Mesquite gum was found by chromatography on diethylaminoethyl cellulose to contain one essentially homogeneous polysaccharide, which contained as constituent monosaccharides D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galactose, L-arabinose and (presumably L-)rhamnose. The Cleavage products from the methylated gum were identified as 2,3,4-tri- and 2,3-di-0-methylglucuronic acid, 2,3,4-tri-0-methylrhamnose, 2,3,5-tri- and 2,3,5-di-0-methylarabinose and 2,3,5,6-tri-, 2,4-di- and 2-0-methylgalactose, while the methylated arabinose-free degraded gum gave rise to 2,3,4-tri-0-methylglucuronic acid, and 2,3,4,6-tetra-, 2,3,4-, 2,4,6- and 2,3,6-tri- and 2,4-di-0-methylgalactose as cleavage products. On the basis of the results of Smith degradation studies on the degraded gum, the distribution of linkages within the galactan core of the polysaccharide was discussed.

The acidic oligosaccharides characterised as partial acid hydrolysis products of the gum were 6-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-D-galactose, 6-O-(4-0-methyl-2,3,4-tri-0-methylglucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-2,3,4-tri-0-methylglucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-D-glucopyranosyluronosy1uronic acid)-(1 + 6)-0-β-D-galactopyranosyl-β-D-galactose and 6-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-1-0-D-galactopyranosyl-(1 + 4)-0-β-D-galactopyranosyl-(1 + 3)-D-galactose. After partial methanalysis of the methylated gum, evidence was obtained for the presence of the following methylated and partially methylated aldobiouronic acids: 6-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-2,3,4-tri-0-methylgalactose, 6-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-2,4-di-0-methylgalactose, 6-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-2-0-methylgalactose and 4-O-(2,3,4-tri-0-methylglucopyranosyluronic acid)-2,3,5-tri-0-methylgalactose. On the basis of the present results the structural significance of the different uronic acid linkages in the gum was discussed and the importance of results of other workers assessed.

After identification of the neutral oligosaccharides released on mild hydrolysis of the gum, the main structural features of the arabinose-rich periphery of the molecule were recognised as α-L-Araf1-(82L-Araf1-(84L-Araf183L-Ara and α-L-Araf1α6G-Galplα3L-Araf1α3L-Ara (1+3)-L-Ara.

The configuration of the linkages contained in the aldobiouronic acids formed as partial hydrolysis products of Araucaria bidwillii gum were unambiguously determined and the question of the occurrence of anomerisation during acetolysis was discussed.

As a result of degradative studies on methylated cherry gum the distribution of the different types of linkages involving uronic acids in the polysaccharide was discussed.
TO DOROTHY
CONTENTS

Introduction

Origin of plant gums 1
Chemistry of plant polysaccharides 3
Classification of gums and mucilages 17
Galacturonans and Galacturonorhamnans 19
Gluconomannans 28
Galactans 34
Chemistry of mesquite gum 55

Part I

Mesquite Gum

Discussion

Introduction 59
Purification and fractionation 61
Acidic oligosaccharides released during partial acid hydrolysis 62
Methylation of mesquite gum 77
Partial methanolysis of methylated mesquite gum 80
Studies on arabinose-free degraded gum
  Autohydrolysis of mesquite gum 92
  Smith degradation of carboxyl-reduced degraded gum A 99
Structure of acid-labile peripheral units 104
Conclusions 128
Contents (cont'd.)

Experimental

General methods
Purification and fractionation
Partial acid hydrolysis
Methylation of gum
Partial methanolysis of methylated gum
Autohydrolysis of gum
Methylation of degraded gum A
Carboxyl-reduced degraded gum A
Smith degradation of carboxyl-reduced
  degraded gums
Investigation of oligosaccharides
  released during autohydrolysis

Part II

Araucaria Bidwillii Gum

Introduction
Discussion
Experimental

Part III

Cherry Gum

Chemistry of Cherry gums
Discussion
<table>
<thead>
<tr>
<th>Contents (cont'd.)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry gum (cont'd.)</td>
<td>Page</td>
</tr>
<tr>
<td>Methanolysis of methylated cherry gum</td>
<td>246</td>
</tr>
<tr>
<td>Partial methanolysis of methylated cherry gum</td>
<td>249</td>
</tr>
<tr>
<td>Partial hydrolysis of methylated cherry gum</td>
<td>254</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>258</td>
</tr>
<tr>
<td>Bibliography</td>
<td>259</td>
</tr>
</tbody>
</table>
COMMON MESQUITE

(Prosopsis juliflora, var. velutina)

photo by author,
Arizona, 1969.
INTRODUCTION

ORIGIN OF PLANT GUMS

The term "gum" is applied in general to high molecular weight compounds which form high viscosity suspensions or solutions at low concentrations. Compounds which fit this description include high molecular weight hydrocarbons, rubbers and certain synthetic polymers with hydrophobic properties. More specifically, the name "exudate gum" is used to describe the highly viscous secretions, mainly containing polysaccharides, from the surfaces of some trees and fruits. It is in this context that gums will be discussed in this thesis.

Plant gums have for a long time been used as a food by the natives of Africa, Asia, India and Australia. Today gums are industrially important because they impart viscosity to aqueous solutions and endow them with advantageous physical properties. They are used in the paper, printing and textile industries, as well as for additives to pharmaceuticals and confectionery.

The gum is secreted from the tree as a sticky, mobile syrup which dries to form a clear yellow or brown glassy mass, termed a nodule. A very wide range of plants produce such secretions, although the amount may vary considerably. Some trees, such as those of the various species of Acacia, belonging to the Leguminosae family, produce gum in such copious
quantities that it may be harvested on a commercial basis. Other plant families producing gums include the Anacardiaceae, Combretaceae, Meliaceae and Rosaceae.

Exudation of gums is stimulated by environmental conditions, mechanical injury to bark or invasion of the plant by micro-organisms. Gums have been observed to be produced in greatest quantity by injured plants, growing in unfavourable conditions, mainly elevated hot and dry areas. Formation of the gum nodules then acts as a shield against loss of moisture or further microbiological attack on the plant.

Although the polysaccharides present in exudate gums are not found in the interior of the plants producing them, structurally similar polysaccharides are produced by botanically related species and these polysaccharides may therefore be considered to be true products of plant metabolism.
CHEMISTRY OF PLANT POLYSACCHARIDES

The principal components of exudate gums are the neutral salts, usually the calcium, magnesium, sodium or potassium salts, of complex acidic polysaccharides. These polysaccharides contain D-glucuronic acid or D-galacturonic acid (or sometimes both) as the acidic constituent, together with two or more of the following neutral monosaccharides: D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose, L-fucose. Some of these sugars may also be present as their mono-O-methyl derivatives. The gum acid may be isolated by pouring an acidified aqueous solution of the gum into an organic solvent such as ethanol or acetone. The precipitated polysaccharide may be further purified by reprecipitation or dialysis and obtained as a solid by freeze-drying an aqueous solution or by drying the precipitate by solvent exchange.

Other acidic polysaccharides, such as pectins, occur naturally with a proportion of their uronic acid residues present as the methyl ester. Saponification yields the parent acidic polysaccharide. Some gums, particularly those of the Sterculia and Khaya genera, are partially acetylated, and as a result are not completely water soluble. De-acetylation and resultant solution of these polysaccharides may be achieved by treatment with alkali. However, caution must be exercised in the use of alkali in the preparation of polysaccharides, since degradation or modification may take place. (1,2)
To a surprisingly large extent, exudate gums frequently contain only one polysaccharide. In the cases where gums contain more than one polysaccharide, the types of heterogeneity encountered fall into several different classes. First there is the gross heterogeneity as found, for example, in gum tragacanth and Khaya senegalensis gum. Gum tragacanth has been shown to contain tragacanthic acid, a complex fucosxylogalacturonan, together with an arabinogalactan. (3) Khaya senegalensis gum has been fractionated into a polysaccharide similar to those obtained from other Khaya gums, and as a minor component, a structurally unrelated galactan. (4)

A somewhat less pronounced heterogeneity is exhibited by the gum of Anogeissus leiocarpus. This has been separated into two components, both of which were shown to contain the same sequences of units. However, in contrast to leiocarpan A, leiocarpan B has a greater proportion of constituent monosaccharides present in the outer chains. (5)

Combretum leonense gum has been separated by chromatography on diethylaminoethyl cellulose into two fractions. These polysaccharide fractions, however, were found to be structurally and physico-chemically similar, except for minor variations in composition. (6) Since branched heteropolysaccharides may exhibit variations in monosaccharide composition, quantitative proportions of the type of linkage in which monosaccharide units are involved, degree of branching and molecular weight, Gibbons (7, 8) has suggested that the use of the
term "polydisperse" be extended to describe macromolecular preparations where the variation of all measurable parameters, such as those mentioned above, is unimodal. It is thus probable that the fractions obtained from *Combretum leonense* gum were indicative of polydispersity rather than heterogeneity in the original gum.

There is no single unambiguous method available for the confirmation of the homogeneity of a polysaccharide preparation. Instead as many methods as possible must be used to determine the absence of heterogeneity. These include ultracentrifugation, (9) electrophoresis and chromatography on ion-exchange or gel-filtration media.

The methods used to fractionate mixtures of polysaccharides on a preparative scale are governed by the need to obtain the relatively large amounts of material required for structural studies. One of the oldest methods used is the graded precipitation of a polysaccharide from solution. Although this may not initially give a complete separation of components, repeated precipitation can lead to good results. Various precipitation techniques are available. *Khaya senegalensis* gum was fractionated by graded precipitation from an aqueous solution of the gum by the gradual addition of an organic solvent. (4) Acidic polysaccharides may be precipitated from aqueous solutions as salts by the addition of copper (10-12) or detergent cations such as cetyltrimethylammonium (Cetavlon) (13) as was used to resolve *Anogeissus leiocarpus* gum into two com-
ponents of different acid content. Neutral polysaccharides, such as western larch arabinogalactan, have been fractionated by precipitation of their boric acid complexes with this latter type of reagent.

A further method of fractionation of acidic polysaccharides involves column chromatography on ion-exchange supports, such as the diethylaminoethyl derivatives of cellulose or Sephadex. These columns in various forms (formate, phosphate, etc.) retain at neutral pH acidic polysaccharides which may then be eluted with electrolytic solutions of varying concentration and pH. Araucaria bidwillii gum was fractionated by this method.

Once a reasonably pure and homogeneous polysaccharide preparation has been obtained, the nature and relative amounts of the constituent sugars may be determined by quantitative chromatographic separation of the monosaccharides released on total acid hydrolysis of the polysaccharide. This approach is not so readily applicable to acidic polysaccharides since the more drastic conditions required to achieve total hydrolysis may cause the decomposition of some of the sugar units present. The acid content of a polysaccharide may be determined by decarboxylation or with the aid of the carbazole reagent.

Structural studies on polysaccharides are based on three main approaches; (a) methylation, (b) partial depolymerisation, (c) periodate oxidation. Methylation involves the conversion of the polysaccharide to its fully methyl etherified derivative. Complete depolymerisation of the product is followed by
separation of the individual methylated sugars. This is accomplished by column chromatography of a comparatively large amount of material and permits the rigorous identification of each component by the formation of suitable crystalline derivatives. Identification of the individual methylated monosaccharides derived from the methylated polysaccharide gives information on the sites of substitution within the polysaccharide as well as the ring structures of the sugars involved, but not on the sequences of sugars nor the anomeric configurations of the glycosidic linkages present. Identification of the cleavage products from a methylated polysaccharide may also be achieved, this time on a micro-scale, by gas-liquid partition chromatography of suitably volatile derivatives, such as methyl glycosides, a much simpler and less time-consuming method, but one which gives satisfactory results. Although this method does not give the same unambiguous identification of products as that obtained by the formation of crystalline derivatives, the components separated by gas chromatography may be further analysed by mass spectrometry to establish their identity.

Information on the sequences of sugar units and the configurations of the linkages present in these sequences may be obtained from analysis of the products of partial depolymerisation of the polysaccharide. One of the commonest methods of achieving this partial breakdown is acid hydrolysis. Although all glycosidic linkages can be cleaved by acid
hydrolysis the rates of their cleavage depend on the nature of
the sugar unit involved. In general, pyranosidic linkages are
more stable to acid hydrolysis than those involving furano-
sides. Variation is, however, encountered within the pyrano-
sides, since 6-deoxy hexopyranosides are hydrolysed as readily
as furanosides. The most stable linkages are those of glyco-
siduronic acids. Within a particular type of sugar unit, the
stability of the glycosidic linkage varies with the position
of substitution. (1 + 6) Linkages between hexopyranose resi-
dues, for instance, are more resistant to hydrolysis than
linkages between similar residues which involve substitution
on secondary hydroxyl groups. Advantage can be taken of this
varying lability to acid hydrolysis to achieve a fairly
selective depolymerisation of many types of polysaccharide.
Thus mild treatment with acid can cleave the furanosidic link-
ages in a polysaccharide while leaving intact the majority of
pyranosidic linkages. Where the polysaccharide involved is
itself acidic, merely refluxing an aqueous solution of the
polysaccharide is sufficient to cause cleavage of furanosidic
bonds. This technique of autohydrolysis is well illustrated
by its application to gum arabic, where prolonged boiling of
an aqueous solution of the gum acid led to the removal of the
peripheral arabinofuranosyl residues and the isolation of a
less complex arabinose-free degraded galactan. Treatment of
acidic polysaccharides with stronger acid leads to the for-
mation of more acid-stable fragments such as aldobiouronic
acids. Application of such graded hydrolysis was used over twenty-five years ago by Smith in studies on gum arabic (20) and Hirst and Jones on damson (21) and cherry gums, (22) but still forms the basis for the controlled fragmentation of polysaccharides, as illustrated in the flow sheet in Figure 1.

The second method of fragmentation of polysaccharides, acetolysis, is an alternative to acid hydrolysis. Since it proceeds by a different reaction mechanism, a different selectivity in the cleavage of glycosidic linkages is often involved. 6-Deoxy hexopyranosides in particular are relatively more stable to acetolysis than acid hydrolysis. Thus it has proved possible to isolate the oligosaccharide 2-0-α-L-fuco-
pyanosyl-D-xylose after acetolysis of tragacanthic acid, thereby preserving a linkage which was readily hydrolysed in aqueous solution. (23) A further example of differing lability to acetolysis and acid hydrolysis is provided by the manno-
pyranosidic linkages in yeast mannan. Acid hydrolysis of the mannan gave 6-0-α-D-mannopyranosyl-D-mannose as the major disaccharide formed. (24) A different oligosaccharide, namely 2-0-α-D-mannopyranosyl-D-mannose, was the major disaccharide observed after acetolysis of the polysaccharide, (25) however, suggesting that the glycosidic linkage attached to a primary hydroxyl group is split more readily than those attached to secondary hydroxyl groups during acetolysis, whereas the re-
verse is true during acid hydrolysis. A disadvantage of
Acetolysis is that anomeration of the glycosidic configuration can take place under the reaction conditions used; thus too great a significance cannot be placed on the configuration of some oligosaccharides obtained.

Additional structural information can be obtained by modifying the polysaccharide so that different "cracking patterns" are obtained on acetolysis or acid hydrolysis. The first of these methods involves the oxidation of the primary sugar hydroxyl group to form a uronic acid unit, thus increasing the stability of the unit's glycosidic linkage to acid hydrolysis. This method is obviously only of practical importance in a molecule with a low proportion of free primary hydroxyl groups. Such an approach was applied to European larch arabinogalactan, resulting in the characterisation of, among others, the aldobiouronic acid 6-O-(L-arabinofuranosyluronic acid)-D-galactose.

The second method involves the reduction of a hexuronic acid to a hexose residue. Acetolysis of carboxyl-reduced gum arabic and carboxyl-reduced Araucaria bidwillii gum has in both cases led to the characterisation of the oligosaccharides 4-O-L-rhamnopranosyl-D-glucose and 0-L-rhamnopranosyl-(1 + 4)-0-D-glucopyranosyl-(1 + 6)-D-galactose, thus showing that in the parent polysaccharides, D-glucuronic acid residues are 4-O-substituted by L-rhamnose units.

The application of some of these fragmentation methods to gum arabic is illustrated in Figure 1.
Arabic acid total hydrolysis → D-Galactose (3.7)
L-Arabinoose (3.0)
D-Glucuronic acid (1.2)
L-Rhamnose (1.1)

Arabic acid autohydrolysis → L-Arabinoose
L-Rhamnose
α-D-Galp-(1→3)-L-Ara
Degraded gum A

Degraded gum A further autohydrolysis → β-D-Galp-(1→3)-D-Gal
Degraded gum B

Degraded gum B partial acid hydrolysis → β-D-Gpa-(1→6)-D-Gal

Arabic acid acetylation reduction acetolysis → L-Rhap-(1→4)-D-G
β-D-Galp-(1→3)-D-Gal
L-Rhap-(1→4)-D-Gpa-(1→6)-D-Gal

Fragmentation of Gum Arabic

Figure 1
Another method of polysaccharide fragmentation which has been investigated involves alkaline degradation, initiated at the reducing end. On treatment with oxygen-free alkali, 3-0-substituted reducing sugars lose their substituent and are converted to metasaccharinic acids. (30) 3,6-Di-0-substituted reducing sugars are converted, after elimination of the substituent at C (3), to the corresponding 6-0-substituted metasaccharinic acids, which are then stable to alkali.

Application of this method to Japanese larch arabinogalactan A resulted in a peeling reaction, initiated at the reducing end, taking place part of the way along the (1 → 3) linked galactan backbone, releasing the side-chains terminated by a stable 6-0-substituted metasaccharinic acid. Treatment of these degradation products with hypochlorite gave oligosaccharides terminated by 5-0-substituted 2-deoxy-β-threopentose residues. Two such oligosaccharides, namely 5-0-β-D-galactopyranosyl-2-deoxy-β-threopentose and 0-β-D-galactopyranosyl-(1 → 6)-0-β-D-galactopyranosyl-(1 → 5)-2-deoxy-β-threopentose were partially characterised. This sequence of reactions is illustrated in Figure 2.
A reaction sequence such as this may be of value in the structural determinations of polysaccharides. However, the individual steps require to be studied in greater detail, with the aid of model compounds, before this method can become generally applicable to polysaccharides which contain suitable sequences of linkages.

The other widely-used method of structural investigation of polysaccharides is based on periodate oxidation. Analytical procedures involving the estimation of oxidant consumed, formic acid and formaldehyde liberated during oxidation and the pro-
portions of sugar residues remaining unoxidised, provide valuable quantitative data, which is, however, seldom interpretable unambiguously without other structural information. Important degradation procedures are those leading to the isolation of the unoxidised regions of the polysaccharides. The most valuable reaction sequence of this type is known as the Smith degradation (32) and involves reduction of periodate-oxidised polysaccharides followed by graded acid hydrolysis of acyclic acetals under conditions which cause little or no hydrolysis of glycosidic linkages. The products formed during the Smith degradation will depend on the relative distribution of non-oxidised sugar residues in the parent polysaccharide. Non-oxidised sugar residues which are adjacent to oxidised residues are isolated as glycosides of glycerol, erythritol, threitol or less frequently glyceraldehyde. Such oligosaccharide glycosides may be conveniently separated by common chromatographic methods. Those polysaccharides which contain substantial blocks of contiguous non-oxidised residues give rise to degraded polysaccharides of less complex structure. Since 3-0-substituted units are necessarily resistant to periodate oxidation, the Smith degradation is of particular value in assessing the distribution of (1 → 3) linkages in branched polysaccharides, and its use may be exemplified by studies on the galactan framework of the degraded gum formed on autohydrolysis of gum arabic. Methylation of the degraded gum
followed by hydrolysis gave 2,3,4,6-tetra-O-methyl-D-galactose (1 mole), 2,3,4-tri-O-methyl-D-galactose (5 moles), 2,4-di-O-methyl-D-galactose (3 moles) and 2,3,4-tri-O-methyl-D-glucuronic acid (3 moles). The three structures (i, ii and iii) in Figure 3 appeared the most likely. Application of the Smith degradation to the degraded gum resulted in the isolation of a mixture of galactose-containing oligosaccharides which were shown by methylation to contain only (1 \( \rightarrow 3 \)) linkages, thus pointing to structure (i) as the most nearly correct.
Possible Structures of Degraded Gum Arabic

Figure 3
Classification of Gums and Mucilages

Attempts have been made to classify plant gums and mucilages on many different bases. Increasingly, however, classification may now be made on the basis of the chemical structure of the polysaccharide. In this connection, an attempt was made to classify according to the hexuronic acid present, since many exudate gums contain D-glucuronic acid while D-galacturonic acid is a more usual constituent of acidic mucilages. (34) However, there are gums, such as cholla gum and gum tragacanth, which contain D-galacturonic acid, while other gums, the Khaya gums, Combretum leonense gum (6) and a mucilage from cress seeds (35) contain both hexuronic acids.

The presently accepted basis for the classification of complex plant polysaccharides is the nature of the structural units found in the interiors of the molecules. However, our knowledge of the detailed chemistry of some of the mucilages does not allow them to be classified satisfactorily as yet by this system. Similarly, although much work has been done on certain exudate gums, the nature of their main chains is not yet apparent, and they therefore cannot be unambiguously placed in the following classification.

The following table gives an indication of some of the groups into which plant heteropolysaccharides may be placed.
### Table 1

**Classification of Plant Heteropolysaccharides**

<table>
<thead>
<tr>
<th>Group</th>
<th>Type</th>
<th>Example</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>xylan</td>
<td>arabinoxylan</td>
<td>rye flour arabinoxylan</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>glucuronoxylan</td>
<td>sapote gum</td>
<td>37, 38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wood hemicelluloses</td>
<td></td>
</tr>
<tr>
<td>mannan</td>
<td>galactomannan</td>
<td>soy-bean galactomannan</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>glucomannan</td>
<td>iris seed glucomannan</td>
<td>40</td>
</tr>
<tr>
<td>galacturonan</td>
<td>galacturonorhamnan</td>
<td>pectic substances</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Khaya gums</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sterculia gums</td>
<td>42</td>
</tr>
<tr>
<td>glucuronomannan</td>
<td>glucuronomannan</td>
<td>gum ghatti</td>
<td>43-46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anogeissus leiocarpus gum</td>
<td>5, 47-49</td>
</tr>
<tr>
<td>galactan</td>
<td>arabinogalactan</td>
<td>Western larch arabinogalactan</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>arabinoglucuronogalactan</td>
<td>Acacia gums</td>
<td></td>
</tr>
</tbody>
</table>


Galacturonans and Galacturonorhamnans

The main characteristic of members of these types is that their basal chains contain α(1 → 4) linked D-galacturonic acid residues, interspersed to varying degrees with units of L-rhamnopyranose, as in Figure 4.

\[ +4\)-α-D-GalpA-(1 → 4)-α-D-GalpA-(1 → 2)-α-L-Rhap- \]

Figure 4

Three main families of exudate gums have been identified as belonging to these types, all containing varying amounts of D-glucuronic acid, found mainly as the non-reducing end groups of short side-chains.

Tragacanthic acid, the main polysaccharide component of gum tragacanth (Astragalus gummifer) is the only recognised member of the first family. The main chain of this polysaccharide is composed almost entirely of D-galacturonic acid, interrupted only very occasionally by units of L-rhamnose, as indicated by the isolation of the aldobiouronic acid 2-Ω-(α-D-galactopyranosyluronic acid)-L-rhamnose as a minor partial acid hydrolysis product. The side-chains contain units of D-xylene, 2-Ω-α-L-fucopyranosyl-D-xylene or 2-Ω-β-D-galactopyranosyl-D-xylene, while D-glucuronic acid is only present in trace amounts and may terminate some side-chains. A partial structure is indicated in Figure 5.
Figure 5

Tragacanthic acid
The Khaya gums, which occur naturally as partially acetylated polysaccharides, represent the second family of polysaccharides. Compared to tragacanthic acid, they contain more L-rhamnose in their main chains and carry entirely different side-chains. Khaya ivorensis gum is perhaps the most fully studied member of this family, and has been shown to contain the structural units 4-0-(4-0-methyl-\(\alpha\)-D-glucopyranosyluronic acid)-\(\beta\)-galactose and \(\beta\)-galactopyranose attached as side-chains to the \(\beta\)-rhamnopyranose residues present in the main chain, as in the partial structure in Figure 6.
Figure 6

Khaya gum
The third family is represented by gums of the Sterculia genus and also the gum from the botanically unrelated Cochlospermum gossypium. Like the Khaya gums, these gums also occur naturally partially acetylated. Studies on Sterculia urens gum have shown that the galacturonorhamnan chains consist mainly of alternating residues of the two sugars, and that adjacent galacturonic acid units can only occur at infrequent intervals. The isolation of the oligosaccharide \( \beta-(\beta-D\text{-glucopyranosyluronic acid})-(1 \rightarrow 3)-\beta-(\alpha-D\text{-galactopyranosyluronic acid})-(1 \rightarrow 2)\alpha\text{-rhamnose} \) as a partial hydrolysisis product of the polysaccharide indicates that \( D\text{-glucuronic acid} \) is linked directly to the main chain. Characterisation of the aldobiouronic acid \( 4\beta-(\alpha-D\text{-galactopyranosyluronic acid})\alpha\text{-galactose} \) as another cleavage product indicates the presence of a further structural feature. Partial structures for the Sterculia gums are represented in Figure 7.
The similarity of tragacanthic acid to the pectins is immediately obvious since both contain long sequences of D-galacturonic acid residues in their main chains, interrupted occasionally by units of L-rhamnose. Pectins with a backbone composed entirely of D-galacturonic acid must be relatively rare, since most give rise to the aldobiouronic acid 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose on partial hydrolysis.

The acidic polysaccharides isolated from soy-beans\(^{(54,55)}\) have been found to contain similarities to all three polysaccharide families described above. Fragments have been isolated which indicate the presence in the main chains of blocks of D-galacturonic acid residues, alternating units of D-galacturonic acid and L-rhamnose and sequences of at least two contiguous L-rhamnose units. These features are contained in the structure in Figure 8.
Figure 8
A similarity of these soy-bean polysaccharides to tragacanthic acid occurs in the side-chains, since the oligosaccharides 2-0-α-L-fucopyranosyl-D-xylose, 3-0-β-D-xylopyranosyl-D-galacturonic acid and 2-0-β-D-galactopyranosyl-D-xylose, in addition to chains of β(1 → 4) linked D-galactopyranose units, have been identified as the products of acelolysis and enzymic hydrolysis of soy-bean polysaccharides.
Glucuronomannane

The three polysaccharides so far identified as belonging to this group all contain a backbone made up from an alternating sequence of D-glucuronic acid and D-mannose units. The first member, gum ghatti, the gum exudate from Anogeissus latifolia (Combretaceae) was originally thought to have a main chain of D-galactose units, interspersed with L-arabinose residues, on account of the two polymer homologous series of oligosaccharides released on partial hydrolysis, namely β-D-Galp1-(α6-β-D-Galp)n and β-D-Galp1-(α6-β-D-Galp1)n →3-L-Ara. \(44\) In addition the aldobiouronic acids 6-0-(β-D-glucopyranosyluronic acid)-D-galactose and 2-0-(β-D-glucopyranosyluronic acid)-D-mannose were identified. \(43\) Evidence that mannose was present in the interior of the polysaccharide came from the isolation, after partial hydrolysis of the degraded gum obtained after two successive applications of the Smith degradation, of the oligosaccharide 6-0-β-D-galactopyranosyl-D-galactose, 3-0-β-D-galactopyranosyl-L-arabinose and 3-0-L-arabinopyranosyl-D-mannose. \(46\)

Further evidence for the now accepted structure of the gum (Figure 9) came from the identification of the alternating tetrasaccharide 0-(β-D-glucopyranosyluronic acid)-(1 + 2)-0-D-mannopyranosyl-(1 + 4)-0-(β-D-glucopyranosyluronic acid)-(1 + 2)-D-mannose as a partial hydrolysis product of the gum. \(5\)
\[ \begin{align*}
\text{R} & \quad 6 \\
\text{β-D-GpA} & \quad 3 \\
\text{L-Arap} & \quad 1 \\
(R+3-β-D-Galp)_n & \quad 6 \\
\text{β-D-GpA} & \quad 1 \\
\end{align*} \]

or less frequently

\[ \begin{align*}
\text{L-Araf} & -(1 \rightarrow 2) \text{-L-Araf} \\
\text{L-Araf} & -(1 \rightarrow 3) \text{-L-Araf} \\
\text{L-Araf} & -(1 \rightarrow 5) \text{-L-Araf} \\
\end{align*} \]

\text{Gum Chatti}

\text{Figure 9}
The other two polysaccharides of this family come from the gum exudate of the tree Anogeissus leiocarpus. Fractionation of the gum with cetyl trimethylammonium bromide gave rise to two distinct though structurally related polysaccharides, termed leiocarpan A and B.\(^{(5)}\) Leiocarpan A contains \(\beta\)-glucuronic acid, \(\beta\)-mannose and \(L\)-arabinose and, in contrast to gum ghatti, relatively large amounts of \(\beta\)-xylose and very little \(\beta\)-galactose. Proof of the alternating sequence of \(\beta\)-glucuronic acid and \(\beta\)-mannose in the backbone came from the isolation after acetylosis of the carboxyl-reduced gum of the following series of oligosaccharides.\(^{(48)}\)

\[
\begin{align*}
\beta-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Man} \\
\alpha-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-G} \\
\beta-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-G} \\
\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Man} \\
\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Man} \\
\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-C} \\
\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-Cp-}(1 \rightarrow 2)-\beta\text{-Manp-}(1 \rightarrow 4)-\beta\text{-G}
\end{align*}
\]

The partial structures for leiocarpan A are given in Figure 10.
\[-4)\beta-D-Gpa-(1 \rightarrow 2)\alpha-D-Manp-(1 \rightarrow 4)\beta-D-Gpa-(1 \rightarrow 2)\alpha-D-Manp-
\]

\[\begin{array}{c}
R \rightarrow 3 \\
6 \\
1 \\
D-Xylp \\
\end{array}
\]

\[\begin{array}{c}
R = L-Araf- \\
L-Araf-(1 \rightarrow 3)-L-Araf- \\
L-Arap-(1 \rightarrow 3)-L-Araf-
\end{array}
\]

\[D-Gpa-(\rightarrow 6-D-Galp-) \rightarrow 3-L-Arap-
\]

Leiocarpan A

Figure 10
Leiocarpan B has been shown to have a structure basically similar to leiocarpan A. However, since D-galactose is a much more important constituent in polysaccharide B, the galactose-containing side-chains may be expected to be more extended than in leiocarpan A.

The gum exudate from Encephalartos longifolius has been shown by partial hydrolysis to contain units of the aldobiouronic acids 2-0-(β-D-glucopyranosyluronic acid)-D-mannose, 6-0-(β-D-glucopyranosyluronic acid)-D-galactose and 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose. A mixture of higher oligosaccharides containing D-glucuronic acid and D-mannose was also isolated, and since methylation data suggested these arose from interior chains, it seems likely that this polysaccharide also belongs to the glucuronomannan group.

Another exudate gum, that of Virgilia oroboides, similarly gives rise to the aldobiouronic acid 2-0-(β-D-glucopyranosyluronic acid)-D-mannose as a partial hydrolysis product. However, since there is no information on the nature of the sugar units contained within the main chains, this gum may not be considered a member of the group under consideration.

One major group of gums giving the aldobiouronic acid 2-0-(β-D-glucopyranosyluronic acid)-D-mannose as a partial hydrolysis product are those from the species Prunus. Recent investigations by a Czechoslovakian group on various types of cherry gums as well as those from apricot and
blackthorn (62) showed that these gums on partial hydrolysis gave in addition higher oligosaccharides containing \( \beta \)-glucuronic acid and \( \beta \)-mannose. Although it was thought these fragments originated from the interior chains of the molecules, there was insufficient evidence on the sequence of linkages involved to indicate whether these polysaccharides belonged to the type of glucuronomannan already described in detail. There are, however, considerable similarities between Prunus gums and gum ghatti, for instance. The neutral oligosaccharides characterised as partial hydrolysis products of gum ghatti, namely 3-0-\( \beta \)-\( \beta \)-galactopyranosyl-\( \beta \)-galactose, 6-0-\( \beta \)-\( \beta \)-galactopyranosyl-\( \beta \)-galactose, \( \beta \)-Glp-\([(1 + 6)-\( \beta \)-Glp-]* \((1 + 3)-L\)-Ara, as well as the aldobiouronic acid 6-0-(\( \beta \)-\( \beta \)-glucopyranosyluronic acid)-\( \beta \)-galactose, have also been characterised, if sometimes somewhat tentatively, as cleavage products of various Prunus gums. The additional similarities indicated by results from the methylated gums suggest that these polysaccharides may indeed belong to the same type. A further investigation of aspects of the structure of cherry gum forms a minor part of the work presented in this thesis.
**Galactans**

Members of this family contain a branched core of β-D-galactopyranose residues, mutually joined by (1 → 3) and (1 → 6) linkages. In those polysaccharides whose structures have been studied in detail, the distribution of linkages is such that the former type predominate in the interior while the latter are concentrated in the outer chains, as in Figure 11.

![Figure 11](image)

To this basic structure are then attached the peripheral units. These are composed mainly of L-arabinose, but can also include L-rhamnose, D-glucuronic acid (or its 4-O-methyl ether) and occasionally other units of D-galactose.

(1) **Larch arabinogalactans**

The simplest polysaccharides in the group are the arabinogalactans isolated from the heartwood of various species of Larch. Studies on Japanese larch arabinogalactan A(63) showed that it contained L-arabinose and D-galactose with only a
faint trace of acidic sugars. Methylation of the polysaccharide resulted in the identification of the following methylated sugars: 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, and 2,4-di-O-methylgalactose in the ratios 3:2:1:4. There were in addition, approximately equal amounts of 2,3,5- and 2,3,4-tri- and 2,5-di-O-methylarabinose. Mild hydrolysis of the polysaccharide gave the oligosaccharide 3-0-β-L-arabinopyranosyl-L-arabinose, while two successive Smith degradations gave an arabinose-free galactan containing mainly (1 → 3) linkages. This latter observation, taken together with methylation and partial hydrolysis data, indicates that the arabinose is present in the polysaccharide as single unit L-arabinofuranosyl or double unit 3-0-β-L-arabinopyranosyl-L-arabinofuranosyl side-chains, in equimolar proportions. In an attempt to find out more about the linkages involved in the side-chains, the primary hydroxyl groups in the arabinogalactan were protected by triphenylmethylation and the polysaccharide derivative methylated. After removal of the protecting groups, the polysaccharide was oxidised. Analysis of the acidic fraction derived from the subsequent hydrolysis showed it contained mainly 2,3,4-tri-O-methylgalacturonic acid and 2,3,4-tri- and 2,4-di-O-methylgalactose. Further methylation afforded 2,3,4-tri-O-methylgalactose as the major neutral sugar, pointing to the presence of the following structural units in the polysaccharide

$$\beta-\text{D-Galp}-(1 \rightarrow 6)-\beta-\text{D-Galp}^-$$, $$\beta-\text{D-Galp}-(1 \rightarrow 6)-\beta-\text{D-Galp}^-_3$$
From these results, the following partial structure can be proposed for Japanese larch arabinogalactan A (Figure 12)

\[\begin{align*}
+3)\beta-D-Galp \cdot (1 + 3)\beta-D-Galp \cdot (1 + 3)\beta-D-Galp & \rightarrow 3)\beta-D-Galp \\
\text{6} & \rightarrow 6 \\
\uparrow & \uparrow \\
\text{1} & \rightarrow 1 \\
R & \beta-D-Galp \\
\text{6} & \rightarrow 6 \\
\uparrow & \uparrow \\
\beta-D-Galp & \beta-D-Galp \\
\end{align*}\]

\[R = \text{\textit{L}-Araf1- or } \beta-\text{\textit{L}-Arap-(1 + 3)-\text{\textit{L}-Araf1-}}\]

Japanese Larch Arabinogalactan A

Figure 12
Recent Smith degradation studies on tamarack larch arabinogalactan confirm that this polysaccharide also contains a backbone of predominantly $\beta(1 \rightarrow 3)$ linked $\beta$-galactopyranose residues, although earlier authors had claimed the backbone contained both $(1 \rightarrow 3)$ and $(1 \rightarrow 6)$ linkages. Similar claims have been put forward for the presence of both linkages in the backbone of the arabinogalactan from jack pine, although they are not supported by direct evidence. In general, Smith degradation studies on polysaccharides of this type show that with each successive degradation the polymer approximates more and more to a completely linear $(1 \rightarrow 3)$ linked galactan. Moreover, since in the majority of cases only two successive degradations have been performed, it is likely that the few remaining $(1 \rightarrow 6)$ linkages arose from the residual stubs of $(1 \rightarrow 6)$ linked side-chains, rather than representing branch points involving $(1 \rightarrow 3)$ linked chains.

The presence in most larch arabinogalactan extracts of two polysaccharides has been observed. These were first separated by Bouveng and Lindberg in the case of western larch arabinogalactan by fractional precipitation with cetyltrimethylammonium hydroxide-boric acid mixtures into polysaccharides $A$ and $B$. Investigation has shown that both contain similar structural units, but that while arabinogalactan $A$ has a number-average molecular weight of 95,000, that of polysaccharide $B$ is 9,000. In addition, poly-
saccharide B has been found to contain fewer L-arabinofuranosyl units. Studies on the arabinogalactan extract from the annular rings of a 700 year old western larch (68) have shown that the number-average molecular weight of the mixture decreased from 65,500 to 13,000 over a 650 year span, and that the proportion of polysaccharide B in the mixture increased with age. The pH and acetyl content of the wood of this tree were both found to decrease with age. In the light of these observations it was suggested that acetyl groups in the wood were gradually hydrolysed, liberating acetic acid, which was then responsible for a gradual acid-catalysed depolymerisation, over very many years, of arabinogalactan A to form the artefact, arabinogalactan B.

Other coniferous wood galactans, such as those from mountain larch (69) and maritime pine (70) have galactose-containing side chains which are terminated by D-glucuronic acid. The only exudate gum so far found from a coniferous tree, that from the bunya pine (Araucaria bidwillii) (71) has a more complex structure and in many respects resembles gum arabic, an exudate from a species of Acacia. These gums will now be discussed.
(2) *Acacia* gums

In general, gums from the *Acacia* species contain L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid (sometimes also as its 4-O-methyl ether). Their structures are considerably more complicated than the arabinogalactans already described, and will be discussed in terms of (a) linkages involving hexuronic acid residues, (b) the galactan core and (c) the nature of the peripheral side-chains.

Four different aldobiouronic acids have been characterised as partial hydrolysis products of *Acacia* gums, involving two basic types of linkage, namely

6-O-(β-D-glucopyranosyluronic acid)-D-galactose
6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose
4-O-(α-D-glucopyranosyluronic acid)-D-galactose
4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

Table 2 indicates the gums in which these oligosaccharides have been recognised.

<table>
<thead>
<tr>
<th><em>Acacia</em> gum</th>
<th>4-0Me GA1β6Gal</th>
<th>4-0Me GA1α4Gal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pycnantha</td>
<td>+</td>
<td>+</td>
<td>72</td>
</tr>
<tr>
<td>senegal</td>
<td>+</td>
<td>+</td>
<td>73</td>
</tr>
<tr>
<td>mearnsii</td>
<td>+</td>
<td>+</td>
<td>74</td>
</tr>
<tr>
<td>nubica</td>
<td>+</td>
<td>+</td>
<td>75</td>
</tr>
<tr>
<td>nilotica</td>
<td>+</td>
<td>+</td>
<td>76</td>
</tr>
<tr>
<td>drepanolobium</td>
<td>+</td>
<td>+</td>
<td>77</td>
</tr>
<tr>
<td>seyal</td>
<td>+</td>
<td>+</td>
<td>78</td>
</tr>
</tbody>
</table>
There is as yet no evidence to indicate whether these aldobiouronic acids occupy different structural environments within a polysaccharide or are equivalent, with both types terminating chains of (1 → 6) linked D-galactose residues.

It has long been observed that the proportions of L-rhamnose in *Acacia* gums often closely parallel those of D-glucuronic acid. Since rhamnose residues have so far only been found as end-groups, it was suggested that they were joined by (1 → 4) linkages to D-glucuronic acid units. The presence of such a linkage has been confirmed in the case of gum arabic (*Acacia senegal*) by the isolation of the oligosaccharide 4-O-L-rhamnopyranosyl-D-glucose after acetolysis of the carboxyl-reduced gum. (28)

While all *Acacia* gums have a galactan core containing (1 → 3) and (1 → 6) linkages, there is increasing evidence that the arrangement of these linkages is more complex than was at one time believed. It was originally thought that the galactan interior had a simple comb-like structure, as illustrated in Figure 13, containing a single main chain of (1 → 3) linkages to which were attached side-chains of (1 → 6) linked D-galactose residues.

---

**Figure 13**

[Diagram showing the structure of the galactan core with (1 → 3) and (1 → 6) linkages and side-chains of D-galactose units.]

---
Recent Smith degradation studies on gum arabic, however, have shown that after five successive degradations, the polysaccharide isolated, although basically a (1 → 3) linked galactan, still contained 3,6-di-0-substituted D-galactose residues. 6-0-substituted D-galactose units were still present after a total of seven such degradations. Since all the arabinose-containing side-chains were removed after four degradations, these results imply that (1 → 6) linkages are present within the periodate-resistant (1 → 3) linked galactan core and that this core contains a degree of multiple branching, as in Figure 14, rather than being a single unbranched chain.

\[ +3)\beta-D-Galp-(1 \rightarrow 3)\beta-D-Galp-(1 \rightarrow 3)\beta-D-Galp-(1 \rightarrow 3)\beta-D-Galp-(1 \rightarrow 6) \]
\[ \beta-D-Galp \]
\[ 3 \rightarrow 1 \]
\[ \beta-D-Galp \]
\[ 3 \rightarrow 1 \]
\[ \beta-D-Galp \]

Figure 14

Chains of (1 → 6) linked D-galactose residues, some of which are terminated by uronic acids, are then attached to this basic backbone. To the glucuronogalactan thus formed are joined the peripheral arabinose-containing chains, as in the diagram in Figure 15.
A similar degradative approach has been applied to the gum from *A. arabica*. It was found that during the course of four successive sequences of the Smith degradation procedure, the polysaccharide was fragmented to a considerably greater extent than was *A. senegal* gum under similar conditions. These results were interpreted as indicating the presence of short sequences of periodate-vulnerable \((1 + 6)\) linkages between areas of multiply branched \((1 + 3)\) linkages. It is thus apparent that the basic galactan core is not the same for all gums of the *Acacia* species.

Variation can also occur in the frequency of attachment of the \((1 + 6)\) linked \(\beta\)-galactose chains to the \((1 + 3)\) linked framework. In *A. senegal* gum the majority of \((1 + 3)\) linked \(\beta\)-galactose units carry such side-chains, Figure 16(a), whereas in *A. pycnantha* only about half the \((1 + 3)\) linked residues carry \((1 + 6)\) linked side-chains, Figure 16(b).
Variation in frequency of attachment of side-chains to sequences of (1→3) linked D-galactose units in (a) *A. senegal*, (b) *A. pycnantha*.

All *Acacia* gums contain peripheral chains of varying complexity. These invariably contain L-arabinofuranose units and may also include L-arabinopyranose and D-galactopyranose residues. Oligosaccharides which have been identified as originating from this region of various gums are

(i) 3-0-β-L-arabinopyranosyl-L-arabinose
(ii) 3-0-β-L-arabinofuranosyl-L-arabinose
(iii) 2-0-β-L-arabinofuranosyl-L-arabinose
(iv) 3-0-α-D-galactopyranosyl-L-arabinose
Either disaccharides (i) or (ii) or both have been recognised as products of partial depolymerisation of all *Acacia* gums investigated in sufficient detail, and while oligosaccharide (iii) has only been isolated so far from *A. nilotica* gum, the presence of a similar linkage in other gums may be inferred from the identification of 3,5-di-O-methylarabinose as a cleavage product from the methylated derivatives of several other gums. Although methylation evidence indicates that arabinopyranose units generally occur as end-groups, the recognition of 3,4-di-O-methylarabinose as a minor cleavage product from some methylated gums indicates that occasionally arabinopyranose units may be 2-O-substituted. 2,3-di-O-methylarabinose has also been recognised as a product of depolymerisation of several methylated *Acacia* gums. There is, however, no indication as to whether this represents a 4-O-substituted arabinopyranose or a 5-O-substituted arabinofuranose unit in the original polysaccharide.

D-Galactopyranose residues in the periphery are not known to occur as anything other than end-groups.

Table 3 indicates the peripheral linkages known to be present in various *Acacia* gums. As can be seen, a considerable variety of arabinosyl linkages occurs in most of the gums, although little is known about the sequences involved above the disaccharide level. Indirect evidence can be gained on the lengths of these arabinose-containing chains.

---

*Note:* The text is extracted from a document discussing the chemical analysis of *Acacia* gums, focusing on the identification of specific sugars and their linkages. The document highlights the presence of disaccharides and oligosaccharides as products of partial depolymerisation, along with the inference of similar linkages in other gums through methylation evidence. It also notes the presence of arabinopyranose units in various positions, including 2-O-substitution, and discusses the lack of D-galactopyranose in the periphery of *Acacia* gums.
from Smith degradation studies. For instance, all arabinose was removed from *A. senegal* gum after four successive Smith degradations, indicating that in this gum some peripheral chains contain at least four interlinked arabinose residues. Similar studies on *A. drepanolobium* gum, however, showed that after five successive Smith degradations there was still a large amount of arabinose left in the degraded gum. Methylation studies on the degraded gum indicated that a considerable proportion of the residues of this sugar were present as 2-0-substituted arabinofuranosyl units, suggesting that chains of at least eight arabinose residues were present in the original gum, with the possibility of much longer chains being present. In contrast, four successive Smith degradations were sufficient to remove all arabinose from *A. seyal* gum. In this case, however, methylation studies on the gum have indicated the presence of 2-0-substituted L-arabinopyranose residues. Since these could be oxidised by periodate, there remains the possibility of longer arabinose-containing chains being present in this polysaccharide. There is thus considerable variation in the detailed structure of the peripheral chains between individual gums from the *Acacia* genus.

Comparison of the cleavage products from the methylated gums and their methylated arabinose-free degraded derivatives indicates that the arabinose-containing side-chains can be either linked (1 + 6) to 3-0-substituted or (1 + 3) to 6-0-
substituted D-galactopyranose residues, the relative proportions of these two types of linkage varying from gum to gum.

The possible partial structure of gum arabic (Acacia senegal) is represented in Figure 17.
<table>
<thead>
<tr>
<th></th>
<th>pycnantha</th>
<th>senegal</th>
<th>nilotica</th>
<th>arabica</th>
<th>mearnsii</th>
<th>seyal</th>
<th>drapanolobium</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Galp1α3Ara</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arap1β3Ara</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Araβ1β3Ara</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Araβ1β2Ara</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Araf1-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Araβ-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2Araf1-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2Araβ-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3Araf1-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4 or -5Araβ-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ref. 72 79, 85, 86 81 80 74 84 83

A - oligosaccharides released on mild hydrolysis

B - linkages indicated by methylation analysis
Possible units represented by R:

\[ \alpha-D-Galp-(1 \rightarrow 3)-L-Araf- \]

\[ L-Araf- \]

\[ L-Araf-(1 \rightarrow 3)-L-Araf- \]

\[ \beta-L-Arap-(1 \rightarrow 3)-L-Araf- \]

\[ L-Araf-(1 \rightarrow 3)-L-Araf-(1 \rightarrow 3)-L-Araf- \]

\[ \beta-L-Arap-(1 \rightarrow 3)-L-Araf-(1 \rightarrow 3)-L-Araf- \]

*Acacia senegal gum*

**Figure 17**
(3) **Other gums**

Certain other gums have been shown to be based on an inner framework of $\beta$-galactose residues. The gum from *Citrus limonia* (lemon gum)\(^{(87)}\) has been found to contain $\beta$-galactose $L$-arabinose, $L$-rhamnose, $D$-glucuronic acid and its 4-methyl ether. The aldobiouronic acids $6-O-(\beta-D$-glucopyranosyluronic acid)$-D$-galactose, $4-O-(D$-glucopyranosyluronic acid)$-D$-galactose and $4-O-(4-O$-methyl-$\alpha$-$D$-glucopyranosyluronic acid)$-D$-galactose as well as the neutral disaccharides $3-O$-$\beta$-$L$-arabinopyranosyl-$L$-arabinose and the $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 6)$ linked $D$-galactobioses were recognised as the products of partial depolymerisation, all similar to fragments obtained from *Acacia* gums. An additional structural feature recognised was contained in the sequences of arabinofuranosyl residues, containing $(1 \rightarrow 5)$ linkages, which were terminated by units of the aldobiouronic acid $4-O-(4-O$-methyl-$\alpha$-$D$-glucopyranosyluronic acid)$-$L$-arabinopyranose. Methylation of the gum and degraded gum indicated the interior of the molecule contained $(1 \rightarrow 3)$ and $(1 \rightarrow 6)$ linked $D$-galactose residues. Application of the Smith degradation, however, caused considerable fragmentation of the polysaccharide, leading the authors to suggest that the galactan framework is of the branch-on-branch type involving sequences both of $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 6)$ linked $D$-galactose units, as in the diagrammatic representation in Figure 18.
Investigations on cholla gum have shown that it also contains a framework of $\beta$-galactose residues. The gum also contains $L$-rhamnose, $L$-arabinose, $D$-xylose and $D$-galacturonic acid, rather than $D$-glucuronic as in other galactan-type polysaccharides. The major aldobiouronic acid identified as a partial hydrolysis product was $3-O-(\beta-D$-galactopyranosyluronic acid)$-D$-galactose, although the presence of a second one namely $O-(\beta-D$-galactopyranosyluronic acid)$-L$-rhamnose, has been claimed. Mild hydrolysis of the gum gave a degraded polysaccharide containing $D$-galacturonic acid and $D$-galactose. Methylation studies on the degraded gum indicated the presence
of 3,6-di-, 3- and 6-0-substituted galactose units, with galacturonic acid present as end-groups only. Periodate oxidation of this degraded gum followed by reduction and complete hydrolysis gave galactose, glycolaldehyde, glycerol and glyceric acid. Although conclusive evidence on the distribution of linkages within the degraded gum is still lacking, one of several possible structures is indicated in Figure 19.
Degraded cholla gum

Figure 19
$\beta$-Xylose appears to be present only as end-groups in the acid-labile periphery, a major structural feature of which is contained in the trisaccharide identified as $O-\beta-\beta$-xylo-pyranosyl-(1 $\rightarrow$ 5)-$\alpha$-$L$-arabinofuranosyl-(1 $\rightarrow$ 3)-$L$-arabinose. There is no information as yet on the structural significance of the $L$-rhamnose residues, nor the aldobiouronic acid with which it is associated. The possibility of rhamnose being contained within a main chain in the style of the galacturonorhamnan family already described would, however, appear remote, both from the low rhamnose content of the gum (2-3%), and its virtual elimination from the polysaccharide under mild degradative conditions.

Comparison of the structural features exhibited by various members of the galactan family indicates that a whole structural spectrum is involved. This ranges from the relatively simple coniferous wood galactans, such as the larch arabinogalactans with little or no acid content and the more highly acidic polysaccharides from mountain larch and maritime pine, to the more complex exudate gums such as Araucaria bidwillii and the extremely complex Acacia gums. Since the wood polysaccharides have basically simpler structures than the exudate gums, it has been suggested that true arabinogalactans may be the precursors of some plant gums, and that the later stages of biosynthesis might involve the addition of $\beta$-glucuronic acid, $L$-rhamnose
and considerably more $\alpha$-arabinose, as well as perhaps an increase in the extent of branching of the galactan interior. However, there has as yet been found no instance of a tree producing a complex gum and also containing a structurally simpler arabinogalactan.
Chemistry of Mesquite Gum

Mesquite gum is the exudate from the mesquite tree (*prosopsis juliflora*), a small tree or bush found in the southwestern United States and Mexico. It is the neutral salt (mainly calcium) of a complex acidic polysaccharide. Analytical work, mainly by Anderson, established the main constituents as L-arabinose (51%), D-galactose (19%) and a uronic acid (13%), together with moisture (11%). The uronic acid was subsequently identified as 4-0-methyl-D-glucuronic acid by White and Smith. The free gum acid, mesquitic acid, has an equivalent weight of 1350, has a methoxyl content of 2.9% and shows $\alpha_D +60^\circ$ in water. The structure of mesquite gum has been extensively investigated by the late F. Smith and by E. V. White, whose results, while generally in agreement, differed on the nature of the linkages involving uronic acids.

In a series of studies, Smith found that hydrolysis of the gum under mild conditions liberated L-arabinose, forming an essentially arabinose-free degraded gum. Hydrolysis of this degraded gum under more vigorous conditions, followed by separation of the acidic sugars gave a mixture of two monomethyl aldobiouronic acids. Methylation of this mixture resulted in the characterisation, after hydrolysis, of 2,3,4-tri-0-methylglucuronic acid and 2,3,6- and 2,3,4-tri-0-methylgalactose. Also isolated from this methylation mixture was the crystalline amide of the methyl glycoside
hexamethyl ether of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. From these results it was concluded that the aldobiouronic acids present in the partial hydrolysate of the gum were 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose (1) and 4-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-galactose (2)

![Chemical structures](image)

Methylation studies on the gum by Smith (94) and White (95) both indicated that the constituent methylated sugars in the methylated polysaccharide were 2,3,5-tri-O-methyl-D-arabinose, 3,5-di-O-methyl-D-arabinose, 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid in the proportions
1:3:2:1. From the methylated arabinose-free degraded gum, White (96) characterised 2,4-di-0-methyl-D-galactose and a tri-0-methyl-D-galactose fraction composed of the 2,3,4-(63%) and 2,4,6- (37%) isomers.

After partial methanolysis of the methylated gum, (97) White obtained, on distillation of the light petroleum insoluble component, an oligosaccharide fraction which on further methanolysis yielded the methyl ester methyl glycosides of 2,3,4-tri-0-methyl-D-glucuronic acid and methyl 2,4-di-0-methyl-D-galactoside. Methylation of the fraction yielded 2,4,6-tri-0-methyl-D-galactose on depolymerisation. From these results it was concluded that there was present in the methanolysate of the methylated polysaccharide the methyl ester methyl glycoside of the methylated aldobiouronic acid 3-0-(2,3,4-tri-0-methyl-D-glucopyranosyluronic acid)-2,4-di-0-methyl-D-galactose.

From these studies, mesquite gum appears to be structurally related to the galactan group of polysaccharides, in that it contains a branched core of (1 → 6) and (1 → 3) linked D-galactose residues. The acid-labile periphery of the molecule would appear to be composed entirely of (1 → 2) linked L-arabinofuranose units. One major area of uncertainty in the structure of the gum lies in the nature of the aldobiouronic acid linkages present, since Smith obtained evidence for the presence of (1 → 6) and (1 → 4) linked units while White’s results suggested the presence of (1 → 3) linked aldobiouronic acid. It was hence decided to reinvestigate mesquite gum with a view to resolving this
particular apparent contradiction, and also to obtain evidence for the detailed sequences of linkages involved in the galactan core and to examine in more detail the linkages present in the arabinose-rich peripheral chains.
PART I

MESQUITE GUM
DISCUSSION

Introduction

As part of the present study into the structure of mesquite gum, it was decided to investigate three main structural aspects, namely the nature of the linkages involving uronic acids in the polysaccharide, the sequence of linkages contained within the galactan core, and the nature of the acid-labile peripheral chains. Information on the nature of the linkages involving the uronic acid residues was gained by studying the acidic oligosaccharides released during partial acid hydrolysis of the gum, and the methylated acidic oligosaccharides released during partial methanolysis of the methylated gum. The interior galactan core of the molecule was investigated by studying the polysaccharides obtained after successive Smith degradations of the degraded gum which had been formed by the removal, under conditions of mild hydrolysis, of the acid-labile peripheral units of the gum. Finally analysis of the neutral oligosaccharides released during mild hydrolysis of the gum gave information on the nature of the acid-labile peripheral chains themselves. These procedures are summarised in the following flow sheet.
Gum → \textit{partial hydrolysis} → acidic oligosaccharides

\textit{methylated gum} → methylated acidic oligosaccharides

autohydrolysis

degraded gum \textit{A} + \textit{L}-arabinose + neutral oligosaccharides

carboxyl-reduced degraded gum \textit{A}

\textit{Smith degradation}

degraded gum \textit{B}

\textit{Smith degradation}

degraded gum \textit{C}
Purification and Fractionation

Purified mesquitic acid was obtained by precipitation by pouring an acidified aqueous solution of the crude gum into ethanol. Paper chromatographic examination of the hydrolysis products of the polysaccharide showed that in addition to the neutral monosaccharide constituents previously identified, rhamnose was present as a minor component.

The homogeneity of the gum acid was investigated by chromatography on O-(2-diethylaminoethyl)-cellulose, prepared in the phosphate form. The polysaccharide was desorbed by elution of the column with solutions containing increasing concentrations of phosphate buffer at pH 6 and finally with a 0.5 M-potassium chloride solution. Although a number of peaks were obtained in the elution pattern, the majority of the sample (75%) was eluted with 0.25 M-phosphate, with smaller amounts at lower and higher concentrations. Analysis of samples from these peaks with the carbazole reagent indicated that the polysaccharide fractions all had approximately similar uronic acid contents. There was in addition a small quantity of material (<5%) eluted with 0.005 M-phosphate which contained no acidic constituents. From the elution pattern obtained from the fractionation, it would thus appear that there is no obvious heterogeneity in the polysaccharide. The complexity of the elution pattern, however, suggests that there may be a considerable distribution of molecular size.
Acidic Oligosaccharides Released During Partial Acid Hydrolysis

Acidic oligosaccharides formed during partial acid hydrolysis of mesquite acid were separated from the neutral components of the hydrolysate by ion-exchange chromatography on diethylaminoethyl Sephadex. After removal of neutral sugars by elution of the column with water, acidic sugars were desorbed from the column with formic acid solutions. The fractions obtained were further separated by preparative scale paper chromatography in appropriate solvent systems into five homogeneous acidic oligosaccharides, together with three other fractions which contained mixtures of higher oligosaccharides but which could not be further fractionated preparatively.

Oligosaccharide I

This oligosaccharide was identified as 4-0-(4-0-methyl-\(\alpha\)-D-glucopyranosyluronic acid)-D-galactose. Hydrolysis gave galactose and 4-0-methylglucuronic acid. Estimation with the carboxyl reagent indicated the sugar had a uronic acid
content of 48%. The methanolysis products from the methylated
disaccharide were examined by gas-liquid chromatography and
had the retention times of the methyl glycosides of 2,3,4-
tri-O-methylglucuronic acid and 2,3,6-tri-O-methylgalactose.
Methanolysis of the methylated glycitol gave 1,2,3,5,6-penta-
O-methylgalactitol and the methyl glycosides of 2,3,4-tri-O-
methylglucuronic acid.

The position of the linkage was confirmed by methylation
of the carboxyl-reduced disaccharide, prepared by sodium boro-
hydride reduction of the derived methyl ester methyl glyco-
sides. The cleavage products from the methylated oligosac-
charide were identified as 2,3,4,6-tetra-O-methyl-D-glucose,
as the crystalline sugar, and 2,3,6-tri-O-methyl-D-galactose,
after formation of the derived crystalline aldonolactone.

An α-D-glucuronosidic configuration was suggested by
the specific rotation, $[\alpha]_D +94^\circ$. This value was somewhat
lower than previous values for the same oligosaccharide,
i.e., $[\alpha]_D +102^\circ$, (98) but this discrepancy may be explained
by a slight contamination of the sample with oligosaccharide
II. The α-linkage was confirmed by n.m.r. spectroscopy of the
disaccharide glycitol in deuterium oxide. The anomeric pro-
ton was present as a doublet situated at $\tau 4.84$ (J 3c/s),
values characteristic of a β-anomeric proton. (99)
This aldobiouronic acid was characterised as 6-0-
(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose.
It was chromatographically identical to an authentic sample
and gave galactose and 4-0-methylglucuronic acid on hydro-
lysis. The cleavage products from the methylated disac-
charide were identified by g.l.c. as the methyl glycosides
of 2,3,4-tri-0-methylglucuronic acid and 2,3,4- and 2,3,5-
tri-0-methylgalactose. Methanalysis products from the
methylated glycitol were 1,2,3,4,5-penta-0-methylgalactitol
and the methyl glycosides of 2,3,4-tri-0-methylglucuronic
acid. That the specific rotation, [α]_D +20°, was higher
than previous values reported for the aldobiouronic acid
6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose,
[α]_D +1°(74) was due to a slight contamination with oligo-
saccharide I. Methylation of the aldobiouronic acid
furnished the crystalline methyl ester methyl glycoside
hexamethyl ether.
This oligosaccharide was shown to be the aldobiouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. It was chromatographically similar to an authentic sample and gave galactose and glucuronic acid on hydrolysis. Hydrolysis of the methyl glycosides of the carboxyl-reduced disaccharide gave galactose and glucose. The uronic acid content was found to be 46% by means of the carbazole reagent. The cleavage products from the methylated oligosaccharide and its derived glycitol were found by g.l.c. to be the same as those obtained from oligosaccharide II. In addition, methylation of this aldobiouronic acid yielded the same crystalline derivative. The specific rotation, $[\alpha]_D^0$, was fully consistent with β-D-glycosidic linkage. The characterisation of this oligosaccharide thus demonstrates that D-glucuronic acid is present in mesquite gum in addition to its 4-methyl ether.
On the basis of the following experimental evidence, this oligosaccharide was assigned the structure \( \beta-\text{o}-\text{D}-\text{glucopyranosyluronic acid})-(1 \rightarrow 4)\beta-\text{D}-\text{galactopyranosyl}-(1 \rightarrow 3)\text{D}-\text{galactose.} \) Hydrolysis of the sugar gave \( \beta-\text{o}-\text{D}-\text{glucopyranosyluronic acid})-\text{D}-\text{galactose,} \) \( \beta-o-\text{methylglucuronic acid and galactose.} \) Hydrolysis of the glycitol gave similar products, together with galactitol. Estimations with the carbazole reagent gave a uronic acid content of 30%, while comparison of the colorimetric reactions of the sugar and derived glycitol with the phenol-sulphuric acid reagent \((100)\) indicated a degree of polymerisation \((\text{D.P.})\) of three.

Examination of the methanolsysis products from the methylated trisaccharide by g.l.c. indicated the presence of the methyl glycosides of \(2,3,4-\text{tri-o-methylglucuronic acid} \) and \(2,3,6- \) and \(2,4,6-\text{tri-o-methylgalactose.}\) Some of
the peaks of the two last-mentioned methylated sugars were not completely resolved on the columns used, so additional evidence was sought on the position of the linkage to the reducing galactose unit.

\[
\begin{align*}
\text{C H}_2\text{O} \quad \text{HO} \quad \text{0} \\
\text{H}_2\text{O} \quad \text{MeOH/HCl} \\
\text{COLD} \quad \text{HO} \quad \text{CQ H} \\
\text{OH} \quad \text{HOCH}_2\text{O} \quad \text{OH} \\
\text{P} \quad \text{R} \\
\text{4} \\
\text{K} \quad (4\text{Me})\text{GpA 1α4 Galp 1-}
\end{align*}
\]

The first series of experiments was based on the ability of sugars to form furanosides under kinetic control upon treatment with cold methanolic hydrogen chloride for limited periods. The selective formation of a methyl furanoside from an oligosaccharide containing a 3-0-substituted reducing D-glucose unit has formed the basis of its separation from an isomeric oligosaccharide, also formed on partial hydrolysis of nigeran, (101) which oligosaccharide contained a 4-0-substituted D-glucose reducing group and remained unchanged. Treatment of the oligosaccharide (4) with cold methanolic hydrogen chloride resulted in the formation of a new non-reducing sugar derivative (5), probably a
mixture of methyl furanosides, thus pointing to the presence of a 3-\(\beta\)-substituted reducing group in oligosaccharide 4. Evidence in support of this contention was obtained by the following experiments.

The suspected mixture of methyl furanosides (5) was oxidised with one mole of sodium metaperiodate and reduced with sodium borohydride to give compound 6. The identity of this product was confirmed by hydrolysis, which gave 4-\(\beta\)-(4-\(\beta\)-methyl-\(\alpha\)-D-glucopyranosyluronic acid)-D-galactose, 4-\(\beta\)-methylglucuronic acid, galactose and arabinose, and by g.l.c. of the cleavage products from the methylated derivative, which showed the presence of the methyl glycosides of 2,3,4-tri-\(\beta\)-methylglucuronic acid, 2,3,6-tri-\(\beta\)-methylgalactose and 2,5-di-\(\beta\)-methylarabinose. The detection of arabinose as a constituent of product 6 thus proved that the methyl furanoside of oligosaccharide IV had originally been formed during the treatment with methanolic hydrogen chloride, while the identification of 2,5-di-\(\beta\)-methylarabinose as a cleavage product of the methylated derivative of 6 was further proof of a (1 + 3) linkage to the terminal galactose residue in the original trisaccharide.

The second approach to the nature of the linkage to the reducing galactose unit in oligosaccharide IV involved treatment of the oligosaccharide (4) with one mole of lead tetra-acetate. Under these conditions, oxidation takes
place preferentially at the C(1) - C(2) bond of the reducing unit of the molecule with the formation of the formyl ester of the degraded sugar (7).

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \xrightarrow{\text{Pb(OAc)}_4} \\
\text{HO} & \quad \xrightarrow{\text{OH}^-} \\
\end{align*}
\]

\[\text{R} \quad \xrightarrow{\text{(4Me)GpAla4Galpl}} \]

Chromatographic examination of the oxidation products confirmed that a new reducing oligosaccharide had indeed been formed, while hydrolysis gave 4-0-(4-0-methyl-\(\alpha\)-D-glucopyranosyluronic acid)-D-galactose, galactose and lyxose, the expected degradation product from a reducing galactose unit.

Treatment of the oxidised trisaccharide (7) with oxygen-free lime water over an extended period gave another reducing oligosaccharide (8), which, however, gave the same products as 7 on hydrolysis, indicating that the only change had been the removal of the formyl ester substituent. This stability of oligosaccharide 8 to alkali indicated that the lyxose reducing end-group was 2-0-substituted, further confirmation of this being provided by the unreactivity of the
oligosaccharide 8 with the tetrazolium spray reagent. It follows from these results that the linkage in the original trisaccharide IV involved C(3) of the galactose reducing end group.

The relatively low molecular rotation of oligosaccharide IV, \([\text{M}]_D +290^\circ\), compared to that of the aldobiouronic acid 4-0-(4-0-methyl-\(\alpha\)-\(\beta\)-glucopyranosyluronic acid)-\(\beta\)-galactose derived from it suggests that the inter-galactose linkage has a \(\beta\)-configuration.

Oligosaccharide V

\[
\text{This oligosaccharide was assigned the structure } 6-0-(4-0-\text{methyl-}\beta-\(\beta\)-glucopyranosyluronic acid)-(1 \text{ + 6})-0-\beta-\(\beta\)-galactopyranosyl-(1 + 6)-\(\beta\)-galactose. Hydrolysis gave } 6-0-(4-0-\text{methyl-}\beta-\(\beta\)-glucopyranosyluronic acid)-\(\beta\)-galactose, 4-0-\text{methyl-glucuronic acid and galactose. Carbazole estimation of uronic acid gave an acid content of 29\%. D.P. measurements conducted similarly to those described for oligosaccharide IV indicated the sugar was a trisaccharide.}
Analysis by g.l.c. of the cleavage products from the methylated oligosaccharide showed products with the retention times of the methyl glycosides of 2,3,4-tri-0-methylglucuronic acid and 2,3,4- and 2,3,5-tri-0-methylgalactose. Similar investigation of the methylated glycitol gave 1,2,3,4,5-penta-0-methylgalactitol and the methyl glycosides of 2,3,4-tri-0-methylglucuronic acid and 2,3,4-tri-0-methylgalactose.

The paper chromatographic identification of 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-α-D-galactose as a partial hydrolysis product of oligosaccharide V indicated that the glucuronosidic linkage had the β-anomeric configuration, while the low molecular rotation of the oligosaccharide, [M]_D +10°, suggested that the inter-galactose linkage was also of the β-D-configuration. This was confirmed by the isolation, by preparative scale paper chromatography of the partial acid hydrolysis products from carboxyl-reduced oligosaccharide V, of an oligosaccharide with chromatographic mobility and specific rotation similar to those of 6-0-β-D-galactopyranosyl-D-galactose. Methanalysis products from the methylated disaccharide were identified by g.l.c. as the methyl glycosides of 2,3,4,6-tetra- and 2,3,4- and 2,3,5-tri-0-methylgalactose.
Oligosaccharide fractions VI, VII, VIII

These oligosaccharide fractions were chromatographically homogeneous but ionophoresis showed each fraction to contain more than one component. Furthermore, each fraction was shown to contain a mixture of different types of oligosaccharide, since in each case partial acid hydrolysis gave both 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose (II) and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose (III), and cleavage of the methylated oligosaccharide glycitolis gave both 1,2,3,4,5- and 2,3,4,5,6-penta-O-methyl-galactitolis. Although separation of the individual components of each oligosaccharide fraction was not considered to be practicable, some information on the nature of the components of the fractions was obtained by examination of the cleavage products from the methylated derivatives of the oligosaccharides and the oligosaccharide glycitolis, and, in the case of oligosaccharide fractions VI and VII, of the oligosaccharides formed on degradation with alkali. In the latter instance it was assumed that oligosaccharides containing 3-O-substituted reducing groups would be rapidly degraded as outlined in Figure 20.
The aethylation results are summarised in Table 4.
Table 4

Methylation products from oligosaccharide fractions VI, VII, VIII

<table>
<thead>
<tr>
<th>Methylated sugar</th>
<th>Oligosaccharide fraction VI</th>
<th>Oligosaccharide fraction VII</th>
<th>Oligosaccharide fraction VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>2,3,4-Me$_3$ glucuronic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4,6-Me$_4$ galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4-Me$_3$ galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,4,6-Me$_3$ galactose</td>
<td>tr</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,5-Me$_3$ galactose</td>
<td>+</td>
<td>tr</td>
<td>+</td>
</tr>
<tr>
<td>2,5,6-Me$_3$ galactose</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2,4-Me$_2$ galactose</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4,5-Me$_5$ galactitol</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>1,2,4,5,6-Me$_5$ galactitol</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

a methylation of sugar n.d. not detected
b methylation of glycitol tr trace
c methylation of products of alkaline degradation

From the methylation results, it can be seen that the most obvious differences between these oligosaccharides and the ones already characterised are the presence of galactose non-reducing end-groups, implying branched oligosaccharides, and of (1+3) linked galactose units. Since both these features were removed upon treatment of oligosaccharide fractions VI and VII with alkali, it is likely
that 3-0-substituted D-galactose residues are present as reducing end-groups, and that the branching is associated with these units. It is probable that the structures listed in Figure 21 are all contained in oligosaccharide fraction VI, with higher oligosaccharides of the same general type being contained in fractions VII and VIII.

\[
\begin{align*}
\text{Gal} & \quad 6 & \text{Gal} 1 + 3 \text{Gal} & \quad \text{Gal} 1 + 3 \text{Gal} \\
6 & \quad + & 6 & \quad + & 6 \\
+ & \quad 1 & + & \quad 1 \\
+ & \quad \text{Gal} & + & \quad \text{Gal} \\
6 & \quad + & 6 & \quad + & 6 \\
+ & \quad 1 & + & \quad 1 \\
1 & \quad \text{Gal} & 1 & \quad \text{Gal} \\
6 & \quad (4\text{Me})\text{GA} & (4\text{Me})\text{GA} & (4\text{Me})\text{GA} \\
(4\text{Me})\text{GA} & \quad + & \quad + & \quad + & \quad + \\
+ & \quad 1 & \quad 1 & \quad 1 & \quad 1 \\
\end{align*}
\]

Probable Oligosaccharides contained in Fraction VI Figure 21

The results of this partial hydrolysis of mesquite gum thus confirm the identity of the aldobiouronic acid 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose characterised in earlier work by Smith(93) and also provide definite proof of the structure of the second aldobiouronic acid, 4-0-(4-0-methyl-α-D-glucopyranosyluronic acid)-D-galactose. Isolation of the third aldobiouronic acid, namely 6-0-(β-D-glucopyranosyluronic acid)-D-galactose, demonstrates for the first time that D-galacturonic acid is present in the polysaccharide in addition to its 4-methyl ether.
From the isolation of the trisaccharide V and the higher oligosaccharides it can be seen that 4-0-methyl-D-glucuronic acid units are present as non-reducing end-groups, joined by \( \beta(1 \rightarrow 6) \) linkages to D-galactose residues connected by \( \beta(1 \rightarrow 6) \) linkages, and that these in turn are linked to 3-0-substituted galactose residues.

The trisaccharide 0-(4-0-methyl-\( \alpha \)-D-glucopyranosyl-uronic acid)-(3, + 4)-0-0--ga1actopyranosyl-(1 + galactose was the only higher oligosaccharide characterised which gave rise to the aldobiouronic acid 4-0-(4-0-methyl-\( \alpha \)-D-glucopyranosyluronic acid)-D-galactose. The (1 \( \rightarrow 3 \)) linkage contained in the trisaccharide must therefore represent the major mode of linkage of the aldobiouronic acid unit to the adjacent galactose residue in the polysaccharide.
Methylation of Mesquite Gum

Mesquite gum was initially methylated with 30% sodium hydroxide solution and methyl sulphate and finally with methyl iodide and silver oxide until infra-red spectroscopy indicated that no more hydroxyl groups remained. A portion of the methylated gum was methanolysed and the products were examined by gas-liquid chromatography. A quantity of the methylated polysaccharide was reduced with lithium aluminium hydride in tetrahydrofuran. The methanolysis products from the reduced methylated gum were similarly examined by g.l.c.

Table 5

Methanolysis products from methylated and reduced methylated gum

<table>
<thead>
<tr>
<th>Cleavage products</th>
<th>Methylated gum</th>
<th>Reduced Methylated Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-Me₃ glucuronic acid</td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>2,3-Me₂ glucuronic acid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2,3,4-Me₃ glucose</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,4-Me₃ rhamnose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,5-Me₃ arabinose</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>3,5-Me₂ arabinose</td>
<td>+++++</td>
<td>++++++</td>
</tr>
<tr>
<td>2,5-Me₂ arabinose</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2,3-Me₃ arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4,6-Me₄ galactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4-Me₃ galactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me₃ galactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,4-Me₂ galactose</td>
<td>++++</td>
<td>+++++</td>
</tr>
</tbody>
</table>
The methanolysate from the methylated gum was hydrolysed and the methylated sugars were converted to the corresponding aldonolactones by treatment with bromine water. C.l.c. indicated the presence of the following compounds:

- 2,3,5-tri-O-methyalarabinolactone
- 3,5-di-O-methyalarabinolactone
- 2,5-di-O-methyalarabinolactone
- 2,3-di-O-methyalarabinolactone
- 2,3,4,6-tetra-O-methylergalactonolactone
- 2,3,6-tri-O-methylergalactonolactone

From a subsequent methanolysis of the methylated gum (partial methanolysis III), neutral sugars, insoluble in light petroleum were hydrolysed and separated preparatively by thick paper chromatography. Two sugars were isolated as crystalline compounds, namely 2,4-di-O-methyl-D-galactose and 2-O-methyl-D-galactose. No mono-O-methylerabinooses were encountered.

These results confirm earlier studies by White (95) and Smith (94) who identified 2,3,5-tri- and 3,5-di-O-methylerabinoses, 2,4-di-O-methylgalactose and 2,3,4-tri-O-methylglucuronic acid as cleavage products from the methylated gum. In addition 2,5-di-O-methylerabinose together with smaller amounts of 2,3,4-tri-O-methylrhamnose, 2,3-di-O-methylerabinose, 2,3,4,6-tetra-, 2,3,4- and
2,3,6-tri-0-methylgalactose and 2,3-di-0-methylglucuronic acid have now been shown to be present. The structural significance of the 2-0-methylgalactose is uncertain since it could arise as a product of under-methylation of a 3,6-di-0-substituted galactose unit. The axial C(4) hydroxyl group of a 3-0-substituted D-galactopyranose unit is known to be sometimes difficult to methylate. No evidence was obtained that L-arabinose residues are involved as branch points. However, the identification of 3-di-0-methylarabinoses indicates that the arabinose-containing peripheral chains are considerably more complex than was previously thought. D-Glucuronic acid residues are present mainly as end-groups, although the identification of small amounts of 2,3,4-tri-0-methylrhamnose and 2,3-di-0-methylglucuronic acid could indicate that in some cases D-glucuronic acid units are 4-0-substituted by (presumably L-)rhamnopyranose residues, as has been shown to be the case in gum arabic (28) and Araucaria bidwillii gum (15). Direct evidence, however, is lacking.
Partial Methanolysis of Methylated Mesquite Gum

During an investigation of the partial methanolysis products of methylated gum, E. V. White\(^{(97)}\) obtained a fraction which he characterised as the partly methylated aldobiouronic acid 3-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-2,4-di-O-methyl-D-galactose (9)

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{MeO} \\
\text{COOH} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\text{MeO} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\text{H} & \quad \text{MeO} \\
\end{align*}
\]

Although during the course of the partial acid hydrolysis studies on mesquite gum, (1 + 4) and (1 + 6) linked aldobiouronic acids had been characterised, no evidence had been found to indicate the presence of a (1 + 3) linked aldobiouronic acid, as implied by White’s results. Accordingly, it was decided to re-investigate the partial methanolysis products of the methylated gum with a view to clarifying this particular situation.

During the course of his experiments White depolymerised the methylated polysaccharide by heating under reflux with methanolic 4% hydrogen chloride for five hours. The
acidic sugars in the methanolysate were saponified and precipitated as their barium salts. These acidic sugars were re-methyl esterified and fractionally distilled under reduced pressure to give a fraction which yielded a crystalline amide upon treatment with methanolic ammonia. A fraction giving the same crystalline derivative was also obtained by concentrating the partial methanolysate to a syrup, extracting the syrup with hot light petroleum and fractionally distilling the petroleum insoluble residue. Examination of both fractions gave 2,3,4-tri-O-methyl-D-glucuronic acid and 2,4-di-O-methyl-D-galactose, characterised at a crystalline level, as cleavage products. Further methylation of these fractions gave a syrup which furnished a different crystalline amide and gave 2,4,6-tri-O-methyl-D-galactose, identified by a crystalline derivative, as a cleavage product. As a result of these experiments the structure (9) was assigned to the fraction.

There was, however, no confirmation that the fraction obtained by either method was homogeneous and hence no certainty that the methylated sugars characterised were derived from the same compounds as furnished the crystalline amides.

A further fraction was also obtained from the partial methanolysate of the methylated degraded gum which gave the same products as those from the corresponding fraction.
obtained from the methylated whole gum.

Partial Methanolysis I

In the present investigation, a sample of the methylated gum was methanolyised under the same conditions as employed by White. Acidic sugars present in the methanolysate were saponified and separated from the neutral component by chromatography on diethylaminoethyl Sephadex A-25. The methanolysis products obtained from these acidic sugars were found by g.l.c. to be the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid, 2,3,4- and 2,3,6-tri- and 2,4-di-O-methylgalactose. Methylation of these acidic sugars resulted in the characterisation of their cleavage products as 2,3,4-tri-O-methylglucuronic acid, 2,3,4,6-tetra-, 2,3,6-, 2,4,6-, 2,3,4-tri- and 2,4-di-O-methylgalactose.

The occurrence of tetra-O-methylgalactose as a methylation product of the mixture of acidic sugars implies the presence of branched oligosaccharides amongst these acidic sugars. Indeed similar products were obtained from methylation of the mixtures of tetra- and pentasaccharides present in oligosaccharide fractions VI and VII obtained from partial acid hydrolysis of the gum. It is thus apparent that the methanolysis conditions used by White were not sufficiently vigorous to give complete breakdown to aldobiouronic acids alone.
Partial Methanolysis II

A further sample of methylated gum was heated in methanolic 4% hydrogen chloride at 100° for five hours, and acidic sugars were isolated in the same way as after partial methanolysis I. G.l.c. examination of a methanolysate of the acidic component showed that it contained the same constituents as before. The cleavage products obtained after further methylation were 2,3,4-tri-O-methylglucuronic acid and 2,3,6-, 2,4,6- and 2,3,4-tri-O-methylgalactoses. Thus although breakdown of the methylated polysaccharide had obviously proceeded further than during the first partial methanolysis, the presence of a (1 + 3) linked aldobiouronic acid as a unit in the gum could not be ruled out.

Partial Methanolysis III

In a final partial methanolysis, the remainder of the methylated gum was reacted under White's conditions, and the methanolysate, after neutralisation, was evaporated to a syrup. This syrup was then repeatedly extracted with hot light petroleum. A portion of the petrol-insoluble residue was fractionally distilled under reduced pressure (0.005 mm Hg) to give three fractions.

Fraction 1 (b.p. 88-160°) Gas-liquid chromatography of this fraction and its methanolysate showed it contained the
methyl glycosides of 3,5- and 2,5-di-0-methylarabinose.

**Fraction 2 (b.p. 160-180°)** This fraction contained mainly methyl 2,4-di-0-methylgalactosides together with small amounts of the two arabinosides found in the first fraction.

**Fraction 3 (b.p. 180-210°)** G.l.c. of this fraction showed it to contain methyl 2,4-di-0-methylgalactoside. A small quantity of the fraction was methanolyzed and additional constituents of the fraction were found to be the methyl glycosides of 2,3,4-tri-0-methylglucuronic acid together with smaller amounts of 2,3,6- and 2,3,4-tri-0-methylgalactoses. A further quantity of the fraction was methylated and the cleavage products were identified as 2,3,4-tri-0-methylglucuronic acid, 2,3,4,6-tetra-, 2,3,6-, and 2,4,6- and 2,3,4-tri-0-methylgalactose.

Fraction 3 thus corresponds to that investigated by White, since it gave tri-0-methylglucuronic acid and 2,4-di-0-methylgalactose as cleavage products and 2,4,6-tri-0-methylgalactose as a cleavage product after further methylation. Treatment of fraction 3 with methanolic ammonia failed, however, to yield a crystalline amide.

From the identification of methyl 2,4-di-0-methylgalactosides upon g.l.c. examination of fraction 3, it was obvious that the fraction contained a considerable proportion of neutral sugars. The acidic component of the fraction was therefore saponified and separated from the
neutral sugars by ion-exchange chromatography on diethyl-
aminoethyl Sephadex A-25. In this way was obtained fraction
3A, containing acidic sugars, and fraction 3B, composed of
neutral sugars in the approximate ratio 1:2.

Examination of Fraction 3A

Examination of the methanolysis products showed the
presence of the methyl glycosides of 2,3,4-tri-O-methyl-
glucuronic acid, 2,3,6- and 2,3,4-tri- and 2,4-di-O-methyl-
galactose. A sample of fraction 3A was methylated and the
cleavage products were found to be:

<table>
<thead>
<tr>
<th>Relative Amount</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>2,3,4-tri-O-methylglucuronic acid</td>
</tr>
<tr>
<td>+</td>
<td>2,3,6-tri-O-methylgalactose</td>
</tr>
<tr>
<td>++</td>
<td>2,4,6-tri-O-methylgalactose</td>
</tr>
<tr>
<td>+</td>
<td>2,3,4-tri-O-methylgalactose</td>
</tr>
</tbody>
</table>

Partial separation of fraction 3A was observed on thin-
layer chromatography in solvent (2). It was considered
probable that this partial separation was based on differences
in the degree of etherification, and that isomeric oligo-
saccharides of the same degree of substitution, e.g. 10 and
11, would not be resolved. In an attempt to effect a

\[
\begin{align*}
\text{10:} & \quad \begin{array}{c}
\text{MeO} \\
\text{CH}_{2}\text{OH}
\end{array} \\
\text{11:} & \quad \begin{array}{c}
\text{MeO} \\
\text{R-O-CH}_{2} \\
\text{OH}
\end{array}
\end{align*}
\]

\[R = 234 \text{Me}, \text{GA 1-}\]
separation of partially methylated acidic oligosaccharides carrying free primary hydroxyl groups (as in 10), fraction 3A was treated with triphenylmethyl chloride in pyridine. In the event no products of increased chromatographic mobility due to tritylation were observed. However, preparative layer chromatography of the mixture gave three sub-fractions.

Sub-fraction 3A(I) \((R_f 0.65)\)

The methanolysate of a quantity of the sub-fraction was found by g.l.c. analysis to contain the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid, 2,3,6- and 2,3,4-tri-O-methylgalactose, plus traces of the glycosides of 2,4-di-O-methylgalactose. Methylation of a sample followed by g.l.c. of the methanolysate resulted in the identification of the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid and 2,3,6- and 2,3,4-tri-O-methylgalactoses.

Sub-fraction 3A(II) \((R_f 0.40)\)

This sub-fraction gave on methanolysis the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid and 2,4-di-O-methylgalactose together with a trace of methyl 2,3,4-tri-O-methylgalactoside. A further quantity of this sub-fraction was methylated and the cleavage products were identified as 2,3,4-tri-O-methylglucuronic acid and 2,3,4-tri-O-methylgalactose.
Sub-fraction 3A(III) (R_f 0.25)

Methanolysis of a sample of the sub-fraction gave rise to the methyl glycosides of 2,3,4-tri-0-methylglucuronic acid and 2,4-di-0-methylgalactose. Hydrolysis gave 2,4-di- and 2-0-methylgalactose. The sub-fraction was methylated and the cleavage products identified as 2,3,4-tri-0-methylglucuronic acid and 2,3,4-tri-0-methylgalactose.

The remaining material on the thin-layer plates was examined, but no 2,4,6-tri-0-methylgalactose was identified as a cleavage product after further methylation.

Examination of Fraction 3B

Examination of this fraction by g.l.c. indicated the presence of methyl 2,4-di-0-methylgalactosides. The fraction was methylated and the methanolysis products identified by g.l.c. as the methyl glycosides of 2,3,4,6-tetra- and 2,3,4-, 2,4,6- and 2,3,6(tr)-tri-0-methylgalactose.

As a result of the investigations on the acidic sugars contained in fraction 3A, evidence was obtained for the presence of several methylated aldobiouronic acids. The identification of 2,3,4- and 2,3,6-tri-0-methylgalactose as cleavage products from sub-fraction 3A(I) suggests this sub-fraction contained the fully methylated aldobiouronic acids (12) and (13).

\[ 2,3,4 \text{ me}_3 \text{GA} (1 \rightarrow 4) 2,3,6 \text{ me}_3 \text{Gal} \ (12) \]

\[ 2,3,4 \text{ me}_3 \text{GA} (1 \rightarrow 6) 2,3,4 \text{ me}_3 \text{Gal} \ (13) \]
The small amounts of 2,4-di-O-methylgalactose identified as a cleavage product of sub-fraction 3A(I) undoubtedly arose from the partially methylated aldobiouronic acid (14), since methylation of the sub-fraction indicated the presence of only (1 + 4) and (1 + 6) linkages. Compound (14) was identified as the major constituent of sub-fraction 3A(II) since 2,4-di-O-methylgalactose was recognised as a cleavage product of the sub-fraction while 2,3,4-tri-O-methylgalactose was the only neutral sugar formed on cleavage of the sub-fraction after methylation.

2,3,4 me₃ GA (1 + 6) 2,4 me₂ Gal (14)

2,3,4 me₃ GA (1 + 6) 2 me Gal (15)

Aldobiouronic acid (13) was present as a minor component in sub-fraction 3A(II), since 2,3,4-tri-O-methylgalactose was recognised as a minor cleavage product of the sub-fraction. Sub-fraction 3A(III) contained a mixture of partially methylated aldobiouronic acids (14) and (15), since while both 2,4-di- and 2-O-methylgalactoses were identified as cleavage products of the sub-fraction, only 2,3,4-tri-O-methylgalactose was recognised as a cleavage product after methylation. No direct evidence was obtained that the oligosaccharides described were in fact disaccharides, although the cleavage products obtained after methylation of the sub-fractions contained approximately equal quantities of tri-O-methylglucuronic acid and tri-O-methylgalactose,
as judged from the respective peak areas on the gas-liquid chromatograms. Moreover, since fraction 3 was the first fraction obtained from the distillation to contain acidic sugars, it is likely that these sugars included considerably more disaccharides than trisaccharides.

The nature of the linkages thus found in these products from the partial methanolysis of the methylated gum confirms the results of the partial acid hydrolysis, which identified uronic acids involved in only (1 → 4) and (1 → 6) linkages. The identification of the fully methylated aldobiouronic acid (12), when considered in conjunction with that of the trisaccharide IV, namely 0-(4-0-methyl-α-D-glucopyranosyluronic acid)-(1 → 4)-0-β-D-galactopyranosyl-(1 → 3)-D-galactose, obtained from partial acid hydrolysis of the gum, provides valuable information on the mode of linkage of the aldobiouronic acid unit 4-0-(4-0-methyl-α-D-glucopyranosyluronic acid)-D-galactose within the polysaccharide, since it shows that the aldobiouronic acid must be present as an unsubstituted side chain linked to the 3-position of another D-galactose residue. Since 2,4,6-tri-0-methylgalactose was not recognised as a cleavage product of the methylated gum, it follows that this second D-galactose unit must also be 6-0-substituted, as in structure (16).
The alternative structure (17) which could also give rise to acidic oligosaccharide IV may thus be rejected.

\[
\text{(4Me)} \text{GA} 1 \rightarrow 4 \text{Gal} 1 \rightarrow 3 \text{Gal} 1
\]

The methylated aldobiouronic acids (13) and (14) indicate that 4-0-methyl-D-glucuronic acid residues may be joined by (1 + 6) linkages to both branched and unbranched D-galactose residues. The partially methylated aldobiouronic acid (15) is perhaps significant only in that it shows that 2-0-methylgalactose, whether or not a product of undermethylation, is associated with the (1 + 6) as opposed to (1 + 4) linked aldobiouronic acids.

Although several methylated aldobiouronic acids were thus recognised as products of partial depolymerisation of methylated mesquite gum, no evidence was obtained for the presence of one containing a (1 + 3) linkage, as claimed by White. Fraction 3, the fraction from the distillation corresponding most closely to the one he investigated did indeed contain 2,3,4-tri-0-methylglucuronic acid and 2,4-di-0-methylgalactose as the main acidic and neutral components, and gave 2,4,6-tri-0-methylgalactose as a cleavage product after further methylation, although the 2,3,4-tri-0-methyl isomer was present in considerably greater amount.
In this instance, the 2,4,6-tri-0-methylgalactose is likely to have arisen in two ways. The major proportion of this sugar undoubtedly arose from the methylation of neutral partially methylated oligosaccharides present in the distillate, since it is known that the methanolysis conditions used were not sufficiently vigorous to give complete breakdown of the methylated gum to aldobiouronic acids alone.

The second explanation, and the one which accounts for the presence of a small amount of 2,4,6-tri-0-methylgalactose as a cleavage product after methylation of the acidic fraction 3A is that this sugar arose from the 3-0-substituted galactose unit known to be present in the trisaccharide 0-(4-0-methyl-α-D-glucopyranosyluronic acid)-(1+4)-0-β-D-galactopyranosyl-(1+3)-D-galactose isolated during the partial acid hydrolysis studies, and not from a disaccharide.
Previous studies by both White (96) and Cunneen and Smith (93) had indicated that it was possible to remove most of the L-arabinose from mesquite gum by mild acid hydrolysis to leave an essentially arabinose-free degraded gum. In order to obtain more detailed information on the nature of the inner core of the polysaccharide, a similar approach was used in the course of these investigations, using the technique of autohydrolysis to cleave the acid-labile peripheral units.

Using conditions previously determined by trial experiments, mesquitic acid was subjected to autohydrolysis to give degraded gum A in a yield of 27% by weight. Hydrolysis showed this degraded gum contained only traces of arabinose. The degraded polysaccharide was methylated and the cleavage products examined by gas-liquid chromatography. In this way the methyl glycosides of the sugars listed in Table 6 were identified.
Table 6

Cleavage products from methylated degraded gum A

<table>
<thead>
<tr>
<th>methylated sugar</th>
<th>relative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-Me$_3$ rhamnose</td>
<td>(tr)</td>
</tr>
<tr>
<td>2,3,5-Me$_3$ arabinose</td>
<td>(tr)</td>
</tr>
<tr>
<td>3,5-Me$_2$ arabinose</td>
<td>(tr)</td>
</tr>
<tr>
<td>2,5-Me$_2$ arabinose</td>
<td>(tr)</td>
</tr>
<tr>
<td>2,3,4,6-Me$_4$ galactose</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4-Me$_3$ galactose</td>
<td>+++++</td>
</tr>
<tr>
<td>2,4,6-Me$_3$ galactose</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me$_3$ galactose</td>
<td>(tr)</td>
</tr>
<tr>
<td>2,4-Me$_2$ galactose</td>
<td>+++++</td>
</tr>
<tr>
<td>2,3,4-Me$_3$ glucuronic acid</td>
<td>+++</td>
</tr>
</tbody>
</table>
The preparation of a degraded gum which was essentially arabinose-free, and the identification of cleavage products obtained from the methylated degraded gum, show that mesquite gum is made up of a 4-\(\alpha\)-methylglucuronogalactan core, surrounded by an acid-labile periphery containing mainly \(L\)-arabinofuranose residues. The methylation results from the degraded gum indicate that within the core, (1 \(\rightarrow\) 6) and (1 \(\rightarrow\) 3) inter-galactose linkages are involved. Since partial hydrolysis results have already indicated that 4-\(\alpha\)-methyl-D-glucuronic acid residues terminate chains of (1 \(\rightarrow\) 6) linked D-galactose units, the (1 \(\rightarrow\) 3) linkages must predominate in the interior of the galactan core. A possible structure for the degraded gum A, based on the relative proportions of the methylation products, is given in the structure in Figure 22. More evidence will be presented for the distribution of (1 \(\rightarrow\) 3) linkages during discussion of the Smith degradation studies.
Partial structure of degraded gum A

Figure 22
Table 7

Comparison of Products from Methylated Gum and Methylated Degraded Gum A

<table>
<thead>
<tr>
<th>methylated sugar</th>
<th>methylated gum</th>
<th>methylated degraded gum A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-Me₃ rhamnose</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>2,3,5-Me₃ arabinose</td>
<td>+++</td>
<td>tr</td>
</tr>
<tr>
<td>3,5-Me₂ arabinose</td>
<td>+++++</td>
<td>tr</td>
</tr>
<tr>
<td>2,5-Me₂ arabinose</td>
<td>++</td>
<td>tr</td>
</tr>
<tr>
<td>2,3-Me₂ arabinose</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>2,3,4-Me₃ glucuronic acid</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2,3-Me₂ glucuronic acid</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>2,3,4,6-Me₄ galactose</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4-Me₃ galactose</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>2,4,6-Me₃ galactose</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me₃ galactose</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>2,4-Me₂ galactose</td>
<td>+++++</td>
<td>+++</td>
</tr>
</tbody>
</table>

tr = trace
n.d. = not detected
Comparison of the nature and the relative amounts of the cleavage products from methylated mesquite gum and the methylated degraded gum A (Table 7) provides evidence for the hydroxyl groups exposed during formation of the degraded gum. The almost complete removal of arabinose residues during the formation of the degraded gum, taken together with the considerable increase in the proportion of 2,3,4-tri-O-methylgalactose relative to that of 2,4-di-O-methylgalactose as constituents of the methylated degraded gum clearly indicates that the partial structure in Figure 23 is a major structural feature of mesquite gum

\[ \overset{+6}{\text{Galp}}_1 \overset{3}{\text{Galp}}_2 \overset{R}{\text{Galp}}_3 \quad (R = \text{[Araf]}_n) \]

Figure 23

The appearance of significantly greater quantities of 2,3,4,6-tetra- and 2,4,6-tri-O-methylgalactose as cleavage products of the methylated degraded gum than of methylated mesquite gum points to the hydrolysis of other types of glycosidic linkage [Figure 24, A \( \rightarrow \) A', B (or B') \( \rightarrow \) B"] during the formation of the degraded gum. However, in view of the relatively low recovery of the degraded gum (27% by weight of the parent gum) and of the recognition of galactose-containing oligosaccharides as hydrolysis products,
it is apparent that some galactopyranosidic linkages were broken during the autohydrolysis. The transformations $A \to A'$ and $B$ (or $B'$) $\to B''$, therefore, cannot be unambiguously ascribed to the cleavage of arabinosidic linkages.

\[ \xymatrix{ -3 \text{Galp} \ar[r] & -3 \text{Galp} \ar[r] & -3 \text{Galp} \\
\text{R} \ar[r] & \text{R}' \ar[r] & \text{(A')} \\
\text{(A)} & \text{(A')} \\
\text{R''} \ar[r] & \text{3 Galp} \ar[r] & \text{Galp} \\
\text{R} \ar[r] & \text{R}' \ar[r] & \text{(B'')} \\
\text{(B)} & \text{(B'')} \\
\text{R'} \ar[r] & \text{6 Galp} \ar[r] & \text{(B')} \\
\text{(B')} } \]

Figure 24
**Smith Degradation of Carboxyl-reduced Degraded Gum A**

Degraded gum A was converted to its carboxyl-reduced derivative (carboxyl-reduced degraded gum A) by treatment of the acetylated 2-hydroxyethyl ester with lithium borohydride after the method of Rees and Samuel.\(^{(102)}\)

Hydrolysis of the reduced polysaccharide gave galactose and 4-O-methylglucose, together with small amounts of glucose and arabinose, but no trace of acidic components.

Smith degradation, involving periodate oxidation and reduction with sodium borohydride followed by mild hydrolysis of the carboxyl-reduced polysaccharide gave degraded gum B and low-molecular weight cleavage products, which included mainly glycerol and non-reducing oligosaccharides containing galactose and traces of arabinose.

Partial hydrolysis of degraded gum B led to the identification by paper chromatography of 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose as cleavage products. Visual inspection of the chromatograms indicated that the former was present in proportionally greater amounts.

A sample of degraded gum B was methylated using sodium hydride and methyl iodide in methyl sulfoxide as described by Sandford and Conrad.\(^{(103)}\) The methylated polysaccharide was finally treated with methyl iodide and silver oxide to ensure complete methylation. The cleavage products detected...
Degraded gum B was oxidised with sodium metaperiodate in a similar manner to gum A. After twenty-four hours, the consumption of periodate was 0.78 mol per sugar residue. After reduction and mild hydrolysis of the oxidised polysaccharide, degraded gum C was obtained by precipitation in ethanol. Partial hydrolysis of the degraded gum C gave 3-O-β-D-galactopyranosyl-D-galactose, together with a small amount of 6-O-β-D-galactopyranosyl-D-galactose. Periodate oxidation of gum C gave a final consumption of periodate of 0.25 moles per mole of sugar residue. The polysaccharide was methylated as before and the cleavage products, identified by g.l.c. of the methyl glycosides, are listed in Table 8.

Table 8
Relative proportions of cleavage products
from methylated degraded gums B and C

<table>
<thead>
<tr>
<th>Cleavage products</th>
<th>gum B</th>
<th>gum C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Ne4 galactose</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>2,4,6-Ne3 galactose</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>2,3,4-Ne3 galactose</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2,4-Ne2 galactose</td>
<td>1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

From these results it can be seen that the polysaccharides obtained after successive Smith degradations of carboxyl-
reduced degraded gum A approximated more and more to a
β(1 → 3) linked β-D-galactan. The β-linkage was assigned on
the basis of the low specific rotations of degraded gums
B and C ([α]_D +18°, +19° resp.), and of the paper chromatog-
graphic identification of the partial hydrolysis product
3-O-β-D-galactopyranosyl-β-D-galactose which was chromatog-
graphically distinguishable from the corresponding α-isomer.
Degraded gum C consumed 0.25 moles of periodate per mole of
sugar residue. Bearing in mind that 2,3,4,6-tetra- and
2,3,4-tri-O-methylgalactoses were identified as cleavage
products from methylated degraded gum C in the ratio 3:1,
and that both these methylated derivatives would originate
from units within the polysaccharide which would consume 2
moles of periodate per sugar residue, it can be calculated
that approximately 1 in 10 of the β-D-galactose units carries
a branch point. The most likely structure for degraded
gum C is indicated in the partial structure in Figure 25.
Comparison of the partial structure for degraded gum C (Figure 25) and degraded gum A (Figure 22) now permits a more extended partial structure to be proposed for the 4-0-methylglucuronogalactan framework of mesquite gum (Figure 26)

\[
\begin{align*}
-\text{Galp} & \ 1 \ + \ 3 \ \text{Galp} \ 1 \ + \ 3 \ \text{Galp} \ 1 \ + \ 3 \ \text{Galp} \ 1 \ + \ 3 \ \text{Galp} \ 1 \ + \ 3 \ \text{Galp} \ 1 \\
\uparrow & \ 1 \\
\text{Galp} & \ 3 \\
\uparrow & \ 1 \\
\text{Galp} & \ 3 \\
\uparrow & \ 1 \\
\text{Galp} & \\
\end{align*}
\]

Degraded Gum C

Figure 25

\[
\begin{align*}
\text{R} & \ + \ 6 \ \text{Galp} \\
\uparrow & \ 1 \\
\text{R} & \ + \ 6 \ \text{Galp} \\
\uparrow & \ 1 \\
\text{R} & \ + \ 6 \ \text{Galp} \\
\end{align*}
\]

\[
R = (4\text{Me})\text{GpA} \ 1 \ + \ 6 \ \text{Galp} \ 1 \ + \ 6 \ \text{Galp} \ 1 \\
\]

less frequently \( (4\text{Me})\text{GpA} \ 1 \ + \ 6 \ \text{Galp} \ 1 \ + \ 6 \ \text{Galp} \ 1 \)

(4Me) GpA 1 + 4 Galp

4-0-Methylglucuronogalactan framework of Mesquite Gum

Figure 26
On the basis of the partial structure in Figure 26 it can be seen that two successive Smith degradations would remove completely the side-chains containing (1→6) linked β-galactose units. Thus the identification of 2,4-di-0-methylgalactose as a significant cleavage product from methylated degraded gum C, together with the recognition of 6-0-β-β-galactopyranosyl-β-galactose as a partial hydrolysis product of gum C, confirms that (1→6) linkages are contained within the (1→3) linked galactan framework, in addition to being present as side-chains.
Structure of Acid-labile Peripheral Units

In order to obtain information on the nature of the acid-labile periphery of mesquite gum, the oligosaccharides released during autohydrolysis of the gum were investigated. Mesquitic acid was heated under reflux in aqueous solution for a period of time similar to that employed during the previous autohydrolysis. In this instance, however, the degraded polysaccharide recovered was found to contain an appreciable amount of $L$-arabinose, and was consequently refluxed for a further period. The degraded gum subsequently isolated by precipitation with ethanol contained only traces of $L$-arabinose. The two supernatant solutions obtained after precipitation of the degraded gums were combined and the resultant solution separated into acidic and neutral fractions by ion-exchange chromatography on diethylaminoethyl Sephadex A-25. This step-wise autohydrolysis, however unintentional, was undoubtedly responsible for the formation in comparatively good yield of the large number of neutral tri-, tetra-, and pentasaccharides subsequently examined.

Examination of neutral oligosaccharides

The mixture of neutral sugars was applied to a charcoal-Celite column and monosaccharides were eluted with water. Paper chromatographic examination identified these as galactose, arabinose and rhamnose. The column was then eluted with water containing an increasing proportion of ethanol.
Fractions were collected as appropriate and further separated into homogeneous oligosaccharide fractions by filter sheet chromatography in suitable solvents. The oligosaccharides characterised will be described in terms of groups of structurally related compounds, rather than in order of separation, in order to present a more readily understandable picture.

**Group 1 oligosaccharides A and B**

**Oligosaccharide A**

This oligosaccharide was characterised as 3-O-α-L-arabinopyranosyl-L-arabinose on the basis of the following observations. Hydrolysis of the oligosaccharide gave only arabinose, while comparison of the colorimetric reactions obtained with the phenol-sulphuric acid reagent before and after borohydride reduction \(^{(21)}\) indicated the sugar had a degree of polymerisation of 2. G.l.c. of the methanolation products of the methylated disaccharide showed the presence of the methyl glycosides of 2,3,5-tri- and 2,5-di-O-methylarabinose. The methylated oligosaccharide glycitol gave as methanolation products methyl 2,3,5-tri-O-methylarabinoside and a tetra-O-methy1arabinitol, either 1,2,4,5- or 1,3,4,5-tetra-O-methy1arabinitol since these isomers were not resolved on the g.l.c. columns used. Oligosaccharide A furnished a crystalline phenylazoszone whose ultra-violet spectrum was characteristic of a disaccharide phenylazoszone. Periodate oxidation of the phenylazoszone released 1.1 moles of formaldehyde, while no mesoxaldehyde 1,2-bisphenylhydrazone was
detected, thus pointing to the presence of a $3-O$-substituted pentose phenyllosazone.

The chromatographic and electrophoretic mobilities of oligosaccharide A were closely similar to values quoted by Stephen (51) who tentatively characterised $3-O-\alpha-L$-arabinofuranosyl-$L$-arabinose as a cleavage product of Virgilina oroboides gum. The specific rotation of oligosaccharide A, $[\alpha]_D^0$, was likewise similar to the value reported by Stephen and was indicative of an $\alpha-L$-arabinosidic linkage.

**Oligosaccharide B**

Oligosaccharide B was chromatographically and electrophoretically indistinguishable from $3-O-\beta-L$-arabinofuranosyl-$L$-arabinose, and the following observations provided support for the identity of the two compounds. The sugar, which gave arabinose only on hydrolysis, had a degree of polymerisation of two and gave a positive staining reaction with the tetrazolium spray, indicating the absence of a (1→2) linkage. 2,3,5-Tri- and 2,5- and 2,4-di-$O$-methylarabinose were identified as cleavage products from the methylated oligosaccharide by g.l.c. of the derived methyl glycosides and aldonolactones. Methanolysis of the methylated oligosaccharide glycitol furnished methyl 2,3,5-tri-$O$-methylarabinofuranosides and a ($1,2,4,5$- or $1,3,4,5$-) tetra-$O$-methylarabinitol.

Oligosaccharide B furnished a crystalline phenyllosazone with an ultra-violet spectrum characteristic of a disaccharide
phenylosazone. The presence of a \((1 + 3)\) linkage in the phenylosazone was indicated by the formation on periodate oxidation of 0.85 mole of formaldehyde but of no mesoxaldehyde 1,2-bisphenylhydrazone. This derivative had a melting point \((215-217^\circ)\) higher than those previously reported \((200^\circ(104)\) and \(198^\circ(105)\) for the phenylosazone of 3-\(O\)-\(\beta\)-arabinofuranosyl-\(\alpha\)-arabinose. The non-identity of the phenylosazone with that from oligosaccharide A (m.p. 212-213\(^\circ\)) was indicated by depression of the melting point \((205^\circ)\) on admixture of the two phenylosazones.

The evidence thus indicates that the disaccharide is a 3-\(O\)-\(\beta\)-arabinofuranosyl-\(\alpha\)-arabinose, while the specific rotation, \([\alpha]_D +125^\circ\), suggests that the glycosidic linkage has the \(\beta-\alpha\)-configuration. Although this value is somewhat higher than other values quoted for 3-\(O\)-\(\beta\)-arabinofuranosyl-\(\alpha\)-arabinose, i.e. \([\alpha]_D +94^\circ(104), +89^\circ(72)\), the discrepancy may be attributed to a minor impurity, probably a trisaccharide, whose presence was indicated by the identification of 2,3-di-\(O\)-methylarabinose as a minor cleavage product from both the methylated sugar and glycitol.
Group 2 oligosaccharides C–G

The main experimental data for these oligosaccharides is summarised in the following table.

Table 9

<table>
<thead>
<tr>
<th>oligosaccharide:</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.P.:</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Stability to alkali:</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oligosaccharides formed on</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) partial hydrolysis of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oligosaccharide:</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>(ii) partial hydrolysis of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oligosaccharide glycitol</td>
<td>C</td>
<td>-</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>methanolysis products from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>methylated derivatives:</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>2,3,4,6 Me₃ galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,5 Me₃ arabinose</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1,2,4,5/1,3,4,5 Me₅ arabinitol</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

a - methylation of oligosaccharide

b - methylation of oligosaccharide glycitol

C Gal₃plα3Ara
D Gal₃plα3Araf1–3Ara
E Araf₃α 6Gal₃plα3Ara
F Araf₃α6Gal₃plα3Ara1–3Ara
G higher members of series
Oligosaccharide C

This sugar was chromatographically indistinguishable from 3-\(\alpha\)-\(\alpha\)-galactopyranosyl-\(\beta\)-arabinose. The nature of the linkage was confirmed by the identification of cleavage products from both the methylated sugar and glycitol while the specific rotation, \([\alpha]_D^{\circ} +154^\circ\), was characteristic of the \(\alpha\)-\(\beta\)-galactopyranosidic linkage. The identity of oligosaccharide C and 3-\(\alpha\)-\(\alpha\)-galactopyranosyl-\(\alpha\)-arabinose was confirmed by the preparation of identical crystalline phenyl-o-sazones. Additional confirmation that a (1 + 3) linkage was present came from periodate oxidation of the phenylosazone of oligosaccharide C, which gave 1.0 mole of formaldehyde but no mesoxaldehyde 1,2-bisphenylhydrazone.

Oligosaccharide D

This oligosaccharide was assigned the structure 3-\(\alpha\)-\(\alpha\)-galactopyranosyl-(1 + 3)-\(\alpha\)-arabinofuranosyl-(1 + 3)-\(\alpha\)-arabinose. The sequence of sugar units was indicated by the recognition of 3-\(\alpha\)-\(\alpha\)-galactopyranosyl-\(\beta\)-arabinose as a partial hydrolysis product of both the sugar and derived glycitol. While the cleavage products from the methylated oligosaccharide were consistent with the above structure, the additional presence of a small amount of 3,5-di-\(\alpha\)-methylarabinose as a cleavage product cast some doubt on the nature of the linkage to the reducing arabinose residue.
That this residue was not 2-0-substituted and was most probably 3-0-substituted was shown by the degradation of the oligosaccharide with alkali.

**Oligosaccharide E**

Oligosaccharide E was characterised as O-α-L-arabinofuranosyl-(1 + 6)-O-α-D-galactopyranosyl-(1 + 3)-L-arabinose. The sequence and linkages of the constituent sugars were indicated by the cleavage products from both the methylated oligosaccharide and derived glycitol, and confirmed by the recognition of 3-O-α-D-galactopyranosyl-L-arabinose as a partial hydrolysis product of the oligosaccharide but not of the oligosaccharide glycitol. The low molecular rotation of the oligosaccharide, \([\text{M}]_D^{+} 284^\circ\), compared with that of oligosaccharide C (\([\text{M}]_D^{+} 480^\circ\)) suggests that the additional L-arabinofuranosidic linkage has the α-configuration.

**Oligosaccharide F**

The oligosaccharide was assigned the structure O-α-L-arabinofuranosyl-(1 + 6)-O-α-D-galactopyranosyl-(1 + 3)-O-α-L-arabinofuranosyl-(1 + 3)-L-arabinose, which was consistent with the cleavage products from both the methylated oligosaccharide and the derived glycitol, and with the partial hydrolysis products of the sugar and its glycitol. The presence of a 3-0-substituted arabinose reducing unit in the oligosaccharide was confirmed by the ready degradation
of oligosaccharide F with alkali. The small amounts of 3,5- and 2,3-di-O-methy larabinose present as cleavage products from the methylated derivatives presumably arose from impurities. Comparison of the molecular rotation, \([M]_D +172^\circ\), with that of oligosaccharide E suggests that the \((1 \rightarrow 3)\) linkage to the reducing \(\alpha\)-arabinose residue may have the \(\alpha\)-configuration.

**Oligosaccharide fraction G**

Paper chromatography suggested this fraction was not homogeneous, while identification of both galactopyranose and arabinofuranose as non-reducing end groups indicated that the fraction most probably contained a mixture of higher oligosaccharides structurally related to oligosaccharides E and E, particularly as no evidence was obtained for branched oligosaccharides.
**Group 3 oligosaccharides H-K**

Experimental data for these oligosaccharides is contained in the table below.

<table>
<thead>
<tr>
<th>Table 10</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>oligosaccharide:</td>
<td>H</td>
<td>I</td>
<td>J</td>
<td>K</td>
</tr>
<tr>
<td>contains:</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
</tr>
<tr>
<td>D.P.:</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Oligosaccharides formed on partial hydrolysis:

<table>
<thead>
<tr>
<th>methylated derivatives:</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5 Me₂ arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3,5 Me₂ arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3,4 Me₂ arabinose</td>
<td>+</td>
<td>n.d.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1,3,4,5/1,2,4,5 Me₄ arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **a** - methylation of oligosaccharide
- **b** - methylation of oligosaccharide glycitol
- n.d. - not detected with certainty

H $\text{Araf}_1\text{B2Ara}$

I $\text{Araf}_1\text{B2Araf}_1\text{B2Ara}$

J $\text{Araf}_1\text{B2Araf}_1\text{B2Araf}_1\text{B2Ara}$

K $\text{Araf}_1\text{B2Araf}_1\text{B2Araf}_1\text{B2Araf}_1\text{B2Ara}$
Oligosaccharide U

Oligosaccharide U was characterised as 2-0-β-L-arabinofuranosyl-β-L-arabinose on the basis of the following observations. The sugar was found to be a disaccharide on the basis of D.P. measurements and the cleavage products from the methylated glycitol. The nature of the linkage was indicated by g.l.c. of the cleavage products from the methylated oligosaccharides, as their methyl glycosides, and subsequently confirmed by the isolation of these products as crystalline derivatives.

Although these experiments provided conclusive proof of the nature of the linkage involved, before their results became known, additional experiments were performed on the oligosaccharide. Treatment of the oligosaccharide with alkali resulted in the chromatographic separation of two oligosaccharides. One was identical to oligosaccharide U while the other was identified as 2-0-β-L-arabinofuranosyl-β-L-ribose and presumably formed by epimerisation about C(2) of the reducing unit of the disaccharide. Oligosaccharide U was treated with one mole of lead tetra-acetate. No new products were observed and borohydride reduction of the sugar after treatment with lead tetra-acetate, followed by hydrolysis, gave only arabinose and arabinitol. Since lead tetra-acetate most readily cleaves the bond between carbon atoms (1) and (2), provided they carry free hydroxyl groups, its failure in this instance to degrade the oligosaccharide
indicates that the reducing unit is 2-0-substituted.

The arabinofuranosidic linkage was assigned a \( \beta-L \)-configuration on the basis of the high specific rotation, \([\alpha]_D^{+101^\circ}\).

**Oligosaccharides, I, J and K**

These oligosaccharides were assigned structures as members of the polymer homologous series \( 0-\beta-L \)-arabinofuranosyl-(1-\([\alpha]+2\)-0-\(\beta-L \)-arabinofuranosyl-(1-\(_n\)+2)-\(L \)-arabinose \( [I, n=1; J, n=2; \text{and } K, n=3] \) on the basis of the following observations. Each oligosaccharide gave no stain with the tetrazolium spray, indicative of the presence of 2-0-substituted reducing groups, and gave on partial acid hydrolysis arabinose and lower oligosaccharides of the series including 2-0-\(\beta-L \)-arabinofuranosyl-\(L \)-arabinose (oligosaccharide H). The identification of cleavage products from the methylated oligosaccharides and methylated oligosaccharide glycitols indicated that arabinofuranose was involved in \((1+2)\) linkages only. Comparison of the molecular rotations of the oligosaccharides showed that each additional arabinofuranose residue had the \( \beta-L \)-configuration.
Group 4  oligosaccharides L-Q

Some of the experimental data for these oligosaccharides is included in Table 11.

Table 11

<table>
<thead>
<tr>
<th>oligosaccharide:</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>P</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>contains:</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
<td>Ara</td>
</tr>
<tr>
<td>D.P.:</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

oligosaccharides formed on
(i) treatment with alkali:  
(ii) partial hydrolysis of  
oligosaccharide:  
(iii) partial hydrolysis of  
oligosaccharide glycitol:

methanolysis products from methylated derivatives:

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>a</th>
<th>b</th>
<th>a</th>
<th>b</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me₃ arabinose</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3 Me₂ arabinose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5 Me₂ arabinose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,5 Me₂ arabinose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,4,5/1,3,4,5 Me₄ arabinitol</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,5 Me₄ arabinitol</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a - methylation of oligosaccharide

b - methylation of oligosaccharide glycitol
<table>
<thead>
<tr>
<th></th>
<th>Araflβ3Ara</th>
<th>Araflβ2Ara</th>
<th>Araflβ2Araflβ2Araflβ2Ara</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Araflβ3Ara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Araflβ4Ara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Araflβ4Ara</td>
<td>H Araflβ2Ara</td>
<td>I Araflβ2Araflβ2Ara</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>Araflβ2Araflβ1+4Araflβ3Ara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>Araflβ2Araflβ2Araflβ1+4Araflβ3Ara</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Oligosaccharide L

Oligosaccharide L was chromatographically indistinguishable from 3-α-β-L-arabinopyranosyl-L-arabinose, but the observed specific rotation, \([\alpha]_D +156^\circ\), although consistent with that of a disaccharide containing a β-L-arabinopyranosidic linkage, was lower than those previously reported. The disaccharide furnished a phenylosazone of the correct melting point (m.p. 233-234°, lit. 234°(106)) and ultra-violet spectrum. Periodate oxidation of the phenylosazone gave formaldehyde but no mesoxaldehyde 1,2-bisphenylhydrazone in accordance with the proposed structure.

Oligosaccharides M and N

These structurally similar oligosaccharides were assigned the structure 4-α-β-L- and 4-β-β-L-arabinofuranosyl-L-arabinopyranose respectively on the basis of their specific rotations, \([\alpha]_D +6^\circ\) and \(+86^\circ\), and of the following experiments which are additional to those summarised in Table 11. Both disaccharides were oxidised with bromine water, and methylation of the derived aldobionic acids, followed by hydrolysis, furnished components with the retention times of 2,3,5-tri-O-methyalarabinose and 2,3,5-tri-O-methyalarabinolactone. Oligosaccharide M afforded a phenylosazone, which was isolated in a pure but non-crystalline state by thin-layer chromatography and had a typical ultra-violet spectrum. The presence of a (1 + 4) linkage was further indicated since periodate oxidation
of the phenylazone gave no mesoxaldehyde 1,2-bisphenylhydrazone and only a trace of formaldehyde.

**Oligosaccharide 0**

The structure \( \alpha-L\)-arabinofuranosyl-(1 + 4)-\( O-\beta-L\)-arabinopyranosyl-(1 + 3)-\( L\)-arabinose was assigned to this oligosaccharide. The sequence of linkages was indicated by the cleavage products obtained from the methylated oligosaccharide and methylated oligosaccharide glycitol, and confirmed by the recognition of 3-\( O-\beta-L\)-arabinopyranosyl-\( L\)-arabinose as a partial hydrolysis product of the oligosaccharide but not of the oligosaccharide glycitol. Comparison of the molecular rotation of the oligosaccharide, \([\eta]_D +165^\circ\), with that of oligosaccharide L(3-\( O-\beta-L\)-arabinopyranosyl-\( L\)-arabinose), \([\eta]_D +443^\circ\), suggests that the \( L\)-arabinofuranosidic linkage in oligosaccharide 0 has the \( \alpha\)-configuration.

**Oligosaccharide P**

This oligosaccharide was assigned the structure \( \beta-L\)-arabinofuranosyl-(1 + 2)-\( O-\beta-L\)-arabinofuranosyl-(1 + 4)-\( O-\beta-L\)-arabinopyranosyl-(1 + 3)-\( L\)-arabinose. This sequence of linkages was consistent with the cleavage products obtained from the methylated oligosaccharide and the methylated oligosaccharide glycitol, which indicated that the reducing unit was 3-\( O\)-substituted. The involvement of the non-reducing arabinofuranose end-group in a (1 + 2) linkage was
confirmed by the identification of 2-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinofuranosyl-
\(\alpha\)-arabinose as a product of alkaline degradation of oligo-
saccharide P.

**Oligosaccharide Q**

Oligosaccharide Q was assigned the structure 2-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinofuranosyl-(1\(\rightarrow\)2)-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinofuranosyl-(1\(\rightarrow\)2)-\(\alpha\)-
arabinofuranosyl-(1\(\rightarrow\)4)-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinopyranosyl-(1\(\rightarrow\)3)-
arabinose on the basis of the following observations. 2,3,5-Tri-, 3,5-, 2,3- and 2,5-di-\(\alpha\)-methy larabinose in the relative proportions of 1:2:1:1 were identified as cleavage products from the methylated oligosaccharide by g.l.c. of their derived methyl glycosides and aldonolactones. Comparison of these products with the cleavage products from the methylated oligosaccharide glycitol indicated that the reducing arabinose residue was 3-\(\alpha\)-substituted. This conclusion was supported by the recognition of 3-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinopyranosyl-\(\alpha\)-arabinose together with oligosaccharides 0 and P as products of partial hydrolysis of the oligosaccharide but not of its glycitol. Further evidence for the sequence of linkages in oligosaccharide Q was provided by the liberation of the trisaccharide I [2-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinofuranosyl-(1\(\rightarrow\)2)-\(\alpha\)-\(\beta\)-\(\alpha\)-arabinofuranosyl-(1\(\rightarrow\)2)-\(\alpha\)-arabinose] upon degradation of the oligosaccharide with alkali.
**Group 5 oligosaccharides R, S and T**

**Oligosaccharide R**

This oligosaccharide was chromatographically identical to $3\alpha$-D-galactopyranosyl-D-galactose. It contained only galactose and gave as cleavage products from the methylated derivative 2,3,4,6-tetra- and 2,4,6- and 2,5,6-tri-O-methylgalactose.

**Oligosaccharide S**

Oligosaccharide S was chromatographically indistinguishable from $6\alpha$-D-galactopyranosyl-D-galactose and gave only galactose on hydrolysis. The cleavage products from the methylated oligosaccharide were 2,3,4,6-tetra- and 2,3,4-tri-O-methylgalactose, while those from the methylated oligosaccharide glycitol were 2,3,4,6-tetra-O-methylgalactose and 1,2,3,4,5-penta-O-methylgalactitol. The specific rotation of the oligosaccharide, $[\alpha]_D^\circ +23^\circ$ was consistent with the presence of a $\beta$-D-glycosidic linkage.

**Oligosaccharide T**

This oligosaccharide was chromatographically identical to $\alpha$-D-galactopyranosyl-(1 + 6)-D-galactopyranosyl-(1 + 6)-D-galactose. The sugar, which gave only galactose on hydrolysis, had a degree of polymerisation of three and furnished 2,3,4,6-tetra- and 2,3,4- and 2,3,5-tri-O-methylgalactose as cleavage products from the methylated derivative.
The cleavage products from the methylated oligosaccharide glycitol were identified as 2,3,4,6-tetra- and 2,3,4-tri-O-methylgalactose and 1,2,3,4,5-penta-O-methylgalactitol. The molecular rotation of the sugar, \([\chi]_D^{+90^\circ}\), indicated the presence of \(\beta-D\)-glycosidic linkages within the oligosaccharide.

Although a considerable number of oligosaccharides, containing a variety of different linkages, were characterised as cleavage products formed during mild hydrolysis of mesquite gum, all the linkages were consistent with the types of linkage inferred from the cleavage products of the methylated polysaccharide. However the identification of six different arabinobiose raises the question of whether acid-catalysed reversion could have been responsible for the formation of some of them. 3-\(\beta-L\)-Arabinopyranosyl-\(\beta-L\)-arabinose has indeed been shown \(^{106}\) to be a major product of the acid-catalysed reversion of \(\beta-L\)-arabinose. Another typical reversion product, however, namely 4-\(\beta-L\)-arabinopyranosyl-\(\beta-L\)-arabinose was not encountered in these investigations. These reversion products were, however, formed from \(\beta-L\)-arabinose under thermodynamic control, whereas the experimental conditions employed in the autohydrolysis, involving exposure to a mildly acidic solution for a comparatively short period, might have favoured the synthesis under kinetic control of different oligosaccharides. The possi-
bility of kinetically controlled transglycosylation having occurred must also be considered, in view of the presence throughout the autohydrolysis of a comparatively high concentration of polymeric material. However, in view of the correspondence already noted between the linkages in the oligosaccharides and those indicated by the methylation results, it is unlikely that any of the linkages were formed by reversion or transglycosylation.

While the linkages identified in the disaccharides may thus be representative of those occurring in the polysaccharide, the isolation of two pairs, differing in anomeric configuration, does suggest that anomerisation may have taken place. Of the arabinobioses containing the \((1 \rightarrow 4)\) linkage, the \(\alpha\)- and \(\beta\)-anomers were isolated in the molar ratio of 2:1. Consideration of the molecular rotation of oligosaccharide 0 \((\text{Ara}_1(1\rightarrow4)\text{Ara}_2(1\rightarrow3)\text{Ara})\) suggested the \((1 \rightarrow 4)\) linkage had an \(\alpha\)-configuration. The minor \((1 \rightarrow 4)\) linked arabinobiose, involving the \(\beta\)-configuration, could thus be a product of anomerisation. The two arabinobioses A and B containing \((1 \rightarrow 3)\) linkages were isolated in equimolar amounts. Comparison of the molecular rotations of oligosaccharides E \((\text{Ara}_1(1\rightarrow3)\text{Gal}_1(1\rightarrow3)\text{Ara})\) and F \((\text{Ara}_1(1\rightarrow6)\text{Gal}_1(1\rightarrow3)\text{Ara}_1(1\rightarrow3)\text{Ara})\) indicates that in oligosaccharide F, the \((1 \rightarrow 3)\) linkage to the reducing arabinose residue may have the \(\alpha\)-configuration. If then either of the two \((1 \rightarrow 3)\) linked
disaccharides is a product of anomerisation, it is more likely to be the one involving the \(\beta-L\)-linkage.

The four groups of arabinose-containing oligosaccharides discussed earlier would appear to be derived from two basic structural sequences. The first involves a combination of Groups 1 and 2 to form structure (18), from which the various oligosaccharides in the two groups were derived.

(18) \(L\)-Araf\(\beta\)\(6\)D-Galp\(\alpha\)3\(L\)-Araf\(\alpha\)3\(L\)-Araf\(\alpha\)3\(L\)-Araf1-
derived \(D\)-Galp\(\alpha\)3\(L\)-Ara (C) \(L\)-Araf\(\alpha\)3\(L\)-Ara (A)
oligosaccharides \(L\)-Araf\(\alpha\)\(6\)D-Galp\(\alpha\)3\(L\)-Ara (E)
\[D\]-Galp\(\alpha\)3\(L\)-Araf\(\alpha\)3\(L\)-Ara (D)
\(L\)-Araf\(\alpha\)\(6\)D-Galp\(\alpha\)3\(L\)-Araf\(\alpha\)3\(L\)-Ara (F)

Similarly the oligosaccharides of Groups 3 and 4 can be derived from the single structure (19).

(19) \(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\alpha\)\(4\)\(L\)-Arap\(\beta\)\(3\)\(L\)-Ara-
derived \(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Ara (J)
oligosaccharides \(L\)-Arap\(\beta\)\(3\)\(L\)-Ara (L) \(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Ara (I)
\(L\)-Araf\(\alpha\)\(4\)\(L\)-Arap (M) \(L\)-Araf\(\beta\)\(2\)\(L\)-Ara (H)
\(L\)-Araf\(\alpha\)\(4\)\(L\)-Arap\(\beta\)\(3\)\(L\)-Ara (O)
\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\alpha\)\(4\)\(L\)-Arap\(\beta\)\(3\)\(L\)-Ara (P)
\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\beta\)\(2\)\(L\)-Araf\(\alpha\)\(4\)\(L\)-Arap\(\beta\)\(3\)\(L\)-Ara (Q)

One of the interesting features revealed by these structures is that although the oligosaccharides 3-\(O\)-\(\alpha\)-\(D\)-galactopyranosyl-\(L\)-arabinose and 3-\(O\)-\(\beta\)-\(L\)-arabinopyranosyl-\(L\)-arabinose
have been characterised as partial hydrolysis products of various gums of the galactan group, the presence of isolated \( \delta \)-galactopyranose and \( \Lambda \)-arabinopyranose residues within chains of \( \Lambda \)-arabinofuranose units has not been previously observed.

From the isolation of pentasaccharide K, containing (1 \( \rightarrow \) 2) linked \( \Lambda \)-arabinofuranose residues, and the implication from oligosaccharides P and Q that the (1 \( \rightarrow \) 2) linked oligosaccharides are terminated by arabinopyranosylarabinose units in the polysaccharide, it would appear that there are chains containing at least seven \( \Lambda \)-arabinose residues present in the gum.
**Examination of acidic oligosaccharides**

The acidic oligosaccharides released during the autohydrolysis were fractionated preparatively by filter-sheet chromatography in suitable solvents. Examination of the oligosaccharides showed that they were similar to those characterised as partial acid hydrolysis products, namely 6-O-[(β-D-glucopyranosyluronic acid)-D-galactose (III), 6-O-[(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose (II), 4-O-[(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose (I) and the trisaccharide 0-[(4-O-methyl-β-D-glucopyranosyluronic acid)-(1 → 6)-O-β-D-galactopyranosyl-(1 + 6)-D-galactose (V). The other trisaccharide, 0-[(4-O-methyl-α-D-glucopyranosyluronic acid)-(1 → 4)-O-β-D-galactopyranosyl-(1 + 3)-D-galactose (IV), however, was not encountered on this occasion. This latter observation reinforces previous suppositions about the position of this fragment within the polysaccharide, since its formation would require the cleavage of two relatively resistant galactopyranosidic linkages during the autohydrolysis, compared with the cleavage of only one galactopyranosidic linkage required for the release of the other oligosaccharides, as in Figure 27.
Figure 27
Higher acidic oligosaccharides which contained arabinose were separated from the autohydrolysis products. Methylation indicated that the arabinose was present as non-reducing end-groups. This was confirmed by hydrolysis of the derived glycitols, which showed neither the disappearance of arabinose, nor the formation of arabinitol. It is thus apparent that $L$-arabinose is associated with the acidic oligosaccharides only in the form of side-chains, as in Figure 28(a), rather than being an integral part of a galactan chain (b), as is found in gum ghatti.

\[
\begin{align*}
\text{Gal} & \quad 1- \\
& \quad 6 \\
& \quad + \\
& \quad 1 \\
\text{+ Araf} & \quad + 3 \text{ Gal} \\
& \quad 6 \\
& \quad + \\
& \quad 1 \\
\text{Gal} & \quad 6 \\
& \quad + \\
& \quad 1 \\
4\text{MeCA} & \quad \text{GA} \\
\text{(a)} & \quad \text{(b)}
\end{align*}
\]

Figure 28
Conclusions

As a result of these investigations, mesquite gum has been shown to be a complex acidic polysaccharide containing 4-O-methyl-D-glucuronic acid, D-glucuronic acid, D-galactose, L-arabinose and (presumably L-) rhamnose. It has features in common with other members of the arabinogalacturono-galactan family, such as the *Acacia* gums, in that it is composed of a glucuronogalactan core, involving (1 + 6) and (1 + 3) inter-galactose linkages, with glucuronic acid or its 4-methyl ether occupying terminal positions, which core is surrounded by a periphery of chains containing mainly arabinofuranose units.

**Inner galactan core**

Methylation studies on both the whole gum and degraded gum A indicated the presence of both (1 + 6) and (1 + 3) linkages within the basic galactan framework of the polysaccharide. Evidence as to the distribution of these linkages within the molecule was obtained from Smith degradation studies on the degraded gum. The results of these studies indicated that the polysaccharides obtained after successive applications of the Smith degradation contained increasing proportions of (1 + 3) linkages. However, evidence was obtained that in degraded gum C, 1 in every 10 of the galactose residues was present as a branch point, involving a (1 + 6) linkage. These results are qualitatively similar to those obtained from similar investigations on other complex
acidic polysaccharides of this type, such as gum arabic, and reinforce the view that the galactan frameworks of many gums are more complex than was originally believed.

Earlier studies on gums of the arabinogalactan family suggested that the galactan area of the polysaccharides contained relatively simple comb-like structures, as in Figure 29(a), where a single main chain of (1 → 3) linked galactose residues carried side-chains of galactose units mutually involved in (1 → 6) linkages.

---

chains of (1 → 3) linked β-galactose units

(1 → 6) linkages

chains of (1 → 6) linked β-galactose units

---

Figure 29(a)

The studies on the degraded gum C obtained from mesquite gum, however, indicate that the (1 → 3) linked backbone of the molecule, instead of being a single "main chain", contains a degree of multiple branching, involving occasional (1 → 6) linkages. Side-chains of galactose units containing (1 → 6) linkages are attached to this multiply branched framework, as in Figure 29(b).
Figure 29(b)

Both the (1 → 3) and (1 → 6) β-galactopyranosidic linkages within the galactan framework of the polysaccharide have the β-anomeric configuration, as indicated by the recognition of oligosaccharides (20–22) as cleavage products formed on hydrolysis of the gum under mild conditions, and the low optical rotations of degraded gums A, B and C.

(20) β-Galp1β3p-β-Gal
(21) β-Galp1β6p-β-Gal
(22) β-Galp1β6p-β-Galp1β6p-β-Gal
Linkages involving uronic acids

Three aldobiouronic acids, (23-25), involving two basic types of linkage, were formed on partial hydrolysis of mesquite gum, together with the trisaccharides (26) and (27).

(23) (4Me)D-GpAαD-Gal
(24) (4Me)D-GpAβ6D-Gal
(25) D-GpAβ6D-Gal
(26) (4Me)D-GpAαD-Galpβ3D-Gal
(27) (4Me)D-GpAβ6D-Galpβ6D-Gal

The isolation of the two trisaccharides suggests that the aldobiouronic acids containing (1→4) and (1→6) linkages originated from structurally distinct areas of the polysaccharide.

More information on the nature of the linkages involving uronic acids came from an examination of the products of partial depolymerisation of the methylated gum which indicated that the structural units (28-30) are present in the polysaccharide.

(28) D-GpA1→6D-Galp1–
(29) D-GpA1→6D-Galp1–
(30) D-GpA1→4D-Galp1–

Consideration of structures (26) and (30) shows that terminal units of 4-0-methyl-α-D-glucuronic acid are joined (1→4) to unbranched D-galactose residues which in turn
are linked (1 → 3) to 6-O-substituted α-D-galactopyranose units, as in structure (31), since results from studies on the methylated gum showed that all the 3-O-substituted α-D-galactose units were also 6-O-substituted.

\[
\begin{align*}
\text{(31)} & \quad (4\text{Me})\beta-D-Gal\alpha4\beta-D-Gal\beta3\beta-D-Galp \\
& \quad \uparrow \\
& \quad 1 \\
\end{align*}
\]

It is therefore likely that while units of aldobiouronic acids (24) and (25), containing (1 → 6) linkages, terminate chains of α-D-galactose residues involved in (1 → 6) linkages, units of aldobiouronic acid (23), containing (1 → 4) linkages, are attached as side-chains thereto, as in structure (32).

\[
\begin{align*}
\text{(32)} & \quad (4\text{Me})\alpha-D-GpA(1 \rightarrow 4)\beta-D-Galp(1 \rightarrow 3)\beta-D-Galp \\
& \quad \uparrow \\
& \quad 1 \\
\end{align*}
\]

No direct evidence was obtained to indicate the position of rhamnose in the structure of mesquite gum. However, the identification of 2,3,4-tri-O-methylrhamnose and 2,3-dimethylglucuronic acid as cleavage products from the methylated gum suggests rhamnose could be joined by a (1 → 4)
linkage to glucuronic acid, as in gum arabic (28) and Araucaria bidwillii gum. (15)

Structure of arabinose-containing peripheral chains

The L-arabinose-rich peripheral chains have been found to be considerably more complex than was at one time supposed, since they contain (1 + 2), (1 + 3) and (1 + 4) inter-arabinose linkages, as well as isolated units of D-galactopyranose. The principal features of the peripheral chains are long sequences containing at least five units of β(1 + 2) linked L-arabinofuranose residues. These are joined by a (1 + 4) linkage to single L-arabinopyranose units which are in turn linked (1 + 3) to other L-arabinose residues, presumably containing furanose ring forms. Such sequences are contained in structure (33). A second type of peripheral side-chain is illustrated by structure (34), where a D-galactopyranose unit, 6-O-substituted by an L-arabinofuranose residue, is joined to a series of 3-O-substituted L-arabinofuranose units.

(33) L-Araf1β[1-2β-Araf]n1α4L-Arap1β3L-Ara
(34) L-Araf1α6D- Galp1α3L-Araf1α3L-Ara

No evidence for branching was found among the neutral oligosaccharides investigated. The absence of branching in the arabinose-containing chains was further supported by results from the methylated polysaccharide, where examination of the cleavage products failed to detect mono-O-methyl-
arabinoses. Examination of these cleavage products also indicated that L-arabinopyranose units, if present, did not occur as non-reducing end-groups. This conclusion was supported by the isolation of a series of neutral oligosaccharides (0, P and Q) after partial depolymerisation of the polysaccharide which demonstrated that L-arabinopyranose is present as isolated units within chains of L-arabinofuranose units. The presence of both L-arabinopyranose and D-galactopyranose as isolated units within chains of L-arabinofuranose units represents structural features not previously recognised in polysaccharides of this type.

The position of linkage of these arabinose-containing side-chains to the galactan portion of the polysaccharide was established by comparison of the methylated sugars and their relative amounts identified on depolymerisation of the methylated gum and methylated degraded gum A (Table 7). The major difference in the galactose derivatives was found to be a considerable increase in the proportion of 2,3,4-tri-O-methylgalactose relative to 2,4-di-O-methylgalactose, in the degraded gum. This indicates that the majority of the arabinose-containing chains are linked (1→3) to 6-O-substituted D-galactopyranose units.

The conclusions drawn from these observations on structural features of mesquite gum are summarised in the partial structure in Figure 30.
Figure 30
EXPERIMENTAL

General Methods

Paper chromatography of sugars was carried out on Whatman No. 1 and Whatman No. 4 paper. The chromatograms were developed using the following solvent systems (v/v).

A ethylacetate : pyridine : water (10:4:3)
B ethylacetate : acetic acid : formic acid : water (18:3:1:4)
C ethylacetate : acetic acid : formic acid : water (18 :8:3:9)
D ethylacetate : pyridine : acetic acid : water (5:5:1:3)
E butan-1-ol : ethanol : water (4:1:5, upper layer)
F butan-2-one : water : concentrated ammonia (200:17:1)
G butan-2-one : acetic acid : water (9:1:1, saturated with boric acid)
H a 3:2 mixture of solvent B and butan-2-one : water : formic acid (200:17:1).

After development for the required time, the chromatograms were air dried and the sugars were located by treatment of the chromatograms with one of the following spray reagents.

(a) Aniline oxalate. Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol and heating them at 120-130° for about five minutes.

(b) Alkaline silver nitrate. This was used to detect non-reducing sugars and other polyhydroxy compounds. Because
of its great sensitivity, this reagent was used to detect trace quantities.

(c) Alkaline permanganate-periodate. (108) This spray was used to detect both sugars and sugar alcohols.

(d) Periodate-Schiff. (109) This reagent detects sugars which carry an α-glycol group and is particularly useful when formaldehyde is formed as a product of periodate oxidation.

(e) Aniline-xylose. (110) This spray was used to detect acidic sugars.

(f) Triphenyltetrazolium chloride (111) was used to detect reducing sugars unsubstituted at C(2).

The definition of the symbols used in the description of the chromatographic mobilities of sugars are as follows:

\[ R_f = \frac{\text{rate of movement of sugar}}{\text{rate of movement of solvent front}} \]

\[ R_{\text{Gal}} = \frac{\text{rate of movement of sugar}}{\text{rate of movement of } \alpha-\text{galactose}} \]

\[ R_G = \frac{\text{rate of movement of sugar}}{\text{rate of movement of } 2,3,4,6-\text{tetra-}0\text{-methyl-}D\text{-glucose}} \]

Preparative paper chromatographic separations of sugars were carried out on Whatman 3MM filter sheets which had been washed with water for three days in a Soxhlet extractor. The positions of the sugars on the developed chromatograms were determined by cutting off and spraying narrow strips. Appropriate bands of the filter sheet were then cut out and the sugars were eluted with water.
Paper electrophoresis \((112)\) of sugars was conducted on Whatman No. 1 paper in 0.05 M borate buffer (pH 10) at a potential of 350 volts for 4-8 hours. Reducing sugars were detected by spraying the dried electrophoretograms with aniline oxalate reagent containing 10% glacial acetic acid and heating at 120-130° for five minutes.

Alternatively electrophoresis of acidic sugars was carried out in water : acetic acid : pyridine (289:10:1) solution using a potential of 500 volts for about two hours.

The value for \(M_0\) refers to the rate of movement of sugars on electrophoresis in borate buffer relative to that of \(\beta\)-glucose.

Thin layer chromatography. This was most frequently carried out on microscope slides coated with silica gel. Silica gel (kieselgel G nach Stahl., Merck) was added to water (4 ml) with shaking until a creamy suspension was obtained (sufficient for about 12 plates). Part of the suspension was poured onto a clean microscope slide and smeared over the surface. By tilting and gently agitating the plate a thin uniform layer of silica was obtained. The plate was left at room temperature to set, activated by heating at 105° for 30 minutes and stored in a desiccator.

Preparative scale chromatography was carried out on 20 x 20 cm plates. Bands were detected by removing a strip of silica adhered to the edge of another plate which
had been coated with a paste made from silicone vacuum grease and silica gel, and by treating with the appropriate spray reagent.

Thin layer chromatograms were developed in the following solvents.

1. benzene : ethanol (2:1)
2. benzene : methanol (6:1)
3. toluene : ethanol : water (270:30:1)

Sugars were detected by the spray reagents already described or by the anisaldehyde-sulphuric acid reagent. The plates were sprayed lightly with a solution of anisaldehyde (1 ml) and AnalaR sulphuric acid (1 ml) in ethanol (20 ml). On heating, sugars gave dark spots on a light background.

**Column Chromatography**

(a) **Cellulose columns** were packed as a slurry in acetone. The packed column was washed with acetone containing an increasing amount of water, water, and finally with the solvent to be used.

(b) **Diethylaminoethyl (D.E.A.E.) Sephadex columns** were used to fractionate mixtures containing acidic sugars. 0-(2-Diethylaminoethyl)-Sephadex A-25 was allowed to swell in water overnight, fine material was washed out by decantation and the residue washed alternately twice with 0.5 N sodium hydroxide and twice with 0.5 N hydrochloric acid before being generated in the formate form by stirring with
15% formic acid. The column was packed and washed free
from formic acid with water.

(c) Diethylaminoethyl cellulose. (113) The ion-exchange
cellulose was washed alternately with 0.5 M hydrochloric
acid and 0.5 M sodium hydroxide by stirring for several
minutes, centrifuging and decanting the supernatant solution.
The cellulose was then washed free from acid (or base) with
water and packed in a column as a slurry. The column was
generated in the phosphate form by elution with 0.5 M
sodium dihydrogen phosphate (buffered at pH 6 by the addi-
tion of sodium hydroxide). Finally the column was equili-
brated by elution with 0.005 M sodium dihydrogen phosphate
solution at the same pH. The polysaccharide was dissolved
in a little water and allowed to soak into the column over-
night. The rate of elution of the column thereafter was
adjusted to 40/50 ml per hour.

(d) Charcoal-Celite columns (114) were used to fraction-
ate mixtures of neutral oligosaccharides. Celite was al-
lowed to stand overnight in concentrated hydrochloric acid,
filtered and washed free from chloride with water. Charcoal
was washed six times with boiling distilled water and fine
particles were decanted. The mixture of charcoal : Celite
(1:1) was packed into a column as a water slurry and equili-
brated with water before use.

(e) Duolite A4 columns. Duolite A4 was washed altern-
ately with 0.5 M hydrochloric acid and 0.5 M sodium hydroxide,
fine material being removed by decantation each time. The resin was finally generated in the hydroxide form, packed in the column and equilibrated with water.

Gas-liquid partition chromatography (g.l.c.) \( (115) \) was conducted qualitatively on a "Pye Argon Chromatograph", using the method of Bishop and Cooper. \( (116) \) Separations were achieved using various stationary liquid phases supported on acid washed Celite (80-100 mesh). The liquid phases used were:

(a) 3% by weight of neopentylglycoladipate polyester.
(b) 5% by weight of polyethyleneglycoladipate polyester.
(c) 3% by weight of XE-60 on Gas-chrom P.
(d) 10% by weight of polyphenyl ether.

Operating temperatures were (a) 150\(^\circ\)C, (b) 175\(^\circ\)C, (c) 125\(^\circ\)C, (d) 200\(^\circ\)C.

The retention times \( (T) \) of the peaks are given relative to the peak of methyl 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-D-gluco-pyranoside.

Evaporations were carried out under reduced pressure on a rotary film evaporator at or below 40\(^\circ\)C.

Optical rotations were measured at room temperature in aqueous solution (unless otherwise stated) at 589 \(\mu\) (sodium D-line) on a Perkin-Elmer model 141 polarimeter.

Deuterations for nuclear magnetic resonance spectroscopy were achieved by concentrating the sugar three times on a rotary
evaporator from deuterium oxide.

Hydrolyses

(a) Hydrolysis with sulphuric acid.

Small scale hydrolyses were conducted by heating 1-5 mg of the sample in 0.5-2 ml N sulphuric acid in a sealed tube at 100°. The duration of the hydrolysis varied depending on the nature of the sugar and the degree of depolymerisation required. The time required to achieve complete depolymerisation varied from four hours for a neutral oligosaccharide to overnight for acidic oligo- or polysaccharides. The hydrolyses were neutralised by raising the pH initially with saturated barium hydroxide solution and finally with barium carbonate until neutralisation was complete. Insoluble barium salts were removed at the centrifuge and the supernatant was deionised with Amberlite IR 120 (H) cationic exchange resin. After filtration the solution was concentrated to a syrup which was examined by paper chromatography.

(b) Hydrolysis with hydrochloric acid.

The sugar was heated at 100° in N hydrochloric acid for the required length of time. The solution was neutralised with silver carbonate and insoluble salts were removed at the centrifuge. By treatment of the supernatant with hydrogen sulphide, colloidal silver was converted to silver sulphide which was removed by filtration through
glass fibre paper. The filtrate was then concentrated to a syrup.

**Deionisation.** Removal of cations from sugar solutions was achieved by treatment with Amberlite IR 120 (H) cationic exchange resin. Anions were removed with Amberlite IR 45 (OH) anionic exchange resin.

**Alkali degradations** were effected by dissolving the sugar (1-5 mg) in saturated calcium hydroxide solution (2-5 ml). Oxygen was removed by bubbling nitrogen through the solution for 1/2 hour. The flask was then tightly stoppered and left for the required length of time. The solution was neutralised with anionic exchange resin, filtered and the filtrate was concentrated.

**Methanolyses** were carried out by heating the sample (1-2 mg) in dry methanolic 4% hydrogen chloride (0.5-1 ml) in a sealed tube at 100°. The length of time varied from four hours for methylated neutral oligosaccharides to overnight for methylated acidic oligosaccharides. The solution was cooled, neutralised with silver carbonate and centrifuged. The centrifugate was carefully concentrated to give the methyl glycosides.

**Acetates** for gas-liquid chromatography were prepared by heating the sugar or glycoside (2-3 mg) for 1-1/2 hours in a sealed tube at 100° with anhydrous sodium acetate (2 mg) and acetic anhydride (0.5 ml). The solution was mixed with water and concentrated to a syrup. Traces of water were
removed by distillation with acetone and the residue was extracted with chloroform.

**Small scale methylations**

(a) Haworth methylation. (117)

The oligosaccharide (5 mg) was dissolved in water (2 ml) and methyl sulphate (0.25 ml) and 30% sodium hydroxide solution (0.50 ml) were added dropwise over a period of one hour. The vigorously stirred solution was kept in an ice bath while oxygen was excluded from the reaction by a stream of nitrogen. Additions of methyl sulphate (1 ml) and 30% sodium hydroxide (2 ml) were made on the following five days, each addition taking four hours. Twenty-four hours after the final addition, the solution was heated at 100° for one hour to destroy any remaining methyl sulphate. After the pH of the solution had been adjusted to 4 with dilute sulphuric acid, sodium sulphate was precipitated by pouring into ethanol (8 vols.). The precipitate was removed at the centrifuge and washed with ethanol. The supernatant and washings were combined, the pH adjusted to 8 and the solution concentrated to small volume. The pH was re-adjusted to 4, the solution was extracted with chloroform and the combined extracts, after drying over anhydrous sodium sulphate were evaporated to dryness.

(b) Kuhn methylation. (118)

The oligosaccharide (0.5-2 mg) was dissolved in N,N-
dimethylformamide (0.2 ml) and shaken in the dark in a sealed tube with methyl iodide (0.3 ml) and silver oxide (0.1 g) at room temperature for 48 hours. The reaction mixture was diluted with chloroform, filtered and the residue was washed with chloroform. The combined filtrate and washings were concentrated to dryness, the N,N-dimethylformamide being removed by azeotropic distillation with redistilled toluene under reduced pressure.

Demethylations were effected by one of two methods, depending on whether (a) or not (b) the methylated sugar or its methyl glycoside was soluble in dichloromethane.

(a) The sugar was treated in dichloromethane with boron trichloride at -70° for 1/2 hour. The solution was allowed to evaporate overnight at room temperature and borate was removed by distillation with methanol.

(b) The sugar (4 mg) was heated with hydriodic acid (1 ml) in a sealed tube at 100° for eight minutes. After dilution with water, the solution was neutralised with silver carbonate. The removal of insoluble material and decolorisation of the solution was accomplished by filtration through charcoal. Colloidal silver was precipitated as the sulphide upon treatment with hydrogen sulphide, and after filtration, the solution was concentrated to a syrup.
Reductions

(a) Glycitol reductions. (121)

Sodium or potassium borohydride (10 mg) was added to the sugar (2-4 mg) dissolved in water (0.5 ml) and the solution was left overnight. Excess borohydride was destroyed and cations were removed by treatment with Amberlite IR 120 (H) cationic exchange resin. Boric acid was removed by repeated distillation with methanol.

(b) Carboxyl reductions.

(i) Aldobiouronic acid. The aldobiouronic acid was converted to the derived methyl ester methyl glycosides by standing the sugar overnight in methanolic 1% hydrogen chloride, before refluxing the solution for 3/4 hour. After neutralisation of the solution with silver carbonate and concentration, the sugar was taken up in water and treated with borohydride as described in (a) above. This gave the methyl glycosides of the corresponding derived neutral disaccharide.

(ii) Methylated polysaccharide. Lithium aluminium hydride (100 mg) in tetrahydrofuran (3 ml) was added to a solution of methylated polysaccharide (100 mg) in tetrahydrofuran (5 ml). After being left for 30 minutes at room temperature, the solution was stirred under reflux for 3 hours. Aqueous ethyl acetate was added to destroy excess hydride and dilute sulphuric acid was then added until the solution was pH 4. The reduced methylated polysaccharide was extracted with chloroform.
Small scale periodate oxidations of methylated sugars were performed according to the method of Bauer and Lemieux. (122) Methoxyl contents were measured by the semi-micro Zeisel method. (123)

Spectrophotometric determinations

(a) Total sugar content was determined by the phenol-sulphuric acid method. (124)

(b) Degree of polymerisation (D.P.) was estimated by comparing the sugar concentrations in the oligosaccharide and oligosaccharide glycitol as determined by the phenol-sulphuric acid method.

(c) Uronic acid content was measured with the aid of the carbazole reagent. (17)

(d) Sodium metaperiodate consumption was measured by the method of Aspinall and Ferrier. (125)

(e) Formaldehyde produced during the periodate oxidation of phenylosazones was measured as described by Hough et al. (126)

Formation of derivatives

(a) Phenylosazones of oligosaccharides were prepared by dissolving phenylhydrazine hydrochloride (100 mg), sodium acetate (200 mg) and sodium bisulphite (30 mg) in water (0.5 ml). The solution was warmed until all the solid had dissolved and filtered to remove tarry material. The sugar (50 mg) was added and the test tube plugged with glass wool and placed in a boiling water bath. The phenylosazone
(c) **Methyl sulfoxide** was redistilled over calcium hydride.

(d) **Methyl iodide** was redistilled over silver oxide.

(e) **Pyridine** was redistilled twice over phosphorous pentoxide and stored over potassium hydroxide pellets.

(f) **Light petroleum** was shaken overnight with concentrated sulphuric acid (10%, v/v), washed free from acid with distilled water, dried over anhydrous sodium sulphate and redistilled.

(g) **Formamide** was redistilled using a splash bulb and stored over molecular sieve (type 4A).

(h) **Methanol** was refluxed with magnesium and iodine for 1/2 hour, then distilled.
Purification and Fractionation of Mesquite Gum

Mesquite gum was obtained as hard vitreous amber-yellow nodules, embedded with bark, twigs and other foreign material. The nodules (50 g) were dissolved by standing in water (150 ml) overnight. Insoluble material was removed by filtration through muslin and the solution was made 5% acid with dilute hydrochloric acid. After stirring for five minutes the polysaccharide was precipitated by pouring into ethanol (6 vol.). This procedure was repeated and the precipitate was redissolved in water (200 ml) and freeze-dried to give purified mesquitic acid (28 g). Hydrolysis of a small sample followed by paper chromatographic examination of the hydrolysate showed that the neutral monosaccharide constituents of the gum were mainly arabinose and galactose, together with a small amount of rhamnose.

A column of O-(2-diethylaminoethyl)-cellulose (30 g, 3 x 30 cm) was prepared in the phosphate form by elution with 0.5 M sodium dihydrogen phosphate (2L, pH 6) followed by 0.005 M sodium dihydrogen phosphate (1L, pH 6). The polysaccharide (470 mg) was washed onto the column with water (50 ml) and left overnight to adsorb. The column was eluted with batches of solution (pH 6) of sodium dihydrogen phosphate of the following concentrations.
(a) 0.005 M (900 ml)  
(b) 0.05 M (600 ml)  
(c) 0.10 M (400 ml)  
(d) 0.15 M (500 ml)  
(e) 0.20 M (800 ml)  
(f) 0.25 M (900 ml)  
(g) 0.30 M (300 ml)  
(h) 0.35 M (300 ml)  
(i) 0.45 M (250 ml)  

The column was finally eluted with 0.5 M potassium chloride (250 ml).

Fractions were collected every 20 ml, and the sugar concentration was measured by the phenol-sulphuric acid method, using a standard graph based on mesquitic acid to calculate the concentration. The elution pattern is represented on the graph in Figure 31. The majority of the polysaccharide (75%) was eluted with 0.25 M sodium dihydrogen phosphate. Estimation of the uronic acid content of the polysaccharide eluted at each peak was made by the carbasol method. All the peaks were found to contain polysaccharide of similar acid content to unfractionated mesquitic acid, except the first peak, which had no acid content.
D.E.A.E.-cellulose chromatography of Mesquitic acid

Graph of concentration of eluted sugar versus concentration of eluant.
Partial Acid Hydrolysis

Mesquitic acid (20 g) was hydrolysed by refluxing in 0.5 M sulphuric acid (2%) for 1-1/2 hours. The solution was cooled and neutralised by the addition of barium carbonate. The precipitate was removed by centrifugation, washed with water (500 ml) and the combined solution and washings were reduced in volume (200 ml). After deionisation with Amberlite IR 120 (H) resin, the solution was further concentrated and applied to a column of D.E.A.E. Sephadex A-25 (formate form, 3 x 20 cm). After the sugars had been allowed to adsorb overnight, the column was eluted with water (1.5 l) to remove neutral sugars.

The column was then eluted with water containing an increasing concentration of formic acid. Fractions (30 ml) were collected every 1/2 hour, with samples from every third tube being concentrated and examined by paper chromatography. Appropriate fractions were combined and evaporated to dryness.

<table>
<thead>
<tr>
<th>Eluant</th>
<th>Fraction</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.05 M formic acid</td>
<td>1</td>
<td>0.2 g</td>
</tr>
<tr>
<td>(3%)</td>
<td>2</td>
<td>1.2 g</td>
</tr>
<tr>
<td>0.05 M formic acid</td>
<td>3</td>
<td>1.7 g</td>
</tr>
<tr>
<td>(1.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fraction 1. Paper chromatographic examination in solvents B and C indicated the presence of acidic oligosaccharides I, II, IV, V and VI. The fraction was not investigated further.

Fraction 2. This fraction was separated by thick paper chromatography in solvent C into oligosaccharides I (100 mg), II (300 mg), IV (87 mg), V (190 mg), VI (150 mg), VII (140 mg), and VIII (22 mg).

Fraction 3. Part of this fraction was separated by chromatography in solvent B into oligosaccharides I (105 mg), II (180 mg), III (150 mg) and V (20 mg).

Oligosaccharide I \( [\alpha]_D ^+94^\circ (c \ 0.5) \)

\[ R_{\text{Gal}} (B) \ 0.75, \quad M_G \ 0.69 \]

Acid hydrolysis gave galactose and 4-O-methylglucuronic acid. Estimation of uronic acid by the carbazole method gave an acid content of 48%.

The oligosaccharide (3 mg) was methylated by the Kuhn procedure. Examination of the methanolysate by gas-liquid chromatography showed the presence of the methyl glycosides of

2,3,4-tri-O-methylglucuronic acid* \( T(a) \ 2.29, \ 3.01 \)
2,3,6-tri-O-methylgalactose \( T(a) \ 2.81, \ 3.64, \ 4.00. \)

The oligosaccharide (150 mg) was converted into the derived methyl ester methyl glycosides by standing overnight

* present as methyl ester
in methanolic 1% hydrogen chloride (10 ml), then refluxing the solution for 3/4 hour. After neutralisation with silver carbonate, the solution was filtered and evaporated to a syrup. This syrup was redissolved in water and treated overnight with excess sodium borohydride. Remaining borohydride was destroyed with Amberlite IR 120 (H) resin and the reduced oligosaccharide was methylated with 30% sodium hydroxide solution and methyl sulphate. The methylated disaccharide (105 mg) was hydrolysed in HCl hydrochloric acid at 100° for six hours. The solution was neutralised with silver carbonate and concentrated to a syrup which was fractionated on a cellulose column (2 x 35 cm) using light petroleum (b.p. 100-120°)-butan-1-ol (7:3) saturated with water as eluant. Two fractions were obtained.

Fraction (a) 45 mg. This was chromatographically identical to 2,3,4,6-tetra-O-methyl-D-glucose. The sugar, recrystallised from acetone, had m.p. 79-80° and mixed m.p. 79-80° (with sample, m.p. 81-82°).

Fraction (b) 32 mg. The sugar was chromatographically identical to 2,3,6-tri-O-methyl-D-galactose. It was converted by oxidation with bromine water into 2,3,6-tri-O-methyl-D-galactonolactone which, after recrystallisation from ether, had m.p. 98-99° and mixed m.p. 97-98° (with sample, m.p. 97-98°).

Oligosaccharide I (3 mg) was reduced with sodium
borohydride to give the derived glycitol which was methylated by the Kuhn procedure. The methylated product was methanolysed and the following compounds were identified by g.l.c.: - 1,2,3,5,6-penta-0-methylgalactitol T(b) 2.70
2,3,4-tri-0-methylglucuronic acid T(b) 2.40, 3.13, the latter product as its methyl ester methyl glycosides.

A further quantity of the oligosaccharide (50 mg) was similarly converted to the glycitol and the product, after proton exchange with deuterium oxide, was examined by nuclear magnetic resonance spectroscopy. The signal due to the anomeric proton was observed as a doublet at $\tau$ 4.84 ($J = 3c/s$).


dOligosaccharide II. $[\alpha]_D^{+20} = 1.0$

$R_{\text{Gal}} (B) 0.65, M_G 1.06$

The sugar was chromatographically identical to 6-0-(4-0-methyl-3-glucopyranosyluronic acid)-D-galactose, although there was also a minor contamination with oligosaccharide I. Hydrolysis of the oligosaccharide gave galactose and 4-0-methylglucuronic acid.

The oligosaccharide was methylated on a small scale by the Kuhn procedure and the methanolysis products were investigated by g.l.c. The methyl glycosides of the following sugars were detected,

2,3,4-tri-0-methylglucuronic acid* T(a) 2.31, 3.03
2,3,5-tri-0-methylgalactose T(a) 3.95
2,3,4-tri-0-methylgalactose T(a) 6.53.

* present as methyl ester
The derived oligosaccharide glycitol (2 mg) was similarly methylated and the product was methanolysed. Investigation of the methanolysate by g.l.c. showed the presence of the methyl ester methyl glycosides of 2,3,4-
tri-O-methylglucuronic acid, \( T(a) 2.27, 2.94 \), and 1,2,3,4,5-
penta-O-methylgalactitol, \( T(a) 3.83 \).

The oligosaccharide was methylated initially with sodium hydroxide and methyl sulphate and later with methyl iodide and silver oxide. The product crystallised and after recrystallisation from chloroform-light petroleum (b.p. 100-120°) had m.p. 89-90° and mixed m.p. 90° [with a sample of the methyl ester methyl glycoside hexamethyl ether of 6-\( \beta \)-galactosyluronic acid-\( \beta \)-galactose, m.p. 90-91°].

Oligosaccharide III. \([\alpha]_D \ (c \ 2.0)\)

\[ R_{\text{Gal}} \ (B) \ 0.26, \ N_{\text{G}} \ 1.14 \]

This oligosaccharide was chromatographically homogeneous and identical to 6-\( \beta \)-galactosyluronic acid-\( \beta \)-galactose. Hydrolysis followed by paper chromatographic examination of the products in solvent D showed the presence of galactose and glucuronic acid. Borohydride reduction of the derived methyl ester methyl glycosides followed by hydrolysis gave galactose and glucose. Estimation of uronic acid content in the oligosaccharide with the carboxole reagent gave an acid content of 46\%. 

The oligosaccharide (2 mg) was methylated by the Kuhn procedure. Examination of the methanolysate by g.l.c. revealed the presence of the methyl glycosides of

2,3,4-tri-O-methylglucuronic acid*  T(a) 2.28, 2.98  T(b) 2.37, 3.07
2,3,5-tri-O-methylgalactose  T(a) 3.87  T(b) 4.20
2,3,4-tri-O-methylgalactose  T(a) 6.41  T(b) 6.98.

The methanolysate products from the methylated oligosaccharide glycitol were examined by g.l.c. Detected were the methyl ester methyl glycosides of

2,3,4-tri-O-methylglucuronic acid T(a) 2.27 2.98 T(b) 2.40, 3.12
and 1,2,3,4,5-penta-O-methylgalactitol T(a) 3.82 T(b) 3.76.

The oligosaccharide (75 mg) was methylated to give the methyl ester methyl glycoside hexamethylether of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. The product, recrystallised from chloroform-light petroleum (b.p. 100-120°), had m.p. 86-87° and mixed m.p. 86-88° (with sample, m.p. 90-91°).

Oligosaccharide IV.  [α]_D +56° (c 1.0)

R_Gal (B) 0.24,  M_G 0.72.

Hydrolysis gave galactose and 4-O-methylglucuronic acid. Partial hydrolysis gave in addition 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. Borohydride reduction of the oligosaccharide (2 mg) followed by partial hydrolysis of the product gave galactose, galactitol and 4-O-(4-O-...
methyl-\(\alpha-D\)-glucopyranosyluronic acid)-\(D\)-galactose.

Estimation of uronic acid content by the carbasole method gave an acid content of 30.5%. Comparison of the colorimetric intensities of the reactions of the sugar and derived glycitol with the phenol-sulphuric acid reagent indicated that the oligosaccharide had a degree of polymerisation of three.

The oligosaccharide (5 mg) was methylated by the Haworth method. Examination of the methanolysis products by g.l.c. identified the presence of the methyl glycosides of

- \(2,3,4\)-tri-\(D\)-methylglucuronic acid \(T(a) 2.27, 2.98 T(b) 2.36, 3.07\)
- \(2,3,6\)-tri-\(D\)-methylgalactose \(T(a) 2.75 (3.49, 4.01) T(b) 4.07\)
- \(2,4,6\)-tri-\(D\)-methylgalactose \(T(a) 3.49, 4.01 T(b) 3.89, 4.44\).

The methanolysate was reduced with lithium aluminium hydride and methanolysed. Peaks with the retention times of the methyl glycosides of the following sugars were detected by g.l.c.

- \(2,3,4\)-tri-\(D\)-methylglucose \(T(b) 2.47, 3.52\)
- \(2,3,6\)-tri-\(D\)-methylgalactose \(T(b) 3.03, 4.06, (4.47)\)
- \(2,4,6\)-tri-\(D\)-methylgalactose \(T(b) 3.90, 4.47\)

Degradation to arabinose:

The oligosaccharide (20 mg) was dissolved in methanolic 4% hydrogen chloride (2 ml) and the change in optical rotation with time noted.
The reaction was stopped after 2-3/4 hours by the addition of silver carbonate and the solution was filtered and evaporated to dryness. Paper chromatographic examination of the product showed that it contained some starting material together with a major component at \( R_{Gal} \) (B) 0.46 which was unreactive to aniline oxalate spray.

A portion of this product (5 mg) was dissolved in water (2 ml) and periodic acid (3 mg) was added. The solution was kept at 5° for one hour, when excess periodate was precipitated by the addition of barium hydroxide solution. The solution was centrifuged and the supernatant was neutralised with Amberlite IR 120 (H) resin. After filtration, potassium borohydride (15 mg) was added to the solution which was then left overnight. After destruction of remaining borohydride with Amberlite IR 120 (H) resin, the solution was concentrated and boric acid was removed by repeated distillation with methanol. Half of this oxidised product was hydrolysed in 2 N sulphuric acid at 100° for two hours. Paper chromatographic examination of the hydrolysate in solvents A and B showed the presence of arabinose, galactose, 4-O-methylglucuronic acid and 4-O-(4-O-methyl-\( \alpha \)-D-glucopyranosyluronic acid)-D-galactose. The remainder of the periodate oxidised product was methylated by the Kuhn procedure. The methanolysis products were investigated by g.l.c. which showed the presence of the
methyl glycosides of the following sugars

2,3,4-tri-0-methylglucuronic acid T(a) 2.32, 3.01 T(b) 2.34 (3.06)
2,3,6-tri-0-methylgalactose T(a) 2.81, 3.61, 4.11 T(b) 3.79, 4.11, 4.48
2,5-di-0-methylarabinose T(a) 1.48 T(b) 1.80

Degradation to lyxose

The oligosaccharide (12 mg) was dissolved in water (0.5 ml) and glacial acetic acid (1 ml) was added. A solution of lead tetra-acetate (10 mg) in acetic acid (1 ml) was then added and the solution was left for 15 minutes. Lead was precipitated by treatment with hydrogen sulphide gas and the solution, after filtration, was concentrated. Chromatographic examination of the product showed the presence of a small amount of starting material, together with a much larger component with $R_{Ga1}$ (B) 1.13, staining pink with aniline oxalate spray. Hydrolysis of a small sample of the product gave lyxose, galactose and 4-O-(4-O-methyl-a-D-glucopyranosyluronic acid)-a-D-galactose.

The remainder of the tetra-acetate treated sugar was dissolved in oxygen-free saturated lime water (15 ml) and left for 10 days. The solution was neutralised with Amberlite IR 120 (B) resin, filtered and concentrated. Paper chromatographic examination showed the presence of only one component, $R_{Ga1}$ (B) 0.59, reactive to aniline oxalate spray. This sugar, which had a negative reaction with the
tetrazolium spray reagent, gave on hydrolysis lyxose, galactose and 4-\(\beta\)-\(\beta\)-glucopyranosyluronic acid)-\(\beta\)-galactose.

<table>
<thead>
<tr>
<th>Oligosaccharide V.</th>
<th>([\alpha]_D +2^\circ\ (c 1.0))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R_{Gal}) (B)</td>
<td>0.15</td>
</tr>
<tr>
<td>(M_G)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Hydrolysis of a sample (2 mg) gave galactose, 4-\(\beta\)-methyl-glucuronic acid and 6-\(\beta\)-(4-\(\beta\)-methyl-\(\beta\)-glucopyranosyluronic acid)-\(\beta\)-galactose. Formation of the derived methyl ester methyl glycosides followed by borohydride reduction and hydrolysis gave galactose and 4-\(\beta\)-methylglucose. Estimation of uronic acid in the oligosaccharide by the carbazole method gave an acid content of 29%. Comparison of the intensities of the colorimetric reactions of the sugar and derived glycitol with the phenol-sulphuric acid reagent indicated that the sugar was a trisaccharide.

The oligosaccharide (2 mg) was methylated by the Kuhn procedure. Examination of the methanolysis by g.l.c. showed the presence of the methyl glycosides of

- 2,3,4-tri-\(\beta\)-methylglucuronic acid \(T(a)\) 2.28, 2.98
- 2,3,4-tri-\(\beta\)-methylgalactose \(T(a)\) 6.41
- 2,3,5-tri-\(\beta\)-methylgalactose \(T(a)\) 3.87.

The derived oligosaccharide glycitol was similarly methylated and the cleavage products investigated by g.l.c. The following components were identified (reducing sugars as their methyl glycosides).
Isolation of 6-0-β-D-galactopyranosyl-β-D-galactose:

The oligosaccharide (50 mg) was dissolved in methanolic 1% hydrogen chloride (15 ml) and the solution, after standing overnight, was refluxed for one hour. After neutralisation and filtration, the solution was concentrated and the resulting syrup was treated overnight with a solution of sodium borohydride (25 mg) in water (15 ml). After destruction of borohydride and removal of borate, the solution was concentrated and the product was hydrolysed in 0.5 N sulphuric acid at 100° for one hour. Filter sheet chromatography of the hydrolysate in solvent B resulted in the isolation of a fraction (6 mg) which was chromatographically identical to 6-0-β-D-galactopyranosyl-β-D-galactose. This oligosaccharide, $R_{\text{Gal}}$ (B) 0.31, $[\alpha]_D^{25}$ (c 0.5), gave galactose on hydrolysis. The sugar (2 mg) was methylated by the Kuhn method and the methanolysis products were examined by g.l.c. The methyl glycosides of the following sugars were detected.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>$T(b)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-0-methylgalactose</td>
<td>1.78</td>
</tr>
<tr>
<td>2,3,4-tri-0-methylgalactose</td>
<td>6.87</td>
</tr>
<tr>
<td>2,3,5-tri-0-methylgalactose</td>
<td>4.19</td>
</tr>
</tbody>
</table>

Oligosaccharide VI. $R_{\text{Gal}}$ (B) 0.06, $R_{\text{Gal}}$ (C) 0.30

This fraction was homogeneous on paper chromatography.
Electrophoresis in borate buffer, however, gave two components, $M_G$ 0.78, 0.92. Partial hydrolysis gave galactose, $6-O-(\beta-D$-glucopyranosyluronic acid)$-\alpha$-galactose, $6-O-(4-O$-methyl-$\beta-D$-glucopyranosyluronic acid)$-\alpha$-galactose and the corresponding trisaccharide.

**Oligosaccharide VII.** $R_{Gal}$ (B) 0.02, $R_{Gal}$ (C) 0.20

Partial hydrolysis gave products similar to those obtained from oligosaccharide VI, together with a spot corresponding to oligosaccharide VI.

**Oligosaccharide VIII.** $R_{Gal}$ (B) 0.01

Although the sugar was apparently chromatographically and electrophoretically homogeneous, partial hydrolysis gave products similar to those obtained from oligosaccharide VII together with that oligosaccharide.

Oligosaccharides VI, VII and VIII, together with their derived glycitols, were each methylated on a small scale by the Kuhn procedure. The cleavage products, identified by g.l.c. of the methyl glycosides, are listed in Table 12. Oligosaccharides VI and VII were also treated with oxygen-free saturated lime water for seven days. After neutralisation and concentration of the solutions, the products were methylated by the Kuhn method. The cleavage products from these derivatives are included in Table 12.
### Table 12

**Cleavage Products from Methylated Derivatives of Oligosaccharides VI, VII, VIII**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Oligosaccharide</th>
<th>VI</th>
<th>VII</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>a</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>b</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>c</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>a</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,5 Me₃ galactose</td>
<td>b</td>
<td>+</td>
<td>tr</td>
<td>+</td>
</tr>
<tr>
<td>2,5,6 Me₃ galactose</td>
<td>c</td>
<td>+</td>
<td>tr</td>
<td>+</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>a</td>
<td>n.d.</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>1,2,3,4,5 Me₃ galactitol</td>
<td>b</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1,2,4,5,6 Me₃ galactitol</td>
<td>c</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* a: Kuhn methylation of sugar
* b: Kuhn methylation of sugar glycitol
* c: Kuhn methylation of products of alkaline degradation.
Methylation of Mesquite Gum

The crude gum (30 g) was dissolved in water (100 ml) by standing overnight. Bark and other insoluble material were filtered off and the gum was methylated by adding methyl sulphate (200 ml) and 30% sodium hydroxide (400 ml) dropwise over 4 hours. The solution was stirred vigorously overnight, then cooled on an ice-bath and almost neutralised with dilute sulphuric acid. The precipitated inorganic salts were filtered off and the volume of the solution was reduced to 100 ml. The gum was then subjected to a further methylation using the same quantities of reagents, with sufficient acetone being added to maintain solution. Sodium sulphate was this time removed by precipitation with methanol. After the fifth such methylation the solution was heated on a boiling water bath to expel acetone and the partially methylated gum, which came out of solution, was filtered off. It was redissolved in acetone/water solution (1/1, 200 ml) and methylated again. This process was repeated twice.

A small sample of the methylated gum was then suspended in dilute sulphuric acid and extracted with chloroform. The extract was dried over sodium sulphate, concentrated, and the methylated gum acid was precipitated by pouring into light petroleum (b.p. 60-80°). [Found: OMe, 34.5%]

The partly methylated polysaccharide was redissolved in a water-acetone mixture and treated twice more with Haworth reagents. A small sample was purified as before.
The bulk of the polysaccharide was then similarly purified, dissolved in acetone-water and neutralised with silver carbonate. This solution was filtered, concentrated and freeze-dried. Yield: 19.7 g.

The polysaccharide was dissolved in methyl iodide (200 ml), silver oxide (20 g) was added in four batches and the solution was refluxed for eighteen hours. The solution was cooled and filtered and the residue was thoroughly washed with chloroform. After concentration of the combined solutions, the polysaccharide was precipitated in light petroleum. [Found: OMe, 40.8%]. The polysaccharide was again treated with methyl iodide (150 ml) and silver oxide (8 g) to give the methylated gum (15 g), \([\alpha]_D +59^\circ \ (c \ 0.8\ \text{in} \ CHCl_3)\), [Found: OMe, 43.1%] whose infra-red spectrum showed negligible hydroxyl absorption.

A sample (15 mg) of methylated gum was methanolysed in methanolic 4% hydrogen chloride at 100° overnight. After neutralisation with silver carbonate, the methanolysate was examined by gas-liquid partition chromatography. Peaks with the retention times of the methyl glycosides of the following sugars were obtained. (In those cases where the retention time may be attributed to more than one sugar, the T value is given in parenthesis).
<table>
<thead>
<tr>
<th>Sugars</th>
<th>column a</th>
<th>column b</th>
<th>column d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ rhamnose</td>
<td>0.46</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td>0.49, 0.63</td>
<td>0.54, 0.72</td>
<td>0.49, (0.63)</td>
</tr>
<tr>
<td>3,5 Me₂ arabinose</td>
<td>0.83, 1.98</td>
<td>1.05, 2.40</td>
<td>(0.63), 0.84</td>
</tr>
<tr>
<td>2,5 Me₂ arabinose</td>
<td>1.49</td>
<td>1.79</td>
<td>0.71, 1.08</td>
</tr>
<tr>
<td>2,3 Me₂ arabinose</td>
<td>1.19</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.77</td>
<td></td>
<td>1.59</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.54</td>
<td>7.02</td>
<td>2.55, 2.82</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose</td>
<td>2.74, 3.54, 4.07</td>
<td>3.95, 4.52</td>
<td></td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>13.55, 15.47</td>
<td></td>
<td>3.66, 4.21</td>
</tr>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.28, 2.99</td>
<td>2.40, 3.13</td>
<td>1.75, 2.18</td>
</tr>
<tr>
<td>2,3 Me₂ glucuronic acid</td>
<td></td>
<td>7.7, 8.45, 10.26</td>
<td></td>
</tr>
</tbody>
</table>

A portion of the glycosides was hydrolysed in H₂SO₄ (5 ml) at 100° for twelve hours. The solution was neutralised with barium hydroxide solution and barium carbonate, filtered, deionised with Amberlite IR 120 (H⁺) resin and reduced in volume (5 ml). Bromine (10 drops) was added and the mixture was kept in the dark for three days. Excess bromine was removed by aeration and the solution was neutralised with silver carbonate. After the filtration, the solution was evaporated.
to dryness and the products were extracted from the residue with acetone. The mixture of aldolactones was examined by g.l.c.

Table 14

Aldolactones Derived from Cleavage Products of Methylated Mesquite Gum

<table>
<thead>
<tr>
<th>Aldolactones</th>
<th>T (column b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5 Me₂ arabonolactone</td>
<td>1.74</td>
</tr>
<tr>
<td>3,5 Me₂ arabonolactone</td>
<td>5.41</td>
</tr>
<tr>
<td>2,5 Me₂ arabonolactone</td>
<td>14.8</td>
</tr>
<tr>
<td>2,3 Me₂ arabonolactone</td>
<td>8.09</td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactonolactone</td>
<td>6.80</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactonolactone</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Reduction of Methylated Mesquite Gum

The methylated polysaccharide (150 mg) was dissolved in tetrahydrofuran (8 ml) and lithium aluminium hydride (200 mg) in tetrahydrofuran (8 ml) was added slowly. The reaction mixture was stirred at room temperature for 30 minutes, then under reflux for a further 3 hours. Excess lithium aluminium hydride was destroyed by the addition of ethylacetate, water was added and the mixture was acidified (pH 4) by the addition of 2 M sulphuric acid. The reduced polysaccharide was
extracted into chloroform (5 x 50 ml), the extracts were concentrated and the reduced methylated polysaccharide (50 mg) was precipitated by pouring into light petroleum (20 vol.). [Found: OMe, 39.4%], [α]D +61° (c 1, CHCl3).

A sample of the reduced methylated gum was methanolysed, and on examination of the methanolysate by g.l.c., peaks with the retention times of the methyl glycosides of the sugars listed in Table 15 were obtained.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>column a</th>
<th>column b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me3 rhamnose</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>2,3,5 Me3 arabinose</td>
<td>0.47, 0.63</td>
<td>0.54, 0.72</td>
</tr>
<tr>
<td>3,5 Me3 arabinose</td>
<td>0.83, 2.02</td>
<td>1.02, 2.39</td>
</tr>
<tr>
<td>2,5 Me3 arabinose</td>
<td>1.53, 2.86</td>
<td>1.80, 3.21</td>
</tr>
<tr>
<td>2,3 Me3 arabinose</td>
<td>1.23</td>
<td>1.48</td>
</tr>
<tr>
<td>2,3,4,6 Me4 galactose</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me3 galactose</td>
<td>6.82</td>
<td>7.02</td>
</tr>
<tr>
<td>2,3,6 Me3 galactose</td>
<td>2.86, 3.54</td>
<td>3.09, 4.08, 4.55</td>
</tr>
<tr>
<td>2,4 Me2 galactose</td>
<td>14.4, 16.4</td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me3 glucose</td>
<td>2.29, 3.36</td>
<td>3.58</td>
</tr>
</tbody>
</table>
Partial Methanolysis of Methylated Mesquite Gum

Partial Methanolysis I

Methylated mesquite gum (250 mg) was refluxed in methanolic 4% hydrogen chloride (10 ml) for five hours. The solution was cooled, neutralised with silver carbonate, filtered and concentrated. The syrup was taken up in water (15 ml), the pH was adjusted to 12 with dilute sodium hydroxide solution and left for two hours. The solution was then extracted with chloroform in a liquid-liquid extractor overnight.

The aqueous solution was neutralised with Amberlite IR 120 (H) resin, concentrated and applied to a column (10 x 1 cm) of D.E.A.E. Sephadex A-25 (formate form). The neutral fraction was eluted with water (100 ml) and the acidic fraction was then desorbed by elution with 0.5 M formic acid (100 ml), concentrated to a syrup and dried in vacuo. A sample of the acidic fraction was methanolyzed and the methyl glycosides of the following sugars were identified by g.l.c.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.27, 2.99</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.40</td>
</tr>
<tr>
<td>2,3,6/2,4,6 Me₃ galactose</td>
<td>3.47, 3.95</td>
</tr>
<tr>
<td>2,4 Me₃ galactose</td>
<td>13.6, 15.6</td>
</tr>
</tbody>
</table>
A further sample of the acidic fraction was methylated by the Kuhn method, methanolysed and the methanolysate was examined by g.l.c. The methyl glycosides of the following sugars were identified.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.38, 3.07</td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.80</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.90</td>
</tr>
<tr>
<td>2,3,6/2,4,6 Me₃ galactose</td>
<td>3.89, 4.49</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>15.5, 17.8</td>
</tr>
</tbody>
</table>

A portion of this methanolysate was acetylated and the products were re-examined by g.l.c. Peaks with the retention times of the methyl glycosides of the following sugars were obtained.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(c)</th>
<th>Relative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.36, 3.10</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.83</td>
<td>1</td>
</tr>
<tr>
<td>2,3,6 Me₃ 4 Ac galactose</td>
<td>3.92, 4.43</td>
<td>1</td>
</tr>
<tr>
<td>2,4,6 Me₃ 3 Ac galactose</td>
<td>6.41, 8.64</td>
<td>3</td>
</tr>
<tr>
<td>2,3,4 Me₃ 6Ac galactose</td>
<td>7.30</td>
<td>7.5</td>
</tr>
</tbody>
</table>

**Partial Methanolysis II**

Methylated mesquite gum (250 mg) was heated with methanolic 4% hydrogen chloride (10 ml) in a sealed tube for five hours in a boiling water bath. The solution was neutralised
with silver carbonate, filtered and concentrated. The syrup was taken up in dilute sodium hydroxide (pH 12) and after two hours the solution was extracted with chloroform on a liquid-liquid extractor. The aqueous solution was finally separated into acidic and neutral fractions by chromatography on D.E.A.E. Sephadex.

A portion (3 mg) of the acidic fraction was methanolysed. G.l.c. analysis of the methanolysate identified the methyl glycosides of the following sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me&lt;sub&gt;3&lt;/sub&gt; glucuronic acid</td>
<td>2.40, 3.10</td>
</tr>
<tr>
<td>2,3 Me&lt;sub&gt;2&lt;/sub&gt; glucuronic acid</td>
<td>7.77, 8.40, 10.2</td>
</tr>
<tr>
<td>2,3,6 Me&lt;sub&gt;3&lt;/sub&gt; galactose</td>
<td>3.70, 4.06, 4.52</td>
</tr>
<tr>
<td>2,3,4 Me&lt;sub&gt;3&lt;/sub&gt; galactose</td>
<td>6.92</td>
</tr>
<tr>
<td>2,4 Me&lt;sub&gt;2&lt;/sub&gt; galactose</td>
<td>15.5, 17.9</td>
</tr>
</tbody>
</table>

The methanolysate was subsequently acetylated. G.l.c. indicated the presence of the methyl glycosides of these sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me&lt;sub&gt;3&lt;/sub&gt; glucuronic acid</td>
<td>2.36, 3.12</td>
</tr>
<tr>
<td>2,3,6 Me&lt;sub&gt;3&lt;/sub&gt; 4 Ac galactose</td>
<td>3.96, 4.47</td>
</tr>
<tr>
<td>2,3,4 Me&lt;sub&gt;3&lt;/sub&gt; 6 Ac galactose</td>
<td>7.35</td>
</tr>
</tbody>
</table>

A further quantity (5 mg) of the acidic fraction was methylated by the Kuhn procedure and methanolysed. Examination of the methanolysate by g.l.c. showed the presence of the
methyl glycosides of the following sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.40, 3.10</td>
</tr>
<tr>
<td>2,3,6/2,4,6 Me₃ galactose</td>
<td>4.17, 4.50</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.92</td>
</tr>
</tbody>
</table>

The methanolysate was acetylated and re-examined by g.l.c. The following sugars were identified as their methyl glycosides.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(c)</th>
<th>Relative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.36, 3.12</td>
<td></td>
</tr>
<tr>
<td>2,3,6 Me₃ 4 Ac galactose</td>
<td>3.94, 4.46</td>
<td>1</td>
</tr>
<tr>
<td>2,4,6 Me₃ 3 Ac galactose</td>
<td>6.49, 8.66</td>
<td>1</td>
</tr>
<tr>
<td>2,3,4 Me₃ 6 Ac galactose</td>
<td>7.35</td>
<td>3</td>
</tr>
</tbody>
</table>

**Partial Methanolysis III**

Methylated mesquite gum (13 g) was refluxed in methanolic 4% hydrogen chloride (110 ml) for five hours. The solution was then cooled, neutralised with silver carbonate, filtered and concentrated to a syrup. This syrup was extracted five times with hot (ca. 100°) light petroleum (b.p. 100-120°). The petroleum-insoluble residue was taken up in chloroform and concentrated to a syrup (3.0 g).
Examination of petrol-insoluble residue

A portion of the petrol-insoluble residue was treated with dilute sodium hydroxide solution (pH 12) for two hours. After neutralisation with Amberlite IR 120 (H) resin, the solution was concentrated to a syrup which was separated on a column of D.E.A.E. Sephadex A-25 (formate form) into an acidic fraction (340 mg) and a neutral fraction (460 mg).

The neutral fraction was hydrolysed in M hydrochloric acid (25 ml) at 100° for 15 hours, neutralised and concentrated to a syrup. A portion of this syrup (55 mg) was further fractionated by thick paper chromatography in solvent E.

Fraction 1 (2 mg) Rg 0.10. This fraction was chromatographically identical to arabinose.

Fraction 2 (21 mg) Rg 0.21. The sugar was chromatographically identical to 2-0-methyl-β-D-galactose. Treatment of a portion of the sugar (2 mg) with 1 mole of lead tetra-acetate followed by saponification of the reaction products gave only one sugar, which was chromatographically identical to the starting material. The syrup crystallised on seeding with 2-0-methyl-β-D-galactose and after recrystallisation from ethanol had m.p. 148-150° and mixed m.p. 148-151° (with a sample m.p. 149-151°).

Fraction 3 (22 mg) Rg 0.40. This was chromatographically identical to 2,4-di-0-methyl-β-D-galactose and crystallised on standing, m.p. 101-103°. A sample was methanolysed. G.L.C. analysis indicated that in addition to the major component,
there were two minor peaks with T (column a) 3.66 and 4.22. 

**Fraction 4** (3 mg) R<sub>G</sub> 0.65. G.l.c. of the derived methyl glycosides gave the same two peaks as were present in minor amount in the methanolysate of Fraction 3 together with a third peak with retention time identical to that of methyl 2,3,4-tri-O-methylgalactose.

**Fraction 5** (7 mg) R<sub>G</sub> 0.74. The fraction was methanolysed and subsequent g.l.c. analysis showed the presence of the methyl glycosides of 3,5- and 2,5-di-O-methylerabinoses.

**Fractional distillation of petrol-insoluble residue**

A further quantity of the petrol-insoluble residue (1.8 g) was fractionally distilled under reduced pressure (0.005 mm Hg)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight</th>
<th>Distillate temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34 mg</td>
<td>88-160°</td>
</tr>
<tr>
<td>2</td>
<td>393 mg</td>
<td>160-180°</td>
</tr>
<tr>
<td>3</td>
<td>300 mg</td>
<td>180-210°</td>
</tr>
</tbody>
</table>

**Fraction 1** Examination of the fraction by g.l.c. showed the presence of methyl 3,5- and 2,5-di-O-methylerabinosides.

Methanolysis of the fraction gave the same products.

**Fraction 2** G.l.c. analysis showed that this fraction contained the methyl glycosides of 2,4-di-O-methylgalactose, 3,5-di-O-methylerabinose together with a trace of 2,5-di-O-methylerabinoside. After methanolysis of the fraction, small amounts of methyl 2,3,4-tri-O-methylgalactoside and methyl 2,3,4-tri-O-
methylglucuronoside were detected as additional components. **Fraction 3** Gas-liquid chromatography showed the presence in the fraction of methyl 2,4-di-0-methylgalactoside. After methanolysis, the methyl glycosides of 2,3,4-tri-0-methylglucuronic acid 2,3,4- and 2,3,6-tri-0-methylgalactose, in addition to those of 2,4-di-0-methylgalactose were detected by g.l.c.

A sample of Fraction 3 was methylated twice by the Kuhn procedure. After methanolysis, the products were acetylated and examined by g.l.c. The methyl glycosides of the following sugars were detected.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.40, 3.10</td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.82</td>
</tr>
<tr>
<td>2,3,6 Me₃ 4 Ac galactose</td>
<td>3.97, 4.39</td>
</tr>
<tr>
<td>2,4,6 Me₃ 3 Ac galactose</td>
<td>6.40, 8.50</td>
</tr>
<tr>
<td>2,3,4 Me₃ 6 Ac galactose</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Treatment of a portion of Fraction 3 with methanolic ammonia yielded a syrup which failed to crystallise. The remainder of the fraction was saponified in dilute sodium hydroxide solution (pH 12.5) for two hours and after neutralisation and concentration, the fraction was applied to a column of D.E.A.E. Sephadex A-25 (formate form). Neutral sugars (sub-fraction 3B) were eluted with water and
acidic sugars (sub-fraction 3A) were desorbed with 0.5 M formic acid.

**Acid sub-fraction 3A (90 mg)**

Examination of the methanolysis products of the sub-fraction and their derived acetates by g.l.c. indicated the presence of the methyl glycosides of the following sugars.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T(a) (glycosides)</th>
<th>T(c) (derived acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.30, 3.01</td>
<td>2.34, 3.05</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose</td>
<td></td>
<td>3.91, 4.37</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.42</td>
<td>7.20</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>13.2, 15.0</td>
<td></td>
</tr>
</tbody>
</table>

A portion of sub-fraction 3A (2 mg) was methylated and the methanolysis products acetylated. Methyl glycosides of the following sugars were detected by g.l.c.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T(c)</th>
<th>Relative amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.36, 3.06</td>
<td></td>
</tr>
<tr>
<td>2,3,6 Me₃ 4 Ac galactose</td>
<td>3.95, 4.42</td>
<td>++</td>
</tr>
<tr>
<td>2,4,6 Me₃ 3 Ac galactose</td>
<td>6.37, 8.50</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4 Me₃ 6 Ac galactose</td>
<td>7.21</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Fractionation of sub-fraction 3A**

The sub-fraction (75 mg) was taken up in dry pyridine (2 ml), triphenylmethyl chloride (80 mg) was added and the solution was heated at 100° for 1 hour. The reaction products were examined by thin layer chromatography on activated
silica gel using 15% methanol in benzene as solvent and anisaldehyde-sulphuric acid as spray reagent. Three main fractions ultimately turning blue on heating after treatment with the spray were observed. The reaction products were then fractionated by preparative thin layer chromatography using 20 x 20 cm plates in the same system. Bands were detected by removing a strip of silica gel adhered to a glass strip coated with a paste made from silica gel and high-vacuum silicone grease and spraying with anisaldehyde-sulphuric acid. This method detected the presence of three bands, which were removed from the plates and extracted with acetone. These extracts were then filtered and concentrated, to give sub-fractions 3A(I), (II) and (III) which were treated with cold methanolic 1% hydrogen chloride for 18 hours to remove any triphenylmethyl substituents which may have been present.

_sub-fraction 3A(I) \( R_f \) 0.65

Part of the sub-fraction was methanolysed. Analysis of the glycosides and their derived acetates by g.l.c. indicated the presence of the methyl glycosides of these sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b) (glycosides)</th>
<th>T(c) (derived acetates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.38, 3.04</td>
<td>2.36, 3.10</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose</td>
<td>4.01, 4.44</td>
<td>3.91, 4.39</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td></td>
<td>6.70</td>
</tr>
<tr>
<td>2,4 Me₂ galactose (tr)</td>
<td>15.4, 17.2</td>
<td>7.17</td>
</tr>
</tbody>
</table>
The remainder of sub-fraction 3A(I) was methylated and the methanolysis products and their derived acetates were examined by g.l.c. Methyl glycosides of the following sugars were detected.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b) (glycosides)</th>
<th>T(c) (derived acetates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.38, 3.04</td>
<td>2.36, 3.06</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose</td>
<td>4.09, 4.46</td>
<td>3.91, 4.42</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.75</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Sub-fraction 3A(II) \( R_f 0.40 \)

Methanolysis of a sample gave the methyl glycosides of the following sugars on examination by g.l.c.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.37, 3.04</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>1.50, 17.0</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose (tr)</td>
<td>6.66</td>
</tr>
</tbody>
</table>

A further portion of the sub-fraction was methylated and methanolysed. Methyl glycosides of the following sugars were detected on g.l.c. examination of the methanolysate and subsequent acetylation products.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b) (glycosides)</th>
<th>T(c) (derived acetates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.38, 3.05</td>
<td>2.36, 3.08</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.70</td>
<td>7.17</td>
</tr>
</tbody>
</table>
Sub-fraction 3A(III)  \( R_f \) 0.25

The methyl glycosides of the following sugars were detected as methanolysis products of the sub-fraction.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 ( \text{Me}_3 ) glucuronic acid</td>
<td>2.38, 3.05</td>
</tr>
<tr>
<td>2,4 ( \text{Me}_2 ) galactose</td>
<td>15.0, 17.0</td>
</tr>
</tbody>
</table>

Hydrolysis of a sample of the sub-fraction followed by paper chromatographic examination in solvent E showed the presence of 2,4-di- and 2-O-methylgalactose. G.L.C. examination of the methanolysis products and their derived acetates from the methylated sub-fraction gave peaks with the retention times of the methyl glycosides of the following sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(b) (glycosides)</th>
<th>T(c) (derived acetates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 ( \text{Me}_3 ) glucuronic acid</td>
<td>2.36, 3.05</td>
<td>2.37, 3.06</td>
</tr>
<tr>
<td>2,3,4 ( \text{Me}_3 ) galactose</td>
<td>6.71</td>
<td>7.20</td>
</tr>
</tbody>
</table>

The remainder of the material on the thin layer plates was extracted and examined by methylation. The fastest moving material, with \( R_f \) similar to trityl chloride gave small amounts of 2,3,4-tri-O-methylglucuronic acid and 2,3,4- and 2,3,6-tri-O-methylgalactose as cleavage products. The first two sugars were also obtained in trace quantities from the slowest moving material.
Neutral Sub-fraction 3B (160 mg)

Examination of the neutral sub-fraction by gas-liquid chromatography showed the presence of methyl 2,4-di-O-methylgalactoside. Methanolysis gave in addition a small amount of methyl 2,3,4-tri-O-methylgalactoside. A portion of the sub-fraction 3B was methylated and the methanolysis products and their acetates were examined by g.l.c. Methyl glycosides of the following sugars were detected.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(a) (glycosides)</th>
<th>T(c) (derived acetates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.81</td>
<td>1.82</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.41</td>
<td>7.20</td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>3.55, 4.04</td>
<td>6.38, 8.50</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose (tr)</td>
<td>3.95, 4.42</td>
<td></td>
</tr>
</tbody>
</table>
Autohydrolysis of Mesquite Gum

A trial autohydrolysis was conducted by dissolving the purified gum acid (2.40 g) in water (120 ml). The optical rotation was noted and the solution then heated under reflux. Samples (20 ml) were withdrawn every three hours, the optical rotations were measured and high molecular weight material was precipitated in ethanol. After purification by reprecipitation in ethanol, this material was hydrolysed and the products examined by paper chromatography.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>0</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\alpha]_D$</td>
<td>60.0</td>
<td>73</td>
<td>75</td>
<td>75</td>
<td>75.5</td>
<td>77</td>
<td>77.5</td>
</tr>
<tr>
<td>Arabinose in ppt.</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>tr</td>
<td></td>
</tr>
</tbody>
</table>

Purified gum acid (25.5 g) was dissolved in water (1275 ml) and heated under reflux for 24 hours. The solution was then cooled and poured into acidified ethanol (6 L). The precipitated degraded polysaccharide was removed at the centrifuge, taken up in water (100 ml) and reprecipitated in ethanol (500 ml). The precipitate was again removed by centrifugation, redissolved in water and the solution was freeze-dried to give degraded gum A (7.0 g), $[\alpha]_D -3^\circ (c 1.0)$. Found: uronic acid (by carbazole method) 40%. Hydrolysis of the polysaccharide showed there was still a trace of arabinose present.
Degraded mesquite gum A (5 g) was dissolved in water (20 ml) and methyl sulphate (50 ml) and 30% sodium hydroxide solution (100 ml) were added over four hours with vigorous stirring under a stream of nitrogen. After five such additions during five days, the solution was reduced in volume, sodium sulphate was precipitated by the addition of methanol and removed at the centrifuge, and the solution was further concentrated. After two similar methylations, the solution was heated on a boiling water bath for one hour, reduced in volume and sodium sulphate was removed by precipitation with methanol. The solution was concentrated (200 ml) and acidified (pH 4) with dilute sulphuric acid. This solution was then extracted with chloroform (5 x 200 ml). The extracts were combined and concentrated to a syrup which was redissolved in acetone-water (150 ml). The solution was neutralised with silver carbonate, excess carbonate was filtered off, acetone was removed and the resulting aqueous solution was freeze-dried to give partially methylated polysaccharide (2.5 g). Four treatments of this product with methyl iodide and silver oxide furnished methylated degraded gum A (1.2 g) \( [\alpha]_D -5^\circ \) (c 1.0 in CHCl₃), [Found: OMe, 43.6%].

A sample (10 mg) of the methylated degraded gum was methanolyzed and the products were analysed by gas-liquid chromatography. Peaks with the retention times of the methyl glycosides of the sugars listed in Table 16 were obtained.
<table>
<thead>
<tr>
<th>Sugars</th>
<th>column a</th>
<th>column b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₂ rhamnose</td>
<td>0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>2,3,5 Me₂ arabinose</td>
<td>0.47, 0.63</td>
<td>0.55, 0.71</td>
</tr>
<tr>
<td>3,5 Me₂ arabinose</td>
<td>0.83, 1.98</td>
<td>1.02</td>
</tr>
<tr>
<td>2,5 Me₂ arabinose</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.77</td>
<td>1.77</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.48</td>
<td>6.88</td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>3.54, 4.06</td>
<td>3.87, 4.45</td>
</tr>
<tr>
<td>2,3,6 Me₃ galactose</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>13.7, 15.5</td>
<td>15.3, 17.6</td>
</tr>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.28, 2.99</td>
<td>2.38, 3.08</td>
</tr>
</tbody>
</table>

Hydrolysis of a sample of methylated degraded gum A followed by paper chromatographic examination gave only a trace amount of 2-0-methylgalactose.
Formation of glycol ester

Degraded mesquite gum A (9.0 g) was dissolved in water (600 ml). Ethylene oxide (100 ml) was added and the solution was allowed to stand in a stoppered flask at room temperature. After three days the pH had risen from 2 to 7.1. Excess ethylene oxide was removed by evaporation and the glycol ester was precipitated in ethanol, removed by centrifugation, re-dissolved in water and freeze-dried (9.5 g).

Formation of glycol ester acetate

A small-scale acetylation was conducted to establish suitable conditions. Freeze-dried glycol ester (1 g) was dissolved in formamide (25 ml) and the solution stirred at 30° for four hours. The temperature was raised to 40-45° (water bath) and pyridine (33 ml) added dropwise over two hours. After the solution had been cooled again to 30°, acetic anhydride (27 ml) was added dropwise over two hours. The solution was then stirred for a further seventeen hours at room temperature. The reaction mixture was finally poured dropwise, with vigorous stirring, into ice-cold hydrochloric acid (1 L, 2% w/v). The precipitated glycol ester acetate was removed at the centrifuge and washed with water (2 x 150 ml). The acetate was dissolved in acetone and water removed by azeotropic distillation with chloroform. By this process the acetate eventually became soluble in chloroform, and
concentration of the dried chloroform solution (70 ml) was followed by precipitation of the acetate by pouring the solution into light petroleum (500 ml). The product was recovered by filtration and dried in vacuo to give a white powder (1.3 g).

The remainder of the glycol ester (8.4 g) was similarly acetylated to give the product (14.4 g).

**Reduction of glycol ester acetate**

Lithium borohydride (16 g) in tetrahydrofuran (250 ml) was added to glycol ester acetate (15.5 g) in tetrahydrofuran (250 ml) with the immediate formation of a precipitate. The reaction mixture was stirred at room temperature for two hours and then heated under reflux for eighteen hours with continued stirring. Excess lithium borohydride was destroyed by the careful addition of water. The mixture was then acidified (pH 3) by the addition of dilute sulphuric acid, dialysed against running tap water for five days, concentrated (200 ml) and freeze-dried to give carboxyl-reduced degraded gum A (6.5 g). \([\alpha]_D +50^\circ \quad (c 1.0)\).

Hydrolysis of a sample of the gum followed by paper chromatographic examination in solvent A gave galactose and 4-O-methylglucose together with some arabinose and glucose, but no trace of slow-moving acidic components.
Smith Degradation of Carboxyl-Reduced Degraded Gum A

To a solution of sodium metaperiodate (17.12 g) in water (1 l) was added carboxyl-reduced degraded gum A (6.8 g) and the reaction was followed spectrophotometrically.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles periodate consumed</td>
<td>0.030</td>
<td>0.034</td>
<td>0.035</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Moles periodate consumed</td>
<td>0.75</td>
<td>0.85</td>
<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Mole sugar residue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After seven hours the reaction was stopped by the addition of ethylene glycol (3.4 g). The solution was dialysed for five days against running tap water before being treated with potassium borohydride (5 g) which was added in two portions over three days. Excess borohydride was destroyed with Amberlite IR 120 (H) resin, the solution was concentrated and boric acid was removed by codistillation with methanol. The solution was finally concentrated and dried in vacuo to give a syrup (3.6 g).

The syrup in N sulphuric acid (100 ml) was kept at room temperature for three hours. The solution was neutralised with barium hydroxide solution and barium carbonate, the precipitate was removed at the centrifuge and the solution was deionised with Amberlite IR 120 (H) resin. After concentration to small volume, the solution was poured into ethanol (6 vol.). The resulting precipitate was removed by centrifugation, washed with ethanol and redissolved in water. This
solution was freeze-dried to give degraded gum B (1.2 g), 
\([\alpha]_D^{+18^\circ} (c 1.0)\).

The supernatant ethanolic solution was concentrated and 
dried to a syrup (2.0 g). Paper chromatographic examination 
of the syrup showed that it contained mainly glycerol, but no 
reducing sugars. Hydrolysis of the syrup gave small amounts 
of galactose and arabinose.

Degraded gum B

Hydrolysis of a sample gave galactose together with a 
faint trace of arabinose. Partial hydrolysis in 0.5 M sul-
phuric acid at 100° for one hour followed by paper chromato-
graphy in solvents A and B gave in addition 3-O-galactopyrano-
sylgalactose and the corresponding trisaccharide together 
with a relatively smaller amount of 6-O-galactopyranosyl-
galactose.

Methylation of the gum:

Sodium hydride (1.5 g) was washed three times with dry 
light petroleum and stirred with methyl sulphoxide (15 ml) 
under nitrogen for one hour at 30°. Methyl sulphoxide (10 ml) 
was added to the freeze-dried gum (200 mg) in a 100 ml conical 
flask and the methyl sulphinyl carbanion solution (4 ml) was 
added dropwise with stirring. The resulting gel was stirred 
at room temperature for five hours, then methyl iodide (1 ml) 
was added dropwise over a period of ten minutes. The mixture 
was stirred overnight and dialysed against running tap water
for twenty-four hours before the residue was concentrated and extracted with chloroform. The extracts were combined, dried over anhydrous sodium sulphate, and concentrated to a syrup. This syrup was further methylated with silver oxide and methyl iodide before the methylated polysaccharide (83 mg) was finally recovered by precipitation with light petroleum. 

\[ [\alpha]_D^{-24} \text{ (c 1.0 in CHCl}_3 \text{). Found: OMe, 43.8\%. Infra-red spectroscopy confirmed that the polysaccharide was fully methylated.} \]

A sample of the methylated polysaccharide was methanolysed. The methyl glycosides of the sugars listed in Table 17 were identified by gas-liquid chromatography of the methanolysate. The relative amounts of the products were determined by measurement of the peak areas on the chromatograms.

**Table 17**

Cleavage Products of Methylated Degraded Gum B

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Relative Amount</th>
<th>Column a</th>
<th>Column b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6 Me₃ galactose</td>
<td>1.5</td>
<td>1.77</td>
<td>1.77</td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>4</td>
<td>3.53, 4.07</td>
<td>3.88, 4.46</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>1</td>
<td>6.42</td>
<td>6.90</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>1</td>
<td>13.7, 15.6</td>
<td>15.4, 17.7</td>
</tr>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td>tr</td>
<td>0.49, 0.63</td>
<td>0.55, 0.72</td>
</tr>
<tr>
<td>3,5 Me₂ arabinose</td>
<td>tr</td>
<td>0.96, 2.00</td>
<td>1.04, 2.37</td>
</tr>
<tr>
<td>2,5 Me₂ arabinose</td>
<td>tr</td>
<td>0.52, 2.83</td>
<td>(1.77), 3.24</td>
</tr>
</tbody>
</table>
Smith Degradation of Degraded Gum B

Degraded gum B (800 mg) was dissolved in 0.08 M sodium metaperiodate (100 ml) and the uptake of periodate was measured spectrophotometrically.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles periodate consumed</td>
<td>0.0023</td>
<td>0.0028</td>
<td>0.0028</td>
<td>0.0029</td>
<td>0.0035</td>
</tr>
<tr>
<td>Moles periodate consumed</td>
<td>0.52</td>
<td>0.63</td>
<td>0.63</td>
<td>0.65</td>
<td>0.78</td>
</tr>
</tbody>
</table>

After twenty-four hours, the reaction was stopped by the addition of ethylene glycol. Sodium ions were removed by passing the solution through a column of Amberlite IR 120 (H) resin and iodic acid was neutralised with barium carbonate. The solution was filtered, the filtrate again treated with Amberlite IR 120 (H) and concentrated (50 ml). The solution was treated with sodium borohydride (500 mg) overnight, excess borohydride was destroyed with Amberlite IR 120 (H) resin and boric acid was removed by repeated distillation with methanol. The resulting syrup was hydrolysed in 6 sulphuric acid (50 ml) at room temperature for three hours. After neutralisation and deionisation, the solution was concentrated and poured into ethanol (7 vol.). The resulting precipitate was removed by centrifugation, taken up in water and freeze-dried to give degraded gum C (360 mg), \( [\alpha]_D +19^\circ \) (c 1.0).
Degraded Gum C

Total hydrolysis gave galactose while partial hydrolysis gave 3-\(\beta\)-galactopyranosylgalactose and only a small amount of 6-\(\beta\)-galactopyranosylgalactose. The gum (10 mg) was oxidised with sodium metaperiodate and the reaction was followed spectrophotometrically. The final uptake of periodate was 0.25 moles per mole of sugar residue. The polysaccharide (80 mg) was methylated initially with sodium hydride and methyl sulphoxide and finally with methyl iodide and silver oxide. Precipitation from light petroleum furnished fully methylated degraded gum C (30 mg), \([\alpha]_D\) -16° (c 1.0 in CHCl). The methylated gum was methanolysed and the methyl glycosides of the following sugars detected by gas-liquid chromatography.

Table 18

Cleavage Products of Methylated Degraded Gum C

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Relative Amount</th>
<th>Column a</th>
<th>Column b</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2,3,4,6) Me₄ galactose</td>
<td>1</td>
<td>1.77</td>
<td>1.75</td>
</tr>
<tr>
<td>(2,4,6) Me₃ galactose</td>
<td>10</td>
<td>3.50, 4.04</td>
<td>3.83, 4.41</td>
</tr>
<tr>
<td>(2,3,4) Me₃ galactose</td>
<td>0.3</td>
<td>6.42</td>
<td>6.91</td>
</tr>
<tr>
<td>(2,4) Me₂ galactose</td>
<td>1.2</td>
<td>13.5, 15.4</td>
<td>15.4, 17.7</td>
</tr>
</tbody>
</table>
Investigation of Oligosaccharides Released during Autohydrolysis

Mesquitic acid (30 g) was refluxed in water (1.5 L) for twenty-four hours. The solution was then cooled, reduced in volume and degraded polysaccharide was precipitated by pouring into ethanol (6 vol). A sample of the precipitate was purified by reprecipitation and hydrolysed. Paper chromatographic examination of the hydrolysate indicated the continued presence of a substantial proportion of arabinose residues in the degraded gum. The partially degraded gum was again refluxed in water (500 ml) for a further twelve hours and degraded polysaccharide was recovered by precipitation with ethanol. Hydrolysis of a sample of the precipitate indicated the presence of only a small proportion of arabinose in the gum. The precipitate was redissolved in water and freeze-dried to give degraded gum A (7.1 g), [α]D -1° (c 1.0).

The two supernatant solutions obtained after precipitation of the degraded polysaccharides were combined and concentrated to a syrup. This syrup was applied to a column of diethylaminoethyl Sephadex A-25 (30 g, formate form) and allowed to adsorb overnight. Neutral sugars were eluted with water (2 L), the solution was concentrated and dried to a syrup (15 g). Acidic sugars were desorbed by eluting the column with 0.5 M formic acid (1.5 L). Concentration of the solution yielded a syrup (3.5 g).
Examination of Neutral Sugars

Paper chromatographic examination of the neutral sugar fraction showed that it contained components with mobilities identical to the monosaccharides D-arabinose, D-galactose and D-rhamnose, together with others of lower mobility staining both brown and pink with aniline oxalate spray.

The fraction (14 g) was adsorbed on a charcoal-celite column (300 g). Monosaccharides were removed by eluting the column with water (3 L). The column was then eluted with water containing increasing concentrations of ethanol. Fractions (25 ml) were collected every 1/2 hour. Samples (8 ml) from every third tube were treated with Amberlite IR 45 (OH⁻) resin, evaporated to dryness and examined by paper chromatography in solvent B. Appropriate fractions were combined, treated with anion exchange resin and evaporated to dryness.
<table>
<thead>
<tr>
<th>Eluant</th>
<th>Fraction</th>
<th>Weight (mg)</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1</td>
<td>9 g.</td>
<td>arabinose, galactose, rhamnose</td>
</tr>
<tr>
<td>0-5% ethanol (3 L)</td>
<td>1</td>
<td>40</td>
<td>oligosaccharide L</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>70</td>
<td>oligosaccharides L, C</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2-5</td>
<td>oligosaccharides L, C, M, H, S</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>210</td>
<td>oligosaccharides C, M, H, S, A</td>
</tr>
<tr>
<td>5% ethanol (1 L)</td>
<td>5</td>
<td>105</td>
<td>oligosaccharides H, N, R</td>
</tr>
<tr>
<td>5-10% ethanol (3 L)</td>
<td>6</td>
<td>73</td>
<td>oligosaccharides H, N</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>50</td>
<td>oligosaccharides B, T</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24</td>
<td>oligosaccharides B, T, D</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>36</td>
<td>oligosaccharides B, D, F</td>
</tr>
<tr>
<td>10% ethanol (2 L)</td>
<td>10</td>
<td>200</td>
<td>oligosaccharides E, O</td>
</tr>
<tr>
<td>15% ethanol (3 L)</td>
<td>11</td>
<td>135</td>
<td>oligosaccharides I, Q, F, G</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>46</td>
<td>oligosaccharides I, K</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>102</td>
<td>oligosaccharides I, K</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>72</td>
<td>oligosaccharides J, K</td>
</tr>
</tbody>
</table>
Fraction 1. Paper chromatography showed this fraction contained oligosaccharide L, together with a faint trace of arabinose.

Fraction 2. This fraction was sub-fractionated by filter sheet chromatography in solvent B into chromatographically pure oligosaccharide L (24 mg) and oligosaccharide C (24 mg).

Fraction 3. Thick paper chromatography in solvent A yielded oligosaccharides C (87 mg), M (18 mg), H (24 mg) and S (18 mg) together with a mixture which was fractionated in solvent B into chromatographically pure oligosaccharides L (18 mg) and C (10 mg).

Fraction 4. Fractionation in solvent B yielded oligosaccharides C (31 mg), H (71 mg), S (14 mg) together with a mixture (36 mg). Fractionation of this mixture in solvent A gave oligosaccharides M (9 mg) and A (23 mg).

Fraction 5. Thick paper chromatography in solvent A gave oligosaccharides H (42 mg), N (10 mg) and R (6 mg).

Fraction 6. Separation in solvent A gave oligosaccharides H (3 mg), N (2 mg) and three further small fractions which on paper chromatography in solvent B gave several spots each and were not investigated further.

Fraction 7. This was separated in solvent A into two main fractions, oligosaccharides T (6 mg) and B (25 mg). Some arabinose and slower moving streaking material were discarded.
Fraction 8. Fractionation in solvent A gave the chromatographically pure oligosaccharides B (3 mg) and T (4 mg) together with a slightly impure fraction containing mainly oligosaccharide D (4 mg).

Fraction 9. Chromatography in solvent A gave oligosaccharides B (3 mg), D (8 mg) and a mixture which on subsequent chromatography in solvent B gave as a major component oligosaccharide P (5 mg), the minor component being discarded.

Fraction 10. (200 mg) Fractionation in solvent B gave oligosaccharides C (13 mg) and E (32 mg) together with unresolved slower moving material which was not investigated further.

Fraction 11. (135 mg) Separation in solvent B gave four main sub-fractions, oligosaccharides I (26 mg), Q (13 mg), F (5 mg) and a further component, oligosaccharide G (3 mg) which did not appear to be homogeneous.

Fraction 12. (46 mg) This was fractionated in solvent B into oligosaccharides I (12 mg) and K (6 mg).

Fraction 13. (102 mg) Separation in solvent B gave oligosaccharides I (10 mg) and K (6 mg) plus slower moving streaking material.

Fraction 14. (72 mg) Fractionation in solvent B gave oligosaccharides J (6 mg) and K (18 mg) together with unresolved slow moving material.

Later fractions were obtained which could not be resolved into chromatographically distinct components and were not investigated further.
Oligosaccharide A (23 mg) [α]_D 0° (c 0.5)
R_Gal (A) 1.48, R_Gal (B) 1.26, R_Gal (E) 1.23, M_G 0.39.
Hydrolysis gave arabinose only, while partial hydrolysis (0.1 N sulphuric acid at 100° for 1/2 hour) gave arabinose and starting material only. Comparison of the colorimetric reaction obtained with the phenol-sulphuric acid reagent before and after borohydride reduction showed a decrease in intensity of 52%. A sample (2 mg) was twice methylated by the Kuhn procedure and the methanolyse products examined by g.l.c. The methyl glycosides of the following sugars were detected.

2,3,5 tri-O-methylarabinose T(a) 0.48, 0.63 T(b) 0.54, 0.72
2,5 di-O-methylarabinose T(a) 1.49, 2.70 T(b) 1.79, 3.20

Borohydride reduction of the oligosaccharide (2 mg) followed by methylation by the Kuhn method and g.l.c. of the subsequent methanolysate gave peaks with the retention times of methyl 2,3,5-tri-O-methylarabinose T(b) 0.54, 0.72
1,2,4,5/1,3,4,5-tetra-O-methylarabinitol T(b) 0.98.

The phenylosazone derivative (3 mg) was prepared, m.p. 212±3° [Found: C, 55.0; H, 6.6. C_{22}H_{29}O_{7}N_{4} requires C, 57.3; H, 6.1%]

Ultra-violet absorption, M 460 (in ethanol)

λ max. 255 μm, ε 18,000
λ max. 310 μm, ε 9,800
λ max. 394 μm, ε 18,300
Oxidation of the phenyllosazone with sodium metaperiodate gave formaldehyde (1.1 moles). No mesoxaldehyde 1,2-bisphenylhydrazone was formed.

Oligosaccharide B (25 mg) $\lbrack\alpha\rbrack_D ^{+125^\circ} (c 0.5)$

$\alpha_{\text{Gal}} (A) 1.40$, $\alpha_{\text{Gal}} (B) 1.35$, $M_c 0.4$

This oligosaccharide was chromatographically and electrophoretically identical to 3-0-β-α-arabinofuranosyl-α-arabinose. Hydrolysis gave only arabinose. Comparison of the colorimetric reactions obtained on treatment of the sugar and derived glycitol with the phenol-sulphuric acid reagent showed a decrease in intensity of 53%. The sugar gave a positive reaction with the tetrazolium spray, while treatment with oxygen-free saturated lime water for twenty-four hours gave arabinose as the major reducing product, together with a trace of starting material. The methyl glycosides of the following sugars were identified by g.l.c. as cleavage products of the oligosaccharide after methylation by the Kuhn procedure.

2,3,5-tri-0-methy larabinose $T(a) 0.48, 0.64$ $T(b) 0.57, 0.72$

2,5-di-0-methy larabinose $T(a) 1.48, 2.68$, $T(b) 1.74, 3.09$

2,4-di-0-methy larabinose (tr) $T(a) 1.86$ $T(b) 2.18$

2,3-di-0-methy larabinose (tr) $T(a) 1.18$ $T(b) 1.48$

These methyl glycosides were hydrolysed and the products were oxidised with bromine water. G.l.c. of the oxidation products showed the presence of
Methylation of the oligosaccharide glycitol by the Kuhn method followed by g.l.c. of the cleavage products identified

methylation 2,3,5-tri-O-methylarabino-ide T(a) 0.48, 0.64

1,2,4,5/1,3,4,5-tetra-O-methylarabinitol T(a) 0.91

methyl 2,3-di-O-methylarabinoside (tr) T(a) 1.18, 1.48.

The phenylosazone derivative had m.p. 215-7°, mixed m.p. 205° - with the phenylosazone of oligosaccharide A (m.p. 212-3°).

Ultra-violet absorption M 460 (in ethanol)

\[ \lambda_{\text{max}} = 255 \text{ m\u}, \ \varepsilon = 20,100 \]

\[ \lambda_{\text{max}} = 309 \text{ m\u}, \ \varepsilon = 12,100 \]

\[ \lambda_{\text{max}} = 390 \text{ m\u}, \ \varepsilon = 22,200 \]

Oxidation of the osazone with periodate gave formaldehyde (0.85 moles) and no mesoxaldehyde 1,2-bisphenylyhydrzone.

Oligosaccharide C (150 mg) \([\alpha]_D +154^\circ \ (\varepsilon 0.03)\)

R\text{Gal (A)} 0.73, R\text{Gal (B)} 0.52, M\text{G} 0.62

The oligosaccharide was chromatographically identical to 3-\(\beta\)-\(\alpha\)-\(\beta\)-galactopyranosyl-\(\alpha\)-arabinose while hydrolysis gave galactose and arabinose. Alkaline degradation resulted in the disappearance of the oligosaccharide and the liberation of galactose. The oligosaccharide (2 mg) was methylated by the Kuhn procedure and the methanolysis products examined
by g.l.c. The methyl glycosides of the following sugars were detected.

2,3,4,6-tetra-\(\beta\)-methylgalactose  \(T(a)\) 1.76

2,5-di-\(\beta\)-methylarabinose  \(T(a)\) 1.49, 2.73

The oligosaccharide glycitol was similarly methylated and the following cleavage products detected by g.l.c.

methyl 2,3,4,6-tetra-\(\beta\)-methylgalactoside  \(T(a)\) 1.77

1,2,4,5/1,3,4,5-tetra-\(\alpha\)-methylarabinitol  \(T(a)\) 0.91

The phenylosazone derivative of the oligosaccharide had m.p. 232-3\(^\circ\) and mixed m.p. 233\(^\circ\) [with authentic sample (m.p. 235\(^\circ\)].

Ultra-violet absorption  \(M\) 460 (in ethanol)

\[\lambda\ max.\ 257\ \mu\mu,\ \varepsilon\ 18,000\]

\[\lambda\ max.\ 310\ \mu\mu,\ \varepsilon\ 10,800\]

\[\lambda\ max.\ 394\ \mu\mu,\ \varepsilon\ 20,500\]

Oxidation of this derivative with sodium metaperiodate yielded formaldehyde (1.0 moles) and no mesoxaldehyde, 1,2-bisphenylhydrazine.

Oligosaccharide D  (12 mg)

\(R_{Gal}\) (A) 0.53, \(R_{Gal}\) (B) 0.30. Solvent A showed the presence of a small amount of impurity at \(R_{Gal}\) 0.67.

Partial hydrolysis in 0.02 \(\text{M}\) sulphuric acid at 100\(^\circ\) for one hour gave galactose, arabinose and 3-\(\alpha\)-D-galactopyranosyl-\(\alpha\)-arabinose. Similar reducing sugars were detected as partial hydrolysis products of the oligosaccharide glycitol.
Treatment of the oligosaccharide (2 mg) with oxygen-free lime water for 5 days followed by paper chromatographic examination of the products showed galactose and traces of other reducing sugars, but no starting material. The oligosaccharide (1 mg) was methylated by Kuhn's procedure and the product methanolysed. Peaks with the retention times of the methyl glycosides of the following sugars were obtained by g.l.c.

2,3,4,6-tetra-O-methylgalactose \( T(a) 1.74 \)

2,5-di-O-methylarabinose \( T(a) 1.50, 2.70 \)

2,3,5-tri-O-methylarabinose \( (tr) \) \( T(a) 0.50, 0.63 \)

2,3,4-tri-O-methylarabinose \( (tr) \) \( T(a) 0.89 \)

3,5-di-O-methylarabinose \( (tr) \) \( T(a) (0.89), 1.95 \)

Oligosaccharide E (32 mg) \([\alpha]_D^{+64\circ} (c 1.0)\)

\( R_{Gal} (A) 0.72, R_{Gal} (B) 0.24. \) Oligosaccharide D was also present as a very minor impurity.

Partial hydrolysis of the oligosaccharide in 0.02 M sulphuric acid at 100° for one hour gave galactose, arabinose and 3-\(\alpha\)-D-galactopyranosyl-\(\alpha\)-arabinose. Borohydride reduction of the oligosaccharide followed by hydrolysis under similar conditions gave galactose, arabinose and virtually no galactosylarabinose. Treatment of the oligosaccharide and derived glycitol with the phenol-sulphuric acid reagents showed a decrease in the intensity of the reaction with the glycitol of 31% compared to the sugar. The
sugar (2 mg) was methylated by the Kuhn method, methanolyzed and the products were examined by g.l.c. The methyl glycosides of the following methylated sugars were identified.

- 2,3,5-tri-\(\beta\)-methyalarabinose: \(T(a)\) 0.50, 0.64
- 2,5-di-\(\beta\)-methyalarabinose: \(T(a)\) 1.48, 2.67
- 2,3,4-tri-\(\beta\)-methylgalactose: \(T(a)\) 6.40
- 2,3,4,6-tetra-\(\beta\)-methylgalactose (tr): \(T(a)\) 1.76

A sample of the derived glycitol (2 mg) was similarly methylated. The following cleavage products were identified by g.l.c.

- methyl 2,3,5-tri-\(\beta\)-methyalarabinoside: \(T(a)\) 0.40, 0.64 \(T(b)\) 0.54, 0.71
- methyl 2,3,4-tri-\(\beta\)-methylgalactoside: \(T(a)\) 6.40 \(T(b)\) 6.90
- 1,2,4,5/1,3,4,5-tetra-\(\beta\)-methyalarabinitol: \(T(a)\) 0.90 \(T(b)\) 0.97
- methyl 2,3,4,6-tetra-\(\beta\)-methylgalactoside (tr): \(T(a)\) 1.76 \(T(b)\) (1.80)
- methyl 2,5-di-\(\beta\)-methyalarabinoside (tr): \(T(a)\) 1.50 \(T(b)\) (1.80)

**Oligosaccharide F** (5 mg) \([\alpha]_D\) +30° (c 0.5)

- \(R_{Gal}\) (A) 0.69, \(R_{Gal}\) (B) 0.20

Partial hydrolysis of the oligosaccharide gave galactose, arabinose and 3-\(\alpha\)-\(\beta\)-galactopyranosyl-L-arabinose together with a component with chromatographic mobility similar to oligosaccharide E. Hydrolysis of the derived glycitol under similar conditions gave galactose, arabinose, and 3-\(\alpha\)-\(\beta\)-galactopyranosyl-L-arabinose as the only detectable reducing sugars.

A sample of the oligosaccharide (0.5 mg) was methylated
by the Kuhn procedure. The methyl glycosides of the following sugars were detected by g.l.c. as cleavage products of the methylated oligosaccharide.

2,3,5-tri-O-methylarabinose  
T(a) 0.50, 0.65  
T(b) 0.55, 0.72

2,3,4-tri-O-methylgalactose  
T(a) 6.47  
T(b) 6.70

2,5-di-O-methylarabinose  
T(a) 1.50, 2.74  
T(b) 1.80

3,5-di-O-methylarabinose (tr)  
T(a) 0.88, 1.98  
T(b) 1.03, 2.33

2,3-di-O-methylarabinose (tr)  
T(a) 1.21, (1.50)  
T(b) 1.50, (1.80)

Methylation of a similar quantity of the derived glycitol was followed by g.l.c. analysis of the cleavage products.

Methyl glycosides of the following sugars were detected.

2,3,5-tri-O-methylarabinose  
T(a) 0.49, 0.64

2,3,4-tri-O-methylgalactose  
T(a) 6.43

2,5-di-O-methylarabinose  
T(a) 1.49

3,5-di-O-methylarabinose (tr)  
T(a) (0.91), 1.98

2,3-di-O-methylarabinose (tr)  
T(a) 1.21, (1.49)

and 1,2,4,5/1,3,4,5-tetra-O-methylarabinitol  
T(a) 0.91

Oligosaccharide C, (3 mg)

R_{Gal} (B) 0.09. Chromatography in solvent A gave an elongated spot indicating the fraction was not homogeneous. Partial hydrolysis gave galactose, arabinose, 3-0-α-D-galactopyranosyl-α-L-arabinose together with higher oligosaccharides. The methyl glycosides of the following sugars were detected as cleavage products from the methylated derivative.
2,3,5-tri-\(\beta\)-methylarabinose \(T(a)\) 0.50, 0.66

2,3,4,6-tetra-\(\beta\)-methylgalactose \(T(a)\) 1.77

2,3,4-tri-\(\beta\)-methylgalactose \(T(a)\) 6.39

2,5-di-\(\beta\)-methylarabinose \(T(a)\) 1.51, 2.71

3,5-di-\(\beta\)-methylarabinose (tr) \(T(a)\) 0.90, 1.98

2,3-di-\(\beta\)-methylarabinose (tr) \(T(a)\) 1.21, (1.51)

**Oligosaccharide H** (137 mg) \([\alpha]_D^{101} +101^\circ\) (c 1.0)

\(R_{Gal}\) (A) 1.44, \(R_{Gal}\) (B) 1.36, \(R_{Gal}\) (E) 1.23, \(M_G\) 0.29

Hydrolysis of the oligosaccharide gave only arabinose.

Measurement of the sugar content by the phenol-sulphuric acid method before and after reduction showed that 49% of the sugar content was removed by reduction. A sample (2 mg) of the oligosaccharide was methylated by the Kuhn procedure and methanolysed. The methyl glycosides of the following sugars were detected by g.l.c.

2,3,5-tri-\(\beta\)-methylarabinose \(T(a)\) 0.48, 0.63 \(T(b)\) 0.56, 0.73

3,5-di-\(\beta\)-methylarabinose \(T(a)\) 0.83, 1.99 \(T(b)\) 1.04, 2.37

3,4-di-\(\beta\)-methylarabinose \(T(a)\) 1.57, 3.30 \(T(b)\) 1.99, 4.20

A further sample was methylated as before, hydrolysed and the hydrolysate oxidised with bromine water. G.l.c. of the oxidation products showed the presence of the following sugar derivatives.

2,3,5-tri-\(\beta\)-methylarabonolactone \(T(b)\) 1.74

3,5-di-\(\beta\)-methylarabonolactone \(T(b)\) 5.52

3,4-di-\(\beta\)-methylarabonolactone \(T(b)\) 10.70

2,3-di-\(\beta\)-methylarabonolactone (tr) \(T(b)\) 8.14
The sugar (2 mg) was reduced with sodium borohydride and methylated. The methylated glycitol was methanolysed and the following products identified by g.l.c.

methyl 2,3,5-tri-O-methylarabinoside  T(a) 0.48, 0.63
1,2,4,5/1,3,4,5-tetra-O-methylarabinitol  T(a) 0.90

The oligosaccharide (40 mg) was methylated with methyl sulphate and 30% sodium hydroxide solution. The product was finally refluxed in methyl iodide with silver oxide to ensure complete methylation and hydrolysed. The hydrolysis products were fractionated by thick paper chromatography in solvent Z into two main fractions.

**Fraction 1** (12 mg). This fraction was characterised as 2,3,5-tri-O-methyl-L-arabinose by conversion of the sugar to the corresponding derived aldonolactone, followed by treatment of this product with methanolic ammonia which gave, on evaporation of the solvent, 2,3,5-tri-O-methyl-L-arabonamide, m.p. 135-6° and mixed m.p. 135-6° (with sample, m.p. 137-8°).

**Fraction 2** (14 mg). This fraction was identified as 3,4-di-O-methyl-L-arabinose. The sugar was oxidised with bromine water and the derived aldonolactone treated with methanolic ammonia. Evaporation of the solvent gave a syrup which crystallised on seeding with 3,4-di-O-methyl-L-arabonamide and had m.p. 123-5° and mixed m.p. 124-5° (with sample, m.p. 127-8°).
Oligosaccharide H (10 mg) was treated with oxygen-free saturated lime water for 10 days. The solution was then neutralised with Amberlite IR 120 (H) resin, filtered and concentrated. Paper chromatography revealed the presence of two components, both staining pink with aniline oxalate spray. These were separated preparatively by chromatography in solvent B.

Oligosaccharide H1 (4.2 mg) This was chromatographically similar to the starting material and on hydrolysis gave arabinose. A sample was methylated, methanolysed and the products examined by g.l.c. The methyl glycosides of the following compounds were recognised.

2,3,5-tri-O-methylarabinose \( T(a) \) 0.48, 0.64 \( T(b) \) 0.57, 0.73
3,5-di-O-methylarabinose \( T(A) \) 0.83, 2.00 \( T(b) \) 1.05, 2.40
3,4-di-O-methylarabinose \( T(a) \) 1.57, 3.29 \( T(b) \) 2.02, 4.20

Oligosaccharide H2 (2.0 mg) \( R_{Gal} \) (A) 1.68, \( R_{Gal} \) (B) 1.66. Hydrolysis of the sugar gave arabinose and ribose. The remainder of the sample was methylated by the Kuhn method and the methanolysis products examined by g.l.c. Peaks with the retention times of methyl 2,3,5-tri-O-methylarabinoside \( [T(a) \) 0.48, 0.64] together with several other unidentified peaks were obtained.

Oligosaccharide H (5 mg) was treated with lead tetraacetate (1.5 mole) in aqueous acetic acid (1 ml) for twenty minutes. Lead was precipitated by treatment with hydrogen
sulphide and the solution was filtered and concentrated. The syrup was redissolved in saturated lime water. After three hours the solution was neutralised with Amberlite IR 120 (H) resin, filtered and concentrated. Paper chromatographic examination of the resulting syrup showed the presence of only one component, with chromatographic mobility identical to the original oligosaccharide. Hydrolysis of a sample gave only arabinose. A further sample of the tetra-acetate treated sugar was reduced with potassium borohydride and hydrolysed. Paper chromatography showed the presence in the hydrolysate of arabinose and arabinitol only.

**Oligosaccharide I (48 mg)** \( [\alpha]_D^{+98^\circ} \ (c \ 1.0) \).

\[
R_{\text{Gal}} (A) \ 1.29, \ R_{\text{Gal}} (B) \ 0.81
\]

Hydrolysis of the oligosaccharide gave arabinose while partial hydrolysis in 0.02 M sulphuric acid at 100° for one hour gave arabinose and oligosaccharide B. Similar products were observed after partial hydrolysis of the derived glycitol. Oligosaccharide I gave a negative colour reaction with the tetrazolium spray reagent. Comparison of the colorimetric reactions of the oligosaccharide and glycitol with the phenolsulphuric acid reagents showed a decrease in the intensity of the reaction with the glycitol of 30%.

The oligosaccharide (2 mg) was methylated by the Kuhn method and methanolyised. GC examination showed the presence in the methanolsolate of
methyl 2,3,5-tri-0-methylarabinoside T(a) 0.50, 0.65 T(b) 0.55, 0.72
methyl 3,5-di-0-methylarabinoside T(a) 0.84, 1.99 T(b) 1.04, 2.35
Glc. of the methanolysis products of the methylated glycitol
showed the presence of
methyl 2,3,5-tri-0-methylarabinoside T(a) 0.48, 0.66 T(b) 0.55, 0.71
methyl 3,5-di-0-methylarabinoside T(a) 0.83, 1.98 T(b) (1.01), 2.36
1,2,4,5/1,3,4,5-tetra-0-methylarabinitol T(a) 0.90 T(b) (1.01).

**Oligosaccharide J (6 mg)**

R_{Gal} (A) 1.20, R_{Gal} (B) 0.55

Hydrolysis gave arabinose while partial hydrolysis in 0.02 N
sulphuric acid at 100°C for 3/4 hour gave products with the
paper chromatographic mobilities of arabinose, oligosaccharide
H and oligosaccharide I. Comparison of the colorimetric re-
actions of the oligosaccharide and derived glycitol showed a
decrease in the intensity of the reaction with the glycitol
of 25.5%. The oligosaccharide (1 mg) was methylated by the
Kuhn procedure. Examination of the methanolysis products
by g.l.c. showed the presence of the methyl glycosides of the
following compounds.

2,3,5-tri-0-methylarabinose T(a) 0.48, 0.64
3,5-di-0-methylarabinose T(a) 0.83, 1.95
3,4-di-0-methylarabinose (tr) T(a) 1.55
2,3-di-0-methylarabinose (tr) T(a) 1.18, 1.50

The oligosaccharide glycitol (1 mg) was methylated by the
Kuhn method and the following methanolysis products were de-
tected by g.l.c.

methyl 2,3,5-tri-0-methylarabinoside \( T(a) \) 0.48, 0.66
methyl 3,5-di-0-methylarabinoside \( T(a) \) 0.83, 1.95
1,2,4,5/1,3,4,5-tetra-0-methylarabinitol \( T(a) \) 0.91
methyl 2,3-di-0-methylarabinoside \( T(a) \) 1.18, 1.49

Oligosaccharide K (30 mg) \([\alpha]_D^{+121^\circ} \ (c \ 1.0)\)

\( R_{Gal} \ (A) \) 1.14, \( R_{Gal} \ (B) \) 0.32

Hydrolysis gave arabinose only, while partial hydrolysis in 0.02 N sulphuric acid at 100° for 3/4 hour gave, on paper chromatographic examination, arabinose and oligosaccharides H, I and J. The intensity of the colorimetric reaction obtained on treatment of the oligosaccharide glycitol with the phenol-sulphuric acid reagent was 18.5% less than the reaction with a similar weight of oligosaccharide.

The oligosaccharide (2 mg) was methylated by Kuhn's procedure and the methanolysis products examined by g.l.c.

Methyl glycosides of the following sugars were detected.

2,3,5-tri-0-methylarabinose \( T(a) \) 0.49, 0.64 \( T(b) \) 0.55, 0.72
3,5-di-0-methylarabinose \( T(a) \) 0.82, 1.97 \( T(b) \) 1.04, 2.35
3,4-di-0-methylarabinose (tr) \( T(a) \) 1.54, 3.30 \( T(b) \) 1.97, 4.10
2,3-di-0-methylarabinose (tr) \( T(a) \) 1.19, 1.53 \( T(b) \) 1.49, 1.84

The derived glycitol was similarly methylated and the following cleavage products were identified by g.l.c.

methyl 2,3,5-tri-0-methylarabinoside \( T(a) \) 0.50, 0.64
methyl 3,5-di-0-methylarabinoside \( T(a) \) 0.83, 1.94
1,2,4,5/1,3,4,5-tetra-0-methylarabinitol \( T(a) \) 0.88
Oligosaccharide L (94 mg) \([\alpha]_D +156^\circ (c 0.5)\)

\(R_{Gal} (A) 0.86, \ R_{Gal} (B) 0.73, \ M_G 0.61\)

The oligosaccharide was chromatographically identical to 3-\(\text{O}-\beta-L\)-arabinopyranosyl-\(\alpha-L\)-arabinose. Hydrolysis gave arabinose only. Treatment of the sugar before and after borohydride reduction with the phenol–sulphuric acid reagent gave a decrease of 49\% in the intensity of the colorimetric reaction obtained with the glycitol. Treatment with oxygen-free saturated lime water degraded the oligosaccharide, while the sugar gave a positive reaction with the tetrazolium spray reagent.

A sample (2 mg) was methylated by the Kuhn method. The following methanolysis products were detected by g.l.c.

methyl 2,3,4-tri-\(O\)-methylarabinoside \(T(a) 0.88 \ T(b) 1.04\)

methyl 2,5-di-\(O\)-methylarabinoside \(T(a) 1.52, 2.74 \ T(b) 3.20\)

The derived glycitol was methylated and the following cleavage products were identified by g.l.c.

methyl 2,3,4-tri-\(O\)-methylarabinoside \(\}) \ T(a) 0.88 \ T(b) 1.04\)

1,2,4,5/1,3,4,5-tetra-\(O\)-methylarabinitol \(\})

The phenylosazone derivative of the oligosaccharide was prepared, m.p. 233-4\(^\circ\).

Ultra-violet absorption, \(M 460 \) (in ethanol)

\(\lambda \ \text{max.} \ 256 \ \mu\mu, \ \varepsilon 23,100\)

\(\lambda \ \text{max.} \ 309 \ \mu\mu, \ \varepsilon 12,400\)

\(\lambda \ \text{max.} \ 395 \ \mu\mu, \ \varepsilon 22,500\)
Periodate oxidation of the osazone gave formaldehyde (1.1 moles) but no mesoxaldehyde 1,2-bisphenylhydrazone.

**Oligosaccharide M (27 mg) [α]_D +6° (c 0.08)**

<table>
<thead>
<tr>
<th>Component</th>
<th>T(a)</th>
<th>T(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-tri-O-a-methylarabinose</td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>2,3-di-O-a-methylarabinose</td>
<td>1.18</td>
<td>1.45</td>
</tr>
<tr>
<td>2,3,4-tri-O-a-methylarabinose (tr)</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>2,5-di-O-a-methylarabinose (tr)</td>
<td>1.50</td>
<td>2.70</td>
</tr>
<tr>
<td>3,5-di-O-a-methylarabinose (tr)</td>
<td>0.89</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Electrophoresis in borate buffer showed the additional presence of three minor mobile components. Hydrolysis of the oligosaccharide gave arabinose.

The oligosaccharide (2 mg) was methylated twice by the Kuhn procedure. The methylated derivative was methanolysed and the following cleavage products were identified, as methyl glycosides, by g.l.c.

- 2,3,5-tri-O-a-methylarabinose
  - T(a) 0.48, 0.64
  - T(b) 0.55, 0.72
- 2,3-di-O-a-methylarabinose
  - T(a) 1.18, 1.45
  - T(b) 1.48, 1.67, 1.82
- 2,3,4-tri-O-a-methylarabinose (tr)
  - T(a) 0.89
  - T(b) 1.02
- 2,5-di-O-a-methylarabinose (tr)
  - T(a) 1.50, 2.70
  - T(b) 1.82
- 3,5-di-O-a-methylarabinose (tr)
  - T(a) 0.89, 1.97
  - T(b) 1.02, 2.34

A further sample of the oligosaccharide (2 mg) was reduced with sodium borohydride and the derived glycitol was methylated by the Kuhn procedure. The product was hydrolysed and the following compounds were identified by g.l.c.

- 2,3,5-tri-O-a-methylarabinose
  - T(b) 2.23
- 2,3,4-tri-O-a-methylarabinose (tr)
  - T(b) 3.17
- 1,2,3,5/1,2,3,4-tetra-O-a-methylarabinitol
  - T(b) 1.12
- 1,3,4,5/1,2,4,5-tetra-O-a-methylarabinitol (tr)
  - T(b) 1.00
The oligosaccharide (4 mg) was treated with bromine water (10 ml) in the dark for four days. Excess bromine was removed by aeration and the solution was neutralised with silver carbonate. After filtration, the solution was treated with hydrogen sulphide, precipitated silver sulphide was removed by filtration and the filtrate was evaporated to dryness. The oxidised oligosaccharide thus obtained was methylated twice by the Kuhn procedure. The methylated product was taken up in a solution (5 ml) of sodium hydroxide (pH 12). After two hours the solution was acidified with 2 N sulphuric acid (5 ml) and heated at 100° for four hours. After neutralisation and deionisation, the solution was evaporated to dryness, the residue was extracted with acetone and this solution was examined by g.l.c. The products identified were

2,3,5-tri-O-methylarabinose  
T(a) 1.78  T(b) 2.20

2,3,4-tri-O-methylarabinose (tr)  
T(a) 2.49  T(b) 3.14

2,3,5-tri-O-nethylarabonolactone  
T(a) 1.40  T(b) 1.77.

The phenylosazone derivative was prepared but not obtained crystalline. It was purified by t.l.c. in solvent (3).

Ultra-violet absorption (in ethanol)

\( \lambda \) max. 390 m\( \mu \), (c 20,000 assumed)

\( \lambda \) max. 307 m\( \mu \), c 11,700

\( \lambda \) max. 256 m\( \mu \), c 18,900

The phenylosazone (0.306 mg in 1 ml water) (concentration determined by u.v. absorption at 390 m\( \mu \)) was oxidised with
0.003 M sodium metaperiodate (1 ml).

After one hour, periodate consumed 1.8 moles from aldehyde formed 0.046 moles

No mesoxaldehyde 1,2-bisphenylhydrazone was observed, either visually or by U.V. spectroscopy.

**Oligosaccharide** M (12 mg) \([\alpha]_D +86^\circ \ (c 0.5)

\[ \text{R}_{\text{Gal}} (A) 1.28, \text{R}_{\text{Gal}} (B) 1.19, \text{M}_{\text{G}} 0. \]

Hydrolysis of the oligosaccharide gave arabinose while treatment with oxygen-free lime water resulted in the destruction of the oligosaccharide and the appearance of arabinose.

The oligosaccharide (2 mg) was methylated by the Kuhn method. The following components were identified by g.l.c. as cleavage products from the methylated derivative.

- methyl 2,3,5-tri-\(O\)-methylarabinoside \(T(a) 0.49, 0.83 \ T(b) 0.55, 0.72\)
- methyl 2,3-di-\(O\)-methylarabinoside \(T(a) 1.20, 1.47 \ T(b) 1.47, 1.80\)

These glycosides were then hydrolysed and the methylated sugars were oxidised with bromine water. G.l.c. confirmed the identity of the products as

- 2,3,5-tri-\(O\)-methylarabonolactone \(T(b) 1.74\)
- 2,3-di-\(O\)-methylarabonolactone \(T(b) 7.90.\)

A sample of the oligosaccharide (2 mg) was converted to the glycitol and the product was methylated. The methanoly-sis products were identified by g.l.c. as

- methyl 2,3,5-tri-\(O\)-methylarabinoside \(T(b) 0.55, 0.72\)
- 1,2,3,4/1,2,3,5-tetra-\(O\)-methylarabinitol \(T(b) 1.11.\)
The sequence of reactions, involving oxidation and methylation, performed on oligosaccharide \( M \) was also applied to this oligosaccharide (3 mg). G.l.c. examination of the hydrolysis products showed the presence of

- \( 2,3,5\text{-tri-0-methylarabinose} \) \( T(b) \ 2.20 \)
- \( 2,3,5\text{-tri-0-methylarabonolactone} \) \( T(b) \ 1.77 \)

**Oligosaccharide 0 (13 mg) \([\alpha]_D^\circ +40^\circ \ (c \ 1.0)\)**

\( R_{Gal} \ (A) \ 1.32, \ R_{Gal} \ (B) \ 0.78 \)

Partial hydrolysis of the oligosaccharide gave arabinose and a trace of a component with mobility identical to that of \( 3\text{-0-}\beta\text{-D-arabinopyranosyl-}\beta\text{-arabinose} \). Comparison of the colorimetric reactions of the oligosaccharide and derived glycitol with the phenol-sulphuric acid reagent showed a decrease in the intensity of the reaction with the glycitol of 40%.

The oligosaccharide (2 mg) was methylated by the Kuhn method and methanolysed. The methyl glycosides of the following sugars were identified by g.l.c.

- \( 2,3,5\text{-tri-0-methylarabinose} \) \( T(a) \ 0.48, \ 0.61 \)
- \( 2,3\text{-di-0-methylarabinose} \) \( T(a) \ 1.14, \ (1.46) \)
- \( 2,5\text{-di-0-methylarabinose} \) \( T(a) \ (1.46) \)
- \( 3,5\text{-di-0-methylarabinose (tr)} \) \( T(a) \ 0.83, \ 1.90 \)

This methanolysate was hydrolysed and the methylated sugars oxidised with bromine water. The following products were identified by g.l.c.
2,3,5-tri-O-methylarabonolactone T(a) 1.51
2,3-di-O-methylarabonolactone T(a) 6.40
2,5-di-O-methylarabonolactone T(a) 14.20
3,5-di-O-methylarabonolactone (tr) T(a) 4.51

The derived oligosaccharide glycitol was methylated and the following cleavage products were detected by g.l.c.

methyl 2,3,5-tri-O-methylarabinoside T(a) 0.49, 0.62
methyl 2,3-di-O-methylarabinoside T(a) 1.13, 1.41
1,2,4,5/1,3,4,5-tetra-O-methylarabinitol T(a) 0.87
methyl 3,5-di-O-methylarabinoside (tr) T(a) 1.90

Oligosaccharide P (5 mg)

R_Gal (A) 1.12, R_Gal (B) 0.56

The methyl glycosides of the following sugars were detected by g.l.c. as cleavage products from the methylated oligosaccharide.

2,3,5-tri-O-methylarabinose T(a) 0.49, 0.62
2,3-di-O-methylarabinose T(a) 1.17, (1.47)
2,5-di-O-methylarabinose T(a) (1.47)
3,5-di-O-methylarabinose T(a) 0.83, 1.96

The derived glycitol was methylated and methanolyzed.

The products were identified by g.l.c. as

methyl 2,3,5-tri-O-methylarabinoside T(a) 0.48, 0.62
methyl 2,3-di-O-methylarabinoside T(a) 1.14, 1.41
methyl 3,5-di-O-methylarabinoside T(a) 0.81, 1.86
1,2,4,5/1,3,4,5-tetra-O-methylarabinitol T(a) 0.86.
Treatment of a sample of the oligosaccharide (2 mg) with oxygen-free saturated lime water resulted in degradation of the sugar and the appearance of an oligosaccharide with chromatographic mobility identical to that of 2-0-β-L-arabinofuranosyl-L-arabinose (oligosaccharide H).

**Oligosaccharide Q (13 mg) [α]_D^+30° (c 1.0)**

R_Gal (A) 1.02, R_Gal (B) 0.37

Partial hydrolysis gave arabinose and components with chromatographic mobilities similar to those of 3-0-β-L-arabinopyranosyl-L-arabinose and oligosaccharides O and P.

Partial hydrolysis of the derived glycitol gave arabinose as the only detected reducing sugar. Treatment of the oligosaccharide with oxygen-free saturated lime water degraded the sugar and liberated oligosaccharide I (Araf 1+2 Araf 1+2 Ara). Comparison of the intensities of the colorimetric reactions of the sugar and derived glycitol with the phenol-sulphuric acid reagent showed a decrease in the intensity of the reaction with the glycitol of 24%.

The oligosaccharide (2 mg) was methylated by the Kuhn procedure and the product was methanolysed. The methyl glycosides of the following sugars were detected by g.l.c.

- **2,3,5-tri-0-methyalarabinose**
  - T(a) 0.47, 0.62 T(b) 0.57, 0.73
- **3,5-di-0-methyalarabinose**
  - T(a) 0.85, 1.99 T(b) 1.05, 2.30
- **2,5-di-0-methyalarabinose**
  - T(a) (1.44), 2.70 T(b) (1.82), 3.20
- **2,3-di-0-methyalarabinose**
  - T(a) 1.16, (1.44) T(b) 1.52, (1.82)
A further sample of the oligosaccharide was twice methylated by the Kuhn method, the methylated derivative was hydrolysed and the products were oxidised with bromine water. The following sugar derivatives were detected by g.l.c.

2,3,5-tri-0-methylarabonolactone  
T(b) 1.78

3,5-di-0-methylarabonolactone  
T(b) 5.40

2,5-di-0-methylarabonolactone  
T(b) 14.0

2,3-di-0-methylarabonolactone  
T(b) 7.83

The derived oligosaccharide glycitol was twice methylated by the Kuhn method. The product was hydrolysed, and after oxidation with bromine water, the following sugar derivatives were identified by g.l.c.

2,3,5-tri-0-methylarabonolactone  
T(a) 1.43  T(b) 1.72

3,5-di-0-methylarabonolactone  
T(a) 4.45  T(b) 5.35

2,3-di-0-methylarabonolactone  
T(a) 6.35  T(b) 7.89

1,2,4,5/1,3,4,5-tetra-0-methylarabinitol  
T(a) 0.92  T(b) 0.99

Oligosaccharide R (6 mg)

R_Gal (A) 0.43, R_Gal (B) 0.30

This oligosaccharide was chromatographically identical to 3-0-β-D-galactopyranosyl-D-galactose. Hydrolysis gave only galactose. The oligosaccharide (2 mg) was methylated by the Kuhn procedure and the cleavage products identified by g.l.c. as the methyl glycosides of
2,3,4,6-tetra-\(\beta\)-methylgalactose \(T(a) 1.78\) \(T(b) 1.78\)

2,4,6-tri-\(\beta\)-methylgalactose \(T(a) 3.48, 3.98\) \(T(b) 3.84, 4.44\)

2,5,6-tri-\(\beta\)-methylgalactose \(T(a) 4.85\) \(T(b) 4.09\).

**Oligosaccharide S** (32 mg) \([\alpha]_D +23^\circ\) \((c 1.0)\)

\(R_{Gal} (A) 0.38, R_{Gal} (B) 0.31\)

The sugar was chromatographically identical to 6-\(\beta\)-D-galactopyranosyl-\(\beta\)-galactose, while hydrolysis gave only galactose. A sample (5 mg) was methylated by the Haworth procedure. The methanolysis products from the methylated sugar were identified by g.l.c. as

methyl 2,3,4,6-tetra-\(\beta\)-methylgalactoside \(T(a) 1.79\)

methyl 2,3,4-tri-\(\beta\)-methylgalactoside \(T(a) 6.41\).

The cleavage products from the methylated oligosaccharide glycitol were identified by g.l.c. as

methyl 2,3,4,6-tetra-\(\beta\)-methylgalactoside \(T(b) 1.77\)

1,2,3,4,5-penta-\(\beta\)-methylgalactitol \(T(b) 3.72\).

**Oligosaccharide T** (10 mg) \([\alpha]_D +17^\circ\) \((c 1.0)\)

\(R_{Gal} (A) 0.11, R_{Gal} (B) 0.10\).

The oligosaccharide was chromatographically identical to 6-\(\beta\)-D-galactopyranosyl-(1 \(\rightarrow\) 6)-\(\beta\)-D-galactopyranosyl-(1 \(\rightarrow\) 6)-\(\beta\)-galactose. Partial hydrolysis gave 6-\(\beta\)-D-galactopyranosyl-\(\beta\)-galactose, galactose and a trace of arabinose.

Comparison of the colorimetric reaction obtained on treatment of sugar and glycitol with the phenol-sulphuric acid reagent showed a decrease in the intensity of the reaction
obtained with the glycitol of 31%.

Methanolation of the Kuhn methylated oligosaccharide furnished the methyl glycosides of

2,3,4,6-tetra-0-methylgalactose  T(b) 1.80
2,3,4-tri-0-methylgalactose  T(b) 6.85
2,3,5-tri-0-methylgalactose  T(b) 4.13.

The oligosaccharide glycitol was similarly methylated and the following cleavage products were detected by g.l.c.

methyl 2,3,4,6-tetra-0-methylgalactoside  T(a) 1.75  T(b) 1.79
methyl 2,3,4-tri-0-methylgalactoside  T(a) 6.40  T(b) 6.90
1,2,3,4,5-penta-0-methylgalactitol  T(a) 3.90  T(b) 3.72

Examination of the Acidic Sugars

Chromatographic examination of the fraction in solvents B and C showed the presence mainly of oligosaccharides already characterised as partial acid hydrolysis products. The syrup was separated by filter sheet chromatography in solvent C into the following components.

**Fraction 1 (55 mg)**  \( R_{Gal} (B) 0.76 \)

This fraction was chromatographically identical to oligosaccharide I. Borohydride reduction of the derived methyl ester methyl glycosides followed by hydrolysis gave galactose and 4-0-methylglucose.

**Fraction 2 (104 mg)**  \( R_{Gal} (B) 0.67 \)

The fraction was chromatographically identical to oligosaccharide II. Borohydride reduction of the derived methyl
ester methyl glycosides followed by hydrolysis gave galactose and 4-\(O\)-methylglucose. The sugar (3 mg) was methylated by the Kuhn procedure. Gas-liquid chromatography of the methanolysis products gave peaks with the retention times of the methyl glycosides of 2,3,4-tri-\(O\)-methylglucuronic acid (as methyl ester) and 2,3,5- and 2,3,4-tri-\(O\)-methylgalactose. *Fraction 3* (83 mg). This was further fractionated by chromatography in solvent B.

**Sub-fraction 3a** (22 mg) \(R_{Gal} \) (B) 0.25

The sugar was chromatographically identical to oligosaccharide III and on hydrolysis gave glucurone and galactose. Reduction of the derived methyl ester methyl glycosides with borohydride followed by hydrolysis gave glucose and galactose.

**Sub-fraction 3b** (37 mg) \(R_{Gal} \) (B) 0.15

This was identical on paper chromatography to oligosaccharide V. Hydrolysis of the borohydride reduced methyl ester methyl glycosides gave 4-\(O\)-methylglucose and galactose. Partial hydrolysis of the oligosaccharide gave 6-\(O\)-(4-\(O\)-methyl-\(\beta\)-D-glucopyranosyluronic acid)-\(\beta\)-galactose and galactose.

**Fraction 4** (42 mg) \(R_{Gal} \) (B) 0.07. Further fractionated by electrophoresis.

**Sub-fraction 4a** (12 mg)

This sub-fraction was chromatographically similar to oligosaccharide VI and on partial hydrolysis gave galactose,
aldobiouronic acid II and III and the trisaccharide V.

**Sub-fraction 4b (17 mg)**

Partial hydrolysis gave the same products as were obtained from sub-fraction 4a, together with arabinose. Borohydride reduction of sub-fraction 4b followed by hydrolysis gave arabinose, galactose, galactitol and the aldobiouronic acids II and III.

**Fraction 5 (29 mg) R_{Gal} (C) 0.22**

The fraction was homogeneous on paper chromatography, with mobility similar to that of oligosaccharide VII, but was further fractionated by electrophoresis.

**Sub-fraction 4a (8 mg)**

Partial hydrolysis gave galactose, aldobiouronic acids I, II and III and trisaccharide V.

**Sub-fraction 4b (6 mg)**

Hydrolysis gave arabinose, galactose and 6-0-(4-0-methyl-\(\beta\)-D-glucopyranosyluronic acid)-D-galactose. Hydrolysis of the glycitol gave galactitol in addition. The sub-fraction was methylated by the Kuhn procedure and the methanolyis products examined by g.l.c. The methyl glycosides of the following sugars were identified.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>T(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td>0.49, 0.63</td>
</tr>
<tr>
<td>2,3,4,6 Me₄ galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.30, 3.10</td>
</tr>
<tr>
<td>2,3,4 Me₃ galactose</td>
<td>6.36</td>
</tr>
</tbody>
</table>
Sugars (cont'd.)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>3.48, 3.95</td>
</tr>
<tr>
<td>2,5,6 Me₃ galactose</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Sub-fraction 5c (4 mg).

Hydrolysis gave arabinose, galactose and glucuronic acid. Hydrolysis of the glycitol gave the same reducing sugars.
PART II

ARAUCARIA BIDWILLII GUM
ARAUCARIA BIDWILLII GUM

Introduction

The resinous exudate from coniferous woods are generally terpenoid in nature. However that from the Australian bunya pine (Araucaria bidwillii) has been found to contain an additional polysaccharide component. It was the first reported polysaccharide exudate from a coniferous tree, and initial studies (71) showed it was composed of D-galactose, L-arabinose and L-rhamnose in the molar proportions of 13:3.5:1, together with acidic sugars which were later identified as D-glucuronic acid and 4-O-methyl-D-glucuronic acid.

Partial acid hydrolysis of the polysaccharide and separation of the acidic components led to the identification of two aldobiouronic acids. Aldobiouronic acid I was chromatographically identical to 6-0-(β-D-glucopyranosyluronie acid)-D-galactose. Hydrolysis of the sugar gave glucuronic acid and galactose, while hydrolysis of the carboxyl-reduced derivative gave glucose and galactose. The methylated product gave rise to 2,3,4-tri-O-methylglucuronic acid and 2,3,4-tri-O-methylgalactose on examination of the hydrolysate by paper chromatography and the methanolysate by gas chromatography. On the basis of the specific rotation, [α]D 0°, a β-D-glycosidic linkage was assigned to the oligosaccharide.

Oligosaccharide II was chromatographically identical to
6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose and gave 4-O-methylglucuronic acid and galactose on hydrolysis. Hydrolysis of the carboxyl-reduced disaccharide showed 4-O-methylglucose and galactose while periodate oxidation (122) of this hydrolysate gave 2-O-methylerythrose, a characteristic product of 4-O-methylglucose. Methylation gave the same products as aldobiouronic acid I, while the specific rotation, \([\alpha]_D +3^\circ\), was similarly consistent with a β-D-glycosidic linkage.

Under milder conditions of hydrolysis of the polysaccharide, neutral oligosaccharides with the chromatographic mobilities of 6-O-β-D-galactopyranosyl-D-galactose (major), 3-O-β-D-galactopyranosyl-D-galactose (minor) and 3-O-β-L-arabinopyranosyl-L-arabinose (trace) were detected.

Gas chromatographic examination of the methanalysis products from the methylated polysaccharide indicated the presence of the methyl glycosides of the following sugars: 2,3,4-tri-O-methylrhamnose (+), 2,3,5-(+++), 2,3,4-tri- (trace) and 2,5-di-O-methylarabinose (tr), 2,3,4,6-tetra- (++++), 2,3,4-(++) and 2,4,6-tri-(++) and 2,4-di-O-methylgalactose (++++) and 2,3,4-tri-O-methylglucuronic acid. In a separate hydrolysis of the methylated polysaccharide the acidic sugars were separated by ion-exchange chromatography on diethylaminoethyl Sephadex. Gas-liquid chromatography of the subsequent methanalysis identified the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid and 2,3,4-tri-O-
methylgalactose, thus indicating that the fully etherified aldobiouronic acid 6-0-(2,3,4-tri-O-methylglucopyranosyluronic acid)-2,3,4-tri-O-methylgalactose represented the major mode of linkage of acidic sugars in the polysaccharide. In particular, no di-0-methylglucuronic acid was identified.

The nature of the interior of the molecule was investigated by periodate oxidation. Degraded polysaccharides A and B were obtained after two sequences of the Smith degradation, involving periodate oxidation, reduction and mild hydrolysis. Investigation by hydrolysis and methylation indicated that these polysaccharides were approximating increasingly to linear (1→3) linked galactans.

From these studies it was clear that the structure of A. bidwillii gum was typical of the galactan group, and resembled that of gum arabic in many ways, except in the location of the L-rhamnose units which in gum arabic are known to be present as end-groups linked (1→4) to D-glucuronic acid residues. However, failure to detect 2,3-di-0-methylglucuronic acid as a cleavage product of the methylated polysaccharide appeared to rule out a similar arrangement in A. bidwillii gum.

In a subsequent investigation of another sample of the exudate of A. bidwillii, the carbohydrate portion of the gum was found to contain two polysaccharides, a neutral arabinogalactan and, as the major component, an acidic polysaccharide. Investigation of this acidic polysaccharide
indicated that it was of the same structural type as the one studied earlier. However the identification of 2,3-di-O-methylglucuronic acid as a significant cleavage product from the methylated polysaccharide reopened the question of the location of the L-rhamnopyranose end-groups. To establish the position of the L-rhamnose residues in the polysaccharide, an approach similar to that used on gum arabic was taken, namely acetylation of the carboxyl-reduced polysaccharide.

From the mixture of acetylation products a number of oligosaccharides were characterised, among which were

(i) $4\rightarrow L$-rhamnopyranosyl-$D$-glucose
(ii) $6\rightarrow D$-glucopyranosyl-$D$-galactose
(iii) $6\rightarrow (4\rightarrow D$-methyl-$D$-glucopyranosyl)-$D$-galactose
(iv) $0\rightarrow L$-rhamnopyranosyl-$(1 \rightarrow 4)$-$D$-glucopyranosyl-$(1 \rightarrow 6)$-$D$-galactose.

The isolation of oligosaccharides (i) and (iv) thus confirmed that $D$-glucuronic acid residues are $4\rightarrow$-substituted by $L$-rhamnopyranose units in the parent polysaccharide.

Oligosaccharides (ii), (iii) and (iv), however, cast doubt on the configurations of the glucuronosidic linkages in the polysaccharide. These were thought to be of the $\beta$-$D$-configuration, but the specific rotations of oligosaccharides (ii)-(iv) derived from the carboxyl-reduced gum were too high to be consistent with $\beta$-$D$-glucosidic linkages. Indeed, clear
evidence was presented that oligosaccharide (II) contained an α-β-glucosidic linkage.

Since inversion of the configuration of the glycosidic linkage is known to sometimes occur under the conditions used to achieve acetolysis, (39, 127) it seems likely that the configurations of the linkages in the oligosaccharides obtained after acetolysis of carboxyl-reduced A. bidwillii gum are not representative of those in the original polysaccharide. However, as the anomeric configurations of aldobiouronic acids I and II obtained as partial hydrolysis products of the polysaccharide were only inferred from the observed specific rotations, it was decided to reinvestigate these aldobiouronic acids in order to obtain more definite evidence for the configurations of their linkages.
Discussion

The water soluble portion of the gum (15) was subjected to partial hydrolysis with dilute mineral acid. Separation of the products by ion-exchange column chromatography and filter-sheet chromatography furnished two aldobiouronic acids.

Aldobiouronic acid I

This was identified as 6-0-(β-D-glucopyranosyluronic acid)-β-galactose. It was chromatographically identical to an authentic sample and gave glucuronic acid and galactose on hydrolysis. The n.m.r. signals assigned to the anomeric proton (99) (τ 5.39, J 7c/s) in the disaccharide glycitol corresponded to those of a β-β-glucuronoside. Methylation of the aldobiouronic acid furnished the crystalline fully methylated derivative which gave an identical melting point and X-ray powder photograph to those of an authentic sample.

Aldobiouronic acid II

This sugar was characterised as 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-β-galactose. It was chromatographically identical to an authentic sample of that oligosaccharide and gave 4-0-methylglucuronic acid and galactose on hydrolysis. Reduction of the derived methyl ester methyl glycosides with sodium borohydride, followed by hydrolysis, gave 4-0-methylglucose. The identity of the 4-methyl ether was established by chromatography of its periodate oxidation
products (122) which were indistinguishable from those derived from 4-\(O\)-methyl-\(D\)-mannose. Methylation of the aldobiouronic acid gave a crystalline derivative with melting point and X-ray powder photograph identical to those of an authentic sample of the methyl ester methyl glycoside hexamethyl ether of 6-\(O\)-(\(\beta\)-\(D\)-glucopyranosyluronic acid)-\(D\)-galactose. The n.m.r. spectrum of the disaccharide glycitol in deuterium oxide showed a doublet (\(\tau\) 5.39, \(J=7\)c/s) characteristic of an \(\alpha\)-\(D\)-anomeric proton and a three-proton singlet at \(\tau 6.50\) (\(-OCH_3\)).

From these results it is clear that both aldobiouronic acids obtained as partial hydrolysis products of Araucaria bidwillii gum contained \(\beta\)-\(D\)-glucuronosidic linkages. The \(\alpha\)-\(D\)-glycosidic linkages in the corresponding oligosaccharides obtained from acetolysis of the carboxyl-reduced gum must therefore be the result of anomeration during the acid-catalysed depolymerisation. Acetolysis results in the anomeration of a small proportion of the (1 \(\rightarrow\) 4) linked \(\beta\)-\(D\)-mannopyranosyl bonds in glucomannans (127) and galactomannans (39). However, the present studies confirm that anomeration occurs to a considerable extent when (1 \(\rightarrow\) 6) linkages are present.
Experimental

Araucaria bidwillii gum (40 g) was stirred overnight in water (500 ml). Insoluble material was removed by filtration and the polysaccharide (19 g) was precipitated by pouring the filtrate into ethanol (2 l). The precipitate was dried by solvent exchange.

The purified polysaccharide (16 g) was hydrolysed by refluxing in 1 N sulphuric acid (1 l) for four hours. The solution was cooled and neutralised with barium carbonate. Barium sulphate was removed by filtration and the filtrate, after concentration (100 ml), was deionised with Amberlite IR 120 (H) resin. This deionised solution was adsorbed on a column (20 x 3 cm) of diethylaminoethyl Sephadex A-25 (formate form). Neutral sugars were eluted with water and acidic sugars with 0.05 N formic acid (1.5 l). Concentration of the solution of acidic sugars gave a syrup (3.3 g) which was found by paper chromatographic examination to contain mainly glucuronic acid and oligosaccharides I and II. A portion of this syrup (1.5 g) was fractionated by filter sheet chromatography in solvent B to give these two oligosaccharides.

Oligosaccharide I (340 mg) \([\alpha]_D^2 -2^\circ\quad (c 1.0). \quad R_{Gal} (B) 0.23.\]

The oligosaccharide was chromatographically homogeneous and identical to 6-O-((\(\beta\)-D-glucopyranosyluronic acid))-D-galactose. Hydrolysis of a sample (2 mg) followed by paper
chromatographic examination of the hydrolysate showed the presence of glucuronic acid and galactose. The oligosaccharide (50 mg) was reduced to the glycitol with sodium borohydride. After destruction of excess borohydride and removal of borate, the glycitol was concentrated twice from deuterium oxide. N.m.r. spectroscopy of the glycitol in deuterium oxide showed a doublet due to the anomeric proton at 5.39, J-7c/s. The oligosaccharide (100 mg) was methylated with 30% sodium hydroxide and methyl sulphate and later with silver oxide and methyl iodide to give the methyl ester methyl glycoside hexamethyl ether of 6-0-(β-D-glucopyranosyluronic acid)-β-D-galactose, crystallised from chloroform-light petroleum, m.p. 90° and mixed m.p. 89-90° [with authentic sample (m.p. 89-90°)]. The X-ray powder photograph of the derivative was identical to that obtained from an authentic sample.

Oligosaccharide II (160 mg) [α]D 0° (c 1.0). RGal(B) 0.60.

The oligosaccharide was chromatographically homogeneous and identical to 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-β-D-galactose. Hydrolysis of a sample (2 mg) gave galactose and 4-0-methylglucuronic acid. The sugar (40 mg) was converted to the glycitol with sodium borohydride. After proton exchange with deuterium oxide the glycitol was examined by n.m.r. spectroscopy. The signal due to the anomeric
proton was observed as a doublet at $\tau$ 5.39 ($J=7e/s$) while there was a further three-proton singlet (-OCH$_3$) at $\tau$ 6.50. The glycitol was subsequently methanalysed in methanolic 4\% hydrogen chloride at 100° for 18 hours. After neutralisation and concentration, the syrup obtained was treated with sodium borohydride (30 mg) in water (10 ml). After destruction of excess borohydride and removal of borate, the sample was hydrolysed in H$_2$ sulphuric acid at 100° for four hours. Filter sheet chromatography of the hydrolysate yielded a fraction (5 mg) chromatographically identical to 4-0-methylglucose. The periodate oxidation products of this sugar were chromatographically indistinguishable from those obtained from a crystalline sample of 4-0-methyl-D-mannose. Oligosaccharide II was methylated with methyl sulphate and 30\% sodium hydroxide and later with methyl iodide and silver oxide to give the crystalline methyl ester methyl glycoside hexamethyl ether of 6-0-(B-D-glucopyranosyluronic acid)-D-galactose. m.p. 85-87° and mixed m.p. 85-87° [with authentic sample (m.p. 89-90°)]. The X-ray powder photographs of the derivative and authentic sample were identical.
PART III

CHERRY GUM
Chemistry of Cherry Gums

The gum from the English cherry tree (Prunus cerasus) has been extensively investigated by J. K. N. Jones. In initial experiments, he found that the gum acid consisted of L-arabinose (6 moles), D-galactose (2 moles), D-mannose (1 mole) and D-glucuronic acid (1 mole), together with a smaller amount of D-xylose (1.5%). In addition, the aldobiouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was formed as a product of acid hydrolysis of the gum.

The cleavage products from the methylated gum were found to include 2,3,5-tri- and 2,5-di-O-methyl-L-arabinose, 2,4,6-tri- and 2,4-di-O-methyl-D-galactose and 2,3,4-tri- and 2,3-di-O-methyl-D-glucuronic acid. However, because of the difficulty encountered in separating complex mixtures of methylated sugars by fractional distillation of methyl glycosides, other products, notably derivatives of D-mannose and D-xylose, were not identified.

It was found that under mild conditions of hydrolysis, all the L-arabinose and some of the D-xylose residues could be removed from the gum to give a degraded polysaccharide. The cleavage products from the methylated degraded gum were, after column chromatographic separation, identified as 2,3,4-tri- and 2,4-di-O-methyl-D-xylose, 2,3,4,6-tetra-, 2,4,6-tri-, 2,4- and 2,6-di-O-methyl-D-galactose and 2,3,4-tri- and 2,3-di-O-methyl-D-glucuronic
Subsequently the oligosaccharide 3-O-\(\beta-L\)-arabinopyranosyl-\(L\)-arabinose was found to be released on mild hydrolysis of the gum,\(^\text{(130)}\) while a second aldobiouronic acid, 6-O-(\(\beta-D\)-glucopyranosyluronic acid)-\(D\)-galactose, was also reported to be a partial acid hydrolysis product. However, despite these results, the general arrangement of sugar units within the polysaccharide is unknown, beyond the fact that the bulk of the \(L\)-arabinose residues occur as acid labile peripheral units.

The polysaccharides from other species of cherry trees have recently been examined.\(^\text{(58-60)}\) Although composed of similar monosaccharides, these gums all contained considerably larger proportions of \(D\)-galactose and less \(D\)-mannose than the English cherry gum. These other cherry gums gave 2-O-(\(\beta-D\)-glucopyranosyluronic acid)-\(D\)-mannose together with the homologous series of oligosaccharides \(\beta-D-GpAl+(6-\beta-D-Galpl-)_{n}+6-D-Cal\) \((n = 0-3)\) as products of partial hydrolysis. On the basis of these and other observations it was suggested that the structures of these polysaccharides were based on chains of \(\beta(1 \rightarrow 6)\) linked \(D\)-galactopyranose residues, which were 6-O-substituted at their non-reducing ends by \(D\)-glucuronic acid residues (or sometimes the 4-methyl ether), and branched at the 3-positions by single units of \(D\)-xylopyranose and perhaps longer sequences of \(L\)-arabinose. There was,
however, no indication as to the structural significance of $\text{d-mannose}$ in the gums.

The gum from another species of Prunus, namely apricot tree gum$^{(55)}$ was found to contain additional structural features, as represented by the oligosaccharides $\beta-\text{d-GpA}+(6-\beta-\text{d-Galp})_n \rightarrow \text{d-Gal} (n = 0-2)$, $(\text{d-Me}) \beta-\text{d-GpA}-(1 \rightarrow 6)-\text{d-Galp}+\text{l-Ara}$, $\text{d-Gal-} \text{d-Gal-} \text{l-Ara}$ and $\beta-\text{d-Galp}+(3-\beta-\text{d-Galp})_n \rightarrow \text{d-Gal} (n = 0, 1)$ which were characterised as partial hydrolysis products. In addition an acidic oligosaccharide was tentatively characterised as $\text{d-GpA}-(1 \rightarrow 2)-\text{Man}-(1 \rightarrow 2)-\text{Man}$. These oligosaccharides are broadly similar to ones already characterised as partial hydrolysis products of gum ghatti.

In the light of these results, the possibility arises that Prunus gums are in fact structurally related to the glucuronomannan group of polysaccharides as represented by gum ghatti and leiocarp A. Since a quantity of methylated cherry gum (provided by Professor J. K. N. Jones) was available, it was therefore decided to reinvestigate the cleavage products from the methylated gum both with a view to determining the constituent methylated monosaccharides present in the gum, and also, by examining the products of partial depolymerisation, to obtaining more information on the nature of the linkages involving uronic acids within the polysaccharide.
Discussion

A sample of methylated cherry gum was methanolysed and the cleavage products were examined by gas-liquid chromatography. A further sample was methanolysed under milder conditions, and after saponification of the methyl esters of acidic sugars present in the methanolysate with sodium hydroxide solution, the aqueous solution was extracted with chloroform. The chloroform extract was examined by g.l.c. while the aqueous solution, after neutralisation and concentration, was separated into neutral and acidic fractions by ion-exchange column chromatography. Both the acidic and neutral fractions were remethanolysed and examined by g.l.c. After extensive g.l.c. analysis of these fractions, peaks with the retention times of the methyl glycosides of the following methylated sugars were obtained: 2,3,4-tri- and 2,4-di-O-methylxylose, 2,3,5-, 2,3,4-tri- and 2,3-, 2,4- and 2,5-di-O-methylarabinose, 2,3,4-tri-O-methylrhamnose, 2,4,6- and 2,3,4-tri- and 2,4-di-O-methylgalactose, 3,4,6-tri-, 3,4- and 4,6-di-O-methylmannose and 2,3,4-tri- and 2,3-di-O-methylglucuronic acid.

A further quantity of methylated cherry gum was methanolysed under conditions which were thought to be sufficiently vigorous to give breakdown to mainly aldobiouronic acids and monosaccharides. After the methanolysate had been treated with sodium hydroxide solution, the sugars
present in the aqueous solution were adsorbed on a column of D.E.A.E. Sephadex. After elution of neutral sugars with water, acidic sugars were desorbed with formic acid solution. After concentration to small volume, the acidic solution was exhaustively extracted with ether. This extract was found to contain the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid. The acidic sugars remaining in the aqueous solution, Fraction A, were investigated (a) by methanolysis and (b) by methylation followed by methanolysis. The cleavage products from Fraction A were found, by g.l.c. of the methyl glycosides, to be 2,3,4-tri- and 2,3-di-O-methylglucuronic acid, 4,6-di-O-methylmannose and 2,4-di-O-methylgalactose. After methylation of Fraction A, the cleavage products were found to be 2,3,4-tri- and 2,3(tr)-4i-O-methylglucuronide, 3,4,6-tri-O-methylmannose and 2,3,4-tri-O-methylgalactose.

A quantity of Fraction A was hydrolysed and the neutral monosaccharides, after separation from the acidic monosaccharides by ion-exchange column chromatography, were fractionated preparatively by thick paper chromatography into the following components.

Fraction 1: This was chromatographically identical to 2,3,4-tri-O-methylgalactose. After methanolysis of the sample, g.l.c. gave a peak with the retention time of methyl 2,3,4-tri-O-methylgalactoside while methylation of the sample gave methyl 2,3,4,6-tetra-O-methylgalactoside.
**Fraction 2:** By paper chromatography of the sugar and g.l.c. of the derived glycoside, this component was identical to 4,6-di-0-methylmannose. Periodate oxidation of the sugar gave products indistinguishable from those obtained from a synthetic sample of 4,6-di-0-methylmannose while similar oxidation of the derived methyl glycosides followed by hydrolysis gave no trace of the original sugar on subsequent paper chromatography. The sugar was finally treated with one mole of lead tetra-acetate. The product was identified as 3,5-di-0-methylarabinose by g.l.c. both of the derived methyl glycosides and of the derived aldonolactone.

**Fraction 3:** This was chromatographically identical to 2,4-di-0-methylgalactose and gave galactose on demethylation.

**Fraction 4:** The sugar was chromatographically identical to 4-0-methylmannose and on demethylation gave mannose. Oxidation of the sugar with periodate and one mole of lead tetra-acetate in both cases gave products indistinguishable from those obtained by similar treatments of a sample of crystalline 4-0-methyl-α-mannose.

**Fraction 5:** This sugar was chromatographically identical to 2-0-methylgalactose and gave galactose on demethylation. Periodate oxidation of this sugar and 2-0-methylgalactose gave identical products, while treatment with one mole of lead tetra-acetate failed to degrade the sugar.

The remainder of Fraction A was separated by filter
sheet chromatography into two principal fractions, 6 and 7. 

**Fraction 6:** Methanolysis of this fraction gave the methyl glycosides of 4,6-di-Ω-methylmannose and 2,3,4(tr)- and 2,3-di-Ω-methylglucuronic acid. After methylation of the fraction, the methanolysis products were found to consist of approximately equal amounts of the glycosides of 3,4,6-tri-Ω-methylmannose and 2,3,4-tri-Ω-methylglucuronic acid.

**Fraction 7:** Methanolysis of the fraction gave the methyl glycosides of 4,6-di-Ω-methylmannose and 2,3-di-Ω-methylglucuronic acid. The methylated fraction gave as cleavage products 3,4,6-tri-Ω-methylmannose and 2,3,4-tri-Ω-methylglucuronic acid in the ratio of 2:1, together with a smaller amount of 2,3-di-Ω-methylglucuronic acid.

From the relative amounts of sugars present in Fractions 1-5, it is likely that the principal modes of linkage of D-glucuronic acid in cherry gum are represented by the partial structures (i) and (ii). Other structural features probably also present are (iii)-(v).

\[
\begin{align*}
\ldots \text{4)GA 1} & \pm 2 \text{Man 1-} \\
\text{3} & \uparrow \\
\ldots \text{4)GA 1} & \pm 6 \text{Gal 1-} \\
\text{3} & \uparrow \\
\text{(i)} & \\
\uparrow \\
\text{6} \\
\ldots \text{4)GA 1} & \pm 2 \text{Man 1-} \\
\text{3} & \uparrow \\
\ldots \text{4)GA 1} & \pm 6 \text{Gal 1-} \\
\text{3} & \uparrow \\
\text{(iii)} \\
\text{4)GA 1} & \pm 6 \text{Gal 1-} \\
\text{3} & \uparrow \\
\text{(iv)} \\
\text{4)GA 1} & \pm 6 \text{Gal 1-} \\
\text{3} & \uparrow \\
\text{(v)}
\end{align*}
\]
Since the oligosaccharides in Fractions 6 and 7 were present as their methyl glycosides it was not possible to establish their degrees of polymerisation. It is likely, however, that Fraction 6 was a disaccharide and that Fraction 7 was a tri- or tetrasaccharide. The cleavage products from these oligosaccharides and their methylated derivatives suggest that the D-glucuronic acid residues associated with the di-O-substituted D-mannose units are themselves 4-O-substituted, and that a significant structural feature of the gum is represented in the partial structure (vi).

\[ \rightarrow 4 \text{ GA} \rightarrow 2 \text{ Man} \rightarrow 3 \]

(vi)

In an attempt to obtain partially methylated acidic oligosaccharides containing D-mannose, methylated cherry gum was hydrolysed in dilute mineral acid using dioxan as a diluent to maintain the solubility of the polysaccharide. The hydrolysis products were adsorbed on a column of diethylaminoethyl Sephadex, and after neutral sugars had been eluted with water, acidic sugars were desorbed (in comparatively low yield) by gradient elution with formic acid solution. The mixtures of acidic oligosaccharides so obtained were further separated by preparative scale paper chromatography into the following fractions.
Fraction 8: This fraction contained 2,3,4-tri-O-methyl-
glucuronic acid.

Fraction 9: The bulk of this fraction was composed of
2,3-di-O-methylglucuronic acid.

Fraction 10: This fraction also contained some 2,3-di-O-
methylglucuronic acid although the major constituent was
characterised as the partially methylated aldobiouronic acid
6-O-(2,3,4-tri-O-methylglucopyranosyluronic acid)-2-O-
methylgalactose.

The identification of this oligosaccharide both confirms
that the aldobiouronic acid unit 6-O-(D-glucopyranosyluronic
acid)-D-galactose is present in cherry gum, and that the
uronic acid in this unit occupies a terminal position in the
polysaccharide molecule, as represented in structure (vii).

\[
\begin{align*}
\text{GA} \quad & \quad \text{Gal} \\
4 & \quad 3
\end{align*}
\]

(vii)

Fraction 11: From its low chromatographic mobility and the
variety of cleavage products derived from it, this fraction
was thought to contain a mixture of tri- or tetrasaccharides.
The main cleavage products from the fraction were identified
as 2,3,4-tri-O-methylglucuronic acid and 2,4,6-tri-, 2,4-di-
and 2-O-methylgalactose, while 2,3,4-tri-O-methylglucuronic
acid and 2,3,4,6-tetra-, 2,4,6- and 2,3,5-tri- and 2,4-di-O-
methylgalactose were observed after depolymerisation of the methylated fraction. These cleavage products also indicate that these oligosaccharides, some of which must be branched, contain 3-0-substituted galactose residues.

**Fraction 12:** It is likely that this fraction contained a mixture of tetra- or pentasaccharides. In addition to the cleavage products detected from Fraction 11, this fraction gave rise to 2,3,4-tri- and 2,4-di-0-methylxylose. After methylation of the fraction, the cleavage products contained 2,3,4-tri-0-methylxylose as the only xylose derivative, together with other products similar to those derived from methylated Fraction 11. The following structural features are thus present in Fraction 12.

\[
\begin{align*}
\text{GA} & \quad \text{Gal} \\
\rightarrow & \quad 3 \text{ Gal} \\
\rightarrow & \quad 6 \text{ Gal} \\
& \quad 3 \text{ Gal} \\
\rightarrow & \quad 4 \text{ Xyl}
\end{align*}
\]

From the results of the partial hydrolysis of the methylated gum it can be seen that no mannose-containing oligosaccharides were encountered as had been hoped, and it can only be assumed that the hydrolysis conditions employed were insufficiently vigorous to cause the necessary degree of depolymerisation of the polysaccharide. This view is supported
both by the low yield of acidic oligosaccharides isolated and by the comparatively high degree of polymerisation of some of the fragments obtained.

In the acidic oligosaccharides obtained, glucuronic acid was glycosidically linked to galactose units. Furthermore, these uronic acid residues were present exclusively as non-reducing end-groups in the polysaccharide. This observation would, therefore, suggest that the 4-O-substituted glucuronic acid units known to be present in the polysaccharide are involved in glycosidic linkages to mannose residues. Such a conclusion has indeed already been drawn as a result of the investigations on Fractions 6 and 7. It is thus probable that while the glucuronosylgalactose units are present as the ends of the outer chains of the cherry gum molecule, the glucuronosylmannose units are involved in linkages in the interior of the polysaccharide. This is similar to the arrangement of these units in leiocarpan A, as represented in Figure 32.
Indications that the structures of the outer galactose-containing chains of cherry gum and leiocarpan A may be similar comes from examination of Fractions 11 and 12, since the cleavage products from these Fractions showed the presence of linkages similar to those contained within the outer chains of leiocarpan A. The available evidence, however, suggests that in leiocarpan A the β-xylopyranose units are linked to the β-mannose residues in the interior chains, whereas the identification of xylose derivatives in Fraction 12 means that in cherry gum, xylose is linked to the outer galactose-containing chains.
Experimental

Methanolysis of Methylated Cherry Gum

Methylated cherry gum (10 mg) was methanolysed by heating at 100° for 18 hours in methanolic 4% hydrogen chloride. After neutralisation and concentration of the solution, the products were examined by g.l.c. Peaks with the retention times of the methyl glycosides of the sugars listed in Table 20 were obtained.

Table 20

<table>
<thead>
<tr>
<th>Methylated sugars</th>
<th>Retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>column a</td>
</tr>
<tr>
<td>2,3,4 Me₃ rhamnose</td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me₃ xylose</td>
<td>0.40, (0.49)</td>
</tr>
<tr>
<td>2,3,5 Me₃ arabinose</td>
<td>(0.49), 0.63</td>
</tr>
<tr>
<td>2,3,4 Me₃ arabinose</td>
<td>0.83</td>
</tr>
<tr>
<td>2,5 Me₂ arabinose</td>
<td>(1.50), (2.69)</td>
</tr>
<tr>
<td>2,3 Me₂ arabinose</td>
<td>1.18, (1.50)</td>
</tr>
<tr>
<td>2,4 Me₂ arabinose</td>
<td></td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>3.48, 4.02</td>
</tr>
<tr>
<td>2,4 Me₂ galactose</td>
<td>13.1, 15.0</td>
</tr>
<tr>
<td>3,4,6 Me₃ mannose</td>
<td>(2.69)</td>
</tr>
<tr>
<td>3,4 Me₂ mannose</td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.28, 2.99</td>
</tr>
<tr>
<td>2,3 Me₂ glucuronic acid</td>
<td></td>
</tr>
</tbody>
</table>
The methanolysate was subsequently hydrolysed and the products were converted to the corresponding derived aldono-lactones by oxidation with bromine water. The following compounds were identified by g.l.c.

<table>
<thead>
<tr>
<th>Aldonolactones</th>
<th>T (column b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ xylonolactone</td>
<td>3.52</td>
</tr>
<tr>
<td>2,3,4 Me₃ arabinolactone</td>
<td>3.72</td>
</tr>
<tr>
<td>2,3,5 Me₃ arabinolactone</td>
<td>1.74</td>
</tr>
<tr>
<td>2,5 Me₂ arabinolactone</td>
<td>14.75</td>
</tr>
<tr>
<td>2,3 Me₂ arabinolactone</td>
<td>8.05</td>
</tr>
</tbody>
</table>

A further quantity of methylated cherry gum (1 g) was heated under reflux in methanolic 4% hydrogen chloride for 5 hours. After neutralisation and concentration of the solution, the resultant syrup was dissolved in water (50 ml) and the solution was cooled to 0° and made pH 12 with sodium hydroxide. After two hours the pH of the solution was lowered to 10 and the solution was then continuously extracted overnight with chloroform. After drying over anhydrous sodium sulphate the extract was concentrated and examined by g.l.c. Peaks with the retention times of the methyl glycosides of the neutral sugars listed in Table 20 were obtained, together with a small amount of methyl 2,3,4-tri-O-methylgalactoside.
The aqueous solution, after the extraction with chloroform, was neutralised with Amberlite IR 120 (H) resin and after concentration to small volume, was applied to a column of Duolite A4 (OH). Neutral glycosides were eluted with water and the solution was concentrated. Hydrolysis of this fraction showed the presence of mainly 2,4-di-O-methylgalactose and 3,4-di-O-methylmannose on paper chromatographic examination in solvents E and F, together with a considerable amount of material with the mobility of mono-O-methyl hexoses.

The glycosides of acidic sugars were eluted from the Duolite column with N sodium hydroxide (200 ml). After neutralisation with Amberlite IR 120 (H) resin, the solution was evaporated to dryness and a portion of the product was methanolysed. G.l.c. of the methanolysate indicated the presence of the methyl glycosides of the sugars listed in Table 22.

Table 22
Cleavage Products from Acidic Fraction

<table>
<thead>
<tr>
<th>Methylated sugars</th>
<th>Retention times</th>
<th>column a</th>
<th>column a (125°)</th>
<th>column b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4 Me₃ xylose</td>
<td>0.63, 0.49</td>
<td>0.38, 0.48</td>
<td>0.47, 0.59</td>
<td></td>
</tr>
<tr>
<td>2,4 Me₂ xylose</td>
<td>1.16, 1.53</td>
<td>1.13, 1.60</td>
<td>1.41, 1.86</td>
<td></td>
</tr>
<tr>
<td>2,4,6 Me₃ galactose</td>
<td>3.50, 4.04</td>
<td>3.86, 4.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4,6 Me₃ mannose</td>
<td>2.64</td>
<td></td>
<td>(2.97)</td>
<td></td>
</tr>
<tr>
<td>4,6 Me₂ mannose</td>
<td>9.86</td>
<td></td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>2,3,4 Me₃ glucuronic acid</td>
<td>2.30, 2.99</td>
<td></td>
<td>2.38, 2.97</td>
<td></td>
</tr>
<tr>
<td>2,3 Me₂ glucuronic acid</td>
<td>6.7, 7.4, 9.0</td>
<td></td>
<td>7.63, 8.3, 10.1</td>
<td></td>
</tr>
</tbody>
</table>
Partial Methanolysis of Methylated Cherry Gum

Methylated cherry gum (0.50 g) was heated with methanolic 4% hydrogen chloride (40 ml) in a sealed tube in a boiling water bath for 5 hours. After neutralisation and concentration of the solution the product was redissolved in sodium hydroxide solution (50 ml, pH 12) which, after two hours, was extracted with chloroform. The aqueous solution was neutralised with Amberlite IR 120 (H), and after reduction in volume, was applied to column of D.E.A.E. Sephadex A-25 (formate form). The glycosides of neutral sugars were removed by elution with water, while elution with 0.5 M formic acid (300 ml) desorbed acidic compounds. The acid fraction was concentrated (50 ml) and continuously extracted overnight with ether. Both the ethereal and aqueous solutions were concentrated to yield the ether extract (150 mg) and Fraction A (310 mg).

Ether extract

G.l.c. examination of the methanolysis products of both the extract and methylated extract showed that the fraction contained only the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid. Treatment with methanolic ammonia yielded a crystalline amide which, after recrystallisation from ether, had m.p. 175-8°.

Fraction A

After methanolysis of the fraction, the methyl glycosides of the following sugars were detected by g.l.c.
2,3,4-tri-0-methylglucuronic acid  
T(a) 2.29, 2.98

2,3-di-0-methylglucuronic acid  
T(a) 6.50, 7.21, 8.73

4,6-di-0-methylmannose  
T(a) 9.40

2,4-di-0-methylgalactose  
T(a) 13.0, 15.0

After methylation of the fraction the glycosides of the following sugars were detected as cleavage products.

2,3,4-tri-0-methylglucuronic acid  
T(a) 2.29, 2.98  T(b) 2.45, (3.11)

2,3-di-0-methylglucuronic acid(tr)  
T(a) 7.29, 8.81

3,4,6-tri-0-methylmannose  
T(a) 2.67  T(b) 3.11

2,3,4-tri-0-methylgalactose  
T(a) 6.35  T(b) 6.98

Fraction A (200 mg) was hydrolysed in H hydrochloric acid (25 ml) at 100°C for 12 hours. The hydrolysis products were then applied to a column of D.E.A.E. Sephadex A-25 and the neutral sugars were eluted with water. Concentration of this solution yielded a syrup (80 mg) which was separated by filter sheet chromatography, in solvent E into the following fractions.

Fraction 1 (5 mg) Rg (E) 0.73. The sugar was chromatographically identical to 2,3,4-tri-0-methylgalactose, while the derived methyl glycosides had the same retention time on g.l.c. as methyl 2,3,4-tri-0-methylgalactoside. Methylation of the glycosides gave methyl 2,3,4,6-tetra-0-methylgalactoside.

Fraction 2 (28.5 mg) Rg (E) 0.63, [α]D +36° (c 2.0). By g.l.c. of the derived glycosides and paper chromatography of the sugar, the fraction was identical to 4,6-di-0-methyl-
mannose. Demethylation of the sugar gave mannose. The periodate oxidation products of the sugar were chromatographically indistinguishable from those derived from synthetic 4,6-di-\text{O}-methylmannose. The derived glycosides were also treated with periodate. After destruction of the periodate, hydrolysis gave no trace of the original sugar.

The sugar (3 mg), in glacial acetic acid (1 ml), was added to a solution of lead tetra-acetate (10 mg) in glacial acetic acid (1 ml). After 1/2 hour, lead was precipitated by treatment with hydrogen sulphide and the solution was filtered and evaporated to dryness. Methanolysis of the residue gave methyl 3,5-di-\text{O}-methylarabinosid., $T(a)$ 0.82, 1.93, while hydrolysis of this product followed by bromine oxidation gave 3,5-di-\text{O}-methylarabonolactone, $T(b)$ 5.30.

**Fraction 3** (5 mg) $R_g$ (E) 0.49. This fraction was identical by paper chromatography of the sugar and g.l.c. of the derived methyl glycosides to 2,4-di-\text{O}-methylgalactose. Demethylation gave galactose.

**Fraction 4** (6 mg) $R_g$ (E) 0.35. The sugar was chromatographically identical to 4-\text{O}-methylmannose while demethylation gave mannose. Periodate oxidation of the sugar gave identical products to those derived from an authentic sample of the sugar. Oxidation of the sugar with excess lead tetra-acetate for 1/2 hour followed by removal of lead and saponification gave a single product, $R_g$ (E) 0.57, which was chromatographi-
cally identical to that obtained by a similar treatment of 4-O-methylmannose, product 2-O-methylerythrose.

**Fraction 5** (10 mg) \( R_g (E) = 0.25 \). This sugar was chromatographically identical to 2-O-methylgalactose and on demethylation gave galactose. Periodate oxidation gave products identical to those obtained from a crystalline sample while treatment of the sugar with 2 moles of lead tetra-acetate followed by removal of lead and saponification gave the original sugar as the only product.

The remainder of fraction A was separated by filter sheet chromatography in solvent H, using the aniline-xylose spray reagent to detect the components, into two main fractions.

**Fraction 6** (38 mg) \( R_g (H) = 0.60 \). Methanolysis of this fraction gave the methyl glycosides of the following sugars

- 2,3,4-tri-O-methylglucuronic acid \( \text{tr} \) \( T(a) = 2.27, 2.96 \)
- 2,3-di-O-methylglucuronic acid \( T(a) = 6.56, 7.27, 8.85 \)
- 4,6-di-O-methylmannose \( T(a) = 9.60 \).

After methylation of the fraction, the cleavage products were identified by g.l.c. as the methyl glycosides of

- 2,3,4-tri-O-methylglucuronic acid \( T(a) = 2.27, 3.03 \)
- 3,4,6-tri-O-methylmannose \( T(a) = 2.63 \)

in the ratio 1.2:1, as measured by the respective peak areas.

**Fraction 7** (12 mg) \( R_g (H) = 0.33 \). Methanolysis of the fraction gave the methyl glycosides of 2,3-di-O-methylglucuronic acid, \( T(a) = 6.60, 7.29, 8.90 \) and 4,6-di-O-methylmannose, \( T(a) = 9.65 \).
The fraction was methylated and the cleavage products were examined by g.l.c. The methyl glycosides of the following sugars were detected in the ratio 1:0.4:1.8.

2,3,4-tri-O-methylglucuronic acid \( T(a) \) 2.30, 2.98
2,3-di-O-methylglucuronic acid \( T(a) \) 6.61, 7.30, 8.90
3,4,6-tri-O-methylmannose \( T(a) \) 2.67
Partial Hydrolysis of Methylated Cherry Gum

Methylated cherry gum (30 g) was heated for 5 hours under reflux (96°) in N sulphuric acid (1.5 %) containing dioxan (900 ml). After neutralisation with barium carbonate, the solution was filtered and the filtrate was concentrated (100 ml) and deionised with Amberlite IR 120 (H) resin. The deionised solution was then adsorbed on a column of D.E.A.E. Sephadex A-25 (3 x 30 cm, formate form). Neutral sugars were removed by eluting the column with water. The column was then eluted with water containing increasing concentrations of formic acid and the following fractions were collected.

<table>
<thead>
<tr>
<th>Conc. of eluant</th>
<th>Fraction</th>
<th>Weight</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.045 M (2 %)</td>
<td>B</td>
<td>2.1 g</td>
<td>8, 9, 10, 11</td>
</tr>
<tr>
<td>0.045-0.06 M (1.5 %)</td>
<td>C</td>
<td>520 mg</td>
<td>8, 9, 10, 11</td>
</tr>
<tr>
<td>0.06-0.10 M (1.5 %)</td>
<td>D</td>
<td>309 mg</td>
<td>10, 11, 12</td>
</tr>
<tr>
<td>0.10 M (1 %)</td>
<td>E</td>
<td>105 mg</td>
<td>12</td>
</tr>
<tr>
<td>0.15 M (1.5 %)</td>
<td>F</td>
<td>321 mg</td>
<td>12 + immobile</td>
</tr>
</tbody>
</table>

Fractions B-E were separated into individual components by preparative scale paper chromatography in solvent H.

Fraction B (1.0 g) was separated into fractions 8 (800 mg), 9 (45 mg), 10 (10 mg) together with a trace of fraction 11.

Fraction C (250 mg) gave fractions 8 (105 mg), 9 (17 mg), 10 (33 mg) and 11 (30 mg).
Fraction D (50 mg) gave fractions 10 (11 mg), 11 (12 mg) and 12 (9 mg).
Fraction E (100 mg) gave fraction 12 (31 mg) together with material which streaked on the chromatograms and was discarded. 
Fraction F contained mainly chromatographically immobile material together with a component which was chromatographically similar to fraction 12.
Fraction G contained no chromatographically mobile components.

Examination of fractions 8-12
Fraction 8 R<sub>G</sub> (H) 0.96. This fraction was chromatographically identical to 2,3,4-tri-O-methylglucuronic acid and on methanolysis gave only the methyl glycosides of that sugar.
Fraction 9 R<sub>G</sub> (H) 0.70. The fraction was chromatographically identical to 2,3-di-O-methylglucuronic acid. A sample was methanolysed and the products were examined by g.l.c. Peaks with the retention times of 2,3,4(tr)- and 2,3-di-O-methylglucuronic acid and 2,3,4-tri-O-methylgalactose were obtained.
Fraction 10 R<sub>G</sub> (H) 0.58. Methanalysis of the fraction gave the methyl glycosides of 2,3,4-tri- and 2,3(tr)-di-O-methylglucuronic acid while hydrolysis gave mainly 2,3,4-tri-O-methylglucuronic acid and 2-O-methylgalactose together with trace amounts of 2,3-di-O-methylglucuronic acid and 2,4-di-O-methylgalactose. Borohydride reduction of the fraction followed by hydrolysis gave only 2,3,4-tri-O-methylglucuronic acid. The fraction was methylated by the Kuhn procedure and after
methanalysis of the products, the methyl glycosides of the following sugars were detected by g.l.c.

2,3,4-tri-O-methylglucuronic acid $T(a)$ 2.30, 3.05
2,3,5-tri-O-methylgalactose $T(a)$ 3.96
2,3,4-tri-O-methylgalactose $T(a)$ 6.70

Fraction 11 $R_g (H)$ 0.26. The methanalysis products from this fraction were found by g.l.c. to include the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid, 2,4,6- and 2,3,4(tr)-tri-O-methylgalactose. Hydrolysis gave in addition 2,4-di- and 2-O-methylgalactose. The methyl glycosides of the following sugars were detected by g.l.c. as cleavage products from the methylated fraction.

2,3,4-tri-O-methylglucuronic acid $T(b)$ 2.51, 3.33
2,3,4,6-tetra-O-methylgalactose $T(b)$ 1.92
2,4,6-tri-O-methylgalactose $T(b)$ 4.25, 5.03
2,3,5-tri-O-methylgalactose $T(b)$ 4.80
2,3,4-tri-O-methylgalactose $T(b)$ 8.23

In addition 2,4-di-O-methylgalactose was detected by paper chromatography after hydrolysis of the mixture of glycosides.

Fraction 12 $R_g (H)$ 0.08. G.l.c. examination of the methanalysis products from this fraction gave peaks with the retention times of the methyl glycosides of the following methylated sugars.
After hydrolysis of the fraction, 2,4-di- and 2-O-methylgalactose were detected by paper chromatography. The fraction was methylated by the Kuhn procedure. After methanolysis, glycosides of the following sugars were identified by g.l.c.

2,3,4-tri-O-methylglucuronic acid T(a) 2.28, 2.95
2,3,4-tri-O-methylxylose T(a) 0.42, 0.53
2,4-di-O-methylxylose T(a) 1.16, 1.50
2,4,6-tri-O-methylgalactose T(a) 3.50, 4.06
2,3,4-tri-O-methylgalactose T(a) 6.67

2,4-Di-O-methylgalactose was also detected as a cleavage product of the methylated fraction by paper chromatographic examination of the hydrolysis products.
ACKNOWLEDGMENTS

I should like to express my gratitude and sincere thanks to Professor G. O. Aspinall for the advice, encouragement and invaluable guidance he has given throughout the course of these investigations. I should also like to thank Dr. D. A. Rees for his help and Professor Sir Edmund Hirst, C.B.E., for his interest and provision of facilities to carry out this work during the period 1966-68. I am grateful to the Institute of Brewing for financial assistance received during that period. I also thank Trent University, Peterborough, Ontario, for facilities and the National Research Council of Canada for financial assistance for the year 1968-69.
Bibliography (cont'd.)


32. I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith,
    Abs. papers, 135th Amer. Chem. Soc. Meeting, Boston,
    April, 1959, p. 3D.


34. F. Smith, and R. Montgomery, "The Chemistry of Plant Gums
Bibliography (cont'd.)


50. E. V. White, J. Amer. Chem. Soc., (1941), 63, 2871; (1942), 64, 302; 1507; 2838.
Bibliography (cont'd.)


Bibliography (cont'd.)


Bibliography (cont'd.)

110. Block, Durrum, and Zweig, "A manual of paper chromato-
graphy and paper electrophoresis", Academic Press Inc.,


44, 1939.

(1950), 72, 677.


388.


67, 32.

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

(1950), 1702.

121. W. J. Whelan, and K. Morgan, Chem. and Ind., (1955), 33,
1449.

814.

123. R. Belcher, and A. L. Godbert, "Semi-micro Quantitative
Bibliography (cont'd.)


