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THE MOLECULAR STRUCTURE OF EXUDATE GUMS

WITH SPECIAL REFERENCE TO

GUMS OF THE STERCULIA GENUS

by

GEORGE R. SANDERSON

A thesis presented for the degree of Doctor of Philosophy

University of Edinburgh

September, 1967.
TO MY PARENTS AND SYLVIA
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Structural features of Sterculia gums
Relationship of Sterculia gums with other polysaccharides

DISCUSSION

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Partial acetolysis of Sterculia urens gum
NMR spectroscopy of acidic oligosaccharides
Oxidative degradations of 2-O-(\alpha-D-galactopyranosyluronic acid)-L-rhamnose
Preparation and methylation of carboxyl-reduced Sterculia urens gum
Smith degradation of carboxyl-reduced Sterculia urens gum
Partial acetolysis of carboxyl-reduced Sterculia urens gum
Conclusions

EXPERIMENTAL

General methods
Partial acidic hydrolysis
**EXPERIMENTAL (contd.)**

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</tbody>
</table>
INTRODUCTION
INTRODUCTION

General.

The term 'gum', in its broadest sense, refers to both hydrophobic and hydrophilic substances of high molecular weight which usually exhibit colloidal properties when dispersed in an appropriate solvent. Hydrophobic substances often called gums include high molecular weight hydrocarbons and other petroleum products, rubbers, certain synthetic polymers and resinous saps which often exude from evergreens.

More specifically, the term gum applies to plant polysaccharides or their derivatives which are dispersible in either cold or hot water to produce viscous solutions or suspensions. As much as three-quarters of the dry weight of plants may be polysaccharide and, consequently, such substances are of wide occurrence. The most important gums, however, are those which are readily obtainable in large amounts from the plant. Some of these gums are used industrially and, indeed, many have been known since ancient times. One of the chief sources of such polysaccharides is seaweed which furnishes agar, algin and carrageenin while seed gums, such as gum guar and locust bean gum, are also important, particularly from the point of view that the plant which produces the seeds is often grown extensively as a food crop. In contrast to these naturally occurring gums, other gums are obtained from cellulose, one of the main components of the plant cell wall, and starch, a food reserve polysaccharide, by esterification and etherification. Commercially, however, the most important gums are plant exudates and most plant families have been found to include species which exude gums to a greater or lesser degree.

In this context, the term 'exudate gum' strictly refers to those commercially important gums which exude in copious amounts from shrubs or low-growing trees, forming, on exposure to the atmosphere, glossy nodules or flakes which are usually
brown or yellow in colour. These gum producing trees grow predominantly in
Africa or Asia indicating the climatic requirements for their growth.

The most likely function of gum formation is to prevent infection of
the plant tissue and to prevent loss of moisture (1). This is evident from
the fact that tapping of the trees immediately results in the sealing off of
the exposed area by the gum and the tree continues to grow without apparent
damage.

Several suggestions have been made as to the origin of these gums. They
may be products of normal plant metabolism but healthy Acacia trees, grown under
favourable conditions of moisture, soil and temperature do not produce any gum,
while trees which are grown under adverse conditions do secrete gum (2).

A most challenging problem to the chemist is the mode of formation of these
gums. This requires a knowledge of the complex processes whereby they are first
of all synthesised in the plant from simple compounds and then transported as
required from the site of formation to the injured site. Work on the early stages
of biosynthesis concerning the reactions involved in the transformations of
monosaccharides, the building units of polysaccharides, into one another and
into derivatives capable of enzymic polymerisation has been reviewed by J.K.N.
Jones (3) and by Neufeld and Hassid (4). At present, little is known of the
reactions involved in the enzymic polymerisation stage of complex hetero-
polysaccharide biosynthesis although, in this respect, recent advances have
been made in the biosynthesis of complex lipopolysaccharides from the cell wall
of Gram-negative bacteria (5). Clearly, a better understanding of the problem
would be obtained from a knowledge of the structural relationships between
different polysaccharides such as cellulose, hemicelluloses, pectins and gum
exudates and between individual polysaccharides at different stages in their
formation.
Classification of exudate gums.

Until recently, gums were classified according to their behaviour when treated with water. This classification gave rise to two groups of gums. The first group, which included gum arabic, mesquite, damson and cherry gums, dissolved completely in water, while the second group were only partly soluble in water and usually swelled to give very viscous solutions. This latter group was typified by gum tragacanth. This classification was of limited application, however, since many gums did not readily fall into either of these groups.

Another classification was based on the nature of the acidic sugar present in the gum. Acacia gums, including gum arabic, were found to differ in the nature of their neutral sugar components, but each contained $\text{D-}$glucuronic acid while gum tragacanth and linseed and slippery elm mucilages were each found to contain $\text{D-}$galacturonic acid (6). This classification does not, however, include gums composed only of neutral sugars or those seaweed polysaccharides such as agar and carrageenan, which contain sulphate groups. Now it is of little value as it has since been shown that Khaya gums, and Sterculia gums contain both $\text{D-}$glucuronic and $\text{D-}$galacturonic acids (7,8).

At present, plant polysaccharides in general are classified on a structural basis (9,10) and may be divided into groups of related polysaccharides, the members of each group having certain common structural features. In general, the structural feature shared by polysaccharides of a particular group is a similar arrangement of sugar residues in the basal chains of the molecules. Such polysaccharides may however differ quite markedly in other aspects of their molecular structure, notably in the arrangement of the sugar units in the outer chains of branched polysaccharides. Thus, within a particular group, it is not surprising to find polysaccharides which are obtained from vastly
different sources. For example, the most intensively studied groups of structurally related polysaccharides are the xylan, mannan-glucomannan, and arabinogalactan groups. Each of these includes, on the one hand, polysaccharides which are generally regarded as hemicelluloses and, on the other hand, polysaccharides which are more frequently referred to as cereal gums, exudate gums or mucilages. Fig. 1. gives a partial classification of various groups of polysaccharides and also indicates the categories of plant products within which the members of each group are commonly included.
<table>
<thead>
<tr>
<th>POLYSACCHARIDES</th>
<th>H</th>
<th>CG</th>
<th>EG</th>
<th>P</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>(11,12) Xylans</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinoxylans</td>
<td>H</td>
<td>CG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\pm)Ac)(4-O-methyl)Glucuronoxylans</td>
<td>H</td>
<td></td>
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<td></td>
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<tr>
<td>Arabino (4-O-methyl)glucuronoxylans</td>
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<td></td>
<td>EG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex xylans</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11,12) Mannans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>Galactomannans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>((\pm)Ac)Glucomannans</td>
<td>H</td>
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<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
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<td>H</td>
<td></td>
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<tr>
<td>(11,13-11,14) Arabinogalactans</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glucuronoarabinogalactans</td>
<td>H</td>
<td></td>
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<tr>
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<td></td>
<td>EG</td>
<td></td>
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<td>(and other complex acidic polysaccharides)</td>
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<tr>
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<td></td>
<td>H</td>
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<tr>
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<td>EG</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
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<td>P</td>
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<td>EG</td>
<td></td>
<td></td>
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<tr>
<td>Complex galacturonorhamnans</td>
<td></td>
<td></td>
<td>EG</td>
<td>P</td>
<td>M</td>
</tr>
</tbody>
</table>

Fig. 1. A structural classification of plant heteropolysaccharides.

H = hemicellulose; CG = cereal gum; EG = exudate gum; P = pectic substance; M = mucilage.
Application of this classification to exudate gums permits the recognition of three distinct groups of polysaccharides of which sufficient is known to permit reasonably detailed structural formulations. It should be stressed that these groups may need to be extended, or other groups recognized, to accommodate the discovery of new polysaccharides with further variations in structure, or to accommodate well known exudate gums such as mesquite gum (16), cholla gum (16,17) and the various Prunus gums (16). These latter gums have been investigated to some extent but, as yet, evidence is not sufficient to provide a clear indication of which sequences of sugar units comprise the basal chains of the polysaccharides and which are attached as side chains to these interior chains.

Gums of the Acacia genus provide examples of the first group of exudate gums and are referred to as galactans since each polysaccharide contains a branched core of $\beta-D$-galactopyranose residues mutually joined by 1-3' and 1-6' linkages. The 1-3' linkages are found to predominate in the interior chains while the 1-6' linkages are concentrated in the outer chains. These polysaccharides vary to some extent, however, in the degree of branching of the galactan core and also in the sites of attachment of the peripheral L-arabinofuranose and L-rhamnopyranose residues and in the nature of the more complex substituted L-arabinofuranose units. One of the most complex gums of this group and also one of the most thoroughly investigated is Acacia senegal or gum arabic. Work on this gum by Dillon and O'Colla (18) and by Smith (16) established for the first time the mode of distribution of the 1-3' and 1-6' linkages in the branched galactan core. A possible structure for this gum is shown in Fig. 2. While this indicates the main structural features of the gum it should be pointed out that this is not a unique representation of the very complex molecule and is probably an oversimplification.
Fig. 2. Gum arabic.

\[ \begin{align*}
\beta -\text{D-GpA} & \quad \downarrow \\
1 & \quad \downarrow \\
6 & \quad \downarrow \\
R \rightarrow 3\beta -\text{D-Galp} & \\
1 & \quad \downarrow \\
6 & \quad \downarrow \\
\end{align*} \]

\[ \ldots \]

Of the various peripheral acid-labile units (R), only the location of the \( \text{L-rhamnopyranose} \) units is known with certainty. This knowledge was obtained by utilisation of the resistance to cleavage by acetylation of rhamnosidic linkages and the isolation of the oligosaccharides \( 4\alpha-\text{L-rhamnopyranosyl-D-glucose} \) and \( 4\alpha-\text{L-rhamnopyranosyl-(1\rightarrow4)}-\text{D-glucopyranosyl-(1\rightarrow6)}-\text{D-galactose} \) after acetylation of carboxyl-reduced arabic acid (19). (see Fig. 3.)
Fig. 3. Location of \( L \)-rhamnopyranose end groups in arabic acid.

Exudate gums with a highly branched galactan core are not confined to the \textit{Acacia} species and are also obtained from a number of botanically unrelated trees. Coniferous wood arabinogalactans, notably those from larches (20), also have the same type of basal structure. These polysaccharides tend to be less highly branched than the \textit{Acacia} gums and contain a lower proportion of sugar residues other than \( D \)-galactose. On a broad structural basis, however, there is no clear line of demarcation between exudate gums of the galactan group and coniferous wood arabinogalactans.

The second group of exudate gums is the glucuronomannan group. As the name suggests, these gums are made up of inner chains of \( D \)-glucuronic acid and \( D \)-mannose residues, and, like the galactan group, contain peripheral acid-labile pentose units. The first gum to be assigned to this group was gum ghatti from \textit{Anogeissus latifolia} (Combretaceae), which, on partial hydrolysis, yielded
two polymer-homologous series of 1-6-linked β-D-galactose-containing oligosaccharides, one series with and the other without 3-O-substituted L-arabinose reducing units. On the basis of these results, it was first supposed that the gum had a main chain of β-D-galactopyranose residues interrupted at intervals by L-arabinose residues, with units of the two aldobiouronic acids, 2-O-(β-D-galactopyranosyluronic acid)-D-mannose and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, also obtained from partial hydrolysis, attached as side chains. That the gum has, in fact, main chains of D-glucuronic acid and D-mannose residues and a structure indicated in Fig. 4 (21, 22) was shown by further experiments involving two successive degradations by Smith's procedure (23) followed by partial hydrolysis which furnished the oligosaccharides 6-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-L-arabinose, and 3-O-L-arabinopyranosyl-D-mannose (24). These oligosaccharides can only arise from a structure of the type shown.

Evidence that some of the side chains are terminated by D-glucuronic acid is rather indirect, but this would explain the formation of the disaccharide 6-O-(β-D-glucopyranosyluronic acid)-D-galactose on partial hydrolysis. The oligosaccharide 2-O-(β-D-glucopyranosyluronic acid)-D-mannose seems to arise from blocks of alternating D-glucuronic acid and D-mannose residues rather than from isolated disaccharide units since the alternating tetrasaccharide O-β-D-glucopyranosyluronic acid-(1→2)-O-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyluronic acid-(1→2)-D-mannose has recently been isolated (25).

Even more striking evidence for the presence of an alternating backbone of D-glucuronic acid and D-mannose units in certain plant exudates was obtained from studies on the gum from the tree *Anogeissus leiocarpus* (formerly *A. schimperi*). A structural similarity to gum ghatti was realised when partial hydrolysis gave the same neutral and acidic oligosaccharides, although in
R = L-Araf 1..., or less frequently L-Araf 1→2 L-Araf 1...
L-Araf 1→3 L-Araf 1...
L-Araf 1→5 L-Araf 1...

vastly different amounts (26). Fractionation of the gum with cetyltrimethylammonium bromide (27) gave rise to two distinct but structurally related polysaccharides and further studies were carried out on the major component, leiocarpan A. Like gum ghatti, leiocarpan A was found to contain D-glucuronic acid, D-mannose and L-arabinose as main sugars but, in contrast, relatively large amounts of D-xylose and only a very low proportion (ca. 4%) of D-galactose. The main structural features of the polysaccharide are shown in Fig. 5. In
contrast to gum ghatti, in which relatively short main chains of D-glucuronic acid and D-mannose residues are shielded in the interior of the molecule by long side chains, the main chains of leiocarpan A are readily exposed, being substituted at C(3) and C(6) of the D-mannopyranose residues, largely by single L-arabinofuranose and D-xylopyranose side chains. The small amounts of D-galactose residues encountered in leiocarpan A are probably located in side chains similar to but probably shorter than those occurring in gum ghatti.

\[ \begin{align*}
R & = L-Araf \cdots, L-Araf \rightarrow 3 L-Araf \cdots, L-Arap \rightarrow 3 L-Araf \cdots, \\
\beta - D-GpA \rightarrow (6 \beta - D-Galp) \rightarrow 3 L-Arap \cdots \\
\beta - D-Galp \rightarrow (6 \beta - D-Galp) \rightarrow 3 L-Arap \cdots
\end{align*} \]

Conclusive proof that the interior chains are made up of regularly alternating sequences of 4-O-substituted $\beta$-D-glucuronic acid and 2-O-substituted $\alpha$-D-mannopyranose residues was obtained by isolation of the series of neutral oligosaccharides shown in Fig. 6, after acetylolyis of the carboxyl-reduced gum (28).
\[ \beta-D-Gp \rightarrow_1 \rightarrow_2 D-Man \]
\[ \alpha-D-Manp \rightarrow_1 \rightarrow_4 D-Gp \]
\[ Gp \rightarrow_1 \rightarrow_2 Manp \rightarrow_1 \rightarrow_4 Gp \]
\[ Manp \rightarrow_1 \rightarrow_4 Gp \rightarrow_1 \rightarrow_2 Man \]
\[ Gp \rightarrow_1 \rightarrow_2 Manp \rightarrow_1 \rightarrow_4 Gp \rightarrow_1 \rightarrow_2 Manp \rightarrow_1 \rightarrow_4 Gp \]

Fig. 6. **Oligosaccharides from carboxyl-reduced)**

*Leiocarpum A.*

Although certain other plant gums, notably those from the *Prunus* genus, give rise to the aldobiouronic acid, 2-\((\beta-D-glucopyranosyluronic acid)\)-\(D\)-mannose, on partial hydrolysis, it is not yet clear whether these gums can be properly regarded as members of the glucurononamman family of polysaccharides. However, the presence of blocks of \(D\)-glucuronic acid and \(D\)-mannose units in some part of the structure of apricot gum has been demonstrated by Zitko and his co-workers (29) who have isolated and partially characterised, as partial hydrolysis products, a series of oligosaccharides composed of these sugar residues. As yet, the glucurononamman type of structure has not been encountered in plant polysaccharides other than exudate gums.

Gums of the third group of exudate gums are referred to as galacturonans or galacturonorhammams and may be divided into three structurally related sub-groups. Tragacanthic acid, the main polysaccharide component of gum tragacanth, is the only known member of the first sub-group and, at present, it is sufficient to say that this polysaccharide may be regarded as a galacturonan because of the high proportion of \(D\)-galacturonic acid residues in the main chains. Polysaccharides of the other two sub-groups are represented by gums of the *Khaya* and *Sterculia* genera. These polysaccharides contain both \(D\)-galacturonic acid
and L-rhamnose residues in their interior chains and also peripheral units of D-glucuronic acid or its 4-O-methyl ether. The three sub-groups will later be discussed individually in more detail.
Structural features of Sterculia gums.

Gums of the Sterculia genus are of widely different origins and include Sterculia urens gum (India), S. setigera gum (West Africa) and S. caudata gum (Australia). These are grouped with the structurally similar gum from the botanically unrelated Cochlospermum gossypium (Bixineae).

Early work on these gums (8,30-33) was hindered by the inadequacy of the experimental techniques available and difficulties were encountered in the separation of acidic oligosaccharides. However, later investigations (34,35) involving more advanced techniques, notably the application of diethylaminoethyl (DEAE)-Sephadex chromatography to the separation of acidic oligosaccharides, showed that the four gums were closely structurally related and, from a structural point of view, could be considered together.

The main structural units present in the gums may be deduced from the methylated sugars obtained from each gum, which are shown in Table I.

Rhamnose occurs as terminal non-reducing end groups, chain units and branch points while galactose is present as terminal non-reducing end groups and chain units only. D-Glucuronic acid residues occur as non-reducing end group only, in contrast to those of D-galacturonic acid which are present mainly as branch points and, to a lesser extent, as chain units. Terminal non-reducing D-galacturonic acid residues are of infrequent occurrence. These main structural units are summarised in Fig. 7.
### TABLE I

Methylated Sugar Residues in Methylated Gums
(+ indicates approx. relative proportions)

<table>
<thead>
<tr>
<th>Methylated Sugar</th>
<th>S. urens</th>
<th>S. caudata</th>
<th>S. setigera</th>
<th>C. gossypium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me₄Gal</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,6-Me₃Gal</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,4-Me₃Rha</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3,4-Me₂Rha</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3-Me Rha</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4-Me₃GA</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4-Me₃GalA</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>2,3-Me₂GalA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Me GalA</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3-Me GalA</td>
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<td>+++</td>
<td>+++</td>
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</tbody>
</table>

### TABLE II

Acidic Oligosaccharides from Partial Hydrolysis.

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>S. urens</th>
<th>S. caudata</th>
<th>S. setigera</th>
<th>C. gossypium</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) GalA 1→2 Rha</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(II) GalA 1→4 Gal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(III) GA 1→3 GalA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(IV) GA 1→3 GalA 1→2 Rha</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(V) GalA 1→4 GalA 1→2 Rha</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>
The acidic oligosaccharides obtained from partial hydrolysis of each gum are shown in Table II and their structural significance, in terms of structural units (VI)-(XV) (Fig. 7.), is discussed below.

In oligosaccharide (IV), O-(D-glucopyranosyluronic acid)-(1→3)-O-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnose, terminal non-reducing D-glucuronic acid is linked to C(3) of a D-galacturonic acid residue. Structural units (XIII) and (XIV) show that D-galacturonic acid cannot be linked through C(1) and C(3) or C(1) and C(2) alone but must also be linked through C(4). Further, the L-rhamnose residue may exist as a branch point. Hence, partial structure (XVI) (Fig. 8) is a feature of these gums and defines the mode of attachment of the D-glucuronic acid residues as single unit side chains. It is assumed that oligosaccharide (III), 3-O-(D-glucopyranosyluronic acid)-D-galacturonic acid, arises from further hydrolytic cleavage of oligosaccharide (IV) and so its location in the gum is also indicated in partial structure (XVI).

The main product of partial hydrolysis is oligosaccharide (I), 2-O-(D-galactopyranosyluronic acid)-L-rhamnose. Since D-galacturonic acid is only present in very small amounts as terminal, non-reducing units, this oligosaccharide can only be accommodated in partial structure (XVII) and must have
arisen from the inner chains of the molecule. This is also evident from the fact that the same oligosaccharide has been characterised as a hydrolysis product from the degraded polysaccharides obtained from S. urens and S. caudata (36) gums by degradation using Smith's procedure (23).

Another structural feature of the inner chains of the molecule is shown in partial structure (XVIII), which indicates the environment of oligosaccharide (II), \(\text{4-}\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-\text{D-galactose}\). A related oligosaccharide, obtained from partial hydrolysis of each of the gums, is \(\text{2-}\text{O-}\text{acetyl-4-}\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-\text{D-galactose}\) and almost certainly arises from hydrolysis of incompletely de-acetylated gum. \(\text{O-Acetyl groups indeed have a remarkable stability to acidic hydrolysis as is shown by the fact that the acetyl content of certain hardwood xylans, after the drastic conditions of pulping, is in the region of 7-9\%}$. Like oligosaccharide (I), the oligosaccharides \(\text{4-}\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-\text{D-galactose}\) and \(\text{2-}\text{O-}\text{acetyl-4-}\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-\text{D-galactose}\) have also been obtained as partial hydrolysis products from the periodate resistant gum acid from S. caudata gum (36).

A second acidic trisaccharide, (V), \(\text{6-}\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-(1\rightarrow4)-\text{O-}(\text{\text{D}-galactopyranosyluronic acid})-(1\rightarrow2)-\text{L-rhamnose}\), not completely characterised in some cases, has also been isolated and may be accommodated in the partial structure (XIX). This oligosaccharide has also been obtained from the periodate resistant portions of S. urens and S. caudata (36) gums and, like oligosaccharide (I), is possibly a constituent of the main chains of the molecule.
The following oligosaccharides have also been isolated from partial hydrolysis of carboxyl-reduced S. urens gum and tentatively characterised (36).

\[(XX) \quad \beta-D\text{-galactopyranosyl-}L\text{-rhamnose.}\]
\[(XXI) \quad \beta-L\text{-rhamnopyranosyl-}D\text{-galactose.}\]
\[(XXII) \quad 3-\beta-D\text{-galactopyranosyl-}D\text{-galactose.}\]
\[(XXIII) \quad 4-\beta-D\text{-galactopyranosyl-}D\text{-galactose.}\]
\[(XXIV) \quad \beta-D\text{-galactopyranosyl-(1-4)-}\beta-D\text{-galactopyranosyl-(1-2)-L-rhamnose.}\]
\[(XXV) \quad \beta-D\text{-galactopyranosyl-(1-4)-}\beta-D\text{-galactopyranosyl-(1-4)-D-galactose.}\]

Interpretation of these results to give further structural information on the Sterculia gums is difficult since it is not possible to decide whether the galactose residues in the carboxyl-reduced gum came originally from galacturonic acid or galactose residues in the parent gum acid. Attempts to clarify this problem have been made in the present work by comparing the neutral oligosaccharides obtained from partial acetolysis of carboxyl-reduced S. urens gum with those obtained from partial acetolysis of the periodate-resistant polymer, isolated after periodate oxidation of carboxyl-reduced S. urens gum. Work by Nasir-ud-din (37) established that the periodate-resistant portion of the gum acid contained rhamnose, galacturonic acid and
only small amounts of galactose. Hence, the galactose residues in the carboxyl-
reduced periodate resistant polymer must originally have been present to a large extent as galacturonic acid residues in the parent gum acid.

In conclusion, it appears that gums of the *Sterculia* genus have a very complex, highly branched structure. The main chains seem to consist of units of D-galacturonic acid linked through positions C(1) and C(4) and L-rhamnose linked through positions C(1) and C(2), possibly with regions of contiguous D-galacturonic acid residues. D-Galactose residues may also be located in these main chains. The backbone of the molecule is highly substituted and carries a large number of side chains, which may vary from the single unit, D-glucuronic acid, to complex branched side chains having terminal non-reducing units such as D-galactose and L-rhamnose and with D-galacturonic acid, D-galactose and possibly L-rhamnose residues present as chain units and branch points.
Relationship of Sterculia gums with other polysaccharides.

The Sterculia gums occur naturally as partly acetylated polysaccharides and, in the cases discussed, the gum acids, obtained from the parent polysaccharides by de-acetylation with sodium hydroxide or dilute ammonia, have been shown to exist as homogeneous polymers. The Khaya gums, which include *K. grandifolia* (7), *K. senegalensis* (38) and *K. ivorensis* (39) gums, are also partly acetylated in the natural state, but the de-acetylated polymers have been shown to consist of more than one polysaccharide.

The main polysaccharides, however, have many structural features in common with the Sterculia gums and furnish similar fragments when subjected to the same experimental procedures such as partial hydrolysis and methylation. The main chains of these gums appear to have a greater proportion of contiguous D-galacturonic acid residues, but these are again interposed by L-rhamnose residues, giving rise to the oligosaccharide \( 2\,\text{O-} (\alpha\text{-D-galactopyranosyluronic acid})\,\text{L-rhamnose} \) on partial hydrolysis. Non-reducing end groups of D-glucuronic acid (as the 4-O-methyl ether) are also structural features of these gums. These are linked, not to D-galacturonic acid as in the Sterculia gums, but to D-galactose residues which in turn are probably linked to L-rhamnose branch points. Structural features of the Khaya gums are shown in Fig. 9.

Another polysaccharide with certain structural features in common with the Sterculia gums is tragacanthic acid, the only known member of the third sub-group of the galacturonan-galacturonorhamnan exudate gums. In this polysaccharide, the main chains are composed almost entirely of 1-4' linked \( \alpha\text{-D-galacturonic acid} \) residues. However, evidence that these chains are occasionally interrupted by L-rhamnose residues has been obtained by the isolation of \( 2\,\text{O-} (\alpha\text{-D-galactopyranosyluronic acid})\,\text{L-rhamnose} \) as a partial hydrolysis product (40). The side chains attached to the D-galacturonic acid backbone
Fig. 9. Major polysaccharide components of gums from Khaya spp.

appear to be short, consisting of either single $\beta-D$-xylopyranose residues or $2-O-\alpha-L$-fucopyranosyl-$\beta-D$-xylopyranose and $2-O-\beta-D$-galactopyranosyl-$\beta-D$-xylopyranose units. These two oligosaccharides have been obtained as products of partial acetolysis of tragacanthic acid while the single $\beta-D$-xylopyranose side chains have been isolated as the oligosaccharide $3-O-\beta-D$-xylopyranosyl-$\beta-D$-galacturonic acid, which was obtained by enzymic hydrolysis of the degraded tragacanthic acid liberated after controlled hydrolysis of the parent polysaccharide (41). Unlike the Sterculia and Khaya gums, tragacanthic acid contains $D$-glucuronic acid only in trace amounts, although this sugar is again likely to be present as non-reducing end group, probably terminating some of the side chains. The main structural features of the gum are shown in Fig. 10.

It is clear that the galacturonan-galacturonorhamnan group of exudate gums consists of structurally related polysaccharides ranging from tragacanthic acid on the one hand with a high percentage of contiguous $D$-galacturonic acid residues interrupted infrequently by $L$-rhamnose units, to the Sterculia gums on the other with relatively few contiguous $D$-galacturonic acid residues and a much larger proportion of $L$-rhamnose residues inserted in the main chains.
The interior galacturonan chains found in tragacanthic acid are also structural features of pectins. However, the occurrence of pectic galacturonans, as strictly homopolysaccharides, is infrequent and recent work has established that L-rhamnose residues are integral constituents of certain pectic galacturonans since several pectins such as those from lemon peel (42), lucerne (alfalfa) (43), and the bark of Amabilis fir (44) give rise to 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose and higher oligosaccharides containing these sugars on partial hydrolysis. Thus pectic substances are related to the galacturonan-galacturonorhamnan group of exudate gums in that they contain not only similarly linked blocks of D-galacturonic acid residues but also L-rhamnose residues interposed at certain points in the galacturonan chain.

The following oligosaccharides (Fig. 11) have been isolated as partial hydrolysis and/or partial acetolysis products from the pectin-like acidic polysaccharides obtained from soybean cotyledon meal (45) and soybean hulls (46).

These polysaccharides thus combine the structural features of the whole range of galacturonan-galacturonorhamnan exudate gums and provide most striking examples of polysaccharides in which different areas of the galacturonorhamnan chain may contain (a) blocks of galacturonic acid residues (b) sequences of at least two rhamnose residues, and (c) alternating galacturonic acid and
Fig. 11. Fragmentation of the interior chains of soybean polysaccharides.

rhamnose residues. The side chains of tragacanthic acid, namely β-D-xylopyranose, 2-O-β-D-galactopyranosyl-D-xylopyranose, and 2-O-α-L-fucopyranosyl-D-xylopyranose, are also structural features of these soybean polysaccharides.

In contrast to the exudate gums, the soybean polysaccharides also contain linear chains of 1-4' linked β-D-galactopyranose residues. There is no doubt that these galactan chains are integral constituents of an acidic polysaccharide, rather than of a contaminating neutral galactan, since acidic oligosaccharides containing contiguous 1-4' linked β-D-galactopyranose residues linked either directly or through xylose or rhamnose units to a single D-galacturonic acid reducing unit have been isolated as partial degradation products from the acidic polysaccharide from soybean cotyledons. These oligosaccharides, which have not been completely characterised, and the techniques employed in their isolation are shown in Fig. 12.
Acidic polysaccharide from soybean cotyledons

Controlled acid hydrolysis followed by hydrolysis with "pectinase"

\[
\beta-D-Galp_1-(\rightarrow 4\beta-D-Galp_1-)_n \rightarrow 4\beta-D-Galp
\]

\[
\beta-D-Galp_1 \rightarrow 2D-Xyl
\]

\[
\alpha-L-Fucp_1 \rightarrow 2D-Xyl
\]

\[
\text{acetolysis}
\]

Fig. 12. Fragmentation of the side chains of soybean cotyledon polysaccharide.

The structural relationship of the Sterculia gums with other exudate gums and the pectic substances is summarised in Fig. 13.

From the biosynthetic point of view, it is at present unknown whether these complex heteropolysaccharides are synthesised either in a stepwise manner involving first the formation of the main chains followed by apposition of the side chains or in a much more ordered manner involving the polymerisation of pre-formed branched structures. The structural relationships which have been shown to exist, particularly in the main chains, between the plant polysaccharides discussed favour the former alternative as the mechanism of polysaccharide biosynthesis. If this is the case, it would appear that the exudate gums, end products of plant metabolism, may be formed by the apposition of additional sugar residues to polysaccharide structures already present in the plant, possibly as cell-wall constituents.
Fig. 13. Structural inter-relationships between polysaccharides of the galacturonan-galacturonorhamnan group.
DISCUSSION
DISCUSSION

Partial acidic hydrolysis.

In previous investigations (33), the oligosaccharides $2-O-(\alpha-D$-
$galactopyranosyluronic acid)-L$-rhamnose and $O-(D$-glucopyranosyluronic acid)-
$(1-3)-O-(D$-galactopyranosyluronic acid)-(1-2)-L$-rhamnose were obtained as
partial hydrolysis products from Sterculia urens gum (see Table II). In
addition, the oligosaccharide $4-O-(D$-galactopyranosyluronic acid)$-D$-galactose
was partially characterised in admixture with the above named trisaccharide.
These oligosaccharides were obtained as partial hydrolysis products from
S. caudata, S. setigera and C. gossypium gums, which also gave the oligo-
saccharide $3-O-(D$-glucopyranosyluronic acid)$-D$-galacturonic acid and, in
the cases of S. caudata and S. setigera gums, the oligosaccharide $O-(D$-
galactopyranosyluronic acid)-(1-4)$-O-(D$-galactopyranosyluronic acid)-
$(1-2)-L$-rhamnose (see Table II). In order to investigate the possible
occurrence of $3-O-(D$-glucopyranosyluronic acid)$-D$-galacturonic acid and
$O-(D$-galactopyranosyluronic acid)-(1-4)$-O-(D$-galactopyranosyluronic acid)-
$(1-2)-L$-rhamnose and to establish without doubt the occurrence of $4-O-(D$
-galactopyranosyluronic acid)$-D$-galactose as partial hydrolysis products,
a partial hydrolysis of S. urens gum was undertaken.

Sterculia urens gum acid, prepared from the crude gum by deacetylation
with ammonia, was hydrolysed in $\text{N}$-sulphuric acid to give a mixture of
neutral sugars and acidic mono- and oligosaccharides. The hydrolysate
was adsorbed on a DEAE-Sephadex column in the formate form and neutral
sugars were eluted with water. A partial fractionation of acidic oligo-
saccharides was obtained by eluting the column with water containing
increasing concentrations of formic acid. Separation of the resulting oligosaccharide mixtures by filter sheet chromatography gave six oligosaccharides of which oligosaccharides 1, 2 and 5 were obtained as main products, together with smaller amounts of oligosaccharides 3, 4 and 6.

Oligosaccharide 1 was assigned the structure shown below on the basis of the following observations. Hydrolysis gave galacturonic acid and galactose, hydrolysis of the derived glycitol gave galacturonic acid as the only reducing sugar, and reduction followed by hydrolysis of the derived methyl ester methyl glycosides gave galactose only. Also, gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,6-tri-α-methyl-β-D-galactose and 2,3,4-tri-α-methyl-β-D-galacturonic acid. On the basis of the specific rotation ([α]D + 73°) of the oligosaccharide, it was not possible to assign with certainty the configuration of the glycosidic linkage.

Oligosaccharide 1. 4-α-(D-galactopyranosyluronic acid)-D-galactose.

Oligosaccharide 2 was found to be a disaccharide containing galacturonic acid and rhamnose with the latter as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides
formation followed by reduction and hydrolysis. Identification of the methyl glycosides of 3, 4-di-O-methyl-\(\beta\)-rhamnose and 2, 3,4-tri-O-methyl-\(\beta\)-galacturonic acid by gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide is consistent with the structure shown below for the oligosaccharide. The glycosidic linkage was assigned the \(\alpha\)-configuration on the basis of the specific rotation ([\(\alpha\)]\(\text{D}\) + 88°) of the oligosaccharide.

\[\text{Oligosaccharide 2. } \text{2-\(\alpha\)-(\(\alpha\)-galactopyranosyluronic acid)}-\text{L-rhamnose.}\]

Oligosaccharide 5 was found to be a trisaccharide containing glucuronic acid, galacturonic acid and rhamnose with the latter as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides formation followed by reduction and hydrolysis. The oligosaccharide was converted into the methyl ester methyl glycosides with methanolic hydrogen chloride under mild conditions to minimise cleavage of glycosidic linkages, reduced with borohydride to give the methyl glycosides of the corresponding neutral oligosaccharide and methylated. The methyl glycosides of 2,3,4,6-tetra-O-methyl-\(\beta\)-glucose, 2,4,6-tri-O-methyl-\(\beta\)-galactose, and 3,4-di-O-methyl-\(\beta\)-rhamnose were
identified by gas-liquid chromatography of the methanolysis products. The oligosaccharide thus has the following structure.

\[
\text{Oligosaccharide 5.} \quad \alpha-D-(\text{glucopyranosyluronic acid})-(1-3)\beta-D-(\text{galactopyranosyluronic acid})-(1-2)-L-rhamnose.
\]

Oligosaccharide 3 was found to contain galacturonic acid, rhamnose and galactose with galactose as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides formation followed by reduction and hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a trisaccharide containing galacturonic acid, rhamnose, and galactose in the proportions of 1:1:1. Lime-water degradation of the oligosaccharide gave 2-\(\alpha-(\text{galactopyranosyluronic acid})-L-rhamnose\) showing that this disaccharide formed part of the oligosaccharide and also that the reducing galactose unit was substituted at \(C_{(3)}\) or \(C_{(4)}\). These results, together with the identification of the methyl glycosides of 3,4-di-\(\alpha\)-methyl-\(L\)-rhamnose, 2,3,6-tri-\(\alpha\)-methyl-\(D\)-galactose and 2,3,4-tri-\(\alpha\)-methyl-\(D\)-galacturonic acid by gas-liquid chromatography of the methanolysis products.
from the methylated oligosaccharide and the identification of the methyl glycosides of 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-galacturonic acid together with 1,2,3,5,6-penta-O-methyl-D-galactitol by gas-liquid chromatography of the methanolation products from the methylated oligosaccharide glycitol, are consistent with the following structure for the oligosaccharide.

\[
\text{Oligosaccharide 3.}\quad \beta-(D\text{-galactopyranosyluronic acid})-(1-2)-\beta-(L\text{-rhamnopranosyl})-(1-4)-D\text{-galactose.}
\]

Oligosaccharide 4 was found to contain galacturonic acid and rhamnose with galacturonic acid as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides formation followed by reduction and hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a trisaccharide containing galacturonic acid and rhamnose in the proportions of 2:1. Mild acidic hydrolysis of the oligosaccharide gave 2-O-(\(\alpha\)-D-galactopyranosyluronic acid)-L-rhamnose and galacturonic acid while mild acidic hydrolysis of the oligosaccharide glycitol gave the above
named disaccharide only. 2-\(\alpha\)-(\(\alpha\)-D-Galactopyranosyluronic acid)-\(L\)-rhamnose was also obtained by lime water degradation of the oligosaccharide. Thus, the disaccharide 2-\(\alpha\)-(\(\alpha\)-D-galactopyranosyluronic acid)-\(L\)-rhamnose formed part of the oligosaccharide and also the reducing galacturonic acid unit was substituted at \(C(3)\) or \(C(4)\). The oligosaccharide and the oligosaccharide glycitol were converted to the corresponding neutral oligosaccharides, as described for oligosaccharide 5, and methylated. The methyl glycosides of 2,3,4,6-tetra-\(O\)-methyl-\(D\)-galactose, 2,3,6-tri-\(O\)-methyl-\(D\)-galactose and 3,4-di-\(O\)-methyl-\(L\)-rhamnose were identified by gas-liquid chromatography of the methanolysis products from the methylated neutral oligosaccharide. The methyl glycosides of 2,3,4,6-tetra-\(O\)-methyl-\(D\)-galactose and 3,4-di-\(O\)-methyl-\(L\)-rhamnose together with 1,2,3,5,6-penta-\(O\)-methyl-\(D\)-galactitol and also small amounts of the methyl glycosides of 2,3,6-tri-\(O\)-methyl-\(D\)-galactose, assumed to arise from incomplete methylation of galactose residues, were identified by gas-liquid chromatography of the methanolysis products from the methylated neutral oligosaccharide glycitol. These results are consistent with the following structure for the oligosaccharide.

\[
\text{Oligosaccharide 4. } \quad \text{\(\alpha\)-(\(D\)-galactopyranosyluronic acid)-(1-2)-\(\alpha\)-(\(L\)-rhamnopyranosyl)-(1-4)-\(D\)-galacturonic acid.}
\]
Oligosaccharide 6, which was contaminated by small amounts of oligosaccharide 5, was found to be a disaccharide containing glucuronic acid and galacturonic acid with the latter as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides formation followed by reduction and hydrolysis. The oligosaccharide was converted to the corresponding neutral oligosaccharide, as described for oligosaccharide 5, and methylated. The methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose, 2,4,6-tri-O-methyl-D-galactose, and 3,4-di-O-methyl-L-rhamnose (traces) were identified by gas-liquid chromatography of the methanolysis products. The oligosaccharide thus has the following structure, and was assumed to arise from further hydrolytic cleavage of the oligosaccharide \( \text{O-}(\text{D-glucopyranosyluronic acid}) - (1\rightarrow 3) - \text{O-}(\text{D-galactopyranosyluronic acid}) - (1\rightarrow 2) - \text{L-rhamnose.} \)

\[
\text{Oligosaccharide 6. } 3\rightarrow \text{D-glucopyranosyluronic acid}) - \text{D-galacturonic acid.}
\]

Thus, Sterculia urens gum is similar to S. caudata, S. setigera and C. gossypium gums in that the oligosaccharides 4\(\rightarrow \text{D-galactopyranosyluronic acid}) - \text{D-galactose and 3\(\rightarrow \text{D-glucopyranosyluronic acid}) - \text{D-galacturonic acid are obtained as partial hydrolysis products in addition to the oligosaccharides 2\(\rightarrow \text{(\text{\(\rightarrow \text{D-galactopyranosyluronic acid}) - L-rhamnose and}}
\]
\(\text{O-(D-glucopyranosyluronic acid)-(1-3)}\text{-O-(D-galactopyranosyluronic acid)-(1-2)-L-rhamnose. Further, the isolation, in this case, of 4-O-(D-galactopyranosyluronic acid)-D-galactose indicates without doubt the occurrence in } S. \text{ urens gum of regions containing contiguous galacturonic acid and galactose residues. However, in contrast to } S. \text{ setigera and } S. \text{ caudata gums, no evidence was obtained for the presence of } \text{O-(D-galactopyranosyluronic acid)-(1-4)}\text{-O-(D-galactopyranosyluronic acid)-(1-2)-L-rhamnose as a partial hydrolysis product from } S. \text{ urens gum. The structural significance of these partial hydrolysis products, including the oligosaccharides } \text{O-(D-galactopyranosyluronic acid)-(1-2)}\text{-O-(L-rhamnopyranosyl)-(1-4)-D-galactose and } \text{O-(D-galactopyranosyluronic acid)-(1-2)}\text{-O-(L-rhamnopyranosyl)-(1-4)-D-galacturonic acid, from } S. \text{ urens gum is discussed later.} \)
Partial acetolysis.

Acetolysis of *Sterculia urens* gum acetate gave a mixture of neutral sugar acetates and acidic mono- and oligosaccharide acetates which, after deacetylation, were partially fractionated by DEAE-Sephadex chromatography. Paper chromatography of the resulting oligosaccharide mixtures showed the presence of components chromatographically identical to the partial hydrolysis products galacturonic acid, 2-\(\alpha\)-(\(-\beta\)-galactopyranosyluronic acid)-\(\beta\)-rhamnose and 4-\(\alpha\)-(\(-\beta\)-galactopyranosyluronic acid)-\(\beta\)-galactose. In addition, a further oligosaccharide, not detected as a partial hydrolysis product, was found to be present and was isolated after further fractionation by filter sheet chromatography.

This oligosaccharide, oligosaccharide 21, was found to contain galacturonic acid and galactose in the approximate proportions of 1:2 with galactose as reducing unit by hydrolysis, glycitol formation followed by hydrolysis, and methyl ester methyl glycosides formation followed by reduction and hydrolysis. Partial acidic hydrolysis of the oligosaccharide gave 4-\(\alpha\)-(\(-\beta\)-galactopyranosyluronic acid)-\(\beta\)-galactose which was not detected as a partial hydrolysis product from the oligosaccharide glycitol, showing that this disaccharide formed part of the oligosaccharide and also that the galacturonic acid unit in the oligosaccharide was attached to the reducing galactose unit. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-galactose, 2,3,6-tri-\(\alpha\)-methyl-\(\beta\)-galactose and 3,4-di-\(\alpha\)-methyl-\(\beta\)-galacturonic acid. These results are consistent with the following structure for the oligosaccharide.
Oligosaccharide 21. $\alpha$-(D-galactopyranosyl)-(1→2)-$\alpha$-(D-galactopyranosyl-uronic acid)-(1→4)-D-galactose.
NMR spectroscopy of acidic oligosaccharides.

The nuclear magnetic resonance spectra of the acidic oligosaccharides 2-\(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)-\(\alpha\)-rhamnose, 4-\(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)-\(\beta\)-galactose, and \(\alpha\)-(\(\beta\)-glucopyranosyluronic acid)-(1-3)-\(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)-(1-2)-\(\alpha\)-galactose were examined, using deuterium oxide as solvent, in order to determine the configurations of the glycosidic linkages in the oligosaccharides (72). No clear resolution of proton signals could be obtained (see Spectra 1, 3, 5). The spectra of the glycitols of these oligosaccharides were also examined. The spectrum of 2-\(\alpha\)-(\(\alpha\)-galactopyranosyluronic acid)-\(\alpha\)-rhamnitol (Spectrum 2) shows a doublet at 4.7\(\gamma\), corresponding to the anomeric proton of the glycosidic linkage. The coupling constant of 2.8 c.p.s. is consistent with the anomeric proton having an equatorial conformation and confirms that the glycosidic linkage in the glycitol, and hence in the oligosaccharide itself, has the \(\alpha\)-configuration. The spectrum of 4-\(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)-\(\beta\)-galactitol (Spectrum 4) shows a doublet at 4.8\(\gamma\), corresponding to the anomeric proton of the glycosidic linkage. The coupling constant of 3.4 c.p.s. is consistent with the anomeric proton having an equatorial conformation and shows that the glycosidic linkage in the glycitol, and hence in the oligosaccharide itself, has the \(\alpha\)-configuration. No clear resolution of anomeric proton signals was obtained in the spectrum of the trisaccharide glycitol (Spectrum 6).
Oxidative degradations of \( 2-O-(\alpha-D-galactopyranosyluronic acid)-\text{L-rhamnose} \).

(a) using lead tetraacetate

(b) using sodium periodate

A reducing disaccharide containing galactose as non-reducing unit may be degraded to the corresponding \( \alpha \) - or \( \beta \) - anomer of \( 2-O-D-galactopyranosyl-glycerol \) by sequential lead tetraacetate oxidation and borohydride reduction, using the procedure of Charlson, Gorin and Perlin (67). The anomeric galactopyranosyl-glycerols, which are crystalline compounds, may be distinguished by their physical properties or by crystalline derivative formation, and hence the configuration of the glycosidic linkage in the parent disaccharide may be determined. In the case of disaccharides containing galacturonic acid as non-reducing unit, an extra stage in the reaction sequence is required for reduction of the galacturonic acid residue to galactose.

(a) \( 2-O-(\alpha-D-Galactopyranosyluronic acid)-\text{L-rhamnose} \) was converted to the corresponding glycitol by reduction with borohydride, and the acidic glycitol was esterified with methanolic hydrogen chloride under mild conditions to minimise cleavage of the glycosidic linkage. The ester was oxidised with lead tetraacetate to give a glyceraldehyde derivative which was subsequently converted, by simultaneous borohydride reduction of the glyceraldehyde portion of the molecule to glycerol and the esterified galacturonic acid residue to galactose, to the corresponding anomer of \( 2-O-D-galactopyranosyl-glycerol \). The reaction sequence is shown in Fig. 15. Crystalline \( 2-O-D-galactopyranosyl-glycerol \) could not
be isolated from the final reaction mixture. However, the presence of $2-O-\alpha-D$-galactopyranosyl-glycerol in the mixture was established by trimethylsilylation of the mixture and examination of the trimethylsilyl ethers by gas-liquid chromatography. A component with retention time corresponding to that of the trimethylsilyl ether of $2-O-\alpha-D$-galactopyranosyl-glycerol and distinct from that of the trimethylsilyl ether of $2-O-\beta-D$-galactopyranosyl-glycerol was detected. The glycosidic linkage in the disaccharide thus has the $\alpha$-configuration.

(b) In the above sequence of reactions, lead tetraacetate was used as a selective oxidant to effect glycol-cleavage only of the glycitol portion of the disaccharide glycitol methyl ester. Preliminary experiments on model compounds showed that sodium periodate, in low concentrations, could be used to obtain the same selectivity in oxidation as lead tetraacetate, in accordance with the investigations of Clancy and Whelan (73), and $2-O-\beta-D$-galactopyranosyl-glycerol was obtained as a crystalline product after sequential periodate oxidation and borohydride reduction of $2-O-\beta-D$-galactopyranosyl-$D$-erythritol. $2-O-(\alpha-D$-Galactopyranosyluronic acid)-$L$-rhamnose was degraded by a similar procedure to that described in (a) above, using sodium periodate instead of lead tetraacetate as oxidant. Crystalline $2-O-D$-galactopyranosyl-glycerol was not obtained, but acetylation of the final reaction mixture followed by separation of the products by thin-layer chromatography gave crystalline $2-O-\alpha-D$-galactopyranosyl-glycerol hexaacetate. Isolation of this crystalline derivative confirms that the glycosidic linkage in the disaccharide has the $\alpha$-configuration.
On the assumption that \(2-\alpha-(\alpha-D\text{-galactopyranosyluronic acid})-L-rhamnose\) is formed as a hydrolysis product, the \(D\text{-galactopyranosyluronic acid}\) residues in oligosaccharides 3, \(2-\alpha-(\alpha-D\text{-galactopyranosyluronic acid})-(1-2)-\alpha-(L\text{-rhamnopyranosyl})-(1-4)-D\text{-galactose}\), 4, \(2-\alpha-(\alpha-D\text{-galactopyranosyluronic acid})-(1-2)-\alpha-(L\text{-rhamnopyranosyl})-(1-4)-D\text{-galacturonic acid}\), and 5, \(2-\alpha-(\alpha-D\text{-glucopyranosyluronic acid})-(1-3)-\alpha-(\alpha-D\text{-galactopyranosyluronic acid})-(1-2)-L-rhamnose\), obtained from partial hydrolysis, may be assigned the \(\alpha\)-configurations. Further, on the basis of the specific rotation \([\alpha]_D^\circ +81^0\) of oligosaccharide 5, the \(D\text{-glucopyranosyluronic acid}\) residue may be assigned the \(\beta\)-configuration. Since oligosaccharide 6, \(3-\alpha-(\alpha-D\text{-glucopyranosyluronic acid})-D\text{-galacturonic acid}\), is assumed to arise from further hydrolytic cleavage of oligosaccharide 5, the glycosidic linkage in this oligosaccharide may also be assigned the \(\beta\)-configuration.
Fig. 15. Oxidative degradations of
2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose.
Preparation and methylation of carboxyl-reduced gum.

Carboxyl-reduced Sterculia urens gum was prepared by acetylation of the 2-hydroxyethyl ester of the gum followed by reduction with lithium borohydride in boiling tetrahydrofuran (69). The carboxyl-reduced gum, \([\alpha_\mathrm{D}] + 58^\circ\), had uronic acid anhydride content of 0.0%, estimated by the carbazole reagent (64) and also by the decarboxylation method (63), and glucose, galactose and rhamnose but no uronic acids were detected by paper chromatography of the hydrolysate.

In order to establish that there was no significant difference between the parent gum acid and the carboxyl-reduced gum, other than replacement of hexuronic acid residues by the corresponding neutral sugars, the carboxyl-reduced gum was methylated, using sodium hydride and methyl iodide, by the procedure of Sandford and Conrad (70). Gas-liquid chromatography of the methanolation products from the methylated polysaccharide showed the presence of the methyl glycosides of the following sugars.

\[
\begin{align*}
2,3,4,6\text{-tetra-}O\text{-methyl-D-galactose} & \quad A, C \\
2,3,6\text{-tri-}O\text{-methyl-D-galactose} & \quad A, C \\
2,6\text{-di-}O\text{-methyl-D-galactose} & \quad C \\
3,6\text{-di-}O\text{-methyl-D-galactose} & \quad C \\
2,3,4\text{-tri-}O\text{-methyl-L-rhamnose} & \quad A \\
3,4\text{-di-}O\text{-methyl-L-rhamnose} & \quad A \\
3\text{-}O\text{-methyl-L-rhamnose} & \quad A \\
2,3,4,6\text{-tetra-}O\text{-methyl-D-glucose} & \quad C 
\end{align*}
\]

The methylated sugars A were detected as methanolation products from the methylated gum acid (35). The methylated sugars C arise from carboxyl-
reduction and subsequent methylation of hexuronic acid residues which were found to be present in the gum acid (35).
Smith degradation of carboxyl-reduced gum.

Carboxyl-reduced Sterculia urens gum was oxidised with aqueous sodium periodate solution until the uptake of oxidant was constant. The polyaldehyde obtained was reduced with potassium borohydride and the resulting polyalcohol was hydrolysed with dilute sulphuric acid under conditions designed to minimise cleavage of glycosidic linkages. This reaction sequence of periodate oxidation, borohydride reduction and mild acidic hydrolysis applied to polysaccharides is referred to as Smith degradation (23), and gave, in this case, a degraded polysaccharide, referred to as polysaccharide A, together with a mixture of low molecular weight products.

Investigation of the low molecular weight products.

Paper chromatographic examination of the low molecular weight products showed the presence of components with chromatographic mobilities identical to those of glycerol, threitol, glycolic aldehyde and galactose together with a reducing sugar with similar chromatographic mobility to that of 2-O-β-D-galactopyranosyl-L-glyceraldehyde, prepared from 2-O-β-D-galactopyranosyl-D-erythritol by oxidation with a dilute solution of sodium periodate. The products with the chromatographic mobilities of galactose and 2-O-β-D-galactopyranosyl-L-glyceraldehyde disappeared after borohydride reduction and were replaced by products with the chromatographic mobilities of galactitol and 2-O-α-D-galactopyranosyl-glycerol. A partial fractionation of the low molecular weight products was accomplished on Dowex resin (53), and further fractionation by filter sheet chromatography gave components A - G.

Component A, which contained traces of impurities, gave on hydrolysis
galactose and threitol in the approximate proportions of 2:1. Gas-liquid chromatographic examination of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-\beta-methyl-D-galactose and 2,3,6-tri-\beta-methyl-D-galactose, together with 1,3,4-tri-\beta-methyl-D-threitol. A probable structure for the oligosaccharide is shown below.

\[ \text{Component A.} \quad \beta-D-galactopyranosyl-(1-4)-\beta-\alpha-D-galactopyranosyl-(1-2)-D-threitol. \]

Component B was chromatographically pure and gave on hydrolysis galactose and threitol in the approximate proportions of 1:1. Gas-liquid chromatographic examination of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycoside of 2,3,4,6-tetra-\beta-methyl-D-galactose and also 1,3,4-tri-\beta-methyl-D-threitol. The uptake of 2.8 moles of periodate with the release of 0.9 moles of formaldehyde per mole of oligosaccharide is consistent with the structure shown below. The glycosidic linkage was assigned the \( \alpha \)-configuration on the basis of the specific rotation \( [\alpha]_D + 122^\circ \) of the oligosaccharide.
Component B. 2-O-α-D-galactopyranosyl-D-threitol.

Component D was chromatographically similar to 2-O-β-D-galactopyranosyl-L-glyceraldehyde. The oligosaccharide was reduced with borohydride and the product, \([\alpha]_D + 148^\circ\), which was chromatographically identical to 2-O-α-D-galactopyranosyl-glycerol, gave on hydrolysis galactose and glycerol in the approximate proportions of 1:1. The structure of the oligosaccharide is shown below.

Component D. 2-O-α-D-galactopyranosyl-D-glyceraldehyde.

Component C was characterised as galactose by conversion into crystalline galactitol hexaacetate. Components E, F and G were characterised as erythritol (present in trace amounts), glycerol and D-threitol respectively by paper chromatography and gas-liquid chromatography.
of the derived acetates.

Investigation of polysaccharide A.

Polysaccharide A, \([\alpha]_D + 92^\circ\), which was found to be composed solely of galactose and rhamnose residues in the approximate proportions of 1:1, was methylated by the sodium hydride procedure (70). The methyl glycosides of 2,3,4,6-tetra-\(\text{O}\)-methyl-D-galactose, 2,3,6-tri-\(\text{O}\)-methyl-D-galactose, 3,4-di-\(\text{O}\)-methyl-L-rhamnose and 3-\(\text{O}\)-methyl-L-rhamnose were identified by gas-liquid chromatography of the methanolysis products from the methylated polysaccharide. 3-\(\text{O}\)-Methyl-L-rhamnose was present only in small amounts and was assumed to arise from incomplete methylation of L-rhamnose residues. Acetolysis of polysaccharide A gave a mixture of neutral mono- and oligosaccharide acetates which, after deacetylation, were fractionated on charcoal: celite by elution with water containing increasing concentrations of ethanol. Further fractionation by filter sheet chromatography gave oligosaccharides 7 - 12.

Oligosaccharide 7 was obtained in admixture with oligosaccharide 8. These oligosaccharides were assigned the structures shown below on the basis of the following observations. Hydrolysis of the mixture gave galactose, rhamnose and threitol while hydrolysis of the glycitol gave galactose, rhamnose, threitol and galactitol. Gas-liquid chromatography of the methanolysis products from the methylated mixture showed the presence of the methyl glycosides of 2,3,4,6-tetra-\(\text{O}\)-methyl-D-galactose, 2,3,6-tri-\(\text{O}\)-methyl-D-galactose and 2,3,4-tri-\(\text{O}\)-methyl-L-rhamnose together with 1,3,4-tri-\(\text{O}\)-methyl-D-threitol, and gas-liquid chromatography of the
methanolysis products from the methylated glycitol of the mixture showed the presence of the methyl glycosides of 2,3,4,6-tetra-Ω-methyl-D-galactose and 2,3,4-tri-Ω-methyl-L-rhamnose together with 1,3,4-tri-Ω-methyl-D-threitol and 1,2,3,5,6-penta-Ω-methyl-D-galactitol.

Oligosaccharide 7. 4-Ω-L-rhamnopyranosyl-D-galactose.

Oligosaccharide 8. 2-Ω-D-galactopyranosyl-D-threitol.

Oligosaccharide 9 was found to be a disaccharide containing galactose and rhamnose with the latter as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-Ω-methyl-D-galactose and 3,4-di-Ω-methyl-L-rhamnose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed
the presence of the methyl glycoside of 2,3,4,6-tetra-O-methyl-D-galactose together with 1,3,4,5-tetra-O-methyl-L-rhamnitol. The oligosaccharide thus has the structure shown below. The glycosidic linkage was assigned the \( \alpha \)-configuration on the basis of the specific rotation (\( [\alpha]_D^+ + 105^\circ \)) of the oligosaccharide.

Oligosaccharide 9. \( 2-O-\alpha-D\)-galactopyranosyl-L-rhamnose.

Oligosaccharide 10 was found to contain galactose and rhamnose with galactose as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a trisaccharide containing galactose and rhamnose in the proportions of 2:1. Lime-water degradation of the oligosaccharide gave \( 2-O-\alpha-D\)-galactopyranosyl-L-rhamnose showing that this disaccharide formed part of the oligosaccharide and also that the reducing galactose unit was substituted at \( C(3) \) or \( C(4) \). Gas-liquid chromatography of the methanolyis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose while gas-liquid chromatography of the methanolysis products
from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose together with 1,2,3,5,6-penta-O-methyl-D-galactitol. The oligosaccharide thus has the structure shown below. The galactosidic linkage was assigned the $\alpha$-configuration on the assumption that 2-O-$\alpha$-D-galactopyranosyl-L-rhamnose is obtained from the oligosaccharide as a hydrolysis product. In subsequent oligosaccharides, the galactosidic linkage in the sequence ... $\alpha$-D-galactopyranosyl-(1-2)-L-rhamnose ... was assigned the $\alpha$-configuration on the same assumption.

![Oligosaccharide structure](image)

**Oligosaccharide 10.** $\alpha$-D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-D-galactose.

Oligosaccharide 11 was found to contain galactose and rhamnose with rhamnose as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a tetrasaccharide containing galactose and rhamnose in the proportions of 2:2. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose,
2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose together with 1,3,4,5-tetra-O-methyl-L-rhamnitol. Mild acidic hydrolysis of the oligosaccharide glycitol gave, among other products, 2-O-α-D-galactopyranosyl-L-rhamnose, 4-O-L-rhamnopyranosyl-D-galactose, 2-O-α-D-galactopyranosyl-L-rhamnitol, and O-α-D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-D-galactose, showing that the oligosaccharide was composed of alternating galactose and rhamnose residues as shown below.

Oligosaccharide 11. O-α-D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-
(1-4)-O-α-D-galactopyranosyl-(1-2)-L-rhamnose.

Oligosaccharide 12 was found to contain galactose and rhamnose with galactose as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a pentasaccharide containing galactose and rhamnose in the proportions of 3:2. Lime-water degradation of the oligosaccharide gave O-α-D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-
(1-4)-\(\alpha-D\)-galactopyranosyl-(1-2)-\(L\)-rhamnose showing that this tetrasaccharide formed part of the oligosaccharide and also that the reducing galactose unit was substituted at \(C_3\) or \(C_4\). Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-\(O\)-methyl-\(D\)-galactose, 2,3,6-tri-\(O\)-methyl-\(D\)-galactose and 3,4-di-\(O\)-methyl-\(L\)-rhamnose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4,6-tetra-\(O\)-methyl-\(D\)-galactose, 2,3,6-tri-\(O\)-methyl-\(D\)-galactose and 3,4-di-\(O\)-methyl-\(L\)-rhamnose together with 1,2,3,5,6-penta-\(O\)-methyl-\(D\)-galactitol. The oligosaccharide thus has the structure shown below.

Methylation studies on Sterculia urens gum (35) showed that \(D\)-galactose residues were present in the gum essentially as non-reducing end groups and 1-4' linked chain units, and hence no galactose residues resistant to periodate oxidation are present in the gum. This is confirmed by the fact that hydrolysis of the polyalcohol obtained by subsequent periodate
oxidation and borohydride reduction of gum acid, which had been completely deacetylated by successive treatments with ammonia and sodium hydroxide, gave only trace amounts of galactose in addition to rhamnose and galacturonic acid. Thus, in the carboxyl-reduced gum, galactose residues resistant to periodate oxidation must originally have been present in the gum acid as galacturonic acid residues. Therefore, when considering the products from Smith degradation of the carboxyl-reduced gum, it may be assumed that the galactose residues in the oligosaccharides 7 - 12, obtained from polysaccharide A, and also in the low molecular weight products A, B and D were originally present as galacturonic acid residues in the gum acid.

Identification of 2,3,4,6-tetra-0-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose as methanolysis products from methylated polysaccharide A shows that the degraded polymer obtained by Smith degradation of carboxyl-reduced S. urens gum is composed of linear chains of 1-4' linked D-galactose residues and 1-2' linked L-rhamnose residues terminated by D-galactose residues. Also, isolation of oligosaccharides 7 and 9 - 12 from polysaccharide A shows that, in this degraded polymer, the galactose and rhamnose residues alternate. Hence, the parent gum acid must contain large regions of alternating 1-4' linked D-galacturonic acid residues and 1-2' linked L-rhamnose residues which must be branched as shown in partial structure (XX) in order to be resistant to periodate oxidation and also to accommodate the known structural units present in the gum (Fig. 7). Partial hydrolysis products 2-0-(x-D-galactopyranosyluronic acid)-L-rhamnose and 0-(x-D-galactopyranosyluronic acid)-(1-2)-0-(L-rhamnopyranosyl)-(1-4)-D-
galacturonic acid undoubtedly arise from these regions of the gum.

\[
\begin{align*}
(3) & \quad 4 \quad (3) \quad 4 \\
\cdots & \quad 4\alpha-D-\text{GalpA} \quad 1\rightarrow2 \quad L-\text{Rhap} \quad 1\rightarrow4\alpha-D-\text{GalpA} \quad 1\rightarrow2 \quad L-\text{Rhap} \quad 1 \\
= & \quad (2) \\
\cdots & \quad 4\alpha-D-\text{GalpA} \quad 1\rightarrow2 \quad L-\text{Rhap} \quad 1 \\
= & \quad (2) \\
= & \quad (XXI)
\end{align*}
\]

The oligosaccharide \(2\alpha-D-\text{galactopyranosyl-D-glyceraldehyde}\) can only have arisen from oxidation of an \(L-\text{rhamnose}\) residue attached through \(C(2)\) to a branched \(D-\text{galacturonic acid}\) residue and may be accommodated in partial structure (XXI).

Although there is no direct evidence that the \(D-\text{threitol}\) residue in the oligosaccharide \(2\alpha-D-\text{galactopyranosyl-D-threitol}\) arose from oxidation of a 1-4' linked \(D-\text{galactose}\) unit as opposed to a 1-4' linked \(D-\text{galacturonic acid}\) unit which are also present in the gum, albeit in small amounts, it is likely that this oligosaccharide was derived from the same regions of the gum that gave rise to the oligosaccharide \(4\alpha-(\kappa-D-\text{galactopyranosyluronic acid})-D-\text{galactose}\).
Partial acetolysis of carboxyl-reduced gum.

Acetolysis of carboxyl-reduced *Sterculia urens* gum gave a mixture of neutral mono- and oligosaccharide acetates which, after deacetylation, were fractionated on charcoal:celite by elution with water containing increasing concentrations of ethanol. Further fractionation by filter sheet chromatography gave oligosaccharides 13-20.

Oligosaccharides 13, 16 and 18 were found to be respectively identical to oligosaccharides 7, 4-0-L-rhamnopyranosyl-D-galactose, 9, 2-0-\(\alpha\)-D-galactopyranosyl-L-rhamnose, and 10, 0-\(\alpha\)-D-galactopyranosyl-(1-2)-0-L-rhamnopyranosyl-(1-4)-D-galactose, and were characterised by the same experimental procedures.

Hydrolysis of oligosaccharide 14 gave galactose only while hydrolysis of the derived glycitol gave both galactose and galactitol. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-galactose and 2,3,6-tri-0-methyl-D-galactose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycoside of 2,3,4,6-tetra-0-methyl-D-galactose together with 1,2,3,5,6-penta-0-methyl-D-galactitol. The oligosaccharide thus has the following structure.

![Oligosaccharide 14](image)

**Oligosaccharide 14.** 4-0-D-galactopyranosyl-D-galactose.
Hydrolysis of oligosaccharide 15 gave galactose only while hydrolysis of the glycitol gave both galactose and galactitol. The oligosaccharide gave a negative reaction towards triphenyltetrazolium chloride indicating that the reducing galactose residue was substituted at the C(2) position. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-galactose and 3,4,6-tri-0-methyl-D-galactose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycoside of 2,3,4,6-tetra-0-methyl-D-galactose together with an unknown sugar, assumed to be 1,3,4,5,6-penta-0-methyl-D-galactitol. The oligosaccharide thus has the following structure.

Oligosaccharide 15. 2-0-D-galactopyranosyl-D-galactose.

Hydrolysis of oligosaccharide 17 gave galactose only while hydrolysis of the derived glycitol gave both galactose and galactitol. Colorimetric estimations of total sugar in the oligosaccharide before and after glycitol formation showed that the oligosaccharide was a trisaccharide. Lime-water degradation of the oligosaccharide gave 2-0-D-galactopyranosyl-D-galactose, showing that this disaccharide formed part of the oligosaccharide and
also that the reducing galactose unit was substituted at C(3) or C(4). Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-galactose, 3,4,6-tri-0-methyl-D-galactose and 2,3,6-tri-0-methyl-D-galactose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-galactose and 3,4,6-tri-0-methyl-D-galactose together with 1,2,3,5,6-penta-0-methyl-D-galactitol. The oligosaccharide thus has the following structure.

Oligosaccharide 17. 0-D-galactopyranosyl-(1-2)-O-D-galactopyranosyl-(1-4)-D-galactose.

Oligosaccharide 19 was found to contain galactose and rhamnose with rhamnose as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a trisaccharide containing galactose and rhamnose in the proportions of 1:2. Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl glycosides of 2,3,4-tri-0-methyl-L-
rhamnose, 3,4-di-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-D-galactose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4-tri-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-D-galactose together with 1,3,4,5-tetra-O-methyl-L-rhamnitol. The oligosaccharide thus has the following structure.

![Diagram](image)

**Oligosaccharide 19.** \(\text{O-L-rhamnopyranosyl-(1-4)-O-\alpha-D-galactopyranosyl-(1-2)-L-rhamnose.}\)

Oligosaccharide 20 was found to contain galactose and rhamnose with galactose as reducing unit by hydrolysis and glycitol formation followed by hydrolysis. Colorimetric estimations of the relative amounts of each sugar residue both in the oligosaccharide and the oligosaccharide glycitol showed that the oligosaccharide was a tetrasaccharide containing galactose and rhamnose in the proportions of 2:2. Lime-water degradation of the oligosaccharide gave \(\text{O-L-rhamnopyranosyl-(1-4)-O-\alpha-D-galactopyranosyl-(1-2)-L-rhamnose,}\) showing that this trisaccharide formed part of the oligosaccharide and also that the reducing galactose unit was substituted at \(C(3)\) or \(C(4)\). Gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide showed the presence of the methyl
glycosides of 2,3,4-tri-0-methyl-L-rhamnose, 3,4-di-0-methyl-L-rhamnose and 2,3,6-tri-0-methyl-D-galactose while gas-liquid chromatography of the methanolysis products from the methylated oligosaccharide glycitol showed the presence of the methyl glycosides of 2,3,4-tri-0-methyl-L-rhamnose, 3,4-di-0-methyl-L-rhamnose and 2,3,6-tri-0-methyl-D-galactose together with 1,2,3,5,6-penta-0-methyl-D-galactitol. The oligosaccharide thus has the following structure.

Oligosaccharide 20. O-L-rhamnopyranosyl-(1-4)-O-\alpha-D-galactopyranosyl-
(1-2)-O-L-rhamnopyranosyl-(1-4)-D-galactose.

Oligosaccharides 13, 16 and 18 were also obtained as acetolysis products from polysaccharide A and hence must have arisen to a large extent, from the branched galacturonorhamnan core of the molecule. Oligosaccharides 19 and 20 may also have arisen from the same regions of the gum. However, since the oligosaccharides 19 and 20 were not obtained from polysaccharide A, they may originate from rhamnose-terminated side-chains. There is no direct evidence that the D-galactose residues in these two oligosaccharides were originally present as D-galacturonic acid residues in the gum acid and, indeed, some may be present in the gum acid also as D-galactose residues as is suggested from
the partial hydrolysis product, $\alpha$-D-galactopyranosyluronic acid)-
(1-2)-$\alpha$-(L-rhamnopyranosyl)-(1-4)-D-galactose. Since methylation of
the gum acid (35) showed that no 2-$\alpha$-substituted D-galactose residues
were present, the 2-$\alpha$-substituted D-galactose residues in oligosaccharides
15 and 17 must originally have been present in the gum acid as 2-$\alpha$-
substituted D-galacturonic acid residues. This is confirmed by isolation
of oligosaccharide 21, $\alpha$-(D-galactopyranosyl)-(1-2)-$\alpha$-(D-galactopyranosyl-
uronic acid)-(1-4)-D-galactose, as a partial acetolysis product from
the gum acid. On the assumption that oligosaccharide 14, 4-$\alpha$-D-galactopy-
ranosyl-D-galactose, arises from further hydrolytic cleavage of
oligosaccharide 17, the non-reducing D-galactose end group must originally
have been present in the gum acid as D-galacturonic acid and hence the
partial hydrolysis product, 4-$\alpha$-(\(\alpha\)-galactopyranosyluronic acid)-D-
galactose, may be accommodated in partial structure (XXII). On the
basis of the methylation results (35), galactose end groups and galactose
chain units are present in the gum acid in approximately equal amounts.
Galactose chain units have already been encountered attached to galact-
uronic acid and rhamnose as in the oligosaccharides 4-$\alpha$-(\(\alpha\)-galacto-
pyranosyluronic acid)-D-galactose and $\alpha$-(\(\alpha\)-galactopyranosyluronic acid)–
(1-2)-$\alpha$-(L-rhamnopyranosyl)-(1-4)-D-galactose, and so the galactose residue
attached to C(2) of the galacturonic acid residue in partial structure
(XXII) is probably present in the gum as non-reducing end group.

\[
\begin{align*}
\ldots & 4\alpha-D-GalpA 1\rightarrow 4 \quad D-Galp 1 \ldots \\
& \uparrow \\
& D-Galp \\
& (XXII)
\end{align*}
\]
Conclusions.

Methylation studies on Sterculia urens gum (35) showed that the main structural units present in the gum are as follows (see also Fig. 7).

\[
\begin{align*}
\text{D-Gal} & \sim \text{A} \\
\text{D-Gal} & \sim \text{A} \quad \text{D-Gal} \\
\text{L-Rha} & \sim \text{A} \\
\end{align*}
\]

In the light of the present investigations, it is now clear that these structural units arise from two quite distinct regions in the gum, one containing galacturonic acid and rhamnose residues (region A), and the other containing galacturonic acid and galactose residues (region B). Region A contains rhamnose residues both as branch points and chain units as shown in partial structures (XX) and (XXI). The nature of the substituent at C(4) of the rhamnose residues is unknown but this may be a single L-rhamnose unit which would account for the L-rhamnose end groups in the gum, the location of which is, at present, unknown.

\[
\begin{align*}
\cdots & 4\alpha-D-\text{Gal} \rightarrow 2 & L-\text{Rhap} \rightarrow 4 & 4 \xrightarrow{\alpha-D-\text{Gal}} 4 \xrightarrow{L-\text{Rhap}} 1 & \cdots \\
\end{align*}
\]

(XX)

\[
\begin{align*}
\cdots & 4\alpha-D-\text{Gal} \rightarrow 2 & L-\text{Rhap} \cdots \\
\end{align*}
\]

(XXI)
Partial structures (XX) and (XXI) indicate that the galacturonic acid residues in this region of the gum may be branched either at C(2) or C(3). However, isolation of the partial hydrolysis product D-β-D-glucopyranosyl-
uronic acid)-(1-3)-D-α-D-galactopyranosyluronic acid)-(1-2)-L-rhamnose both in the present investigations and in previous work (33) almost certainly indicates that these galacturonic acid residues in fact carry single unit D-glucuronic acid side chains attached at the C(3) position. Thus, partial structures (XX) and (XXI) may be extended to give the partial structures (XXIII) and (XXIV).

\[ \cdots 4\alpha-D-GalpA \xrightarrow{1 \rightarrow 2} L-Rhap \xrightarrow{1 \rightarrow 4} \alpha-D-GalpA \xrightarrow{1 \rightarrow 2} L-Rhap \xrightarrow{1 \rightarrow 4} \cdots \]

\[ \begin{array}{c}
\downarrow \quad \downarrow \\
D-GpA \\
D-GpA
\end{array} \]

(XXIII)

\[ \cdots 4\alpha-D-GalpA \xrightarrow{1 \rightarrow 2} L-Rhap \xrightarrow{1 \rightarrow 4} D-Galp \xrightarrow{1 \rightarrow 4} D-Galp \xrightarrow{1 \rightarrow 4} \cdots \]

\[ \begin{array}{c}
\downarrow \\
D-Galp
\end{array} \]

(XXIV)

In contrast to the galacturonic acid residues in region A, the galacturonic acid residues in region B are substituted at C(2) by single unit galactose side chains as shown in partial structure (XXII).

\[ \cdots 4\alpha-D-GalpA \xrightarrow{1 \rightarrow 2} L-Rhap \xrightarrow{1 \rightarrow 4} D-Galp \xrightarrow{1 \rightarrow 4} D-Galp \xrightarrow{1 \rightarrow 4} \cdots \]

\[ \begin{array}{c}
\downarrow \\
D-Galp
\end{array} \]

(XXII)
Thus, this region of the gum also contains the galactose end groups and the 1-4' linked galactose chain units in the gum, although some of these latter units are encountered attached to rhamnose as in the oligosaccharide \( \text{Q-}^\text{(\(\alpha\)-D-galactopyranosyluronic acid)} \text{-}(1-2)\text{-Q-}^\text{(L-rhamno-pyranosyl)} \text{-}(1-4)\text{-D-galactose}. \)

Although the oligosaccharide \( \text{Q-}^\text{(D-galactopyranosyluronic acid)} \text{-}(1-4)\text{-Q-}^\text{(D-galactopyranosyluronic acid)} \text{-}(1-2)\text{-L-rhamnose} \) was partially characterised as a partial hydrolysis product from the periodate resistant portion of \textit{S. urens} gum in previous work (33), no direct evidence was obtained for the presence of contiguous galacturonic acid residues in \textit{S. urens} gum in the present investigations. In this respect, \textit{S. urens} gum differs from the main polysaccharide components of the \textit{Khaya} gums which contain blocks of 1-4' linked \textit{D}-galacturonic acid residues interspersed at intervals by \textit{L}-rhamnose residues. Also, in the \textit{Khaya} gums, the \textit{D}-glucuronic acid end groups are attached, not to \textit{D}-galacturonic acid residues as in \textit{S. urens} gum, but to \textit{D}-galactose residues.
EXPERIMENTAL
General methods.

**EXPERIMENTAL**

Paper chromatography of sugars was carried out on Whatman No. 1 paper and Whatman No. 4 paper. The following solvent systems were used to develop the chromatograms.

A. ethyl acetate : pyridine : water (10:4:3).


C. ethyl acetate : acetic acid : formic acid : water (18:8:3:9).


F. ethyl acetate : pyridine : water (8:2:1).


I. butan-2-one : acetic acid : water (9:1:1, saturated with boric acid).

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

(a) **Aniline oxalate**. Reducing sugars were detected by spraying the dried chromatograms with a saturated solution of aniline oxalate in methylated spirits and heating at 120° - 130° for about 5 minutes.

(b) **Silver nitrate** (47) was used to detect non-reducing sugars and other polyhydroxy compounds.

(c) **Alkaline permanganate-periodate** (48). Reducing and non-reducing sugars were detected by spraying the dried chromatograms with alkaline permanganate-periodate solution.

(d) **Hydroxylamine-ferric chloride** (49). Esters were located by spraying the dried chromatograms with alkaline hydroxylamine reagent.
(e) Periodate-Schiff (50). Sugars containing glycol groups susceptible to periodate oxidation were detected by spraying the dried chromatograms with an aqueous solution of sodium periodate.

Paper ionophoresis (51) of oligosaccharides was carried out in borate buffer at pH 10 on Whatman No. 1 paper for 4-8 hrs. The dried ionophoretograms were sprayed with the aniline oxalate reagent containing 10% v/v acetic acid, and developed by heating at 120° - 130° for 5 minutes.

The values for $R_{\text{Gal}}$ and $R_{\text{GalA}}$ refer to the rate of movement of sugars on paper chromatograms relative to that of galactose and galacturonic acid respectively.

The value for $M_{\text{Gal}}$ refers to the rate of movement of sugars on paper ionophoretograms relative to that of galactose.

Preparative paper chromatographic separations were carried out on Whatman 3MM filter sheets which had been washed with water in a Soxhlet extractor for 48 hrs.

Column chromatography.

(a) Diethylaminoethyl (DEAE) - Sephadex columns were used to fractionate mixtures of acidic oligosaccharides. DEAE - Sephadex (A25) was allowed to swell overnight in water and then washed three times alternately with 0.5 N sodium hydroxide and 0.5 N hydrochloric acid before being generated in the formate form by stirring with 15% formic acid. The resin was packed in a column and washed free from formic acid with water.

(b) Charcoal-Celite columns (52) were used to separate mixtures of neutral oligosaccharides. Celite was allowed to stand overnight in concentrated hydrochloric acid, filtered, washed free from chloride with water.
and dried at 120 - 130°. Charcoal was washed six times with boiling water, fines being decanted after each washing, and dried at 120 - 130°. The column was packed as a mixture of charcoal : celite (1:1) in 1% ethanol.

(c) Dowex columns (53) were used to fractionate mixtures of reducing and non-reducing sugars. The resin (Dowex 50 W X 8, 200-400 mesh) was used in the barium salt form.

Gas-liquid partition chromatography (GLC) of methyl ethers methyl glycosides (54,55) was carried out on a "Fye Argon Chromatograph", using columns (120 X 0.5 cm.) containing the following stationary liquid phases supported on acid washed Celite (80-100 mesh).

(a) 5% by weight of neopentylglycol adipate polyester.
(b) 10% by weight of butan-1,4-diol succinate polyester.
(c) 15% by weight of polyethylene glycol adipate polyester.
(d) 10% by weight of polyphenyl ether [m-bis-(m-phenoxyphenoxy)-benzene].

Operating temperatures were (a) 150° (b) 175° (c) 175° (d) 200°.

The retention times (T) of the methyl ethers methyl glycosides are quoted relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.

Acetates of glycerol, threitol and erythritol for GLC were prepared by heating the sugar (2 mg.) for 1.5 hrs. in a sealed tube at 100° with anhydrous sodium acetate (2 mg.) and acetic anhydride (0.5 ml.). The solution was filtered, shaken with water until homogeneous and concentrated to a syrup. Traces of water were removed by several evaporations with acetone and the acetates were extracted with chloroform. Chromatography was carried out on a "Fye Argon Chromatograph" using a column of 3% by weight of XE 60 on Gas Chrom P at 150°.
Trimethylsilyl ethers of 2-O-D-galactopyranosyl-glycerols for GLC (56) were prepared by dissolving the sugar (5-100 mg.) in anhydrous pyridine, and adding Hexamethyldisilazane (0.2 ml.) and trimethyl chlorosilane (0.1 ml.). The solution was shaken vigorously for 30 seconds and allowed to stand for 5 minutes or longer prior to chromatography, which was carried out on a "Pye 104 Chromatograph" using a column of 10% by weight of ECNSS/m on Gas Chrom P at 160°.

Small-scale techniques.

Hydrolyses. Samples (2 mg.) were hydrolysed in N-sulphuric acid (0.5 ml.) in a sealed tube at 100° for periods varying from 4 hrs. for neutral oligosaccharides to 18 hrs. for acidic oligosaccharides. The solutions were neutralised with barium carbonate, treated with Amberlite IR 120 (H⁺) resin and concentrated to a syrup which was examined by paper chromatography. Solvent system A was used for the detection of neutral sugars, solvent system B for acidic mono- and oligosaccharides, and solvent system D for the separation of glucuronic and galacturonic acids.

Partial hydrolyses of acidic oligosaccharides were carried out as above for a period of 4 hrs.

Borohydride reductions. Sodium or potassium borohydride (10 mg.) was added to the sugar (2 mg.) dissolved in water (0.5 ml.) and the solution was allowed to stand overnight. Cations were removed by treatment with Amberlite IR 120 (H⁺) resin and boric acid was removed by several evaporations with methanol. Acidic oligosaccharides were converted to the sodium or potassium salt prior to reduction.

Methanolysis (methyl glycoside and methyl ester methyl glycoside formation). The sugar or polysaccharide derivative (2 mg.) was heated with methanolic 4% hydrogen chloride (2 ml.) in a sealed tube at 100° for 4 hours. The
solution was neutralised with silver carbonate.

**Removal of cations and anions** present in small amounts was carried out by treatment with Amberlite IR 120 (H+) and Amberlite IR 45 (OH-) resins.

**Alkaline degradation of oligosaccharides.** A sample (3 mg.) of the oligosaccharide was dissolved in oxygen-free lime-water (1 ml.) for 10 days. Calcium ions were removed with Amberlite IR 120 (H+) resin and the solution was concentrated to a syrup which was examined by chromatography in an appropriate solvent.

**Haworth methylations (57).** The oligosaccharide (5 mg.) was dissolved in water (2 ml.) and methyl sulphate (0.25 ml.) and 30% sodium hydroxide (0.50 ml.) were added dropwise over a period of 1 hour, the reaction being carried out under nitrogen at 0°. Further additions of methyl sulphate (1 ml.) and 30% sodium hydroxide (2 ml.) were made on the 5 subsequent days, each addition taking 4 hours.

24 Hours after the final addition, the reaction mixture was heated at 100° for 1 hour to destroy excess methyl sulphate. The solution was adjusted to pH 4 with dil. sulphuric acid and poured into ethanol (8 vols.). The precipitated sodium sulphate was removed at the centrifuge and washed thoroughly with ethanol (5 X 10 ml.). The supernatant and washings were combined, the pH was adjusted to 8 and the solution was concentrated to a small volume. The pH was adjusted to 4 and the solution was extracted with chloroform. The chloroform extracts were combined, dried over anhydrous sodium sulphate and evaporated to dryness.

**Kuhn methylations (58).** The oligosaccharide (0.5 - 2 mg.) was dissolved in N,N-dimethyl formamide (0.2 ml.) and shaken with methyl iodide (0.4 ml.) and silver oxide (0.2 g.) at room temperature in
the dark in a sealed tube for 48 hrs. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were concentrated and N,N-dimethyl formamide removed by azeotropic distillation with redistilled toluene under reduced pressure.

Oligosaccharides with galactose present as non-reducing end group were given a further treatment as above, using barium oxide (59) in place of silver oxide, for a period of 18 hrs.

**Methoxyl contents** were estimated by the semi-micro Zeisel method (60).

**Optical rotations** were observed at ca 20° in an appropriate solvent using the sodium D-line as monochromatic light source.

**Deuterations** for nuclear magnetic resonance spectroscopy were effected by freeze-drying the sample 3 times in deuterium oxide or by repeated dissolution in and evaporation of deuterium oxide under reduced pressure.

**Evaporations** were carried out under reduced pressure at or below 40°.

**Spectrophotometric determinations.**

**Total sugar content** was determined by the phenol-sulphuric acid method (61).

**Rhamnose** was estimated by the L-cysteine hydrochloride reagent (62).

**Uronic acid anhydride content** was measured (a) by decarboxylation and titrimetric estimation of the carbon dioxide evolved (63), (b) using the carbazole reagent (64).

**Sodium metaperiodate consumption** was measured using the method of Aspinall and Ferrier (65).

**Formaldehyde release** was estimated using the chromotropic acid reagent (66).

**Oligosaccharide composition** and degree of polymerisation were determined by estimation of the relative amounts of constituent sugars present in the oligosaccharide and the oligosaccharide glycitol. Galacturonic acid was
estimated by the carbazole reagent, rhamnose was estimated by the L-cysteine hydrochloride reagent and galactose was determined by difference using the phenol-sulphuric acid reagent to estimate total sugar content.

Purification of solvents was carried out as follows.

(a) Tetrahydrofuran was allowed to stand over sodium wire for one day, filtered and distilled over lithium aluminium hydride.

(b) Methyl sulphate was redistilled and allowed to stand over potassium carbonate until no longer acid to congo red paper.

(c) Methyl iodide was distilled over silver oxide and stored over molecular sieve (type A4).

(d) Pyridine was refluxed for 3 hours over potassium hydroxide, distilled and stored over potassium hydroxide.

(e) Formamide was redistilled using a splash bulb.

(f) Methanol was dried by refluxing for 30 minutes with magnesium and iodine, distilled and stored over molecular sieve (type A4).

(g) Aniline was distilled over zinc dust and stored in the dark.

Deacetylation of crude Sterculia urens gum.

Sterculia urens gum acid was prepared from partly acetylated crude gum nodules as follows.

Finely powdered crude gum (60 g.) was allowed to swell overnight in water (1.8 l.). Concentrated ammonia (360 ml.) was added to the gel and the whole was maintained at 60° for 4 hours with vigorous stirring. The solution was allowed to cool and small amounts of insoluble material were removed by filtration through muslin. The polysaccharide was precipitated by pouring into ethanol (4.8 l.) containing concentrated hydrochloric acid
(480 ml.) and was washed free from chloride ions with ethanol. The white solid was triturated with ether and air-dried.

The partly deacetylated polysaccharide (44.5 g.) was taken up in aqueous ammonia (5%, 1 l.) and the solution was heated at 60° for 1 hour. The clear solution was acidified with concentrated hydrochloric acid and poured into ethanol (3 l.). The deacetylated polysaccharide was removed at the centrifuge, washed several times with ethanol, finally with ether and air-dried.

The polysaccharide (38 g.) was dissolved in aqueous ammonia (1%) to give a 1% w/v solution and excess ammonia was removed under reduced pressure. The solution was dialysed for 5 days against tap water, deionised with Amberlite IR 120 (H⁺) resin, concentrated and freeze-dried to give deacetylated gum acid (30 g.).
Partial acidic hydrolysis.

Deacetylated gum (12 g.) was heated with N-sulphuric acid (500 ml.) on the boiling water bath for 6 hours. The solution was neutralised with barium hydroxide and barium carbonate, and barium salts were removed at the centrifuge and washed with water (3 × 500 ml.). The supernatant and washings were combined and concentrated to a small volume (60 ml.). Barium ions were removed with Amberlite IR 120 (H+) resin and the solution was concentrated to a syrup (9.0 g.).

The syrup was dissolved in water (5 ml.) and adsorbed on a DEAE-Sephadex column (30 g., formate form). Neutral sugars were eluted from the column with water until the eluate gave a negative reaction to the phenol-sulphuric acid reagent. The acidic sugars were eluted successively with 0.05 M formic acid, 0.4 M formic acid and 0.6 M formic acid. Elution with each concentration of formic acid was continued until the eluate gave a negative reaction to the phenol-sulphuric acid reagent. The eluate was collected in fractions (15 ml.) at the rate of 30 ml./hour and samples from every third fraction were concentrated and examined by chromatography in solvent B. Similar fractions were combined and concentrated to a small volume (40 ml.). Formic acid was removed by continuous extraction with ether in a liquid-liquid extractor for 12 hours. The formic acid free solutions were filtered and concentrated and the following fractions were obtained.
Discrete oligosaccharides were obtained from the above fractions by filter sheet chromatography in an appropriate solvent as follows.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>Weight (mg.)</th>
<th>( R_{\text{GalA}} ) values of components (solvent B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-58</td>
<td>866</td>
<td>0.05 ( \frac{M}{0.23, 0.75, 1.00} ) formic acid</td>
</tr>
<tr>
<td>2</td>
<td>59-114</td>
<td>531</td>
<td>0.75, 1.00</td>
</tr>
<tr>
<td>3</td>
<td>115-175</td>
<td>107</td>
<td>0.03, 0.75, 1.00</td>
</tr>
<tr>
<td>4</td>
<td>1-21</td>
<td>58</td>
<td>0.03, 0.13</td>
</tr>
<tr>
<td>5</td>
<td>22-33</td>
<td>601</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>34-40</td>
<td>98</td>
<td>0.20, 0.25</td>
</tr>
<tr>
<td>7</td>
<td>41-77</td>
<td>404</td>
<td>0.00, 0.25</td>
</tr>
<tr>
<td>8</td>
<td>78-102</td>
<td>133</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>285</td>
<td>0.6 ( \frac{M}{0.00, 0.25} ) formic acid</td>
</tr>
</tbody>
</table>

Oligosaccharide 1. 4-O-(D-galactopyranosyluronic acid)-D-galactose.

This oligosaccharide (230 mg.), \( [\alpha]_D + 73^\circ (c 0.83, H_2O) \), \( R_{\text{GalA}} 0.23 \) (solvent B), was chromatographically pure and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid and galactose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and B showed the presence of galacturonic acid only. A sample
of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give galactose only. The oligosaccharide (2 mg.) was methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>column (a)</td>
<td></td>
</tr>
<tr>
<td>2,3,6-tri-(\beta)-methyl-D-galactose</td>
<td>2.83, 3.65, 4.05</td>
</tr>
<tr>
<td>2,3,4-tri-(\beta)-methyl-D-galacturonic acid*</td>
<td>6.15, 6.50</td>
</tr>
<tr>
<td>* present as methyl ester</td>
<td></td>
</tr>
</tbody>
</table>

Oligosaccharide 2. \(2\text{-}\(\alpha\)-D-galactopyranosyluronic acid\)-L-rhamnose. This oligosaccharide (360 mg.), \([\alpha]_D + 88^\circ (c \ 0.73, H_2O), R_{GalA} 0.75\) (solvent B), was chromatographically pure and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and B showed the presence of galacturonic acid only. A sample of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give galactose and rhamnose in the approximate proportions of 1:1. The oligosaccharide (2 mg.) was methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>column (a)</td>
<td></td>
</tr>
<tr>
<td>3,4-di-(\beta)-methyl-L-rhamnose</td>
<td>0.84</td>
</tr>
<tr>
<td>2,3,4-tri-(\beta)-methyl-D-galacturonic acid*</td>
<td>6.15, 6.47</td>
</tr>
<tr>
<td>* present as methyl ester</td>
<td></td>
</tr>
</tbody>
</table>
Oligosaccharide 3. \( \alpha-\left(\beta\text{-galactopyranosyluronic acid}\right)-(1-2)-\alpha-\left(L\text{-rhamnopyranosyl}\right)-(1-4)-\beta\text{-galactose}. \)

This oligosaccharide (35 mg.), \( R_{\text{GalA}} 0.03 \) (solvent B), 0.30 (solvent C), \( M_{\text{Gal}} 0.73 \), was chromatographically and ionophoretically pure and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid, rhamnose and galactose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A, B and I showed the presence of galacturonic acid, rhamnose and galactitol. A sample of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give galactose and rhamnose in the approximate proportions of 2:1. Colorimetric estimations of galacturonic acid (carbazole reagent), rhamnose (cysteine reagent), and total sugar (phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galacturonic acid, rhamnose and galactose were present in the oligosaccharide in the proportions of 1:1:1. A sample of the oligosaccharide was degraded with lime water and chromatography of the product in solvents A and B showed the presence of \( 2-\alpha-\left(\beta\text{-galactopyranosyluronic acid}\right)-L\text{-rhamnose} \) only. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

\[
\begin{align*}
\text{component} & \quad \text{\( t \)} \\
\text{column (c)} \quad & \text{\( T \)} \\
(a) \text{ oligosaccharide} & \quad \\
2,3,6-\text{tri-\( \alpha \)-methyl-\( \beta \)-galactose} & 3.06,3.74,4.11,4.52 \\
3,4-\text{di-\( \alpha \)-methyl-\( L \)-rhamnose} & 0.98 \\
2,3,4-\text{tri-\( \alpha \)-methyl-\( \beta \)-galacturonic acid} & 6.74,7.18
\end{align*}
\]
(b) glycitol

1,2,3,5,6-penta-\(\beta\)-methyl-\(\alpha\)-galactitol 2.69
3,4-di-\(\beta\)-methyl-\(\alpha\)-rhamnose 0.98
2,3,4-tri-\(\beta\)-methyl-\(\alpha\)-galacturonic acid* 6.73, 7.12

* present as methyl ester

Oligosaccharide 4. \(\beta\)-(\(\alpha\)-galactopyranosyluronic acid)-(1-2)-\(\beta\)-(\(\alpha\)-rhamnop-
pyransyl)-(1-4)-\(\alpha\)-galacturonic acid.

This oligosaccharide (25 mg.), \(R_{Gal}A\) 0.13 (solvent B), 0.53 (solvent C),
\(M_{Gal}\) 0.83, was chromatographically and ionophoretically pure and examination
of the hydrolysate in solvents A, B and D showed the presence of galacturonic
acid and rhamnose. Hydrolysis of the derived glycitol and examination of
the hydrolysate in solvents A, B and C showed the presence of galacturonic
acid, rhamnose and galactonic acid. A sample of the oligosaccharide was
converted into the methyl ester methyl glycosides, reduced with sodium
borohydride and hydrolysed to give galactose and rhamnose in the approximate
proportions of 2:1. Colorimetric estimations of galacturonic acid (carbazole
reagent), rhamnose (cysteine reagent), and total sugar (phenol-sulphuric
acid reagent) in the oligosaccharide and the oligosaccharide glycitol
indicated that residues of galacturonic acid and rhamnose were present in
the oligosaccharide in the proportions of 2:1. Samples (2 mg.) of the
oligosaccharide and the oligosaccharide glycitol were partially hydro-
lysed. The former gave galacturonic acid and 2-\(\beta\)-(\(\alpha\)-galactopyranosyluronic
acid)-\(\alpha\)-rhamnose while the latter gave 2-\(\beta\)-(\(\alpha\)-galactopyranosyluronic acid)-
\(\alpha\)-rhamnose as the only reducing sugar. A sample of the oligosaccharide was
degraded with lime-water and chromatography of the product in solvents A
and B showed the presence of 2-\(\beta\)-(\(\alpha\)-galactopyranosyluronic acid)-
\(\alpha\)-rhamnose and traces of starting material. Samples (4 mg.) of the oligosaccharide
and the oligosaccharide glycitol were dissolved in methanolic hydrogen chloride (1%, 2 ml.) and the solutions were allowed to stand overnight at room temperature. The solutions were refluxed for 1 hour, neutralised with silver carbonate and concentrated. The syrups, dissolved in water (0.5 ml.), were reduced with sodium borohydride and the products were methylated by the Haworth procedure. The methanalysis products were examined by gas-liquid chromatography and the following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>Column(a)</th>
<th>Column(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Oligosaccharide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.76</td>
<td>1.81</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.55, 3.96</td>
<td>3.06, 3.74, 4.08, 4.55</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.85</td>
<td>0.98</td>
</tr>
<tr>
<td>(b) Glycitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.81</td>
<td></td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>(traces)</td>
<td>3.07, 3.66, 4.08, 4.56</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>1,2,3,5,6-penta-O-methyl-D-galactitol</td>
<td>2.70</td>
<td></td>
</tr>
</tbody>
</table>

Oligosaccharide 5. O-(D-glucopyranosyluronic acid)-(1-3)-O-(D-galactopyranosyluronic acid)-(1-2)-L-rhamnose.

This oligosaccharide (283 mg.), [α]_D + 81 ° (c 0.90, H_2O), R_GalA 0.20.
(solvent B) was chromatographically and ionophoretically pure and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid, glucuronic acid and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents in solvents A, B and D showed the presence of galacturonic and glucuronic acids only. A sample of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give glucose, galactose and rhamnose in the approximate proportions of 1:1:1. A sample (4 mg.) of the oligosaccharide was dissolved in methanolic hydrogen chloride (1%, 2 ml.) and the solution was allowed to stand overnight at room temperature. The solution was refluxed for 1 hour, neutralised with silver carbonate and concentrated. The syrup, dissolved in water (0.5 ml.), was reduced with sodium borohydride and the product was methylated by the Kuhn procedure. The methanolysis products were examined by gas-liquid chromatography and the following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-glucose</td>
<td>1.01, 1.46</td>
</tr>
<tr>
<td>2,4,6-tri-O-methyl-D-galactose</td>
<td>3.52, 4.04</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**Oligosaccharide 6.** \(3\-O-(D\text{-glucopyranosyluronic acid})\-D\text{-galacturonic acid}.**

This oligosaccharide (31 mg.), \(R_{GaL} 0.25\) (solvent B), gave on hydrolysis glucuronic acid, galacturonic acid and rhamnose (traces). Chromatography of the oligosaccharide in solvent C and ionophoresis in borate buffer showed the presence of two oligosaccharides. The major
component had $R_{\text{GalA}} 0.60$ (solvent C) while the minor component was chromatographically and ionophoretically identical to oligosaccharide 5. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A, B, D and I showed the presence of glucuronic acid, galacturonic acid, galactonic acid and rhamnitol (traces). A sample of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give glucose and galactose in approximately equal proportions together with traces of rhamnose. A sample (4 mg.) of the oligosaccharide was dissolved in methanolic hydrogen chloride (1%, 2 ml.) and the solution was allowed to stand overnight at room temperature. The solution was refluxed for 1 hour, neutralised with silver carbonate and concentrated. The syrup, dissolved in water (0.5 ml.), was reduced with sodium borohydride and the product was methylated by the Kuhn procedure. The methanolysis products were examined by gas-liquid chromatography and the following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>$T$ column (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5,6-tetra-$\alpha$-methyl-D-glucose</td>
<td>1.00, 1.44</td>
</tr>
<tr>
<td>2,4,6-tri-$\alpha$-methyl-D-galactose</td>
<td>3.48, 4.00</td>
</tr>
<tr>
<td>3,4-di-$\alpha$-methyl-L-rhamnose (traces)</td>
<td>0.85</td>
</tr>
</tbody>
</table>
NMR spectroscopy of acidic oligosaccharides.

The nuclear magnetic resonance spectra of the oligosaccharides 2-O-\(\alpha\)-L-galactopyranosyluronic acid)-L-rhamnose, 4-O-(D-galactopyranosyluronic acid)-D-galactose, and O-(D-glucopyranosyluronic acid)-(1-3)-O-(D-galactopyranosyluronic acid)-(1-2)-L-rhamnose and of the glycitols of these oligosaccharides were examined, using deuterium oxide as solvent, and are shown below. Values are relative to the proton resonance at 8.77\(\gamma\) of tertiary butanol, which was used as an internal standard.
Spectrum 1.

2-\textcircled{O}(\alpha-D-galactopyranosyluronic acid)-L-rhamnose.

Spectrum 2.

2-\textcircled{O}(\alpha-D-galactopyranosyluronic acid)-L-rhamnitol.
Spectrum 3.

4-O-(D-galactopyranosyluronic acid)-D-galactose.

Spectrum 4.

4-O-(D-galactopyranosyluronic acid)-D-galactitol.
Spectrum 5.

\[ \text{O-(\text{D-glucopyranosyluronic acid})-(1-3)-O-(\text{D-galactopyranosyluronic acid})-(1-2)-L-rhamnose.} \]

Spectrum 6.

\[ \text{O-(\text{D-glucopyranosyluronic acid})-(1-3)-O-(\text{D-galactopyranosyluronic acid})-(1-2)-L-rhamnitol.} \]
Oxidative degradations of 2-0-(\(\alpha\)-D-galactopyranosyluronic acid)-L-rhamnose.

(a) using lead tetraacetate.

2-0-(\(\alpha\)-D-Galactopyranosyluronic acid)-L-rhamnose (400 mg.), dissolved in water (10 ml.) and neutralised by the addition of 1\% potassium hydroxide, was reduced for 20 hours with potassium borohydride (300 mg.). Potassium ions were removed by passage through a column of Amberlite IR 120 (H\(^+\)) resin and boric acid was removed by several distillations with methanol. The solution was concentrated to a syrup which was dried thoroughly over phosphorus pentoxide. Examination of the syrup by chromatography in solvent B using aniline oxalate spray showed the absence of 2-0-(\(\alpha\)-D-galactopyranosyluronic acid)-L-rhamnose.

The glycitol (288 mg.) was refluxed with methanolic hydrogen chloride (3\%, 10 ml.) for 3 hours and the solution was neutralised with silver carbonate, treated with Amberlite IR 120 (H\(^+\)) resin and concentrated to a syrup which was dried over phosphorus pentoxide. Chromatography of the product in solvent A showed the presence of one component (\(R_{\text{Gal}}\) 2.07) which gave a positive reaction with the hydroxylamine-ferric chloride reagent. Small amounts of another component (\(R_{\text{Gal}}\) 1.07) were detected using silver nitrate reagent.

The product (235 mg.) was dissolved in water (0.8 ml.) and oxidised at room temperature with lead tetraacetate (620 mg., 2.1 molar equivalents) in acetic acid (47 ml.) for 6 hours with shaking. Lead was removed by passage through a column of Amberlite IR 120 (H\(^+\)) resin and the solution was concentrated to a syrup which was dried over phosphorus pentoxide. The product (176 mg.) was dissolved in water (5 ml.) and reduced with potassium borohydride (300 mg.) for 20 hours. After removal of inorganic
ions with Amberlite IR 120 (H⁺) resin and methanol, the solution was concentrated to a syrup which was chromatographed in solvent B. Components with RGal values 0.97, 1.18 and 2.88 were detected.

No crystalline galactopyranosyl-glycerol could be obtained. A sample of the mixture was trimethylsilylated and the products were examined by gas-liquid chromatography, using the trimethylsilyl ethers of 2-α-D-galactopyranosyl-glycerol and 2-β-D-galactopyranosyl-glycerol separately as internal standards. A component with retention time corresponding to that of the trimethylsilyl ether of 2-α-D-galactopyranosyl-glycerol was detected.

(b) using sodium periodate.

**Preliminary experiments.**

Methyl β-D-galactoside (17.3 mg., 89 μmol.) was oxidised with aqueous sodium periodate solution (60 μM, 5.0 l.). Periodate uptake was measured spectrophotometrically at intervals over a period of 24 hours and the results were plotted on a graph (Fig. 14). The experiment was repeated using, instead of methyl β-D-galactoside,

- (B) methyl α-D-mannoside (17.8 mg., 92 μmol.),
- (C) erythritol (7.5 mg., 62 μmol.),
- (D) 2-β-D-galactopyranosyl-D-erythritol (16.1 mg., 57 μmol.),
- (D') 2-β-D-galactopyranosyl-D-erythritol (16.8 mg., 59 μmol.) and 300 μM sodium periodate (1 l.).

The results are shown in Fig. 14.

2-β-D-Galactopyranosyl-D-erythritol (80 mg., 280 μmol., m.p. 175–176°), prepared by the method of Charlson, Gorin and Perlin (67), was
Fig. 14

Sodium periodate oxidation of

(A) Methyl β-D-galactoside
(B) Methyl α-D-mannoside
(C) Erythritol
(D) 2-O-β-D-Galactopyranosyl-D-erythritol
(D') 2-O-β-D-Galactopyranosyl-D-erythritol
dissolved in aqueous sodium periodate (300 \( \mu \text{M}, 1 \text{l.} \)) and the solution was allowed to stand in the dark for 1.25 hours. Periodate uptake was found to be 1.02 moles per mole of sugar and formaldehyde released, measured by the chromotropic acid reagent, was found to be 1 mole per mole of sugar.

Excess periodate was destroyed with ethylene glycol and the solution, after deionisation with Amberlite IR 120 (H\(^+\)) and Amberlite IR 45 (OH\(^-\)) resins, was concentrated to a syrup (65 mg.). The syrup was dissolved in water (5 ml.) and reduced for 20 hours with sodium borohydride (200 mg.) in water (10 ml.). After removal of inorganic ions with Amberlite IR 120 (H\(^+\)) resin and methanol, the solution was concentrated. Chromatography of the product (47 mg.) in solvent B showed the presence, as main product, of a component with chromatographic mobility identical to that of 2-\(\alpha\)-D-galactopyranosyl-glycerol, and also traces of 2-\(\beta\)-D-galactopyranosyl-D-erythritol. Separation of the mixture by chromatography on filter sheets gave 2-\(\alpha\)-D-galactopyranosyl-glycerol (m.p. 129-130\(^\circ\), mixed m.p. 129-131\(^\circ\)) which was crystallised from methanol-ethanol.

**Degradation of 2-O-(\(\alpha\)-D-galactopyranosyluronic acid)-L-rhamnose.**

2-\(\alpha\)-(D-Galactopyranosyluronic acid)-L-rhamnose (360 mg.), dissolved in water (10 ml.) and neutralised with 1% potassium hydroxide, was reduced for 20 hours with potassium borohydride (300 mg.). After removal of inorganic ions with Amberlite IR 120 (H\(^+\)) resin and methanol, the solution was concentrated to a syrup. Chromatography of the syrup in solvent B using aniline oxalate spray indicated the absence of 2-O-(\(\alpha\)-D-galactopyranosyluronic acid)-L-rhamnose. A sample of the product was methylated by the Kuhn procedure and the methanolysis products were examined by gas-
liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,4,5-tetra-O-methyl-D-rhamnitol</td>
<td>1.08</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galacturonic acid*</td>
<td>6.05, 6.34</td>
</tr>
</tbody>
</table>

* present as methyl ester

The glycitol (205 mg.) was dissolved in methanolic hydrogen chloride (1%, 30 ml.) and the solution was refluxed for 1 hour, after standing at room temperature for 18 hours. The solution was cooled, neutralised with silver carbonate, filtered and concentrated. The syrup (166 mg., 465 μmol.) was oxidised with sodium periodate (300 μM, 5.0 l.) and the uptake of periodate was followed spectrophotometrically. After 1.25 hours, the consumption of periodate was 2.25 moles per mole of sugar and excess periodate was destroyed with ethylene glycol. Inorganic ions were removed with Amberlite IR 120 (H⁺) and Amberlite IR 45 (OH⁻) resins and the solution was concentrated to a small volume (10 ml.). Potassium borohydride (300 mg.) in water (5 ml.) was added and the solution was allowed to stand for 18 hours. After removal of inorganic ions with Amberlite IR 120 (H⁺) resin and methanol, the solution was concentrated to a syrup (92 mg.). Chromatography of the syrup in solvent B showed that the main product was chromatographically identical to 2-O-α-D-galactopyranosyl-glycerol and hydrolysis of the syrup gave galactose, glycerol and traces of a component with the chromatographic mobility of erythritol.

The remainder of the syrup was refluxed for 3 hours with acetic anhydride (5 ml.) and anhydrous sodium acetate (40 mg.). The solution
was filtered and cooled and water was added, with shaking, until a homogeneous solution was obtained. The clear solution was concentrated to a syrup which was dissolved in acetone and examined by thin-layer chromatography on Kieselgel in benzene-methanol (4:1). The main component was chromatographically identical to 2-α-D-galactopyranosyl-glycerol hexaacetate, prepared by the method of Dixon, Buchanan and Beddiley (68). Needles of 2-α-D-galactopyranosyl-glycerol hexaacetate (m.p. 99°, mixed m.p. 99°) were crystallised from aqueous acetone after separation of the syrup by preparative thin-layer chromatography.
Preparation of carboxyl-reduced Sterculia urens gum (69).

Sterculia urens gum (15.0 g.) was dissolved in water (1.5 l.), ethylene oxide (300 ml.) was added and the solution was allowed to stand until the pH remained constant at approximately 6.0. The solution was dialysed against tap water for 5 days, concentrated and freeze-dried to give the glycol ester of S. urens gum (12.0 g.).

The ester (5.0 g.) was thoroughly dispersed in formamide (500 ml.) by vigorous shaking overnight. Pyridine (250 ml.) was added dropwise over a period of 1 hour with vigorous stirring at 45°. The mixture was cooled to 30° and acetic anhydride (180 ml.) was added dropwise with stirring over a period of 4 hours. The mixture was stirred for a further 5 hours at 30° and overnight at room temperature. The glycol ester acetate was precipitated by pouring the mixture into ice-cold hydrochloric acid (2 l., 2 N), and removed at the centrifuge. The product was dispersed in acetone and water was removed by azeotropic distillation with chloroform. The acetate (6.8 g.) was precipitated by pouring the resulting chloroform suspension into light petroleum (4 vol., b.p. 40 - 60°), removed at the centrifuge, washed with light petroleum and dried over phosphorus pentoxide and paraffin wax.

Glycol ester acetate (13.5 g.) was dispersed in dry tetrahydrofuran (270 ml.) and lithium borohydride (13.5 g.) in dry tetrahydrofuran (270 ml.) was carefully added to the suspension. The mixture was stirred at room temperature for 2 hours and under reflux for 18 hours. Lithium borohydride was destroyed by the careful addition of water and, after dilution with water (5 vol.), the suspension was acidified with 2 N-sulphuric acid. The clear solution was dialysed against tap water for 4 days, concentrated
and freeze-dried to give carboxyl-reduced S. urens gum (7.0 g.).

The uronic acid anhydride content of the carboxyl-reduced gum, \([\alpha]_D + 58^\circ (c 0.6, H_2O)\), was 0.0% (decarboxylation method) and 0.0% (carbazole method), and hydrolysis of a sample (2 mg.) gave galactose, glucose and rhamnose but no acid-containing material.

The above procedure was repeated to give a total of 29 g. of carboxyl-reduced gum.
Methylation of carboxyl-reduced gum (70).

Sodium hydride (1.5 g.) was washed three times with dry light petroleum (b.p. 40 – 60°) and stirred with dimethylsulphoxide (15 ml.) under nitrogen at 50° for 1 hour. Dimethylsulphoxide (15 ml.) was added to dry carboxyl-reduced gum (300 mg.) in a 100 ml. conical flask and the mixture was stirred with gentle heating until dissolution was complete. The solution was allowed to come to room temperature and the methylsulphinyl carbanion solution (4 ml.) was added dropwise with stirring. The resulting gel was stirred for 5 hours at room temperature and methyl iodide (1 ml.) was added dropwise over a period of 10 minutes so that the temperature did not rise above 25°. The mixture was stirred overnight and dialysed against tap water for 24 hours. The solution was concentrated and the residue was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered and concentrated to a small volume and, on pouring into light petroleum (b.p. 60 – 80°, 8 vol.), yielded the methylated carboxyl-reduced polysaccharide (176 mg.). The product (Found : OMe, 44.8%) was removed at the centrifuge and dried in vacuo.

A sample of the methylated polysaccharide was methanolysed and the methanolysis products were examined by gas-liquid chromatography. The following components were detected as their methyl glycosides.
<table>
<thead>
<tr>
<th>Component</th>
<th>T column (a)</th>
<th>T column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.74</td>
<td>1.75</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>2.67, 3.57, 3.96</td>
<td>2.99, 3.65, 4.01, 4.45</td>
</tr>
<tr>
<td>2,6-di-O-methyl-D-galactose</td>
<td>8.05, 8.84, 10.64</td>
<td>9.21</td>
</tr>
<tr>
<td>3,6-di-O-methyl-D-galactose</td>
<td>8.05, 11.57, 13.24</td>
<td>10.04, 14.79</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.83</td>
<td>0.97</td>
</tr>
<tr>
<td>3-O-methyl-L-rhamnose</td>
<td>-</td>
<td>3.31</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-glucose</td>
<td>1.01, 1.42</td>
<td>1.42</td>
</tr>
</tbody>
</table>
Smith degradation of carboxyl-reduced gum (23).

Carboxyl-reduced gum (20 g.) was dissolved in 0.1 M sodium periodate (2.0 l.) and the solution was allowed to stand in the dark at 20°. After 30 hours the uptake of periodate remained constant at 0.9 moles per mole of sugar and excess periodate was destroyed with ethylene glycol (10 ml.). The solution was allowed to stand overnight, dialysed against tap water for 4 days, and concentrated to a small volume (350 ml.). The periodate-oxidised polysaccharide was reduced for 48 hours by the addition of potassium borohydride (5 g.) in portions (0.5 g.). Potassium ions were removed with Amberlite IR 120 (H+) resin and the solution was concentrated and distilled several times with methanol (6 X 25 ml.) to remove boric acid.

The product (14.9 g.) was dissolved in N-sulphuric acid (300 ml.) and hydrolysed in the cold for 4 hours. The solution was neutralised with barium hydroxide and barium carbonate and barium salts were removed at the centrifuge and washed with water (3 X 500 ml.). The supernatant and washings were combined and concentrated to a small volume (100 ml.). The solution was treated with Amberlite IR 120 (H+) resin and poured into ethanol (500 ml.). The precipitate (polysaccharide A, 2.5 g.) was removed at the centrifuge, washed several times with ethanol, finally with ether and air-dried. Concentration of the supernatant yielded the low molecular weight oxidation products as a syrup (approx. 5.6 g.).

Periode oxidation of Sterculia urens gum.

Sterculia urens gum (500 mg.), prepared from crude gum nodules by deacetylation with ammonia, was dissolved in N-sodium hydroxide (25 ml.)
and the solution was stirred for 14 hours. The alkaline solution was poured into ethanol (100 ml.) acidified with concentrated hydrochloric acid (10 ml.). The precipitated polysaccharide (360 mg.) was removed at the centrifuge, washed several times with ethanol, finally with ether and air-dried. The polysaccharide (100 mg.) was oxidised with 0.1 M sodium periodate (10 ml.) at 20° for 30 hours. Ethylene glycol (0.2 ml.) was added and the solution was allowed to stand overnight. The solution was dialysed for 3 days against tap water and the oxidised polysaccharide was reduced overnight with potassium borohydride (100 mg.). Potassium ions were removed with Amberlite IR 120 (H⁺) resin and the solution was concentrated and distilled several times with methanol (6 X 15 ml.) to remove boric acid. The product (2 mg.) was hydrolysed in N-sulphuric acid for 18 hours at 100° to give galacturonic acid, rhamnose and traces of galactose.
Investigation of low molecular weight products from Smith degradation.

Examination of the low molecular weight products by chromatography in solvents B and H using silver nitrate and periodate-Schiff sprays showed the presence of components with chromatographic mobilities identical to those of glycerol, threitol and glycolic aldehyde together with chromatographically mobile material of higher molecular weight. Chromatography of the mixture in solvent A using silver nitrate and aniline oxalate sprays resolved the higher molecular weight products into components with the following chromatographic mobilities.

A. 0.65
B. 0.86
C. 1.00 (chromatographically identical to galactose.)
D. 1.30 (chromatographically similar to 2-O-β-D-galactopyranosyl-L-glyceraldehyde.)

A sample (2 mg.) of the mixture was reduced overnight with sodium borohydride and the products were examined by chromatography in solvent A. Components A and B were detected but components C and D were absent. A component chromatographically identical to 2-O-α-D-galactopyranosyl-glycerol was detected. Examination of the products in solvent I using alkaline permanganate-periodate spray indicated the presence of a component chromatographically identical to galactitol.

Fractionation of the low molecular weight products.

The syrup was dissolved in water (4.0 ml.) and a sample (1.5 ml.) was applied to a column of Dowex 50W X 8 resin (200-400 mesh, 2.2 X 100 cm.,
Ba\textsuperscript{2+} salt form). Elution was carried out with water at a rate of 6-9 ml./hour and 1.0-1.5 ml. fractions were collected. The contents of every third tube were concentrated and examined chromatographically in solvent B using silver nitrate spray. The following fractions were obtained.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>Weight (mg.)</th>
<th>( R_{\text{Gal}} ) values of components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136-158</td>
<td>52</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>159-187</td>
<td>78</td>
<td>0.00, 0.65(tr)</td>
</tr>
<tr>
<td>3</td>
<td>188-211</td>
<td>87</td>
<td>0.65(+), 0.86(+), 1.30(+)</td>
</tr>
<tr>
<td>4</td>
<td>212-247</td>
<td>74</td>
<td>0.86(tr), 1.00(+), 1.30(+)</td>
</tr>
<tr>
<td>5</td>
<td>248-265</td>
<td>94</td>
<td>2.26(tr), 2.71(++++)</td>
</tr>
<tr>
<td>6</td>
<td>266-285</td>
<td>112</td>
<td>2.71</td>
</tr>
<tr>
<td>7</td>
<td>293-325</td>
<td>73</td>
<td>2.26</td>
</tr>
</tbody>
</table>

(+) indicates the approximate relative amounts estimated visually on paper.
(tr) = traces.

Fractions 1-4 were examined by chromatography in solvent A using silver nitrate and aniline oxalate sprays.
Fractions 5-7 were examined by chromatography in solvent B using silver nitrate and aniline oxalate sprays.

Discrete components were obtained from the above fractions by filter sheet chromatography in an appropriate solvent as follows.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Component</th>
<th>( R_{\text{Gal}} ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>A</td>
<td>A, B, D</td>
<td>0.65, 0.86, 1.30 (solvent A)</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>C</td>
<td>1.00 (solvent A)</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>E</td>
<td>2.26 (solvent B)</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>F</td>
<td>2.71 (solvent B)</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>G</td>
<td>2.26 (solvent B)</td>
</tr>
</tbody>
</table>
Component A.  $\alpha$-D-galactopyranosyl-(1-4)-$\alpha$-D-galactopyranosyl-(1-2)-D-threitol.

This oligosaccharide (11 mg.), $[^\alpha]_D + 106^\circ$ ($C$ 0.5, $H_2O$), $R_{Gal}$ 0.65 (solvent A), contained traces of impurities and examination of the hydrolysate in solvent B showed the presence of galactose and threitol in the approximate proportions of 2:1. The oligosaccharide (2 mg.) was methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following products were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>product</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>column (c)</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-$\alpha$-methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>2,3,6-tri-$\alpha$-methyl-D-galactose</td>
<td>3.03,3.11,4.07,4.50</td>
</tr>
<tr>
<td>1,3,4-tri-$\alpha$-methyl-D-threitol</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Component B.  $\alpha$-D-galactopyranosyl-D-threitol.

This oligosaccharide (17 mg.), $[^\alpha]_D + 122^\circ$ ($C$ 0.6, $H_2O$), $R_{Gal}$ 0.86 (solvent A), was chromatographically pure and examination of the hydrolysate in solvent B showed the presence of galactose and threitol in the approximate proportions of 1:1. The oligosaccharide (2 mg.) was methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following products were detected, the reducing sugar as its methyl glycoside.

<table>
<thead>
<tr>
<th>product</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>column (c)</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-$\alpha$-methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>1,3,4-tri-$\alpha$-methyl-D-threitol</td>
<td>0.39</td>
</tr>
</tbody>
</table>
The remainder of the oligosaccharide was oxidised with 0.1 M sodium periodate and the periodate uptake and formaldehyde released were estimated. 2.8 Moles of periodate were consumed and 0.9 moles of formaldehyde were released per mole of oligosaccharide.

**Component D. 2-O-\(\alpha\)-D-galactopyranosyl-D-glyceraldehyde.**

This oligosaccharide (19 mg.), R\(_{\text{Gal}}\) 1.30 (solvent A), was chromatographically similar to 2-O-\(\beta\)-D-galactopyranosyl-L-glyceraldehyde and gave an orange-yellow colour with aniline oxalate spray. The remainder of the oligosaccharide was reduced with sodium borohydride and a sample of the product, R\(_{\text{Gal}}\) 1.2 (solvent B), [\(\alpha\)]\(_D\) + 148\(^o\) (c 0.4, H\(_2\)O), was hydrolysed to give galactose and glycerol in the approximate proportions of 1:1.

**Component C. D-galactose.**

This sugar (6 mg.), R\(_{\text{Gal}}\) 1.00 (solvent A), was chromatographically identical to D-galactose and remained unchanged after hydrolysis. The remainder of the sugar was reduced with sodium borohydride and the product was heated with anhydrous sodium acetate (2 mg.) and acetic anhydride (0.5 ml.) in a sealed tube at 130\(^o\) for 2 hours. The solution was filtered and cooled and water was added with shaking until a homogeneous solution was obtained. Crystals of galactitol hexaacetate (m.p. 157\(^o\), mixed m.p. 157\(^o\)) separated upon concentration.

**Component E. erythritol.**

This sugar (3 mg.), R\(_{\text{Gal}}\) 2.26 (solvent B), was chromatographically identical to erythritol and remained unchanged after hydrolysis. The remainder of the sugar was acetylated and the product was examined by
gas-liquid chromatography. The product had retention time identical to that of erythritol tetraacetate.

Component F. Glycerol.

This sugar (26 mg.), \( R_{\text{Gal}} 2.71 \) (solvent B), was chromatographically identical to glycerol and remained unchanged after hydrolysis. A sample (2 mg.) of the sugar was acetylated and the product was examined by gas-liquid chromatography. The product had retention time identical to that of glycerol triacetate.

Component G. D-threitol.

This sugar (13 mg.), \( R_{\text{Gal}} 2.26 \) (solvent B), was chromatographically similar to L-threitol and remained unchanged after hydrolysis. A sample (2 mg.) of the sugar was acetylated and the product was examined by gas-liquid chromatography. The product had retention time similar to that of L-threitol tetraacetate.
Investigation of Polysaccharide A from Smith degradation.

Hydrolysis of the polysaccharide, $\left[\alpha\right]_D + 92^\circ (c 0.6, H_2O)$, uronic acid anhydride content 0.0% (carbazole method), gave galactose and rhamnose only. Colorimetric estimations of rhamnose (cysteine reagent), and total sugar (phenol-sulphuric acid reagent) in the polysaccharide and also colorimetric estimations of the relative amounts of galactose and rhamnose detected on paper after total hydrolysis of the polysaccharide and chromatography of the products using aniline hydrogen phthalate reagent (71) showed that the sugar residues galactose and rhamnose were present in the polysaccharide in the approximate proportions of 1:1. A sample (100 mg.) of the polysaccharide was methylated by the sodium hydride procedure (70) and the product was obtained as a syrup. Infra-red spectroscopy of the syrup, using chloroform as solvent, showed only a weak hydroxyl group absorption. A sample of the methylated polysaccharide was methanolyzed and the methanolysis products were examined by gas-liquid chromatography. The following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>component</th>
<th>T column (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.60</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>2.15, 2.42</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.63</td>
</tr>
<tr>
<td>3-O-methyl-L-rhamnose</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Acetolysis of polysaccharide A.

The polysaccharide (2.0 g.) was added with stirring to a mixture of
acetic anhydride (20 ml.), acetic acid (20 ml.) and concentrated sulphuric acid (2 ml.) at room temperature over a period of 1 hour. Stirring was continued for 24 hours and the solution was allowed to stand for a further 96 hours. The solution was poured into water (100 ml.) and the pH was adjusted to 5 with sodium bicarbonate. The acetylated products were extracted with chloroform (6 X 50 ml.) and the chloroform extracts were dried over anhydrous sodium sulphate and concentrated to a syrup. The syrup was dissolved in dry methanol (10 ml.) and 0.5 N barium methoxide was added until the solution was alkaline to phenolphthalein. The solution was left for 48 hours at 0° and poured into water (200 ml.) with stirring until dissolution was complete. The solution was treated with Amberlite IR 120 (H⁺) resin, filtered and concentrated to a syrup (1.9 g.).

The syrup was dissolved in water containing ethanol (1% v/v, 10 ml.), applied to a charcoal:celite column (5 X 25 cm.) and eluted with water containing an increasing proportion of ethanol. The eluate was collected in portions (30 ml.) at a rate of 60 ml. per hour and samples from every third portion were concentrated and examined by chromatography in solvent A. The following fractions were obtained.
### Discrete oligosaccharides

Discrete oligosaccharides were obtained from the above fractions by filter sheet chromatography in an appropriate solvent as follows.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Oligosaccharide</th>
<th>( R_{gal} ) value (solvent A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>A</td>
<td>7 and 8</td>
<td>0.84</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>9, 10</td>
<td>1.22, 0.35</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>11</td>
<td>0.55</td>
</tr>
<tr>
<td>11</td>
<td>E</td>
<td>12</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Oligosaccharides 7 and 8. 4-0-L-rhamnopyranosyl-D-galactose + 2-0-D-galactopyranosyl-D-threitol.

The mixture of oligosaccharides (12 mg.) had R\text{Gal} 0.84 (solvent A) and examination of the hydrolysate in solvents A and I showed the presence of galactose, rhamnose and threitol. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose, rhamnose and galactitol. Chromatography of the mixture in solvent I showed the presence of two oligosaccharides in approximately equal amounts with R\text{Gal} values of 0.29 and 0.63 respectively. Samples (2 mg.) of the mixture and the derived glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

$$\begin{array}{ll}
\text{component} & T \\
\text{column (c)}
\end{array}$$

(a) mixture

\[
\begin{array}{ll}
\text{2,3,4,6-tetra-0-methyl-D-galactose} & 1.79 \\
\text{2,3,6-tri-0-methyl-D-galactose} & 3.05, 3.72, 4.10, 4.52 \\
\text{2,3,4-tri-0-methyl-L-rhamnose} & 0.46 \\
\text{1,3,4-tri-0-methyl-D-threitol} & 0.38
\end{array}
\]

(b) glycitol

\[
\begin{array}{ll}
\text{2,3,4,6-tetra-0-methyl-D-galactose} & 1.77 \\
\text{1,2,3,5,6-penta-0-methyl-D-galactitol} & 2.64 \\
\text{2,3,4-tri-0-methyl-L-rhamnose} & 0.46 \\
\text{1,3,4-tri-0-methyl-D-threitol} & 0.39
\end{array}
\]
Oligosaccharide 9. \( \alpha-D-\text{galactopyranosyl-}L-\text{rhamnose}. \)

This oligosaccharide (47 mg.), \([\alpha]_D^0 + 105^\circ (c 2.0, H_2O), R_{Gal} 1.22 \)
(solvent A), was chromatographically and ionophoretically pure, and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol gave galactose as the only reducing sugar. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolytic products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(O)-methyl-(D)-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>3,4-di-(O)-methyl-(L)-rhamnose</td>
<td>0.98</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(O)-methyl-(D)-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>1,3,4,5-tetra-(O)-methyl-(L)-rhamnitol</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Oligosaccharide 10. \( \alpha-D-\text{galactopyranosyl-(1-2)-O-}L-\text{rhamnopyranosyl-(1-4)-}D-\text{galactose}. \)

This oligosaccharide (45 mg.), \([\alpha]_D^0 + 92^\circ (c 1.9, H_2O), R_{Gal} 0.35 \)
(solvent A), was chromatographically and ionophoretically pure and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose in the approximate proportions of 2:1. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose and rhamnose in the approximate
proportions of 1:1 and galactitol. Colorimetric estimations of rhamnose (cysteine-reagent) and total sugar (phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galactose and rhamnose were present in the oligosaccharide in the proportions of 2:1. A sample of the oligosaccharide was degraded with lime-water and chromatography of the product in solvent A showed the presence of starting material and 2-\(\alpha\)-\(\beta\)-galactopyranosyl-L-rhamnose. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

\[
\text{component} \quad T
\]

(a) oligosaccharide

\[
\begin{align*}
2,3,4,6\text{-tetra-0-methyl-\(\beta\)-galactose} & \quad 1.77 \\
3,4\text{-di-0-methyl-L-rhamnose} & \quad 0.97 \\
2,3,6\text{-tri-0-methyl-\(\beta\)-galactose} & \quad 3.04, 3.74, 4.03, 4.49
\end{align*}
\]

(b) glycitol

\[
\begin{align*}
2,3,4,6\text{-tetra-0-methyl-\(\beta\)-galactose} & \quad 1.77 \\
3,4\text{-di-0-methyl-L-rhamnose} & \quad 0.97 \\
1,2,3,5,6\text{-penta-0-methyl-\(\beta\)-galactitol} & \quad 2.66
\end{align*}
\]

Oligosaccharide 11. \(\alpha\)-\(\beta\)-galactopyranosyl-(1-2)-0-L-rhamnopyranosyl-(1-4)-\(\alpha\)-\(\beta\)-galactopyranosyl-(1-2)-L-rhamnose.

This oligosaccharide (33 mg.), \([\alpha]_D + 106^\circ (c 1.6, H_2O), R_{Gal} 0.55\) (solvent A), 0.87 (solvent E), was chromatographically and ionophoretically
pure and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose, rhamnose and rhamnitol. Colorimetric estimations of rhamnose (cysteine reagent) and total sugar (phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galactose and rhamnose were present in the oligosaccharide in the proportions of 2:2. A sample (5 mg.) of the oligosaccharide glycitol was hydrolysed in 0.05 N sulphuric acid at 100° for 1 hour and the hydrolysate was examined in solvents A and I. The presence of galactose, 2-\(\alpha-D\)-galactopyranosyl-\(L\)-rhamnose, 4-\(\alpha-L\)-rhamnopyranosyl-\(D\)-galactose, 2-\(\alpha-D\)-galactopyranosyl-\(L\)-rhamnitol and a component with chromatographic mobility identical to that of \(\alpha-D\)-galactopyranosyl-(1-2)-\(L\)-rhamnopyranosyl-(1-4)\(D\)-galactose was indicated. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

\[
\begin{array}{l}
\text{component} \\
\text{column (c)} \\
\hline
\text{(a) oligosaccharide} \\
2,3,4,6-tetra-\(O\)-methyl-\(D\)-galactose \quad 1.80 \\
2,3,6-tri-\(O\)-methyl-\(D\)-galactose \quad 3.04, 3.73, 4.08, 4.50 \\
3,4-di-\(O\)-methyl-\(L\)-rhamnose \quad 0.97
\end{array}
\]
component

(b) glycitol

- 103 -

\[
\begin{array}{ll}
2,3,4,6\text{-tetra-0-methyl-D-galactose} & 1.78 \\
2,3,6\text{-tri-0-methyl-D-galactose} & 3.04,3.72,4.06,4.50 \\
3,4\text{-di-0-methyl-L-rhamnose} & 0.98 \\
1,3,4,5\text{-tetra-0-methyl-L-rhamnitol} & 1.07 \\
\end{array}
\]

Oligosaccharide 12. \( O-\alpha-\text{D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-O-\alpha-\text{D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-D-galactose.} \)

This oligosaccharide (15 mg.), \( [\alpha]_D + 110^\circ \) \( (c 1.2, H_2O) \), \( \text{R}_{\text{Gal}} 0.17 \)

(solvent A), 0.33 (solvent E), was chromatographically and ionophoretically pure and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose, rhamnose and galactitol. Colorimetric estimations of rhamnose (cysteine reagent) and total sugar (phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galactose and rhamnose were present in the oligosaccharide in the proportions of 3:2. A sample of the oligosaccharide was degraded with lime-water and chromatography of the product in solvent A showed the presence of starting material and the tetrasaccharide \( O-\alpha-\text{D-galactopyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-O-\alpha-\text{D-galactopyranosyl-(1-2)-L-rhamnose.} \)

Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolyis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.
(a) oligosaccharide

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.76</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.00, 3.68, 4.03, 4.44</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.98</td>
</tr>
</tbody>
</table>

(b) glycitol

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.76</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.01, 3.71, 4.05, 4.45</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.98</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-O-methyl-D-galactitol</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Partial acetolysis of carboxyl-reduced gum.

Carboxyl-reduced gum (7.0 g.) was dispersed in formamide (500 ml.) by vigorous shaking overnight. Pyridine (250 ml.) was added dropwise over a period of 1 hour with vigorous stirring at 45°. The mixture was cooled to 30° and acetic anhydride (180 ml.) was added dropwise with stirring over a period of 4 hours. The mixture was stirred for a further 5 hours at 30° and overnight at room temperature. The carboxyl-reduced gum acetate was precipitated by pouring the mixture into ice-cold hydrochloric acid (2 l., 2 N), and removed at the centrifuge. The product was dispersed in acetone and water was removed by azeotropic distillation with chloroform. The acetate was precipitated by pouring the resulting chloroform suspension into light petroleum (4 vol., b.p. 40-60°), removed at the centrifuge, washed with light petroleum and dried over phosphorus pentoxide and paraffin wax.

Carboxyl-reduced gum acetate (11.2 g.) was added with stirring to a mixture of acetic anhydride (50 ml.), acetic acid (50 ml.) and concentrated sulphuric acid (5 ml.) at room temperature over a period of 4 hours. The solution was allowed to stand for 116 hours and poured into water (250 ml.). The pH of the solution was adjusted to 5 with sodium bicarbonate and the solution was extracted with chloroform (6 X 100 ml.). The chloroform extracts were combined, dried over anhydrous sodium sulphate and concentrated to a syrup. The syrup was dissolved in dry methanol (15 ml.) and 0.5 N barium methoxide was added until the solution was alkaline to phenolphthalein. The solution was left for 48 hours at 0° and poured into water (600 ml.) with stirring until dissolution was complete. The solution was treated with Amberlite IR 120 (H⁺) resin, filtered and concentrated to a
syrup (6.4 g.).

The syrup was dissolved in water containing ethanol (1% v/v, 10 ml.), applied to a charcoal:celite column (5 X 25 cm.) and eluted with water containing increasing concentrations of ethanol. The eluate was collected in portions (30 ml.) at a rate of 60 ml. per hour and samples from every third portion were concentrated and examined by chromatography in solvent A. The following fractions were obtained.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Weight (mg.)</th>
<th>Eluate</th>
<th>R$_{Gal}$ values of components (solvent A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1896</td>
<td>1% ethanol</td>
<td>glucose, galactose, rhamnose</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>1-5% ethanol</td>
<td>0.49, 0.80</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>ethanol</td>
<td>0.50, 0.80, 1.22</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td></td>
<td>0.50, 1.22</td>
</tr>
<tr>
<td>6</td>
<td>215</td>
<td>5% ethanol</td>
<td>0.26, 0.35, 1.22</td>
</tr>
<tr>
<td>7</td>
<td>112</td>
<td>5-10% ethanol</td>
<td>0.00, 0.18, 0.44, 1.07</td>
</tr>
<tr>
<td>8</td>
<td>285</td>
<td>10% ethanol</td>
<td>0.00, 0.14, 0.25, 0.39</td>
</tr>
<tr>
<td>9</td>
<td>101</td>
<td>10-20%</td>
<td>0.00, 0.23</td>
</tr>
<tr>
<td>10</td>
<td>147</td>
<td>ethanol</td>
<td>0.00, 0.19</td>
</tr>
<tr>
<td>11</td>
<td>228</td>
<td>20% ethanol</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Discrete oligosaccharides were obtained from the above fractions by filter sheet chromatography in an appropriate solvent as follows.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Oligosaccharide</th>
<th>( R_{Gal} ) value (solvent A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>A</td>
<td>13</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>14</td>
<td>0.49</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>15, 16</td>
<td>0.50, 1.22</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>17, 18</td>
<td>0.26, 0.35</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>19</td>
<td>1.07</td>
</tr>
<tr>
<td>8</td>
<td>E</td>
<td>20</td>
<td>0.39</td>
</tr>
</tbody>
</table>

**Oligosaccharide 13. 4-O-L-rhamnopyranosyl-D-galactose.**

This oligosaccharide (9 mg.), \( R_{Gal} \) 0.80 (solvent A), was chromatographically and ionophoretically pure, and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvent A showed the presence of rhamnose as the only reducing sugar. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>( T_{column(c)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>0.46</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.04, 3.72, 4.05, 4.50</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>0.45</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-O-methyl-D-galactitol</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Oligosaccharide 14. $4\alpha\text{-D-galactopyranosyl-\text{-galactose.}}$

This oligosaccharide (12 mg.), $R_{\text{Gal}}$ 0.49 (solvent A), was chromatographically and ionophoretically pure, and hydrolysis gave galactose only. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose and galactitol. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>$2,3,4,6$-tetra-$\alpha$-methyl-$\text{-galactose}$</td>
<td>1.76</td>
</tr>
<tr>
<td>$2,3,6$-tri-$\alpha$-methyl-$\text{-galactose}$</td>
<td>3.00,3.70,4.04,4.47</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>$2,3,4,6$-tetra-$\alpha$-methyl-$\text{-galactose}$</td>
<td>1.78</td>
</tr>
<tr>
<td>$1,2,3,5,6$-penta-$\alpha$-methyl-$\text{-galactitol}$</td>
<td>2.65</td>
</tr>
</tbody>
</table>

Oligosaccharide 15. $2\alpha\text{-D-galactopyranosyl-\text{-galactose.}}$

This oligosaccharide (11 mg.), $R_{\text{Gal}}$ 0.50 (solvent A), was chromatographically and ionophoretically pure, and hydrolysis gave galactose only. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose and galactitol. The oligosaccharide gave a negative reaction towards triphenyltetrazolium chloride indicating that the reducing galactose unit was substituted at
the C(2) position. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T (column c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(\text{O}-)methyl-D-galactose</td>
<td>1.78</td>
</tr>
<tr>
<td>3,4,6-tri-(\text{O}-)methyl-D-galactose</td>
<td>4.07, 6.47</td>
</tr>
<tr>
<td>unidentified peaks assumed to be those of</td>
<td></td>
</tr>
<tr>
<td>3,5,6-tri-(\text{O}-)methyl-D-galactose</td>
<td>4.45, 4.86</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(\text{O}-)methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>unidentified peak (with different retention time to that of)</td>
<td></td>
</tr>
<tr>
<td>1,2,3,5,6-penta-(\text{O}-)methyl-D-galactitol</td>
<td></td>
</tr>
<tr>
<td>assumed to be that of</td>
<td></td>
</tr>
<tr>
<td>1,3,4,5,6-penta-(\text{O}-)methyl-D-galactitol</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Oligosaccharide 16. 2-\(\text{O}-\alpha\)-D-galactopyranosyl-L-rhamnose.

This oligosaccharide (15 mg.), \(R_{\text{Gal}}\) 1.22 (solvent A), was chromato graphically and ionophoretically pure, and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol gave galactose as the only reducing sugar. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined
by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-Ω-methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>3,4-di-Ω-methyl-L-rhamnose</td>
<td>0.97</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-Ω-methyl-D-galactose</td>
<td>1.78</td>
</tr>
<tr>
<td>1,3,4,5-tetra-Ω-methyl-L-rhamnitol</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Oligosaccharide 17. \( \beta \)D-galactopyranosyl-(1-2)\( \beta \)D-galactopyranosyl-(1-4)\( \beta \)D-galactose.

This oligosaccharide (20 mg.), \( R_{gal} \) 0.26 (solvent A), 0.52 (solvent E), was chromatographically and ionophoretically pure, and hydrolysis gave galactose only. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose and galactitol. The ratio of total sugar in the oligosaccharide and the oligosaccharide glycitol determined by the phenol-sulphuric acid reagent, was approximately 3:2. A sample of the oligosaccharide was degraded with lime-water and chromatography of the product in solvents A and E showed the presence of starting material and \( 2-D \)D-galactopyranosyl-\( D \)D-galactose. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanalysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.
component (c)

(a) oligosaccharide

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.78</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-galactose</td>
<td>4.07* 6.45</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.01, 3.71, 4.07* 4.46</td>
</tr>
</tbody>
</table>

*The peak at T 4.07 was too intense to be attributed to 2,3,6-tri-O-
methyl-D-galactose alone and was assumed to include that of the methyl
glycoside of 3,4,6-tri-O-methyl-D-galactose with the lower retention time.

(b) glycitol

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.78</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-galactose</td>
<td>4.10, 6.56</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-O-methyl-D-galactitol</td>
<td>2.66</td>
</tr>
</tbody>
</table>


This oligosaccharide (75 mg.), \( R_{Gal} 0.35 \) (solvent A), 0.63 (solvent E),
was chromatographically identical to oligosaccharide 10 (O-α-D-galacto-
pyranosyl-(1-2)-O-L-rhamnopyranosyl-(1-4)-D-galactose). Examination of the
hydrolysate in solvent A showed the presence of galactose and rhamnose in
the approximate proportions of 2:1. Hydrolysis of the derived glycitol
and examination of the hydrolysate in solvents A and I showed the presence
of galactose, rhamnose, and galactitol. Colorimetric estimations of
rhamnose (cysteine reagent) and total sugar (phenol-sulphuric acid reagent)
in the oligosaccharide and the oligosaccharide glycitol indicated that
residues of galactose and rhamnose were present in the oligosaccharide in
the proportions of 2:1. A sample of the oligosaccharide was degraded with
lime-water and chromatography of the product in solvent A showed the presence
of starting material and \(2-\alpha\-D\-galactopyranosyl-L\-rhamnose\). Samples
(2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methyl-
ated by the Kuhn procedure and the methanolysis products were examined
by gas-liquid chromatography. The following components were detected,
reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) oligosaccharide</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(\alpha)-methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>3,4-di-(\alpha)-methyl-L-rhamnose</td>
<td>0.99</td>
</tr>
<tr>
<td>2,3,6-tri-(\alpha)-methyl-D-galactose</td>
<td>3.03, 3.71, 4.07, 4.48</td>
</tr>
<tr>
<td>(b) glycitol</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetra-(\alpha)-methyl-D-galactose</td>
<td>1.77</td>
</tr>
<tr>
<td>3,4-di-(\alpha)-methyl-L-rhamnose</td>
<td>0.99</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-(\alpha)-methyl-D-galactitol</td>
<td>2.66</td>
</tr>
</tbody>
</table>

Oligosaccharide 19. \(\alpha\-L\-rhamnopyranosyl-(1-4)-\(\alpha\-D\-galactopyranosyl-(1-2)-L\-rhamnose\).
This oligosaccharide (11 mg.), \(R_{Gal} 1.07\) (solvent A), 1.15 (solvent E),
was chromatographically and ionophoretically pure, and examination of the
hydrolysate in solvent A showed the presence of galactose and rhamnose.
Hydrolysis of the derived glycitol and examination of the hydrolysate in
solvents A and I showed the presence of galactose, rhamnose and rhamnitol.
Colorimetric estimations of rhamnose (cysteine reagent) and total sugar
(phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galactose and rhamnose were present in the oligosaccharide in the proportions of 1:2. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
<th>Column (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Oligosaccharide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-0-methyl-L-rhamnose</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>3,4-di-0-methyl-L-rhamnose</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>2,3,6-tri-0-methyl-D-galactose</td>
<td>3.05, 3.72, 4.06, 4.51</td>
<td></td>
</tr>
<tr>
<td>(b) Glycitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-0-methyl-L-rhamnose</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>2,3,6-tri-0-methyl-D-galactose</td>
<td>3.03, 3.68, 4.05, 4.48</td>
<td></td>
</tr>
<tr>
<td>1,3,4,5-tetra-0-methyl-L-rhamnitol</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

Oligosaccharide 20. Ω-L-rhamnopyranosyl-(1-4)-Ω-α-D-galactopyranosyl-
(1-2)-Ω-L-rhamnopyranosyl-(1-4)-D-galactose.

This oligosaccharide (38 mg.), R_Gal 0.39 (solvent A), 0.58 (solvent E), was chromatographically and ionophoretically pure, and examination of the hydrolysate in solvent A showed the presence of galactose and rhamnose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A and I showed the presence of galactose, rhamnose and galactitol. Colorimetric estimations of rhamnose (cysteine reagent) and total sugar
(phenol-sulphuric acid reagent) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galactose and rhamnose were present in the oligosaccharide in the proportions of 2:2. A sample of the oligosaccharide was degraded with lime-water and chromatography of the product in solvents A and E showed the presence of starting material and O-L-rhamnopyranosyl-(1-4)-O-O-L-galactopyranosyl-(1-2)-L-rhamnose. Samples (2 mg.) of the oligosaccharide and the oligosaccharide glycitol were methylated by the Kuhn procedure and the methanolyisis products were examined by gas-liquid chromatography. The following components were detected, reducing sugars as their methyl glycosides.

<table>
<thead>
<tr>
<th>component</th>
<th>T column (c)</th>
</tr>
</thead>
</table>

(a) oligosaccharide

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>0.45</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.98</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.01, 3.68, 4.04, 4.46</td>
</tr>
</tbody>
</table>

(b) glycitol

<table>
<thead>
<tr>
<th>Component</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>0.45</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>0.98</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.04, 3.70, 4.06, 4.47</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-O-methyl-D-galactitol</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Partial acetolysis of gum acid.

Gum acid (10 g.) was thoroughly dispersed in formamide (500 ml.) by vigorous stirring overnight, and pyridine (250 ml.) was added in portions (25 ml.) over a period of 1 hour with stirring at 45°. The mixture was cooled to 30° and acetic anhydride (180 ml.) was added dropwise with stirring over 4 hours. The mixture was stirred for a further 5 hours at 30° and overnight at room temperature. The acetate was precipitated by pouring the mixture into ice-cold hydrochloric acid (2 l., 2 N), and removed at the centrifuge. The product was dispersed in acetone and water was removed by azeotropic distillation with chloroform. The resulting chloroform suspension was poured into light petroleum (4 vol., b.p. 40–60°) and the precipitated acetate was removed at the centrifuge, washed with light petroleum, and dried over phosphorus pentoxide and paraffin wax.

The acetate (11.0 g.) was added slowly to the acetolysis mixture (acetic acid, 240 ml., acetic anhydride, 240 ml., concentrated sulphuric acid, 24 ml.) at 0° with vigorous shaking. The mixture was allowed to come to room temperature, shaken for 8 hours until dissolution was complete, and allowed to stand for a further 112 hours. The solution was poured into water (1.5 l.) and the scum which appeared was removed by filtration and extracted with chloroform (4 X 250 ml.). The pH of the supernatant liquid was adjusted to 5 with sodium bicarbonate and the solution was extracted with chloroform (4 X 500 ml.). The chloroform extracts were combined, dried over anhydrous sodium sulphate and concentrated to a syrup. The syrup was dissolved in dry methanol (25 ml.) and 0.5 N
barium methoxide was added until the solution was alkaline to phenolphthalein. The solution was left at \(0^\circ\) for 24 hours and poured into water (1 l.) with stirring until dissolution was complete. The solution was treated with Amberlite IR 120 (H\(^+\)) resin, filtered and concentrated to a syrup (4.5 g.).

The syrup was dissolved in water (5 ml.) and adsorbed on a DEAE-Sephadex column (30 g., formate form). Neutral sugars were eluted from the column with water until the eluate gave a negative reaction to the phenol-sulphuric acid reagent. Acidic sugars were eluted successively with 0.05 M formic acid, 0.4 M formic acid, 0.7 M formic acid and 1.0 M formic acid. Elution with each concentration of formic acid was continued until the eluate gave a negative reaction to the phenol-sulphuric acid reagent. The eluate was collected in fractions (15 ml.) at the rate of 30 ml. per hour and samples from every third fraction were concentrated and examined by chromatography in solvent B. Similar fractions were combined and concentrated to a small volume (40 ml.). Formic acid was removed by continuous extraction with ether in a liquid-liquid extracter for 12 hours, and the formic acid free solutions were filtered and concentrated to give the following fractions.
Filter sheet chromatography of fraction 1 gave the following oligosaccharide.

**Oligosaccharide 21.** \(\beta-(D\text{-galactopyranosyl})-(1\rightarrow2)-\gamma-(D\text{-galactopyranosyl-}
uronic acid)-(1\rightarrow4)-D\text{-galactose.}

This oligosaccharide (18 mg.), \(R_{\text{Gal A}}\) 0.09 (solvent B), 0.43 (solvent C), was chromatographically and ionophoretically pure and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid and galactose. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents A, B and D showed the presence of galacturonic acid, galactose and galactitol. A sample of the oligosaccharide was converted into the methyl ester methyl glycosides, reduced with sodium borohydride and hydrolysed to give galactose only. Samples (2 mg.) of the
oligosaccharide and the oligosaccharide glycitol were partially hydrolysed. The former gave \( 4\text{-}\beta-(\text{D}-\text{galactopyranosyluronic acid})\text{-D}-\text{galactose} \), which was not detected as a partial hydrolysis product from the glycitol. A sample (2 mg.) of the oligosaccharide was methylated by the Kuhn procedure and the methanolysis products were examined by gas-liquid chromatography. The following components were detected as their methyl glycosides.

<table>
<thead>
<tr>
<th>Component</th>
<th>T (column c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-(\beta)-methyl-(\text{D})-galactose</td>
<td>1.76</td>
</tr>
<tr>
<td>2,3,6-tri-(\beta)-methyl-(\text{D})-galactose</td>
<td>2.93, 3.64, 3.99, 4.30</td>
</tr>
<tr>
<td>3,4-di-(\beta)-methyl-(\text{D})-galacturonic acid*</td>
<td>15.66</td>
</tr>
</tbody>
</table>

*present as methyl ester
ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Professor Sir Edmund Hirst, C.B.E., F.R.S., for the provision of laboratory facilities and to Dr. G. O. Aspinall for his advice, encouragement and, above all, great patience. I am also indebted to my colleagues, both past and present, who always gave freely of their time and knowledge, and to the Carnegie Trust for the provision of a scholarship.

George D. Sanderson
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798. Plant Gums of the Genus Sterculia. Part III.1 Sterculia setigera and Cochlospermum gossypium Gums

By G. O. Aspinall, R. N. Fraser, and G. R. Sanderson

Partial hydrolysis of Sterculia setigera and Cochlospermum gossypium gums furnishes similar mixtures of acidic oligosaccharides, including 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-O-(β-D-galactopyranosyluronic acid)-D-galactose, 3-O-(β-D-glucopyranosyluronic acid)-D-galacturonic acid, and 0-(β-D-glucopyranosyluronic acid)-(1→3)-O-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose. The known structural features of the gums are compared with those of other Sterculia gums in the light of these results and of a re-examination of the cleavage products from the methylated polysaccharides.

Earlier studies of Sterculia setigera2 and Cochlospermum gossypium3 gums indicated that the two highly branched polysaccharides, which contained residues of D-galacturonic acid, D-galactose, and L-rhamnose, had several structural features in common. Partial hydrolysis of the two gums furnished similar mixtures of acidic oligosaccharides, and, although the individual components of the mixtures were not separated, some indication of their nature was obtained by methylation of the mixtures. In the case of C. gossypium gum, however, later work4 established the presence of D-glucuronic acid in addition to D-galacturonic acid residues. A further examination of the partial hydrolysis products led to characterisation of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, but no discrete oligosaccharides containing D-glucuronic acid residues could be isolated although the presence of at least one such oligosaccharide in a mixture was demonstrated. Since recent studies of Sterculia urens5 and S. caudata1,6 gums have shown that the polysaccharide components of these gums also contain residues of D-glucuronic and D-galacturonic acids, the partial hydrolysis products of S. setigera gum have now been re-examined together with those from C. gossypium gum.

Cochlospermum gossypium gum has sometimes been erroneously referred to as "karaya gum," a term properly applied to Sterculia urens. In view of possible doubts as to the origin of the commercial sample of gum used in the previous studies,2,4 gum from a botanically authenticated source was used in the present investigation, and we are grateful to Mr. A. G. Kenyon of the Tropical Products Institute for arranging for the supply of this material and an authentic sample of S. urens gum, and also to Professor J. K. N. Jones for generously providing us with some of the batch of Sterculia setigera gum on which previous studies were performed.

The polysaccharides from Sterculia urens, S. setigera, and Cochlospermum gossypium gums were obtained after deacetylation of the native gums with aqueous ammonia. The polysaccharides were methylated and the cleavage products from the methylated polysaccharides were examined by paper chromatography of the sugars and gas chromatography of the methyl glycosides. In addition, the mixtures of acidic sugars formed on hydrolysis of the methylated polysaccharides were treated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed to give mixtures of sugars which were examined by appropriate chromatographic procedures. Neutral methylated hexoses which were detected only after reduction with borohydride were presumed to have arisen from the corresponding methylated hexuronic acids. Table 1 summarises the methylated sugars which have been characterised as cleavage products from the methylated polysaccharides from the three Sterculia gums (S. urens, S. caudata, and S. setigera) and Cochlospermum gossypium gum in the present and previous investigations.

The results in Table 1 show clearly that the various gums contain the same structural units. In the case of Sterculia urens gum no differences in structural units were
observed between the commercial sample previously examined\(^8\) and the present botanically authenticated specimen. Likewise, the botanically authenticated sample of *Cochlospermum gossypium* gum was similar in most respects to the earlier sample of commercial origin.\(^3,4\) The polysaccharides from the various gums probably differ to a relatively small extent in the proportions of the constituent sugars, but methylation studies have failed to reveal any important qualitative differences in the nature and mode linkage of these units.

### Table 1

<table>
<thead>
<tr>
<th>Gum:</th>
<th>Sterculia urens</th>
<th>Sterculia caudata</th>
<th>Sterculia setigera</th>
<th>Cochlospermum gossypium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylated sugar residues in methylated gums</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Sample:</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Methylated sugar residues</td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
</tbody>
</table>

Further evidence for the structural similarities between the polysaccharides from these gums has been obtained by a re-examination of the acidic oligosaccharides formed on partial acid hydrolysis of the polysaccharides from *Sterculia setigera* and *Cochlospermum gossypium* gums. As in the preceding Paper\(^1\) the complex mixtures of oligosaccharides were separated by ion-exchange chromatography on diethylaminomethyl-Sephadex followed as necessary by filter-sheet chromatography. Where sufficient quantities were obtained the oligosaccharides were characterised by the formation of crystalline derivatives, most frequently by gas chromatography of methyl glycosides in admixture with other sugar components. A semi-quantitative estimate of relative proportions based on isolated yields, relative intensities of spots on paper chromatograms, or relative peak intensities on gas chromatograms.

It is now apparent that the gums from the three *Sterculia* species (*S. urens*, *S. caudata*, and *S. setigera*) and the gum from *Cochlospermum gossypium* contain not only the same structural units as the four polysaccharides, but have been characterised by the same structural units, which can be extended to the major type of the gum. The structural units, fragmentation, and relative proportions of the relative results are summarised in Table I.
structural units but also that many of the same sequences of sugar residues are present in the four polysaccharides. Table 2 indicates the acidic oligosaccharides which have now been characterised as partial hydrolysis products of the gums. The various partial structures, which have been proposed for Sterculia urens and S. caudata gums, may now be extended to S. setigera and C. gossypium gums. In order to obtain further structural information on these gums it will be necessary to isolate, as products of partial degradation, fragments containing new sequences of sugar residues, and experiments to modify the relative rates of cleavage of the different glycosidic linkages are in progress.

### Table 2

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Gum</th>
<th>Sterculia urens</th>
<th>Sterculia caudata</th>
<th>Sterculia setigera</th>
<th>Cochlospermum gossypium</th>
</tr>
</thead>
<tbody>
<tr>
<td>GaLA 1 → 2 Rha</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GaLA 1 → 4 Gal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GA 1 → 3 GaLA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GaLA 1 → 4 GalA → 2 Rha</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Experimental

The general experimental procedures were as described in Part II. 1

**Isolation of Polysaccharides from Gums.**—Finely powdered Cochlospermum gossypium gum (50 g.) was deacetylated with aqueous ammonia, as described for Sterculia caudata, and afforded gum acid (34 g.), \([\alpha]_D +66^\circ (c 0.49 \text{ in } H_2O \text{ containing } 1\% \text{ of ammonia}),\) uronic anhydride (by decarboxylation) 40-45%. Likewise, Sterculia urens gum furnished gum acid, \([\alpha]_D +59^\circ (c 0.06 \text{ in } 1\% \text{ NaOH}),\) uronic anhydride, 45%. Sterculia setigera gum was similarly deacetylated. Chromatography of the hydrolysates from each of the gums showed the presence of galactose, rhamnose, galacturonic and glucuronic acids, and complex mixtures of acidic oligosaccharides.

**Partial Hydrolysis of Gum Acids and Separation of Acidic Sugars.**—Gum acid (10 g.) from Cochlospermum gossypium gum was heated in n-sulphuric acid (500 ml.) on a boiling-water bath for 7 hr. and afforded a syrupy hydrolysate (6.81 g.). The mixture of sugars was fractionated by column chromatography on diethylaminoethyl-Sephadex as described in Part II, followed as required by filter-sheet chromatography in solvent B, to give neutral sugars (183 mg.), galacturonic acid (769 mg.), oligosaccharide I (231 mg.), \(R_{Ga}_{Aa} 0.79\) and \([\alpha]_D +93^\circ (c 2.31 \text{ in } H_2O),\) oligosaccharide II (164 mg.), \(R_{Ga}_{Aa} 0.39\) and \([\alpha]_D +70^\circ (c 1.98 \text{ in } H_2O),\) oligosaccharide III (394 mg.), \(R_{Ga}_{Aa} 0.24\) and \([\alpha]_D +120^\circ (c 1.97 \text{ in } H_2O),\) oligosaccharide IV (61 mg.), \(R_{Ga}_{Aa} 0.29\) and \([\alpha]_D +42^\circ (c 3.05 \text{ in } H_2O),\) and oligosaccharide V (300 mg.), \(R_{Ga}_{Aa} 0.20\) and \([\alpha]_D +75^\circ (c 3.00 \text{ in } H_2O).\) In addition, a sugar with the chromatographic mobility of acidic oligosaccharide VI, \(R_{Ga}_{Aa} 0.18,\) from Sterculia caudata gum was present but was not obtained in suitable form for detailed study.

Gum acid (2 g.) from Sterculia setigera gum was hydrolysed similarly to give a syrupy hydrolysate (1-296 g.). Fractionation of the syrup gave neutral sugars (183 mg.), galacturonic acid (245 mg.), oligosaccharide I (71 mg.), \(R_{Ga}_{Aa} 0.80\) and \([\alpha]_D +94^\circ (c 1.42 \text{ in } H_2O),\) oligosaccharide II (31 mg.), \(R_{Ga}_{Aa} 0.39\) and \([\alpha]_D +74^\circ (c 1.55 \text{ in } H_2O),\) oligosaccharide III (79 mg.), \(R_{Ga}_{Aa} 0.24\) and \([\alpha]_D +122^\circ (c 1.39 \text{ in } H_2O) +122^\circ \rightarrow +76^\circ (4 \text{ hr., equil.}) (c 1.39 \text{ in } H_2O \text{ containing } 1\% \text{ of ammonia}),\) oligosaccharide IV (34 mg.), \(R_{Ga}_{Aa} 0.27\) and \([\alpha]_D +42^\circ (c 1.7 \text{ in } H_2O),\) oligosaccharide V (64 mg.), \(R_{Ga}_{Aa} 0.24\) and \([\alpha]_D +80^\circ (c 1.85 \text{ in } H_2O),\) and oligosaccharide VI (15 mg.), \(R_{Ga}_{Aa} 0.17\) and \([\alpha]_D +73^\circ (c 1.5 \text{ in } H_2O).\)

**Examination of Acidic Oligosaccharides.**—Acidic oligosaccharides from both gums were examined by the following reaction sequences: (1) hydrolysis and paper chromatography of the hydrolysate; (2) reduction with sodium borohydride, hydrolysis, and paper chromatography of the hydrolysate; (3) conversion into the methyl ester methyl glycosides with methanolic hydrogen chloride, reduction with sodium borohydride, hydrolysis, and paper chromatography of the hydrolysate; and (4) methylation with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, reduction with lithium aluminium hydride, methanalysis, and gas chromatography of the resulting methyl glycosides on column b. The results are summarised in Table 3. In addition, oligosaccharides I, III, IV, and V from both gums, and
oligosaccharide II from Cochlospermum gossypium gum gave infrared spectra which were identical with those of the corresponding acidic oligosaccharides from Sterculia caudata gum.1

**TABLE 3**

<table>
<thead>
<tr>
<th>Acidic oligosaccharide</th>
<th>Reaction sequence</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>Galacturonic acid, rhamnose</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Galacturonic acid, rhamnitol</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Galactose, rhamnose</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>Galacturonic acid, galactose</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Galacturonic acid, galactitol</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Galactose</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2,3,4-, and 2,3,6-Me&lt;sub&gt;3&lt;/sub&gt; galactose</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>Galacturonic acid, galactose, O-acetylgalactose</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Galacturonic acid, galactitol</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Galactose</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2,3,4- and 2,3,6-Me&lt;sub&gt;3&lt;/sub&gt; galactose</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>Glucuronic acid, galacturonic acid</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Glucuronic acid, galactonic acid</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Glucose, galactose</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2,3,4-Me&lt;sub&gt;3&lt;/sub&gt; glucose, 2,4-Me&lt;sub&gt;2&lt;/sub&gt; galactose</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>Glucuronic acid, galacturonic acid, rhamnose</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Glucuronic acid, galacturonic acid, rhamnitol</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Glucose, galactose, rhamnose</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2,3,4-Me&lt;sub&gt;3&lt;/sub&gt; glucose, 2,4-Me&lt;sub&gt;2&lt;/sub&gt; galactose, 3,4-Me&lt;sub&gt;4&lt;/sub&gt;rhamnose</td>
</tr>
<tr>
<td>VI</td>
<td>1</td>
<td>Galacturonic acid, rhamnose (molar ratio, 2 : 1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Galacturonic acid, rhamnitol</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Galactose, rhamnose</td>
</tr>
</tbody>
</table>

**Oligosaccharide I.** The aldobiouronic acid from both gums was characterised as 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into the methyl glycoside pentamethyl ether dihydrate, which was identified by m. p. 67° and mixed m. p. 67°, and by X-ray powder photograph.

**Oligosaccharide III.** The oligosaccharide (200 mg.) from Cochlospermum gossypium gum was saponified with 4N-sodium hydroxide (10 ml.) on a boiling-water bath for 30 min. The reaction mixture was acidified and distilled, to give acetic acid which was characterised by conversion into 4-nitrobenzyl acetate, m. p. and mixed m. p. 77°.

**Oligosaccharide V.** The acidic trisaccharide (100 mg.) from Cochlospermum gossypium gum was successively methylated to give methylated acidic trisaccharide, reduced with lithium aluminium hydride to give methylated trisaccharide, and hydrolysed to give a mixture (54 mg.) of sugars. The mixture was separated on filter sheets using solvent D, to give 2,4-di-O-methyl-D-galactose (14 mg.), \([\alpha]_D^\text{+} + 90° (c 1·4 \text{ in } H_2O)\), which crystallised as the monohydrate, m. p. 99° and mixed m. p. 99—100°, and a mixture (27 mg.) of sugars which was further separated by ionophoresis in borate buffer to give 3,4-di-O-methyl-L-rhamnose (10 mg.), \([\alpha]_D^\text{+} + 66° (c 1·1 \text{ in } H_2O)\), which was characterised as the aniline derivative, m. p. 135° and mixed m. p. 134—135°.

**Methylation of Gum Acids,—**Samples (ca. 1·5 g.) of gum acids were stirred in suspension in ethereal diazomethane, and the resulting polysaccharide methyl esters were methylated with methyl sulphate and barium hydroxide in dimethyl sulfoxide and NN-dimethylformamide as described by Kuhn and Trischmann.8 Further methylations with methyl iodide and silver oxide furnished (i) methylated Cochlospermum gossypium gum, \([\alpha]_D^\text{+} + 43° (c 1·64 \text{ in } CHCl_3)\) (Found: OMe, 409%), methylated Sterculia urens gum, \([\alpha]_D^\text{+} + 68° (c 1·24 \text{ in } CHCl_3)\) (Found: OMe, 41·9%), and methylated Sterculia setigera gum, \([\alpha]_D^\text{+} + 66° (c 1·26 \text{ in } CHCl_3)\) (Found: OMe, 43·9%).

Samples of the methylated polysaccharides were heated with methanolic hydrogen chloride, and, in each case, examination of the cleavage products by gas chromatography on columns a and b indicated the presence of methyl glycosides of the following sugars (approximate relative proportions in parenthesis): 2,3,4- (+ or ++), 3,4-di- (+ +), and 3-O-methylrhamnose (+ +), 2,3,4,6-tetra- (+ +) and 2,3,6-tri-O-methylgalactose (+ +), 2,3,4-tri-O-methylglucuronic acid (+ +), 2,3,4-tri- (trace or +), and 2,3-di-O-methylgalacturonic acid (+).
Samples (0.2 g.) of the methylated polysaccharides were refluxed with methanolic 4% hydrogen chloride (10 ml.) for 18 hr. The neutralised solutions were concentrated, heated with saturated barium hydroxide (10 ml.) at 60° for 2 hr., and passed through Amberlite resin IR-120(H) to remove barium ions. The concentrated solutions were adsorbed on columns (6 x 1 cm.) of diethylaminoethyl-Sephadex (formate form). Elution with water furnished neutral methyl glycosides, and elution with aqueous 1% formic acid gave acidic methyl glycosides. The neutral methyl glycosides were hydrolysed, and paper chromatography of the resulting mixtures of sugars in solvents A, D, and E showed the above-mentioned neutral sugars together with traces of 2,6-di-O-methylgalactose and rhamnose. The acidic methyl glycosides were heated with methanolic hydrogen chloride, reduced with sodium borohydride, and hydrolysed, and paper chromatography of the resulting mixtures of sugars showed 3,4-di- (+) and 3-O-methylrhamnose (+), 2,3,4-tri- (trace or +), 2,3-di- (+), 2- (+ +), and 3-O-methylgalactose (+ +), and 2,3,4-tri-O-methylglucose (+ + +).

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1 Part II, G. O. Aspinall and R. N. Fraser, preceding Paper.
3 E. L. Hirst and S. Dunstan, J., 1953, 2332.