]_D = +61^\circ \rightarrow +85^\circ \text{ (c = 1.0 in water)} \]
The sugar was chromatographically pure and identical to 2-O-methyl-D-galactose and crystallised out on keeping into large waxy needles. These on recrystallisation from glacial acetic acid, gave with difficulty a few crystals m.p. 146° undepressed on admixture with an authentic specimen.

**Fraction 13.** 0.0422 g.

Chromatography in solvents (A), (B) and (D) showed galactose, arabinose and 2-O-methyl-D-galactose in approximately equal amounts.

**Fraction 14.** Hexamethyl ethers of 6-O-β-D-glucuronosyl-D-galactose + traces neutral sugars.

0.252 g.

Chromatography in solvent (B) showed barium salts at the starting line and traces of free sugars. The fraction was freed from barium by dissolving in water and shaking with Amberlite resin IR-120(H). The filtered solution was taken to dryness.

**Reduction and Hydrolysis of Methylated Aldobiouronic Acid**

The syrup (165 mg.) was refluxed in methanolic hydrogen chloride (3% : 50 ml.) until the rotation was constant (6 hours). The solution was cooled, neutralised with silver carbonate and taken to dryness. The residue of glycosides was heated with barium hydroxide (0.15 N : 10 ml.) for 5 hours at 60° resulting in saponification of the uronic acid ester groups. Barium ions were then removed from the solution /
solution using Amberlite resin IR-120(H) and the acid glycosides were absorbed on a column of Amberlite resin IR-4B(OH).

The neutral methyl glycosides in the aqueous effluent from the resin were taken to dryness (7.2 mg.). These were not further examined.

The methyl aldobiouronoside fragments, absorbed on the Amberlite resin IR-4B(OH) were eluted with N-sodium hydroxide and the free acid immediately regenerated by shaking with Amberlite resin IR-120(H). Taken to dryness, this gave a syrup (102 mg.).

The syrup was refluxed with methanolic hydrogen chloride (3% : 40 ml.) to constant rotation, and the cooled solution was neutralised with silver carbonate, and taken to dryness. The dry ester was dissolved in purified tetrahydrofuran (30 ml.) and lithium aluminium hydride in tetrahydrofuran (240 mg. in 10 ml.) was added dropwise. The reduction was completed by refluxing for 2 hours. The excess hydride was then destroyed by adding water, the organic layer separated and the aqueous layer concentrated to dryness. The residue was extracted several times with chloroform and acetone, and the combined extracts taken to dryness (91 mg.).

The resulting syrup was hydrolysed by heating with N-hydrochloric acid for 4 hours at 100°. The solution was neutralised with silver carbonate and taken to dryness (74.2 mg.). Run in solvent (B) this showed two spots corresponding in R_G and colour to 2:3:4-tri-O-methyl-D-galactose and 2:3:4-tri-O-methyl-D-glucose.
Separation of Methylated Sugars on Cellulose Column

The methylated sugars (74 mg.) were fractionated by partition chromatography on a cellulose column (50 x 2 cm.) using light petroleum (b.p. 100°-120°)-butan-1-ol (1:1) saturated with water as eluant to give two fractions.

**Fraction (i). (26.4 mg.)**

Run in solvent (B), the material was chromatographically pure, corresponding to 2:3:4-tri-O-methyl-D-glucose. It was characterised by conversion to the aniline derivative m.p. 133°-135° after recrystallisation from light petroleum. Mixed m.p. with authentic 2:3:4-tri-O-methyl-N-phenyl-D-glucosylamine gave no depression.

**Fraction (ii). (30.2 mg.)**

\[ [\alpha]_D = +115^\circ \] \hspace{1cm} (c = 0.5 in water)

This fraction was also chromatographically pure, corresponding to 2:3:4-tri-O-methyl-D-galactose. It was characterised as the aniline derivative m.p. 169° after recrystallisation from acetone. Mixed m.p. with authentic 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine gave no depression.

Estimation of formaldehyde release after periodate oxidation on tri-O-methyl-D-galactitols.

It has been found that certain sugars, including 2:3:4-tri-O-methyl-D-galactose produce less than the theoretical yields of formaldehyde after /
after periodate oxidation. However, if the 2:3:4-tri-O-methyl-D-galactose is first reduced to the galactitol form, oxidation gives the expected one mole of formaldehyde per sugar residue.

Thus the molecular proportions of 2:3:4- and 2:4:6-tri-O-methyl-D-galactoses (the latter yields no formaldehyde when oxidised) can be estimated by determining the formaldehyde release after oxidation of the reduced sugar mixture. This was carried out on the trimethyl galactose containing fractions of both the whole and degraded methylated gum hydrolysates.

The method of estimation used was that of O'Dea and Gibbons (135) with the addition that parahydroxybenzaldehyde was added to the reaction mixture prior to the oxidation in order to prevent the liberated formaldehyde combining with the products of oxidation (136).

The dry sugar mixture (5-10 mg.) was reduced by dissolving in a solution of potassium borohydride (20 mg. in 10 ml.) and leaving overnight. The excess borohydride was then destroyed by adding glacial acetic acid. 2-3 mg. Parahydroxybenzaldehyde were added and the solution made up to a known volume.

Freshly prepared sodium metaperiodate-sodium bicarbonate solution was added to an equal volume of sugar solution (about 0.01% w./v.) and the oxidation allowed to proceed in the dark.

At appropriate intervals, portions (1 ml.) were withdrawn, lead dithionate (20% : 1 ml.) added, the solution mixed, and the precipitated lead periodate and iodate removed by centrifugation. 1 ml. of /
of supernatant was mixed with chromotropic acid reagent (9 ml.) and, after 30 minutes, the lead sulphate removed by centrifugation. The supernatant liquid was heated in a glass stoppered tube on a boiling water bath for 40 minutes and the absorption at 570 m\(\mu\) read on a Unicam spectrophotometer.

All operations were carried out away from direct light and blanks run on all determinations. The readings, corrected for blanks, were plotted against the concentration of reduced sugar in \(\mu\) moles/100 ml. w./v. The 2:3-di-\(\beta\)-methyl-\(L\)-arabinose present in the fractions will give, when reduced, 1 mole of formaldehyde per mole of sugar in the same way as the 2:3:4-tri-\(\beta\)-methyl-\(D\)-galactitol and so must be allowed for.

Graph I

![Graph I](image-url)

Graph legend:

- **I** 2:3:4-tri-\(\beta\)-methyl-\(D\)-galactose
- **II** fraction 5 whole gum
- **III** fraction 6 whole gum
- **IV** fraction 6 degraded gum
- **V** fraction 5 degraded gum

Reading vs. concentration \(\mu\) moles/100 ml.
The results from the Graph I are as follows:

Whole methylated gum hydrolysate fraction 5 gave 0.91 moles of formaldehyde per mole of sugar corresponding to

91% of 2:3:4-tri-O-methyl-D-galactose

and 9% of 2:4:6-tri-O-methyl-D-galactose

Whole methylated gum hydrolysate fraction 6 gave 0.64 moles of formaldehyde per mole of sugar. Assuming the presence of 10% of 2:5-di-O-methyl-L-arabinose, this corresponds to

54% of 2:3:4-tri-O-methyl-D-galactose

and 36% of 2:4:6-tri-O-methyl-D-galactose

Degraded methylated gum hydrolysate fraction 5 gave 0.08 moles of formaldehyde per mole of sugar corresponding to

8% of 2:3:4-tri-O-methyl-D-galactose

and 92% of 2:4:6-tri-O-methyl-D-galactose

Degraded methylated gum hydrolysate fraction 6 gave 0.45 moles of formaldehyde per mole of sugar. Assuming the presence of 10% of 2:5-di-O-methyl-L-arabinose this corresponds to

35% of 2:3:4-tri-O-methyl-D-galactose

and 55% of 2:4:6-tri-O-methyl-D-galactose
### SUMMARY OF METHYLATION RESULTS

The quantities of methylated sugars which were obtained by fractionation of the methylated whole and degraded gum hydrolysates were calculated to be approximately as follows:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Whole Gum</th>
<th>Degraded Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4-tri-O-methyl-L-rhamnose</td>
<td>130 mg.</td>
<td>Minor</td>
</tr>
<tr>
<td>2:3:5-tri-O-methyl-L-arabinose</td>
<td>630 mg.</td>
<td>Minor</td>
</tr>
<tr>
<td>2:5-di-O-methyl-L-arabinose</td>
<td>230 mg.</td>
<td>Minor</td>
</tr>
<tr>
<td>3:5-di-O-methyl-L-arabinose</td>
<td>50 mg.</td>
<td>-</td>
</tr>
<tr>
<td>2:5-di-O-methyl-L-arabinose</td>
<td>35 mg.</td>
<td>-</td>
</tr>
<tr>
<td>(? 2-O-methyl-L-arabinose</td>
<td>Minor</td>
<td>-</td>
</tr>
<tr>
<td>2:3:4:6-tetra-O-methyl-D-galactose</td>
<td>640 mg.</td>
<td>590 mg.</td>
</tr>
<tr>
<td>2:4:6-tri-O-methyl-D-galactose</td>
<td>140 mg.</td>
<td>590 mg.</td>
</tr>
<tr>
<td>2:3:4-tri-O-methyl-D-galactose</td>
<td>410 mg.</td>
<td>90 mg.</td>
</tr>
<tr>
<td>2:4-di-O-methyl-D-galactose</td>
<td>1,900 mg.</td>
<td>440 mg.</td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-galactose</td>
<td>70 mg.</td>
<td>90 mg.</td>
</tr>
<tr>
<td>2:3-di-O-methyl-D-galactose</td>
<td>Minor</td>
<td>-</td>
</tr>
<tr>
<td>2-O-methyl-D-galactose</td>
<td>230 mg.</td>
<td>60 mg.</td>
</tr>
</tbody>
</table>

Hexamethyl ether of 6-0-β-D-glucuronosyl-D-galactose 165 mg. 108 mg.
PERIODATE OXIDATION EXPERIMENTS ON DEGRADED ACACIA PICANANTRA GUM

Uptake of Periodate

The degraded polysaccharide (100-200 mg.) was dissolved in water, sodium metaperiodate (250-500 mg.) added, and the volume made up to 10 ml. The stoppered flask was set aside in the dark. A blank was run at the same time omitting the polysaccharide.

At intervals, samples (1 ml.) were withdrawn and to each added saturated sodium bicarbonate (10 ml.), sodium arsenite (5 ml. : 0.1N) and aqueous potassium iodide (1 ml. : 20%). The solution was shaken and stood in a stoppered flask in the dark for 10 minutes, before the excess arsenite was titrated with standard iodine (~ 0.05 N) using starch at the end-point.

Results. 125.0 mg. degraded polysaccharide. Normality Iodine soln. = 0.05236 N.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>49</th>
<th>80</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titre (corrected for blank)</td>
<td>0.82</td>
<td>2.15</td>
<td>3.30</td>
<td>5.50</td>
<td>3.72</td>
<td>3.85</td>
</tr>
</tbody>
</table>

The results suggest that oxidation over and above the normal glycol cleavage (which seems complete after about 48 hours) is taking place. Exterpolation of the approximately straight line part of Graph II corrects for this and from the graph the moles of periodate consumed per residue \((C_6H_{10}O_5) = 1.01\)
Degradation of periodate oxidised polysaccharide

This was carried out according to Barry's method (26) using phenylhydrazine and glacial acetic acid.

Degraded *Acacia pycnantha* gum (3.5 g.) and sodium metaperiodate (8.5 g.) were dissolved in water (100 ml.) and left in the dark until the oxidation was complete (48 hours). An aqueous solution of lead acetate was added until lead periodate and iodate were completely precipitated, and these were removed at the centrifuge. The lead ions remaining in solution were removed by precipitation as lead sulphate with dilute sulphuric acid, and centrifugation.

The centrifugate was heated on a boiling water bath with freshly distilled phenylhydrazine (17 ml.) and glacial acetic acid (10 ml.) for 5 hours, and the product was repeatedly extracted with ether. The aqueous solution was then made 0.2N with respect to sulphuric acid and heated for 40 minutes on a boiling water bath. After cooling, it/
it was neutralised with Amberlite resin IR-4B(OH), extracting with ether, concentrated to about 50 ml., and ethanol (3 vols.) added to precipitate the remaining polysaccharide (degraded polysaccharide A). Yield = 1.0 g.

**Partial Hydrolysis of Degraded Polysaccharide (A)**

Degraded polysaccharide (A) (17.6 mg.) was dissolved in sulphuric acid (0.5 N : 20 ml.) and heated on a boiling water bath. Samples (2 x 0.5 ml.) were removed at intervals and for one the reducing power estimated with Somogyi's reagent (20) (Graph III). The titre at which the curve levels out (1.12 ml.) corresponds to the sugar content being 100% monosaccharide. Half this value (0.56 ml.) will give the approximate time of maximum disaccharide content (56 minutes).

![Graph III](image)

The other set of samples were neutralised with Amberlite resin IR-4B(OH), alcohol added to precipitate any remaining polysaccharide which was removed by centrifugation and the centrifugates taken to dryness. The resulting syrups were examined by paper chromatography in /
in solvent (A) against authentic samples of 3- and 6-0-β-D-galactopyranosyl-D-galactose. All showed galactose and 5-0-β-D-galactopyranosyl-D-galactose, the latter reaching a maximum concentration after about an hour. None of the 6-0-β-D-galactopyranosyl-D-galactose was detected.

The remainder of the degraded polysaccharide (A) (0.9 g.) was hydrolysed by heating with sulphuric acid (0.5 N : 75 ml.) on a boiling water bath for 1 hour. The cooled solution was neutralised with Amberlite resin IR-4B(OH), taken to small volume and ethanol (3 volumes) added. The precipitated polysaccharide was removed by centrifugation, washed with ethanol and re-hydrolysed. The neutralised supernatants and ethanol washings were combined and taken to dryness. Yield = 0.35 g.

The syrup (0.35 g.) was dissolved in the minimum volume of water and poured on to a charcoal:celite column (1:1, 100 g.).

Elution with water gave galactose alone (212 mg.)

Elution with water containing 5% ethanol gave a syrup (31 mg.) which, when run on chromatograms in solvent (A), showed a spot corresponding to 5-0-β-D-galactopyranosyl-D-galactose ($R_{Gal} = 0.60$) with a faint trace of a slower moving material probably 6-0-β-D-galactopyranosyl-D-galactose ($R_{Gal} = 0.40$). The fraction crystallised readily on being taken to dryness and, after recrystallisation from ethanol : water, had m.p. 145°- 147° undepressed on admixture with an authentic sample of 3-0-β-D-galactopyranosyl-D-galactose. Also the X-Ray powder photograph of the crystals was identical to that of the disaccharide.
disaccharide.

Elution with water containing 10% ethanol gave a syrup (7 mg.). Chromatography in solvent (A) showed spots near the starting line, and a faster moving material \( (R_{\text{gal}} = 0.35) \). Partial acid hydrolysis of this fraction gave spots corresponding to galactose, 3-\(\beta\)-D-galactopyranosyl-D-galactose and a trace of the above sugar \( (R_{\text{gal}} = 0.35) \). This fraction thus seems to contain the series of oligosaccharides made up of galactose residues with 1:3 linkages between them and the spot \( (R_{\text{gal}} = 0.33) \) is probably the trisaccharide 0-D-galactopyranosyl-(1\(\rightarrow\)3)-0-D-galactopyranosyl-(1\(\rightarrow\)3)-D-galactose.
DISCUSSION

The sample of *Acacia pycnantha* gum used in the present work was examined electrophoretically using glass fibre paper (16,17,18) and was found to be essentially homogeneous. However, a minor component (approximately 5% of the total) was detected in another sample of the gum, and Lewis and Smith (18) have reported similar findings in further samples of the gum.

The main methods of attack used in the structural investigation of *Acacia pycnantha* gum were partial acid hydrolysis, methylation, and periodate oxidation studies.

Three fragments of the molecule were isolated under different conditions of acid hydrolysis, and an arabinose free degraded gum was recovered from the reaction mixture.

This degraded gum and the whole gum were methylated and hydrolysed and the products separated and examined. The structure of the degraded gum was further investigated by oxidation with sodium meta-periodate. Finally the main structural features of *Acacia pycnantha* gum were worked out from a comparison of the various results.

A summary of the work is given on p. 94.
SUMMARY OF WORK CARRIED OUT ON ACACIA PYCNANTHA

\[
(0.01 \text{N H}_2\text{SO}_4 \text{ at } 95^\circ \text{ for } 4 \text{ hours}) \rightarrow 3-O-L\text{-arabofuranosyl-L-arabinose}
\]

\[
\phi \rightarrow (N \text{ H}_2\text{SO}_4 \text{ at } 95^\circ \text{ for } 7\frac{1}{2} \text{ hours}) \rightarrow 6-O-\beta-D\text{-glucuronosyl-D-galactose}
\]

\[
\phi \rightarrow (0.1 \text{N H}_2\text{SO}_4 \text{ at } 95^\circ \text{ for } 8 \text{ hours}) \rightarrow 3-O-\beta-D\text{-galactopyranosyl-D-galactose} + L\text{-arabinose} + L\text{-rhamnose} + D\text{-galactose}
\]

Acacia *pycnantha Gum

\[
(0.01 \text{N H}_2\text{SO}_4 \text{ at } 100^\circ \text{ for } 14 \text{ hours}) \rightarrow \text{Arabinose free degraded gum}^*
\]

(Periodate oxidation followed by phenylhydrazine treatment of oxidised polysaccharide)

\[\downarrow\]

Further degraded polysaccharide

\[
(0.5 \text{N H}_2\text{SO}_4 \text{ at } 100^\circ \text{ for } 1 \text{ hour}) \rightarrow 3-O-\beta-D\text{-galactopyranosyl-D-galactose} + D\text{-galactose}
\]

\[
\phi \quad \text{Work previously carried out by Hirst and Perlin.}
\]

\[
\star \quad \text{The modes of linkage of the sugar residues in the gum and in the arabinose free degraded gum were established by identification of the products of hydrolysis of the derived methylated polysaccharides.}
\]
PARTIAL ACID HYDROLYSIS STUDIES

*N. pycnanthe* gum was first studied in 1954 by Hirst and Perlin (23). They found that most of the gum was readily soluble in water with about 3% forming a dense gel. The soluble portion was isolated as a white powder which on complete hydrolysis gave \( \text{D-galactose} \) (65%), \( \text{L-arabinose} \) (27%), \( \text{L-rhamnose} \) (1-2%) and about 5% \( \text{D-glucuronic acid} \).

Partial acid hydrolysis using 0.1N-sulphuric acid at 95° for 8 hours liberated galactose containing oligosaccharides in addition to the three neutral monosaccharides, and from the reaction mixture, was isolated a disaccharide which Perlin (23) characterised as \( 3\text{-O-D-galactopyranosyl-D-galactose} \) by means of lead tetra-acetate oxidation studies.

Partial hydrolysis of the gum under the more drastic conditions of N-sulphuric acid at 95° for 7½ hours, splits all the linkages except the uronic acid glycoside bonds which are relatively resistant to acid hydrolysis. An aldobiouronic acid was obtained from the hydrolysate and characterised by methylation and hydrolysis as \( 6\text{-O-D-galacturonic acid} \) as \( 3\text{-O-D-galactopyranosyl-D-galactose} \).

In the present work the hydrolysis of the gum under milder conditions was studied.

When the gum was heated in 0.01N-sulphuric acid at 100°, the first sugar to be released into the hydrolysate was arabinose and it was almost completely removed from the molecule after 5-6 hours. This ease of removal of the arabinose suggested that it is present in the molecule in the labile furanose form. The second sugar to be hydrolysed off was the /
the rhamnose and, after about 6 hours heating, small amounts of galactose appeared in the hydrolysate. After 14 hours almost all the arabinose and rhamnose was free in solution, so that the polysaccharide recovered from the reaction mixture at this stage was virtually arabinose and rhamnose free and yet had lost little of the galactose. Thus there was recovered a degraded gum which appears to be the more acid resistant nucleus of the molecule, made up of residues of galactose and glucuronic acid. The acid labile arabinose units seem, from the above experiments, to occur in the outer parts of the molecule. More prolonged heating gave oligosaccharides and considerable amounts of galactose.

Under the milder conditions of 0.01N-sulphuric acid at 35°, the whole gum liberated a sugar which appeared in solution after about ½ hour, reached a maximum concentration at 4 hours, and disappeared after approximately 7½ hours heating.

A quantity of this material was isolated almost pure (containing ca. 1% of galactose and arabinose) by chromatography on a cellulose column, of the neutralised reaction mixture after 4 hours heating.

The optical rotation \([\alpha]_D = + 89^\circ\), and chromatographic mobility of the material was similar to those reported for 3-O-\(L\)-arabofuranosyl-\(L\)-arabinose by Andrews, Hough and Powell (132).

The suspected disaccharide was readily hydrolysed by acid to give arabinose alone and the absence of the material in the later stages of the hydrolysis showed that it was not an acid reversion product (21,22). Periodate oxidation liberated 0.37 moles of formaldehyde per mole of sugar and, after reduction with potassium borohydride, 1.54 moles of formaldehyde per
per mole of sugar. This indicated the presence of a 3-O-substituted
arabinose residue, and, as confirmation of the structure, the sugar was
methylated and hydrolysed. Chromatography of the hydrolysate showed
2:3:5-tri-O-methyl-L-arabinose, 2:5-di-O-methyl-L-arabinose and 2:4-di-O-
methyl-L-arabinose. The component methylated sugars were separated on
filter sheets and each characterised by formation of crystalline deriva-
tives. The combined dimethyl fractions were approximately equimolar to
the trimethyl arabinose, which is consistent with the methylation and
hydrolysis products from the disaccharide 3-O-L-arabofuranosyl-L-arabinose
since in solution, the reducing end group arabinose will be in equili-
brum between furanose and pyranose forms (XI). The disaccharide grouping
will, however, probably occur in the molecule joined to the nucleus by a
furanosyl linkage because of the very mild acid conditions in which it was
isolated.

\[
\text{(XI)}
\]

The partial acid hydrolysis studies, therefore, showed that the
three following structural features occur in the molecule of Acacia
pycnantha gum

\[
\text{Araf} \ 1 - 3 \ \text{Araf} \quad \text{Gal} \ 1 - 3 \ \text{Gal} \quad \text{Gl Acid} \ 1 - 6 \ \text{Gal}
\]
METHYLATION AND PERIODATE OXIDATION STUDIES

The Structure of Degraded Gum

The degraded gum was methylated with dimethyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide giving a fully methylated polysaccharide with a methoxyl content of 45%. This methylated degraded gum was hydrolysed, and the mixture of methylated sugars was fractionated by chromatography on cellulose. The weights of the main sugars collected were as follows:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Weight</th>
<th>Moles</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4:6-tetra-(\beta)-methyl-D-galactose</td>
<td>0.59 g.</td>
<td>1.6</td>
</tr>
<tr>
<td>2:4:6-tri-(\beta)-methyl-D-galactose</td>
<td>0.59 g.</td>
<td>1.7</td>
</tr>
<tr>
<td>2:4-di-(\beta)-methyl-D-galactose</td>
<td>0.44 g.</td>
<td>1.4</td>
</tr>
</tbody>
</table>

These were obtained pure and characterised by means of crystalline derivatives. In addition small amounts of 2:6-di-\(\beta\)-methyl-D-galactose and 2-D-methyl-D-galactose were obtained pure and characterised in the same way.

Traces of other sugars were detected chromatographically but were not characterised. In addition there was evidence for the presence of 2:3:4-tri-\(\beta\)-methyl-D-galactose in two fractions, both of which had 2:4:6-tri-\(\beta\)-methyl-D-galactose as the main component.

The isolation of the 2:3:4:6-tetra-\(\beta\)-, 2:4:6-tri-\(\beta\)- and 2:4-di-\(\beta\)-methyl-D-galactoses in approximately equimolecular proportions showed that the degraded gum is made up of a highly branched framework of 1:3 and 1:6 linked galactopyranose residues. A number of possible structures can be put forward for the main repeating unit for the degraded gum, and some of...
of these are given below (Structures XII to XV).

The methylated aldobiouronic acid, recovered from the column by washing with water, was characterised as the hexamethyl ether of 6-\(\alpha\)-\(\beta\)-\(\alpha\)-glucuronosyl-\(\alpha\)-galactose by conversion to the methyl ester methyl glycoside, reduction with lithium aluminium hydride and hydrolysis to give approximately equal amounts of 2:3:4-tri-\(\alpha\)-methyl-\(\alpha\)-glucose and 2:3:4-tri-\(\alpha\)-methyl-\(\alpha\)-galactose. The glucuronic acid residues must therefore occur in the degraded gum as end groups attached to C atom 6 of galactose residues (Structure XVI). However, there is no evidence, as yet, to decide whether the aldobiouronic acid groups are attached directly to the main chain or whether one or more galactose residues are interposed.

\[
\ldots 3 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1\ldots
\]

\[
\begin{array}{c|c|c}
6 & 6 & 3 \\
1 & 1 & 3 \\
\text{Gal} & \text{Gal} & \text{Gal} \\
\end{array}
\]

(Structure XII)

\[
\ldots 6 \text{Gal} 1 - 3 \text{Gal} 1 - 6 \text{Gal} 1 - 3 \text{Gal} 1\ldots
\]

\[
\begin{array}{c|c|c}
3 & 3 & 3 \\
1 & 1 & 3 \\
\text{Gal} & \text{Gal} & \text{Gal} \\
\end{array}
\]

(Structure XIII)

\[
\ldots 3 \text{Gal} 1 - 3 \text{Gal} 1\ldots \ldots 6 \text{Gal} 1 - 6 \text{Gal} 1\ldots
\]

\[
\begin{array}{c|c|c|c|c|c|c}
6 & 6 & 3 & 3 & 3 & 3 & 3 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 \\
\text{Gal} & \text{Gal} & \text{Gal} & \text{Gal} & \text{Gal} & \text{Gal} & \text{Gal} \\
\end{array}
\]

(Structure XIV) (Structure XV)
Gl Acid 1 - 6 Gal 1...

(XVI)

To distinguish between the various possibilities, the degraded gum was oxidised with sodium metaperiodate and further degraded by Barry's method of treatment with phenylhydrazine and glacial acetic acid.

The degraded gum was treated with periodate for 48 hours in the dark, giving complete oxidation. All oxidised sugar residues were then cleaved from the molecule by boiling with phenylhydrazine and glacial acetic acid.

Next the remaining polysaccharide (the various possibilities (XIIIA) to (XVA) are shown below) was recovered from solution by precipitation with ethanol, and partially hydrolysed, giving 3-6-β-D-galactopyranosyl-β-D-galactose as the main disaccharide product, with only a trace of the 1:6 linked galactobiose being detected chromatographically.

\[ \cdots 3 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1 \cdots \]  
(XIIIA)

\[ \cdots 6 \text{Gal} 1 - 3 \text{Gal} 1 - 6 \text{Gal} 1 - 3 \text{Gal} 1 \cdots \]  
(XIII A)

\[ \cdots 3 \text{Gal} 1 - 3 \text{Gal} 1 \cdots \quad 3 \text{Gal} 1 - 6 \text{Gal} 1 \cdots \]  
(XIVA)  
(XVA)

Gal 1  
Gal 1  
Gal 1  
Gal 1

It follows that the main chain, resistant to periodate attack, is composed of 1:3 linked galactopyranosyl residues as required by structure (XII) /
(XII) and that the 1:6 linkages in the degraded gum are those of galactose residues attached as side chains. All possible structures except (XII) would yield, on the above treatment, appreciable amounts of 6-0-β-D-galactopyranosyl-D-galactose. Further evidence for structure (XII) was provided by the detection of a small amount of a 1:3 linked galactotriose and higher homologues. Thus structure (XII) with the attached aldobiose groupings (XV) can be given as the main repeating unit of the degraded gum.

There is no evidence yet as to whether the smaller amounts of other sugars obtained from the hydrolysis of the methylated degraded gum are of structural significance or not. The traces of arabinose and rhamnose methylated sugars were in too small concentration to be of importance but the 2:6-di-0-methyl-D-galactose and the 2-0-methyl-D-galactose formed larger fractions. They may be due to undermethylation and/or demethylation during hydrolysis.

On the other hand the dimethyl ether may be derived from galactose residues linked triply through C atoms 1, 3 and 4:

\[\text{- 1 Gal 3 -} \]
\[\quad 4 \]
\[\quad \]

Similarly the 2-0-methyl-D-galactose would come from double branch points:

\[\text{- 1 Gal 3 -} \]
\[\quad 6 \]
\[\quad 4 \]
\[\quad \]
\[\quad \]

The/
The Structure of Whole Gum

The methylated whole gum was hydrolysed and the mixture of methylated sugars fractionated by partition chromatography on a cellulose column. Preliminary investigation on paper chromatograms has shown that the differences in RF values of the higher methylated fractions were small so that complete separation of all the component methylated sugars was not at first achieved, and several fractions had to be refractionated using smaller cellulose columns. This gave pure specimens of the major components and the following sugars were characterised by the formation of crystalline derivatives:


In addition a small amount of 2-0-methyl-D-galactose was identified and 2 unidentified dimethyl ethers of arabinose (probably the 3:5- and 2:3- isomers) were also present. These sugars were not present in sufficient quantity to be considered as important structural units and, as in the case of the more minor components in the degraded gum, may be the products of incomplete methylation and/or demethylation during hydrolysis.

The methylation results on both the whole and degraded gums are summarised /
summarised below.

**PROPORTIONS OF METHYLATED SUGARS**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Whole Gum (moles)</th>
<th>Degraded Gum (moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4-tri-O-methyl-L-rhamnose</td>
<td>0.2</td>
<td>Minor</td>
</tr>
<tr>
<td>2:3:5-tri-O-methyl-L-arabinose</td>
<td>1.2</td>
<td>Minor</td>
</tr>
<tr>
<td>2:5-di-O-methyl-L-arabinose</td>
<td>0.5</td>
<td>Minor</td>
</tr>
<tr>
<td>3:5-di-O-methyl-L-arabinose</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>2:5-di-O-methyl-L-arabinose</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td>(?) 2-O-methyl-L-arabinose</td>
<td>Minor</td>
<td>-</td>
</tr>
<tr>
<td>2:3:4:6-tetra-O-methyl-D-galactose</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>2:4:6-tri-O-methyl-D-galactose</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>2:3:4-tri-O-methyl-D-galactose</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>2:4-di-O-methyl-D-galactose</td>
<td>3.4</td>
<td>1.4</td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-galactose</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>2:3-di-O-methyl-D-galactose</td>
<td>Minor</td>
<td>-</td>
</tr>
<tr>
<td>2-O-methyl-D-galactose</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

2:3:4-tri-O-methyl-D-gluconic acid Major component Major component

The structure of the main repeating unit of the whole gum was then built up by comparing the nature and amounts of the various methylated sugars isolated from the hydrolysis of the methylated whole and degraded /
degraded gums. This was done by considering:

1. The nature of the acid labile groupings removed in the mild hydrolysis conditions of 0.01N-sulphuric acid at 100°.

2. The points of attachment of these acid labile groupings to the more acid resistant nucleus of galactose and glucuronic acid residues which is the degraded gum.

The nature of these acid labile groupings was found from the methylation results of the whole gum. The acid labile monosaccharides were L-rhamnose and L-arabinose.

The only methyl ether of rhamnose found on hydrolysis of the methylated gum was 2:3:4-tri-0-methyl-L-rhamnose. This means that the rhamnose occurs in the molecule exclusively as end group in the pyranose form.

Apart from the traces of 2:3-di-0-methyl-L-arabinose which can be either in the furanose or pyranose forms, all the methyl ethers of arabinose found can only exist in furanose rings, so that almost all the arabinose will occur in *Acacia pycnantha* gum in the furanose form. This agrees with the ease of removal of the monosaccharide in mild acid conditions.

The most important arabinose derivative was the 2:3:5-tri-0-methyl-L-arabinose, which accounted for about two thirds of all the L-arabinose in the whole gum, indicating that a large proportion of the arabinose occurs as end group arabofuranose.

Most of the rest of the arabinose (i.e. approximately one third /
third of the total) was accounted for by the 2:5-di-Q-methyl-L-arabinose. This is derived from non terminal arabofuranose linked through C atoms 1 and 3.

Since the disaccharide 3-Q-L-arabofuranosyl-L-arabinose has been identified, the simplest assignment of arabinose residues will be equal amounts of:

Araf 1...
and Araf 1 - 3 Araf 1...

These will not be the only arabinose groupings because of the isolation of the small amounts of 2:5- and 3:5-di-Q-methyl-L-arabinose, but they are the most important ones.

Thus the three main acid labile groupings are:

Rgp 1...
Araf 1...
Araf 1 - 3 Araf 1...

In addition, the presence of the 2:3:4:6-tetra-Q-methyl-D-galactose in the methylation products of the whole gum indicated that some galactopyranose residues occur in the molecule as end group.

The isolation of the same fully etherified aldobiouronic acid from both the methylated degraded and undegraded gums showed that no substituents are linked to the acid disaccharide units (XVI).

Thus there occur in the molecule 2 non acid labile groupings.

These /
These are:–

Gal 1...

and Gl Acid 1 - 6 Gal 1...

The points of attachment of the acid labile groupings were found by comparing the proportions of the methyl ethers of galactose in the whole and degraded gums. Since very little galactose and no galactose containing oligosaccharides were among the low molecular weight products of mild acid hydrolysis of the gum, it may be assumed that the galactan framework is substantially unchanged during the formation of the degraded gum.

### Molar Proportions of Methyl Ethers of D-galactose

<table>
<thead>
<tr>
<th>Methyl Ether</th>
<th>Hydrolysis of Methylated Whole gum</th>
<th>Hydrolysis of Methylated Degraded gum</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4:6-tetra-</td>
<td>1.0</td>
<td>1.6</td>
<td>+ 0.6</td>
</tr>
<tr>
<td>2:4:6-tri-</td>
<td>0.2</td>
<td>1.7</td>
<td>+ 1.5</td>
</tr>
<tr>
<td>2:3:4-tri-</td>
<td>0.7</td>
<td>0.5</td>
<td>- 0.4</td>
</tr>
<tr>
<td>2:4-di-</td>
<td>3.4</td>
<td>1.4</td>
<td>- 2.0</td>
</tr>
</tbody>
</table>

The most important feature is the large increase in the proportion of 2:4:6-tri-0-methyl-D-galactose with degradation and the corresponding decrease in 2:4-di-0-methyl-D-galactose.

Thus 2:4-di-0-methyl-D-galactose $\rightarrow$ 2:4:6-tri-0-methyl-D-galactose.

It follows that a large proportion of the acid labile groups are attached to position 6 of galactopyranose residues also linked through 0 atoms 1 and /
Similarly there is a smaller but significant increase in the amount of 2:3:4:6-tetra-\(\beta\)-methyl-\(\alpha\)-galactose and corresponding decrease in 2:3:4-tri-\(\beta\)-methyl-\(\alpha\)-galactose.

i.e. 2:3:4-tri-\(\beta\)-methyl-\(\alpha\)-galactose --- degradation --- 2:3:4:6-tetra-\(\beta\)-methyl-\(\alpha\)-galactose.

In the same way it follows that a number of the acid labile groupings will be attached to C atom 6 of galactose residues also linked through position 1.

\[
\text{degradation} \\
- 1 \text{Gal} 6 - \text{R} \quad \longrightarrow \quad - 1 \text{Gal}
\]

Thus we can now give the main repeating unit of \textit{Acacia pycnantha} gum as:-

\[
\ldots 5 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1 - 3 \text{Gal} 1 \ldots \\
\text{6} \quad \text{6} \quad \text{6} \quad \text{6} \\
\quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad }
repeating unit. Again the glucuronic acid residues occur in the molecule as end groups linked to C atom 6 of galactose units but it is not yet known how the acid disaccharide unit is attached to the galactan framework.

In addition, although the nature and modes of linkages of the main acid labile groupings are known, the particular points of attachment for these units have yet to be found.

Thus most of the main structure features of *Acacia pycnantha* gum are now known and a more detailed comparison can be made with gum arabic.

\[
\begin{align*}
\text{Gal Acid} & \\
1 & \\
| & 6 \\
R - 3 \text{Gal} & \\
1 & \\
| & 6 \\
...3 \text{Gal} & - 3 \text{Gal} 1 - 5 \text{Gal} 1... \\
6 & \\
| & \\
1 & 6 \\
R - 3 \text{Gal} & R - 3 \text{Gal} \\
6 & \\
| & \\
1 & 6 \\
R - 3 \text{Gal} & R - 3 \text{Gal} \\
6 & \\
| & \\
1 & 6 \\
R - 4 \text{Gal Acid} & R - 4 \text{Gal Acid} \\
\end{align*}
\]

\[
R = \text{Araf 1}...
\]
\[
= \text{Rhap 1}...
\]
\[
= \text{Galp 1 - 3 Araf 1}...
\]

and sometimes \[= \text{Arap 1 - 3 Araf 1}... \] (22)
The two gums show similarities in their basic structures, and, like all the *Acacia* gums, contain the same four component sugars (D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid). These are in different relative amounts, gum arabic having greater proportions of rhamnose and glucuronic acid. In addition, both the gums contain highly branched frameworks of galactose residues, in which the main chain is linked 1:3 and the side chains 1:6. However, in the more complex gum arabic each galactose unit in the backbone carries a galactose containing side chain whereas in *Acacia pycnantha* gum such side chains are attached only to approximately every second main chain galactose residue.

The same aldobiouronic acid unit occurs in the side chains of both gums and indeed the acid has been isolated from all the *Acacia* gums so far investigated. In *Acacia pycnantha* gum the acid disaccharide grouping occurs exclusively as end group, but there is evidence that it exists in gum arabic with attached acid labile groupings as well as being in terminal positions. The exact mode of linkage of these units however is not yet known in the case of *Acacia pycnantha* gum.

The same monosaccharides, arabinose and rhamnose, are liberated into solution on mild acid hydrolysis of the two gums, but the more complex acid labile groupings differ. In *Acacia pycnantha* gum only one has been identified and it is 3-0-L-arabofuranosyl-L-arabofuranose. In gum arabic there are two, 3-0-L-arabopyranosyl-L-arabofuranose and 3-0-D-galactopyranosyl-L-arabofuranose groups.

Finally the positions to which the specific acid labile groups are /
are attached are still unknown for both gums but the points of linkage are known and these are different. In gum arabic, the groupings joined to C atom 5 of galactose residues in the outer chains and to C atom 4 of the glucuronic acid units, whereas in \textit{Acacia pycnantha} gum they are linked to C atom 6 of galactose residues in both the backbone and side chains.
SECTION II

THE MOLECULAR STRUCTURE OF EUROPEAN LARCH \( \alpha \)-GALACTAN

EXPERIMENTAL

Oxidation of European larch \( \alpha \)-galactan

The polysaccharide (5 g.) was dissolved in water (150 ml.) and depoisoned by shaking with platinum catalyst (10 mg.). The platinum was removed by centrifugation and the centrifugate added to a solution of sodium bicarbonate (500 mg. in 100 ml.) which had previously been similarly depoisoned.

Platinum catalyst (1 g.) was added to the solution and the reaction mixture, through which was bubbled a constant supply of oxygen, was stirred for fourteen days at 70\(^{\circ}\).

Next the platinum was removed from the cooled solution by centrifugation and the centrifugate reduced in volume to 150 ml. The oxidised polysaccharide was precipitated from the solution by the addition of ethanol (5 volumes), the precipitate dissolved in water and the resulting solution was deionised with Amberlite resin IR-120(H). The free acid polysaccharide was obtained by precipitation with ethanol (5 volumes). Yield = 5.2 g.

The uronic acid content was determined by the method of Kaye and Kent (137) and found to be 7.5%.

Hydrolysis of the oxidised Polysaccharide and the Isolation of Acid Disaccharides.

The free acid polysaccharide (5.0 g.) was dissolved in N-sulphuric /
sulphuric acid (50 ml.) and heated at 100° for 4 hours. The cooled solution was neutralised with di-n-octylmethylamine (5% v./v. in chloroform: 4 extractions) and poured on to a charcoal:celite column (1:1, 15 g.).

Elution in water gave galactose and arabinose.

Elution with 20% ethanol gave a mixture of sugars (330 mg.) all of which were chromatographically slower moving than galactose in solvent (C).

The ethanol eluate was taken to dryness and re-hydrolysed with N-sulphuric acid (50 ml.) at 100° for 2 hours. The neutralised hydrolysate was reduced in volume to a syrup which, when examined chromatographically using solvent (C), showed two main components and several other minor spots. The material was separated on thick papers, which had previously been extracted with 8-hydroxyquinoline in acetone. Two fractions were collected, fraction 1 containing the chromatographically faster moving material, and fraction 2 the slower.

**Examination of the Acid Oligosaccharides.**

**Fraction 1.** 0.045 g.

Paper chromatography in solvent (C) showed a main spot ($R_{Gal} = 0.5$) which was brown pink and a trace of a slower moving brown spot (ca. 2% of total).

The sugar (10 mg.) was converted to the methyl ester methyl glycoside by refluxing with dry methanolic hydrogen chloride (3%: 10 ml.) for 6 hours. The cooled solution was neutralised and taken to a syrup which /
which was reduced by dissolving in a solution of potassium borohydride (20 mg. in 3 ml.) and leaving overnight. The excess of borohydride was then destroyed by the addition of glacial acetic acid and the solution was deionised with Amberlite resins IR-43(OH) and IR-120(H), made N with respect to sulphuric acid and heated for 4 hours at 100°. The neutralised hydrolysate was taken to dryness and chromatography of the resulting syrup in solvents (A) and (C) showed approximately equal quantities of arabinose and galactose.

The sugar (10 mg.) was reduced with potassium borohydride, converted to the methyl ester with methanolic hydrogen chloride, reduced again with borohydride and hydrolysed with N-sulphuric acid at 100° for 4 hours. The neutralised hydrolysate showed arabinose alone on chromatography in solvent (C) and using aniline oxalate as a spray.

These results suggest that fraction 1 is an araburonosylgalactose.

The sugar (2 mg.) was treated with alkaline iodine and the M.W. determined by the method of Chanda, Hirst, Jones and Percival (112). This gave a value of 314 (M.W. of araburonosylgalactose = 328).

The sugar (6 mg.) was oxidised with sodium metaperiodate and the formaldehyde release determined by the method of O'Dea and Gibbons (135) using parahydroxybenzaldehyde (136). The release was 0.06 moles formaldehyde per mole of sugar.

The sugar (10 mg.) was reduced with potassium borohydride, the excess borohydride destroyed with glacial acetic acid and the resulting /
resulting araburonosyl-galactitol oxidised with sodium metaperiodate giving 0.87 moles of formaldehyde per mole of sugar.

These results suggest that the linkage present is 1:5 or 1:6. The 1:6 linkage is much more likely since the reducing group galactose is almost certainly in the stable pyranose form.

**Fraction 2. 0.095 g.**

The chromatographically pure substance had $R_{\text{Gal}} = 0.25$ in solvent (C) and showed up brown when sprayed with aniline oxalate.

The sugar (10 mg.) was converted to the methyl ester methyl glycoside, reduced with potassium borohydride and hydrolysed with N-sulphuric acid giving galactose alone.

Similarly after reduction, conversion to the methyl ester, reduction and hydrolysis, the sugar (10 mg.) gave only galactose. The results suggest that fraction 2 is a galacturonosyl-galactose. The M.W. was determined as for fraction 1 giving 341 (M.W. galacturonosyl-galactose = 358).

The sugar (5 mg.) was oxidised with sodium metaperiodate giving 0.04 moles formaldehyde per mole of sugar.

The sugar (10 mg.) was reduced with potassium borohydride, the excess borohydride destroyed with glacial acetic acid and the galacturonosyl-galactitol thus formed oxidised with sodium metaperiodate yielding 0.85 moles formaldehyde per mole of sugar.

The sugar (55 mg.) was converted to the methyl ester methyl glycoside by refluxing with methanolic hydrogen chloride (3% : 50 ml.) for /
for 6 hours and the methyl glycoside of galactobiose was formed by reduction with potassium borohydride and was isolated (27 mg.) chromatographically by separation on thick papers using solvent (A).

Methyl glycoside (5 mg.) was oxidised with aqueous sodium metaperiodate (0.015 M : 10 ml.) at 35° for 10 hours. The uptake of periodate, measured spectrophotometrically (138), was equivalent to 3.8 moles per mole of sugar.

Methyl glycoside (20 mg.) was dissolved in potassium chloride solution (0.56 M : 50 ml.) and sodium metaperiodate (0.2 M : 20 ml.) added. The solution was shaken and left in the dark. Aliquots (10 ml.) were withdrawn at intervals and the excess of periodate destroyed by the addition of ethylene glycol (1 ml.). The liberated formic acid was titrated with 0.015N-sodium hydroxide using methyl red as an indicator. A blank omitting the polysaccharide was run simultaneously. The release of formic acid after 10 hours was calculated to be 1.8 moles per mole of sugar.
DISCUSSION

European larch (*Larix decidua*)-galactan (see p. 35) is made up of D-galactose and L-arabinose residues in the approximate ratio of 6:1. Mild acid hydrolysis (119) liberates 3-α-D-arabopyranosyl-L-arabinose and methylation, periodate oxidation and further partial hydrolysis studies (117) indicate that the arabinobiose grouping occurs terminally in the molecule as:-

\[
\text{Ara} 1 - 3 \text{Ara} 1...
\]

attached in some way to a galactan framework for which:-

\[
...3 \text{Gal} 1 - 3 \text{Gal} 1...
\]

\[
6 \quad 6
\]
\[
1 \quad 1
\]
\[
\text{Gal} \quad \text{Gal}
\]
\[
6 \quad 6
\]
\[
1 \quad 1
\]
\[
\text{Gal} \quad \text{Gal}
\]

can be given as the main repeating unit, although some galactose residues may be doubly branched:-

\[
\text{Gal} 4 - 3 \text{Gal} 1 - 6
\]

while others may be linked:-

\[
-3 \text{Gal} 1 -
\]
The above studies, however, give no information about the nature of the sugar residue to which the arabinobiose grouping is attached in the molecule, or about the linkage between them.

The normal method of determining the mode of attachment of terminal disaccharide groupings to the main framework of a polysaccharide is partial acid hydrolysis with the subsequent isolation of oligosaccharides made up of the end groupings (or part of them), part of the framework and possibly some intermediate residues. However the ease of hydrolysis of furanosyl relative to pyranosyl linkages makes this method impracticable in this and other cases where the grouping is attached by furanosyl linkage.

However if the polysaccharide is oxidised so that the primary alcohol groups present are converted to carboxylic acid, the easily acid split furanosyl linkages would become acid resistant uronic acid glycoside bonds. The non terminal arabofuranose residues of European larch \(\xi\)-galactan would be oxidised to araburonic acid, while the end group arabopyranose units, with no primary alcohol groups, would be unaffected. Hydrolysis of this oxidised larch \(\xi\)-galactan in conditions harsh enough to split all linkages except the uronic acid glycoside bonds (i.e. N-sulphuric acid at 100° for 4 hours) would then give aldobiouronic acids, an examination of which would reveal the modes of attachment of the arabinobiose to the main framework of the molecule.
In addition galactose residues with free hydroxyl groups at C atom 6 would be oxidised to galacturonic acid units, which, after hydrolysis of the oxidised polysaccharide, would form the non reducing end groups of further aldobiouronic acids.

European larch \( \xi \)-galactan was oxidised by bubbling oxygen through a solution of the polysaccharide. Platinum was used as a catalyst. This treatment gave a polysaccharide with a uronic acid content of 7.5%. The oxidised polysaccharide was then hydrolysed and two acid oligosaccharides were isolated from the hydrolysate.
The chromatographically faster moving material after conversion to the methyl ester methyl glycoside, reduction with potassium borohydride and hydrolysis gave arabinose and galactose. After reduction, treatment with methanolic hydrogen chloride, reduction and hydrolysis, the oligosaccharide gave arabinose only indicating that the non reducing monosaccharide was araburonic acid. The molecular weight was found to be 314, suggesting that this acid oligosaccharide is an araburonosylgalactose (required M.W. = 328).

\[
\begin{align*}
(1) \text{MeOH/HCl} & \quad \text{Galactose} \\
(2) \text{KBH}_4 & \rightarrow \quad \text{Arabinose} \\
(3) \text{H}^+ & \quad \text{(Dulcitol)}
\end{align*}
\]

Similarly, the chromatographically slower moving material (M.W. 341) gave galactose alone after conversion to the methyl ester methyl glycoside, reduction and hydrolysis and also after reduction, treatment with methanolic hydrogen chloride, reduction and hydrolysis showing that it is made up of a galacturonic acid residue attached to a reducing group galactose (required M.W. = 358).

Oxidation with sodium metaperiodate on both aldobiouronic acids liberated no formaldehyde but, after reduction with potassium borohydride, each yielded approximately one mole of formaldehyde per mole of sugar indicating that the linkage in both acid disaccharides is /
is either 1:5 or 1:6. Since the galactose residues are almost certainly in the stable pyranose form, the linkages are 1:6, and since Arap 1 - 3 Araf 1... is the main arabinobiose grouping occurring in the molecule, the arabinose will mainly occur in European larch ξ-galactan attached to the main framework:

\[ \text{Arap 1 - 5 Araf 1 - 6 Galp 1...} \]

However it is not yet known if this grouping is attached directly to the backbone of the molecule or if one or more galactose residues are interposed:

\[ \ldots 3 \text{Gal 1...} \quad \ldots 3 \text{Gal 1...} \]

\[
\begin{array}{c}
\text{Gal} \\
6 \\
| \\
1 \\
Araf \\
3 \\
| \\
1 \\
Araf \\
3 \\
| \\
1 \\
Araf \\
6 \\
| \\
1 \\
Gal \\
1 \\
3 \\
| \\
1 \\
Araf \\
6 \\
| \\
1 \\
Gal
\end{array}
\]

In addition Gal 1 - 6 Gal will occur in the molecule as end group or linked through C atoms 3 or 4 of the non reducing galactose.

Further, the galacturonosylgalactose was reduced with potassium borohydride after conversion to the methyl ester methyl glycoside, and the resulting methyl glycoside of galactobiose was isolated and consumed 3.8 moles of sodium metaperiodate with the liberation of 1.8 moles of formic acid confirming that the linkage present is 1:6.
BIBLIOGRAPHY


(2) E. Anderson and L. Sands, ibid., 1945, 1, 329.

(3) E.L. Hirst, Endeavor, 1951, 10, 106.


(5) E.L. Hirst and J.K.N. Jones, Research, 1951, 4, 411.


(11) S.P. James and F. Smith, J., 1945, 739.


(16) E.J. Bourne, A.B. Foster and P.M. Grant, J., 1956, 4511.


(23) /
(24) J. Jackson, and F. Smith, J., 1940, 78.
(27) F. Smith, J., 1939, 744.
(30) F. Smith, J., 1959, 1724.
(31) J. Jackson and F. Smith, J., 1940, 74.
   Acad., 1953, 55B, 331.
(33) F. Smith and D. Spriestersbach, Amer. Chem. Soc. Meeting,
   Minneapolis, Sept., 1955, Abs. Papers, 15D.
(34) F. Smith, J., 1940, 1035.
(36) A.P. Stephen, J., 1951, 646.
(38) Idem, ibid., 1955, 1428.
   1956, 33, 861.
(40) S. Mukherjee and A.N. Shrivastava, J. Amer. Chem. Soc., 1958,
   80, 2536.
(42) E.L. Hirst and J.K.N. Jones, J., 1958, 1174.
(43) Idem, J., 1946, 506.
(44) Idem, J., 1939, 1482.
(46) /
(47) E.L. Hirst and J.K.N. Jones, J., 1948, 120.
(48) F. Andrew, D.H. Ball and J.K.N. Jones, J., 1953, 4090.
(49) J.K.N. Jones, J., 1939, 558.
(51) Idem, J., 1949, 3141.
(54) A.M. Stephen, J., 1956, 4437.
(63) E. Anderson and H.D. Ledbetter, J. Amer. Pharm. Assoc., 1951, 40, 625.
(64) E.V. White, J. Amer. Chem. Soc., 1953, 75, 257.
(65) Idem, ibid., 1955, 75, 4692.
(66) Idem, ibid., 1954, 76, 4906.
(67) R.J. Mcllroy, J., 1951, 1572.
(69) Idem, ibid., 1954, 15B, 452.
(71) Idem, ibid., 1958, 4408.

B.
124.


(72) E.L. Hirst, E. Percival and R.S. Williams, J., 1958, 1942.


(74) J.K.N. Jones and J.R. Nunn, J., 1955, 3001.


(82) Idem, ibid., 1956, 33, 125.


(84) Idem, ibid., 1947, 69, 622.

(85) Idem, ibid., 1947, 69, 2264.

(86) Idem, ibid., 1948, 70, 367.


(89) F. Smith, J., 1951, 2646.


(95) L. Beuquesne, Compt. rend., 1946, 222, 1056.

(94) R.J. McIlroy, J., 1952, 1918.


(96) E.L. Hirst and S. Dunstan, J., 1953, 2332.


(98) /
(100) S.P. James and F. Smith, J., 1945, 749.
(101) Idem, J., 1945, 746.
(102) E.L. Hirst, J., 1955, 2974.
(106) E. Salkowski, J. Physiol. Chem., 1901, 34, 162.
Idem, ibid., 1902, 35, 240.
(110) A Roudier and L. Eberhard, TAPPI, 1955, 38, 156A.
(111) D.J. Brasch and L.E. Wise, TAPPI, 1956, 39, 581.
(116) H. Mosiman and R. Svedberg, Kolloid Z., 1942, 100, 1.
(120) E.V. White, J. Amer. Chem. Soc., 1941, 63, 2871.
1942, 64, 302, 1507, 2838.
(122) /


   Idem, ibid., 1959, 37, 29.


   1954, 76, 4097.

(126) A.B. Foster, Chem. and Ind., 1952, 1050.

(127) F. Pregl, "Quantitative Organic Microanalysis", 1945, 146.


   1955, 15, 16.


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A. N.

336. The Structure of Acacia pycnantha Gum.

By G. O. Aspinall, E. L. Hirst, and A. Nicolson.

Controlled acid-hydrolysis of the gum liberates L-arabinose and L-rhamnose with the formation of a degraded gum containing residues of D-galactose and D-glucuronic acid. 3-O-L-Arabofuranosyl-L-arabinose has been isolated during the early stages of the hydrolysis. Partial acid-hydrolysis of the fragment remaining after degradation of the periodate-oxidised degraded gum with phenylhydrazine affords 3-O-β-D-galactopyranosyl-D-galactose and only small amounts of a second disaccharide. Hydrolysis of the methylated degraded gum yields 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, 2 : 4 , and 2 : 6-di-, and 2-O-methyl-D-galactose, (2 : 3 : 4-tri-O-methyl-6-D-galactopyranose 2 : 3 : 4-tri-O-methyl-β-D-glucopyranosid)uronic acid, and traces of other sugars. Hydrolysis of the methylated gum affords 2 : 3 : 5-tri- and 2 : 5-di-O-methyl-L-arabinose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4 - and 2 : 4 : 6-tri-, 2 : 4 , and 2 : 6-di-, and 2-O-methyl-D-galactose, 2 : 3 : 4-tri-O-methyl-L-rhamnose, (2 : 3 : 4-tri-O-methyl-6-D-galactopyranose 2 : 3 : 4-tri-O-methyl-β-D-glucopyranosid)uronic acid, and traces of other sugars. It is concluded that the gum is a highly branched polysaccharide containing a framework of D-galactopyranose residues with main chains linked 1 → 3 and with side-chains attached by 1 → 6 linkages; to this branched framework are attached side-chains of L-rhamnopyranose, L-arabofuranose, 3-O-L-arabofuranosyl-L-arabofuranose, and (6-D-galactopyranose β-D-glucopyranosid)uronic acid residues. The structure of the gum is compared with that of gum arabic.

A previous investigation 1 of Acacia pycnantha gum showed that the gum is composed of residues of D-galactose (65%), L-arabinose (27%), L-rhamnose (1—2%), and D-glucuronic acid (5%). Although containing the same sugar units, the gum differs considerably from gum arabic 2,3 and the gums from other Acacia species 4 in the proportions of the constituent sugars. The gum is similar to these gums in giving rise to the aldobiouronic acid (6-D-galactose β-D-glucopyranosid)uronic acid, as a product of partial acid-hydrolysis. A further similarity with gum arabic is the formation of 3-O-β-D-galactopyranosyl-D-galactose as another product of partial acid hydrolysis. In this paper the results of further structural investigations on A. pycnantha gum are reported and in consequence a more detailed comparison of the structure of the gum with that of gum arabic is possible.

Hydrolysis of the gum under controlled conditions resulted in the release of arabinose and rhamnose, and only traces of galactose, with the formation of a stable degraded gum virtually free from arabinose residues. When the mild hydrolysis was arrested at an early stage an arabinose-containing disaccharide was present amongst the products. A quantity of this material was isolated by chromatography on cellulose; it had chromatographic mobility and optical rotation ([α]D +89°) similar to those reported for 3-O-L-arabofuranosyl-L-arabinose isolated from sugar-beet araban. 5 Since the disaccharide was readily hydrolysed by acid it probably contained a furanosyl linkage, and its absence at later stages in the hydrolysis of the gum indicated that it was not an acid reversion product. 6 The presence of a 3-O-substituted arabinose residue was indicated by the formation of ca. 1 mol. of formaldehyde on periodate oxidation of the disaccharide and of 1:54 mols. of formaldehyde on similar oxidation of the derived glycitol. 7 Confirmation of the structure of the disaccharide as 3-O-L-arabofuranosyl-L-arabinose was obtained by the isolation of 2 : 3 : 5-

...
tri- and a mixture of 2 : 4- and 2 : 5-di-O-methyl-L-arabinose on hydrolysis of the methylated disaccharide.

The degraded gum was converted into the fully methylated derivative, hydrolysis of which afforded 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, and 2 : 4-di-O-methyl-D-galactose, and a methylated aldobiouronic acid, together with smaller amounts of 2 : 6-di- and 2-O-methyl-D-galactose. Very small amounts of 2 : 3 : 4-tri-O-methyl-D-galactose and 2 : 3-di-O-methyl-L-arabinose were probably also present in the hydrolysate. The methylated aldobiouronic acid was shown to be (2 : 3 : 4-tri-O-methyl-6-D-galactopyranose 2 : 3 : 4-tri-O-methyl-β-D-glucopyranosid)uronic acid since reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride followed by hydrolysis yielded 2 : 3 : 4-tri-O-methyl-D-glucose and 2 : 3 : 4-tri-O-methyl-D-galactose. It follows from the isolation of the methylated degraded gum of 2 : 3 : 4 : 6-tetra-, 2 : 4 : 6-tri-, and 2 : 4-di-O-methyl-D-galactose in approximately equimolecular amounts that the degraded gum contains a highly branched stable framework of 1 : 3- and 1 : 6-linked D-galactopyranose residues, for which partial structures (I), (II), and other variants may be advanced. The isolation of the fully etherified aldobiouronic acid, (2 : 3 : 4-tri-O-methyl-6-D-galactopyranose 2 : 3 : 4-tri-O-methyl-β-D-glucopyranosid)uronic acid, indicates that aldobiouronic acid units (III) must be attached as side-chains to the galactan backbone, but there is no evidence yet for the positions of linkage of these units. It is not yet known if the minor products of hydrolysis of this methylated polysaccharide are of structural significance.

Evidence in favour of structure (I) for the major part of the degraded gum was obtained by application of Barry's method of degradation \(^8\) to the polysaccharide. Periodate-oxidised degraded gum was treated with phenylhydrazine and partial acid hydrolysis of the residual polymer afforded 3-O-β-D-galactopyranosyl-D-galactose as the main disaccharide product with only traces of the 1 : 6-linked isomer being detected chromatographically. It follows that the main chain is composed essentially of 1 : 3-linked β-D-galactopyranose residues as required by structure (I) and that the 1 : 6-linkages in the degraded gum are those of D-galactose residues attached as side-chains. The isolation of a small amount of a 1 : 3-linked galactotriose and higher homologues provides further evidence in support of this structure. The 1 : 3- and 1 : 6-linked D-galactopyranose residues in Acacia pycnantha gum are, therefore, similarly arranged to those in gum arabic \(^3\) and in larch α-galactan.\(^9\)

The characteristic linkages of the sugar residues in the undegraded gum were established by analysis of the complex mixture of methylated sugars obtained on hydrolysis of the methylated gum. The following sugars were characterised by the formation of crystalline derivatives: 2 : 3 : 5-tri- and 2 : 5-di-O-methyl-L-arabinose, 2 : 3 : 4-tri-O-methyl-L-rhamnose, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4- and 2 : 4 : 6-tri-, and 2 : 4-di-O-methyl-D-galactose, and the methylated aldobiouronic acid, (2 : 3 : 4-tri-O-methyl-6-D-galactopyranose 2 : 3 : 4-tri-
O-methyl-3-0-glucopyranosid]uronic acid. In addition small amounts of 2 : 6-di- and 2-O-methyl-β-galactose were identified, and two unidentified dimethyl ethers of arabinose (probably the 3 : 5- and 2 : 3-isomers) were also present. These sugars were not present in sufficient quantity to be considered as major structural units and some, indeed, may be products of incomplete methylation.

The significance of these results may be assessed by comparing the nature and amounts of the various methylated sugars isolated from the methylated derivatives of the degraded and undegraded gums. Taking the galactan structure (I) with attached aldobiouronic acid units (III) as a working model for the degraded gum, we may consider first the nature of the acid-labile residues removed during the mild acid-hydrolysis, and, secondly, the points of attachment of these groups to the galactan framework. The isolation of the same fully etherified aldobiouronic acid from methylated degraded and undegraded gums indicates that no substituents are linked to the acidic disaccharide units (III).

The only L-rhamnose derivative found on hydrolysis of the methylated gum was the 2 : 3 : 4-trimethyl ether; therefore the small proportions of these sugar residues in the gum occur solely as end groups in the pyranose form. Apart from traces of arabinose residues giving rise to 2 : 3-di-0-methylarabinose and which may be present in either furanose or pyranose form, L-arabinose residues in the gum occur solely in the furanose form, approximately two-thirds in terminal and one-third in non-terminal positions linked 1 → 3. Since 3-O-L-arabinofuranosyl-L-arabinose has been identified as a partial acid hydrolysis product of the gum, the simplest, but not the only, assignment of arabinose residues is of equal proportions of single L-arabinofuranose and of 3-O-L-arabofuranosyl-L-arabofuranose residues attached as side chains to the galactan framework. The points of attachment of the acid-labile residues to galactose residues in the gum follow from a comparison of the proportions of D-galactose methyl ethers formed on hydrolysis of the methylated gum and of the methylated degraded gum. In the absence of galactose and galactose-containing oligosaccharides amongst the low-molecular-weight products of mild acid hydrolysis of the gum it may be assumed that the galactan framework of the gum is substantially undisturbed during the formation of the degraded gum. The most striking difference is the large increase in the proportion of 2 : 4 : 6-tri-O-methyl-β-galactose from the methylated degraded gum and the corresponding decrease in the proportion of 2 : 4-di-O-methyl-β-galactose. It follows that a large proportion of the acid-labile groups are attached to position 6 of 1 → 3 linked D-galactopyranose residues in the main chains of the molecular structure. A second but significant difference is the increase in the proportion of 2 : 3 : 4 : 6-tetra-O-methyl-α-galactose which corresponds approximately with the decrease in the proportion of 2 : 3 : 4-tri-O-methyl-β-galactose. Further acid-labile groups are therefore

![Diagram](image)

**Proportions of methyl ethers of D-galactose.**

<table>
<thead>
<tr>
<th>Methyl ether</th>
<th>Hydrolysis of methylated gum</th>
<th>Hydrolysis of degraded gum</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 : 3 : 4 : 6-Tetra-</td>
<td>1.0</td>
<td>1.6</td>
<td>+0.6</td>
</tr>
<tr>
<td>2 : 3 : 4-Tri-</td>
<td>0.05</td>
<td>0.25</td>
<td>-0.2</td>
</tr>
<tr>
<td>2 : 4 : 6-Tri-</td>
<td>0.2</td>
<td>1.4</td>
<td>+1.2</td>
</tr>
<tr>
<td>2 : 4-Di-</td>
<td>3.4</td>
<td>1.4</td>
<td>-2.0</td>
</tr>
</tbody>
</table>

...3 D-Galp 1 → 3 D-Galp 1 → 3 D-Galp 1 → 3 D-Galp 1 → 3 D-Galp 1 ...

(R = L-Araf 1 → 3 L-Araf 1 → 3 L-Araf 1, or L-Rhap 1 → 3 L-Rhap 1)
linked to position 6 of d-galactopyranose residues present as side chains in the gum. It is not possible to ascribe particular points of attachment to the various types of acid-labile groups, but on the basis of present knowledge the main structural features of Acacia pycnantha gum may be summarised in structure (IV), with aldobiouronic acid units (III) attached in a manner as yet unknown.

Sufficient is now known of the mode and order of linkage of the constituent sugar residues of Acacia pycnantha gum for a broad comparison of its structure to be made with that of gum arabic. The structure (V) for gum arabic is based upon the classic work of Smith and upon more recent investigations in which the presence of the backbone of 1-3-linked galactose residues has been established.

The two gums contain highly branched backbones of galactose residues in which the main chain is linked 1→3 and side-chains are attached by 1→6 linkages, but whereas all the galactose residues in the main chain of gum arabic carry galactose-containing side-chains, such side-chains are attached only to every second galactose residue in the main chain of Acacia pycnantha gum. Both gums contain the same aldobiouronic acid units in the side-chains of the molecule but the precise mode of attachment to the backbone in the latter case is not known. Similarities between the two gums are also shown by the L-arabofuranose and L-rhamnopyranose end groups which are removed by mild acid hydrolysis. The gums differ, however, in the nature of the more complex side-chains also removed under these conditions. In gum arabic these are 3-O-d-galactopyranosyl-L-arabofuranose and in some cases 3-O-L-arabopyranosyl-L-arabofuranose groups, but in A. pycnantha gum they are 3-O-L-arabofuranosyl-L-arabofuranose groups. It may be noted, however, that in both cases the non-terminal L-arabofuranose residues carry substituents at position 3. The most marked differences between the two gums are shown in the positions of linkage to galactose or glucuronic acid residues of the various groups on the periphery of the gum structure. Although it is not known to which positions specific groups are attached, in gum arabic they are linked to C(6) of galactose residues in the outer chains and to C(1) of glucuronic acid residues, whereas in Acacia pycnantha gum they are linked to C(6) of galactose residues in both the main and outer chains.

\[
\begin{align*}
&\text{d-GpA} \\
&\quad\downarrow \\
&\text{R} \rightarrow 3\text{ d-Galp} \\
\quad\downarrow 6 \\
\therefore 3\text{ d-Galp} &\rightarrow 3\text{ d-Galp} \rightarrow 3\text{ d-Galp} \cdots \\
&\quad\downarrow 6 \\
&\quad\downarrow 1 \\
&\text{R} \rightarrow 3\text{ d-Galp} \\
&\quad\downarrow 6 \\
&\text{R} \rightarrow 3\text{ d-Galp} \\
&\quad\downarrow 1 \\
&\text{R} \rightarrow 4\text{ d-GpA} \\
&\text{R} = \text{L-Araf} \cdots, \text{L-Rhap} \cdots, \text{d-Galp} \rightarrow 3\text{ L-Araf} \cdots, \text{or in some cases} 10 \text{ L-Arap} \rightarrow 3\text{ L-Araf} \cdots
\end{align*}
\]

In the absence of any strict proof of homogeneity caution must be exercised in the
interpretation of the results of structural investigations of complex polysaccharides such as the plant gums. There is indeed evidence for a measure of heterogeneity in some samples of gum arabic and of other Acacia gums including A. pycnantha gum. Professor F. Smith (personal communication) informs us that glass-fibre paper ionophoresis in 2N-sodium hydroxide shows his sample of Acacia pycnantha gum to contain a major and a minor component. Glass-fibre paper ionophoresis of the gum sample used by us showed only one component, although another sample of the gum showed a similar degree of heterogeneity to that examined by Professor Smith. There is however no detailed information regarding the nature of the heterogeneity and it is not possible therefore to state whether different fractions from a single gum consist of similarly constituted polysaccharides differing only in some minor respect, or whether there are more fundamental differences of structure. Nevertheless, it is already clear from the comparisons made that the major components of the two gums have important structural features in common. It is indeed possible that just as many polysaccharides of the hemicellulose groups have been shown to be members of the same general family but differing considerably in fine structure, so the vastly more complicated gums from related botanical species may be shown to contain similar basal units of molecular structure upon which are superimposed even wider variations in detailed structure.

**Experimental**

Paper chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate-pyridine-water (10:4:3); (B) butan-1-ol-ethanol-water (4:1:5, upper layer); (C) ethyl acetate-acetic acid-water (3:1:3, upper layer); (D) butan-2-one, half saturated with water; (E) butan-1-ol-benzene-pyridine-water (5:1:3:3, upper layer). Unless otherwise stated, chromatography of methylated sugars was carried out in solvent B, and values refer to the rate of movement relative to 2:3:4:6-tetra-O-methyl-D-glucose in that solvent. Demethylation of methylated sugars was performed by the method of Hough, Jones, and Wadman.

Paper ionophoresis was in borate buffer at pH 10. Optical rotations were observed at 18° ± 2°.

The gum was prepared from crude nodules as described previously, and the sample of methylated gum was that prepared by Hirst and Perlin.

**Partial Acid Hydrolysis and Preparation of the Degraded Gum.**—The gum (1 g.) was heated with 0.01N-sulphuric acid (100 ml.) at 100°. The optical rotation of the solution was observed every hour, and every two hours samples (5 ml.) were withdrawn, neutralised with barium carbonate, filtered, shaken with Amberlite resin IR-120(H) to remove barium ions, and poured into ethanol (3 vol.) to precipitate degraded gum. The supernatant liquor and the hydrolysate from the degraded gum were examined chromatographically in solvent A. The results showed that the arabinose was almost completely removed after 10 hr. and the rhamnose after 14 hr. (solution had [α]D +38°); only small quantities of galactose were released. In a more careful search for oligosaccharides released during the mild acid-hydrolysis, samples were passed through charcoal-Celite columns (1:1; 5 g.). Elution with water removed most of the monosaccharides and elution with 15% ethanol removed an unknown sugar of Rf 1.4 in solvents C and D. This sugar was present in the hydrolysate after 1 hr., in decreased amount after 2 hr., and was no longer present after 4 hr. or at later stages in the hydrolysis.

The gum (29 g.) was heated at 100° with 0.01N-sulphuric acid (1.5 l.) for 14 hr. ([α]D +8° → +38°). The cooled solution was neutralised with Amberlite resin IR-4B(OH), concentrated, and poured into ethanol (4 vol.) to give the degraded gum (15 g.), [α]D +20° (c. 1:2 in H2O) (Found: uronic anhydride, 6%).

**Partial Acid Hydrolysis and Isolation of 3-O-1-Arabinofuranosyl-L-arabinose.**—Further examination of the products of mild acid hydrolysis of the gum showed that the unknown sugar of Rf 1.4 was present in greatest amount after the gum and been heated with 0.01N-sulphuric acid at 85° for 4 hr. The gum (10 g.) was heated at 85° with 0.01N-sulphuric acid (300 ml.) for 4 hr., and the cooled solution was neutralised with Amberlite resin IR-4B(OH), concentrated, and poured into ethanol (3 vol.). The precipitated degraded polysaccharide was removed and the supernatant liquor was concentrated to a syrupy mixture of sugars (2.2 g.), chromatography...
of which showed arabinose and small quantities of rhamnose, galactose, and the sugar of $R_{\text{Gal}}$ 1-4. The syrup was fractionated on cellulose (2.8 x 80 cm.) by use of solvent D to give three fractions. Fraction 1 (17 g.) contained arabinose and traces of rhamnose and the disaccharide ($R_{\text{Gal}}$ 1-4). Fraction 2 (105 mg.) contained the disaccharide with traces (ca. 1%) of galactose and arabinose. Fraction 3 (70 mg.) contained the disaccharide, galactose, and unidentified oligosaccharides.

The disaccharide (fraction 2) had $[\alpha]_D^2 +89^\circ$ (c 1-0 in H$_2$O) and gave only arabinose on hydrolysis. Periodate oxidation $^{15}$ of a sample (10 mg.) gave 0-87 mol. of formaldehyde, identified as the dimedone compound, m. p. 189—190$^\circ$. A second sample (10 mg.) in water (2 ml.) was reduced with potassium borohydride (20 mg.) for 16 hr., and oxidation with periodate afforded formaldehyde corresponding to 1-54 mol. per mol. of glyciol.

Methyl sulphate (2 ml.) and 40% aqueous sodium hydroxide (3 ml.) were added dropwise to the sugar (80 mg.) in water (8 ml.) at 0$^\circ$. Further additions of methyl sulphate (2 x 12 ml. + 6 ml.) and 40% sodium hydroxide (2 x 22 ml. + 9 ml.) were made at room temperature and the reaction was completed by heating the solution on the boiling-water bath for 1 hr. The cooled mixture was extracted with chloroform for 1 hr. to give methylated disaccharide (80 mg.). Hydrolysis of a sample (3 mg.) gave 2: 3: 5-tri-O-methylarabinose, with only traces of products of incomplete methylation. Hydrolysis of the methylated disaccharide (77 mg.) with N-sulphuric acid at 100$^\circ$ for 4 hr., followed by neutralisation with barium carbonate and deionisation, gave a syrup (68 mg.) which was separated on filter sheets with solvent B, to give three fractions. Fraction a (20 mg.), $R_0$ 0-98, was identified as 2: 3: 5-tri-O-methyl-L-arabinose by conversion into 2: 3: 5-tri-O-methyl-L-arabinonamide, m. p. and mixed m. p. 132—133$^\circ$. Fraction b (10 mg.), $R_0$ 0-84, was characterised as 2: 5-di-O-methyl-L-arabinose by conversion into aldonoamine, m. p. and mixed m. p. 123—124$^\circ$. Fraction c (14 mg.), $R_0$ 0-65, was identified as 2: 4-di-O-methyl-L-arabinose by conversion into the aniline derivative, m. p. and mixed m. p. 131$^\circ$.

**Preparation and Hydrolysis of Methylated Degraded Gum.—**The degraded gum (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide, to give methylated degraded gum (31 g.), $[\alpha]_D -35^\circ$ (c 1-0 in CHCl$_3$) (Found: OMe, 45-0%). The methylated degraded gum (29 g.) was suspended in N-hydrochloric acid (300 ml.) at 40$^\circ$ for 8 days. The resulting solution was heated at 100$^\circ$ for 16 hr. (constant rotation), cooled, neutralised with silver carbonate, and, after removal of silver ions as silver sulphide, treated with barium carbonate and concentrated to a syrup (2-4 g.). The syrup mixture of sugars was separated on cellulose (3.5 x 90 cm.) with light petroleum (b. p. 100—120$^\circ$)—butan-1-ol (7: 3; later 1: 1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give ten fractions, and a further fraction was obtained by elution of the cellulose with water.

**Fraction 1.** The syrup (40 mg.), $[\alpha]_D +44^\circ$ (c 0-4 in H$_2$O), contained a mixture of 2: 3: 5-tri-O-methylarabinose ($R_0$ 0-98), 2: 3: 4-tri-O-methylrhamnose ($R_0$ 1-01), and 2: 3: 4: 6-tetra-O-methylgalactose ($R_0$ 0-89). Demethylation gave rhamnose, arabinose, and galactose.

**Fraction 2.** The chromatographically pure sugar (625 mg.), $R_0$ 0-89, crystallised from ether—light petroleum and had m. p. and mixed m. p. 68$^\circ$ (with 2: 3: 4: 6-tetra-O-methyl-D-galactose) and $[\alpha]_D +140^\circ$ +117$^\circ$ (c 1-0 in H$_2$O) (Found: OMe, 52-2. Calc. for C$_{16}$H$_{26}$O$_5$: OMe, 52-3%). The aniline derivative had m. p. and mixed m. p. 194$^\circ$.

**Fraction 3.** The syrup (56 mg.), $[\alpha]_D +90^\circ$ (c 1-1 in H$_2$O), contained tetra-O-methylgalactose ($R_0$ 0-89) and a small quantity of 2: 5-di-O-methylarabinose. Demethylation gave galactose and arabinose. The major component was identified by conversion into 2: 3: 4: 6-tetra-O-methyl-N-phenyl-D-galactosylamine, m. p. 191$^\circ$.

**Fraction 4.** Chromatography of the partly crystalline material (583 mg.), $[\alpha]_D +128^\circ$ +94$^\circ$ (c 0-61 in H$_2$O), showed 2: 4: 6- and/or 2: 3: 4-tri-O-methylgalactose. Recrystallisation from acetone—ether—light petroleum afforded 2: 4: 6-tri-O-methyl-D-galactose, m. p. and mixed m. p. 101$^\circ$ and $[\alpha]_D +120^\circ$ +90$^\circ$ (c 0-71 in H$_2$O) (Found: OMe, 41-8%. Calc. for C$_{16}$H$_{26}$O$_5$: OMe, 41-9%) (aniline derivative, m. p. and mixed m. p. 167—168$^\circ$). A sample (10 mg.) of the fraction in water (10 ml.) was reduced with potassium borohydride (20 mg.) for 16 hr., excess of hydride was destroyed by addition of acetic acid, p-hydroxybenzaldehyde (2—3 mg.) was added, and the solution was made up to a standard volume. Equal volumes of sodium metaperiodate—sodium hydrogen carbonate solution were added to aliquot parts of the reduced sugar solution, and the formaldehyde released was estimated colorimetrically by the method
of O'Dea and Gibbons.16 The formaldehyde formed corresponded to the presence of 8% of the 2 : 3 : 4-trimethyl ether in the fraction. Attempts to characterise the second sugar by fractional crystallisation of the aniline derivatives failed.

**Fraction 5.** Chromatography of the syrup (134 mg.), \([z]_D +103^o (c 1.2 \text{ in } H_2O)\) showed 2 : 4 : 6- and/or 2 : 3 : 4-di-O-methylgalactose and 2 : 3-di-O-methylarabinose. Demethylation gave galactose and arabinose. Treatment of the syrup with aniline furnished the characteristic needles of 2 : 4 : 6-tri-O-methyl-N-phenyl-d-galactosylamine, m. p. and mixed m. p. 165—166°. Concentration of the mother-liquor yielded a mixture, m. p. 146°, of needles and plates (characteristic of 2 : 3 : 4-tri-O-methyl-N-phenyl-d-galactosylamine), but it was not possible to separate the two components. Periodate oxidation of the derived mixture of glycitols gave 0.45 mol. of formaldehyde. If the presence of 10% of di-O-methylarabinose in the fraction is assumed, the fraction contained 2 : 3 : 4- (35%) and 2 : 4 : 6-tri-O-methyl-d-galactose (55%).

**Fraction 6.** The sugar (50 mg.), Rf 0.54, after recrystallisation from chloroform-light petroleum had m. p. and mixed m. p. (with 2 : 6-di-O-methyl-d-galactose monohydrate) 98—99°, and \([z]_D +52^o \rightarrow +82^o (c 0.55 \text{ in } H_2O)\) (Found: OMe, 27.0. Calc. for C_{12}H_{26}O_{14}H_2O: OMe, 27.2%). The aniline derivative had m. p. and mixed m. p. 119°.

**Fraction 7.** Chromatography of the fraction (223 mg.), \([z]_D +105^o \rightarrow +86^o (c 0.88 \text{ in } H_2O)\), showed 2 : 4- and 2 : 6-di-O-methyl-d-galactose, Rf 0.47 and 0.54, in the approximate proportion of 5 : 1. Dimethylation gave galactose. Recrystallisation from acetone containing 1% of water gave 2 : 4-di-O-methyl-d-galactose monohydrate, m. p. and mixed m. p. 101° (aniline derivative, m. p. and mixed m. p. 216—217°). Chromatography of the products of periodate oxidation of the mother-liquor showed unchanged 2 : 4-di-O-methylgalactose and methoxymalondialdehyde (from the 2 : 6-dimethyl ether), but no products characteristic of the 2 : 3-dimethyl ether.

**Fraction 8.** The sugar (212 mg.), Rf 0.47, after recrystallisation from acetone containing 1% of water had m. p. and mixed m. p. (with 2 : 4-di-O-methyl-d-galactose monohydrate) 102° and \([z]_D +123^o \rightarrow +85^o (c 0.68 \text{ in } H_2O)\) (aniline derivative, m. p. and mixed m. p. 218°).

**Fraction 9.** The syrup (47 mg.), \([z]_D +83^o (c 0.47 \text{ in } H_2O)\), contained 2 : 4-di-O-methylgalactose (Rf 0.47) and a small amount of 2-O-methylgalactose (Rf 0.29). Demethylation gave galactose.

**Fraction 10.** The sugar (49 mg.), Rf 0.29 and \([z]_D +63^o \rightarrow +82^o (c 0.49 \text{ in } H_2O)\), after recrystallisation from glacial acetic acid had m. p. and mixed m. p. (with 2-O-methyl-d-galactose) 148—149°.

**Fraction 11.** Removal of barium ions by treatment with Amberlite resin IR—120(H) followed by concentration gave a syrup (108 mg.), which was converted into the methyl ester methyl glycoside by refluxing it with methanolic 3% hydrogen chloride (40 ml.) for 6 hr. Lithium aluminium hydride in tetrahydrofuran was added slowly to a boiling solution of the ester glycoside in tetrahydrofuran, and the mixture was refluxed for 2 hr. Excess of hydride was destroyed by water, the mixture was taken to dryness, and the residue was exhaustively extracted with chloroform and acetone to give a syrup (85 mg.). Hydrolysis of the syrup with N-hydrochloric acid at 100° for 4 hr. gave a mixture of sugars (69 mg.) which was separated on cellulose (50 × 2 cm.) with light petroleum (b. p. 100—120°—butan-1-ol, saturated with water, to give fractions a (23 mg.) and b (28 mg.). Fraction a was characterised as 2 : 3 : 4-tri-O-methyl-d-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 134°. Fraction b, \([z]_D +116^o (c 0.28 \text{ in } H_2O)\), was characterised as 2 : 3 : 4-tri-O-methyl-d-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 169°.

**Degradation of Periodate-oxidised Degraded Gum with Phenylhydrazine.**—Degraded gum (3.5 g.) was oxidised with sodium metaperiodate (8.5 g.) in water (100 ml.) for 48 hr. in the dark (complete oxidation). The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The resulting solution was heated with phenylhydrazine (17 ml.) and glacial acetic acid (10 ml.) at 100° for 5 hr. The cooled solution was repeatedly extracted with ether, made 0.2N with respect to sulphuric acid and heated at 100° for 40 min. to cleave phenylsoazene residues. The cooled solution was neutralised with Amberlite resin IR—4B(OH), extracted with ether, concentrated to 50 ml., and poured into ethanol (150 ml.) to precipitate the polysaccharide residue (1.0 g.).

Small-scale experiments showed that hydrolysis of the degraded polysaccharide with 0.5N-sulphuric acid at 100° for 50 min. gave a solution with 50% of the reducing power obtained on prolonged hydrolysis. Chromatography of the hydrolysate after this period showed galactose
and 3-O-galactopyranosylgalactose. The degraded polysaccharide (0.9 g.) was heated with 0.5N-sulphuric acid (75 ml.) at 100° for 1 hr. The cooled solution was neutralised with Amberlite resin IR-4B(OH), concentrated, and poured into ethanol (3 vol.). The precipitated degraded polysaccharide was rehydrolysed under the same conditions and combination of the soluble hydrolysates afforded a syrup (350 mg.). The syrup (350 mg.) was dissolved in water and poured on charcoal-Celite (1:1; 100 g.). Elution with water afforded galactose (212 mg.). Elution with water containing 5% of ethanol gave a syrup (31 mg.) containing 3-O-galactopyranosylgalactose (Rfial 0-60 in solvent A) and a trace of 0-O-galactopyranosylgalactose (Rfial 0-40). Recrystallisation from ethanol-water gave 3-O-D-galactopyranosyl-D-galactose, identified by m. p. and mixed m. p. 145-147° and by X-ray powder photography. Elution with water containing 10% of ethanol gave a syrup (7 mg.) containing a sugar with Rfial 0-33 in solvent A and higher oligosaccharides. Partial acid-hydrolysis gave galactose and 3-O-galactopyranosylgalactose.

Hydrolysis of Methylated Gum and Separation of Methylated Sugars.—The methylated gum (6-0 g.) was refluxed with methanolic 4% hydrogen chloride (300 ml.) for 18 hr. (constant rotation). Methanol was removed under reduced pressure and the product was heated with 0.5N-hydrochloric acid (300 ml.) on the boiling-water bath for 14 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, then filtered, silver was removed with hydrogen sulphide, and the filtrate was concentrated. Sugars were extracted from the residue with methanol, and the resulting syrup was dissolved in water, neutralised with barium carbonate, filtered, and concentrated to a syrup (5-1 g.). The syrup mixture of sugars was separated on cellulose (80 x 3-5 cm.) with light petroleum (b. p. 100-120°)-butan-1-ol (7:3; later, 1:1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eleven fractions, and a further fraction was obtained by elution of the cellulose with water.

Fraction 1. Chromatography of the syrup (640 mg.) showed 2: 3: 4-tri-O-methylrhamnose (Rf 1-01) and 2: 3: 5-tri-O-methylarabinose (Rf 0-98). The optical rotation [α]D -37° (c 0-64 in H2O) of the syrup corresponded to that of a mixture of 2: 3: 4-tri-O-methyl-L-rhamnose ([α]D +24°) and 2: 3: 5-tri-O-methyl-L-arabinose ([α]D -37°) in the proportions of 23: 77. Demethylation with hydroiodic acid gave rhamnose and arabinose. A portion (360 mg.) of the syrup was fractionated on cellulose (50 x 2 cm.) with light petroleum (b. p. 100-120°)-butan-1-ol, saturated with water, to give four fractions. Fraction 1a (47 mg.), [α]D +24° (c 0-4 in H2O), was characterised as 2: 3: 4-tri-O-methyl-L-rhamnose (aniline derivative, m. p. and mixed m. p. 111°). Fraction 1b (232 mg.), [α]D -28° (c 1-0 in H2O), contained a mixture of 2: 3: 4-tri-O-methyl-L-rhamnose and 2: 3: 5-tri-O-methyl-L-arabinose. Fraction 1c (47 mg.), [α]D -37° (c 0-47 in H2O), was chromatographically pure 2: 3: 5-tri-O-methyl-L-arabinose and afforded 2: 3: 5-tri-O-methyl-L-arabonamide, m. p. and mixed m. p. 135-136°. Fraction 1d (7 mg.), [α]D +100° (c 0-14 in H2O), was 2: 3: 4: 6-tetra-O-methyl-D-galactose (Rf 0-89) which had not been detected previously in the fraction.

Fraction 2. Chromatography of the syrup (717 mg.) showed two components, Rf 0-98 and 0-89, and the optical rotation ([α]D +92° (c 0-71 in H2O) corresponded to that of a mixture of 2: 3: 5-tri-O-methyl-L-arabinose ([α]D -37°) and 2: 3: 4: 6-tetra-O-methyl-D-galactose ([α]D +117°) in the proportion of 16: 84. The major component was identified by conversion into 2: 3: 4: 6-tetra-O-methyl-N-phenyl-D-galactosylamine, m. p. and mixed m. p. 191°.

Fraction 3. Chromatography of the syrup (42 mg.), [α]D +62° (c 0-41 in H2O), showed 2: 3: 4: 6-tetra-O-methyl-D-galactose (identified as the aniline derivative, m. p. and mixed m. p. 189°) and small amounts of 2: 3: 5-tri- and 2: 5-di-O-methylarabinose.

Fraction 4. Chromatography of the syrup (276 mg.), [α]D +4-5° (c 1-1 in H2O), showed di-O-methylarabinose (Rf 0-84) and a small amount of tetra-O-methylgalactose (Rf 0-89). Demethylation gave arabinose and a small amount of galactose. Separation of a portion (160 mg.) on cellulose using light petroleum (b. p. 100-120°)-butan-1-ol (7:3), saturated with water, gave two fractions. Fraction 4a (18 mg.) was 2: 3: 4: 6-tetra-O-methyl-D-galactose (aniline derivative m. p. and mixed m. p. 190°). Chromatography of fraction 4b (122 mg.) showed one sugar (Rf 0-84), but ionophoresis showed 2: 5-di-O-methylarabinose together with a small amount of 3: 5-di-O-methyl-arabinose. Demethylation gave only arabinose. The major component was identified by conversion into 2: 5-di-O-methyl-L-arabonamide, m. p. and mixed m. p. 125-126°.

Fraction 5. Chromatography of the syrup (237 mg.), [α]D +113° (c 1-0 in H2O), showed a tri-O-methylgalactose (Rf 0-73) (Found: OMe, 41-2. Calc. for C14H12O6: OMe, 41-9%).
methylene glycol gave galactose. Treatment of the syrup with ethanolic aniline furnished the aniline derivative of 2 : 3 : 4-tri-O-methyl-D-galactose, m. p. and mixed m. p. 168°. Reduction with potassium borohydride, followed by periodate oxidation, gave 0.91 mol. of formaldehyde per mol. of sugar.

**Fraction 6.** Chromatography of the syrup (351 mg.), \( [\alpha]_D +108^\circ \) (c 1·1 in H\(_2\)O), showed tri-O-methylgalactose \((R_0, 0·73)\) and ca. 10% of 2 : 3 -di-O-methylarabinose \((R_0, 0·67)\). Demethylation gave galactose and a small amount of arabinose. Reaction with ethanolic aniline afforded the aniline derivative of 2 : 3 : 4-tri-O-methyl-D-galactose, m. p. and mixed m. p. 167°, and from the mother-liquors, after repeated fractional crystallisations, the aniline derivative of 2 : 4 : 6-tri-O-methyl-D-galactose, m. p. and mixed m. p. 178—180°. Periodate oxidation of the derived mixture of glycitols gave 0.64 mol. of formaldehyde per mol. of sugar. If 10% of 2 : 3-di-O-methylarabinose is assumed to be present in the fraction, the fraction contained 2 : 3 : 4-di-\( (54\%)\) and 2 : 4 : 6-tri-O-methyl-D-galactose \( (36\%)\).

**Fraction 7.** Chromatography of the syrup (53 mg.), \( [\alpha]_D +109^\circ \) (c 0·53 in H\(_2\)O), showed a mixture of tri-O-methylgalactose \((R_0, 0·73)\) and 2 : 6-di-O-methylgalactose \((R_0, 0·54)\).

**Fraction 8.** The crystalline material (1·885 g.) had \( [\alpha]_D +112^\circ \rightarrow +85^\circ \) (c 0·91 in H\(_2\)O), and recrystallisation from acetone containing 1% of water furnished 2 : 4-di-O-methyl-D-galactose monohydrate, m. p. and mixed m. p. 103° and \( [\alpha]_D +122^\circ \rightarrow +85^\circ \) (c 1·0 in H\(_2\)O) \((\text{Found: OMe, 27·1}^\circ\)).

Calc. for C\(_{18}\)H\(_{26}\)O\(_{5}\)H\(_2\)O: OMe, 27·2\% \((\text{aniline derivative, m. p. and mixed m. p. 217—218}^\circ)\). Chromatography and ionophoresis of the mother-liquor from the above recrystallisation showed a mixture of 2 : 4- and 2 : 6-di-O-methylgalactose. The syrup \((228 \text{ mg.})\) was separated on cellulose \((50 \times 2 \text{ cm.})\) with solvent D as eluant to give three fractions. Chromatography of fraction 8(i) \((39 \text{ mg.})\) showed 2 : 6-di-O-methylgalactose \((R_0, 0·54)\) and a trace of \((i)\) 2-O-methylarabinose \((R_0, 0·38)\). Demethylation gave galactose and a trace of arabinose. Chromatography of the periodate-oxidation products showed methoxymalondialdehyde \((\text{from 2 : 6-di-O-methylhexoses and 2-O-methylpentoses})\).

The main component was characterised as 2 : 6-di-O-methyl-D-galactose monohydrate, m. p. and mixed m. p. 99—100° and \( [\alpha]_D +52^\circ \rightarrow +84^\circ \) (c 0·36 in H\(_2\)O) \((\text{Found: OMe, 27·0}^\circ\)).

Calc. for C\(_{18}\)H\(_{26}\)O\(_{5}\)H\(_2\)O: OMe, 27·2\% \((\text{aniline derivative, m. p. and mixed m. p. 121—122}^\circ)\). Chromatography of fraction 8(ii) \((27 \text{ mg.})\) and its periodate-oxidation products showed 2 : 4- and 2 : 6-di-O-methylgalactose together with a trace of the 2 : 3-dimethyl ether. Fraction 8(iii) \((151 \text{ mg.})\), \( [\alpha]_D +115^\circ \rightarrow +85^\circ \) (c 1·0 in H\(_2\)O), after recrystallisation afforded 2 : 4-di-O-methyl-D-galactose monohydrate, \( [\alpha]_D +85^\circ \) (c 1·0 in H\(_2\)O), and mixed m. p. 102° \((\text{aniline derivative, m. p. and mixed m. p. 216—218}^\circ)\).

**Fraction 9.** Chromatography of the syrup \((108 \text{ mg.})\) and its periodate-oxidation products showed a complex mixture of sugars, including 2 : 3-, 2 : 4-, and 2 : 6-di-O-methylgalactose, 2-O-methylarabinose, and 2-O-methylgalactose, which was not examined further.

**Fraction 10.** The chromatographically pure sugar \((224 \text{ mg.})\), \( R_0, 0·29 \) and \( [\alpha]_D +61^\circ \rightarrow +85^\circ \) (c 1·0 in H\(_2\)O), crystallised from glacial acetic acid and had m. p. and mixed m. p. \((\text{with 2-O-methyl-D-galactose}) 146^\circ\).

**Fraction 11.** Chromatography of the syrup \((42 \text{ mg.})\) showed galactose, arabinose, and 2-O-methylgalactose in approximately equal amounts.

**Fraction 12.** Removal of barium ions by treatment with Amberlite resin IR-120(H) followed by concentration gave a syrup \((165 \text{ mg.})\), which was converted into the methyl ester methyl glycoside by refluxing methanolic \( 3\%\) hydrogen chloride \((50 \text{ ml.})\) during 6 hr. \((\text{constant rotation})\). The cooled solution was neutralised with silver carbonate, filtered and concentrated, and the product was heated with 0·15N-barium hydroxide \((10 \text{ ml.})\) at 60° for 5 hr. After removal of barium ions with Amberlite resin IR-120(H), the acid glycoside was absorbed on Amberlite resin IR-4B(OH). Elution of the resin with \( \times\)-sodium hydroxide and removal of sodium ions gave a syrup \((102 \text{ mg.})\), which was re-esterified by refluxing it with methanoic hydrogen chloride. Lithium aluminium hydride \((240 \text{ mg.})\) in tetrahydrofuran \((10 \text{ ml.})\) was added slowly to a solution of the ester glycoside in tetrahydrofuran \((30 \text{ ml.})\), and the mixture was refluxed for 2 hr. Excess of hydride was destroyed by water, the mixture was taken to dryness, and the residue was extracted exhaustively with chloroform and acetone to give a syrup \((91 \text{ mg.})\). Hydrolysis of the syrup with \( \times\)-hydrochloric acid at 100° for 4 hr. gave a mixture of sugars \((74 \text{ mg.})\), which was separated on cellulose \((30 \times 2 \text{ cm.})\) with light petroleum (b. p. 100—120°)-butan-1-ol, saturated with water, to give fractions \((i)\) \((26 \text{ mg.})\) and \((ii)\) \((30 \text{ mg.})\). Fraction \((i)\) was characterised as 2 : 3 : 4-tri-O-methyl-D-glucose by conversion into the aniline derivative, m. p. and mixed m. p. 133—135°. Fraction \((ii), [\alpha]_D +115^\circ \) (c 0·3 in H\(_2\)O),
was characterised as 2:3:4-tri-O-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 169º.

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2 Smith, J., 1939, 744, 1724; 1940, 1035; Jackson and Smith, J., 1940, 74, 79.
6 Jones and Nicholson, J., 1958, 27.
13 Hirst, J., 1955, 2974.
14 Hough, Jones, and Wadman, J., 1950, 1705.
15 Reeves, J. Amer. Chem. Soc., 1941, 63, 1776.