STRUCTURAL STUDIES ON PLANT GUMS
WITH SPECIAL REFERENCE TO
KHAYA IVORENSIS GUM

by

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TO MY PARENTS
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INTRODUCTION

GENERAL

Plant gums are defined as those substances of plant origin which are obtained as exudates from fruit, trunk or branches of trees spontaneously or after mechanical injury of the plant or after invasion by micro-organisms such as bacteria or fungi. Although this definition would include materials like natural rubber, terpenoid resins, and carbohydrate exudates, all of which are plant exudates, it is only the last group of substances that will be dealt with in this script and will be referred to as "Plant gums", "Exudate gums" or simply as "Gums".

The use of plant gums by man dates as far back as history can recall. To date a very wide spectrum of their use can be found ranging from food\(^1\), cosmetics and pharmaceuticals\(^2\), up to a hair-wave setting agent\(^3\). Their use in corrosion inhibition of brass, aluminium-aluminium alloys, and aluminium-zinc alloys have been reported\(^4\),\(^5\),\(^6\). In admixture with aromatic plastics\(^7\) and hydroxyethylcellulose\(^8\), plant gums have been found to give an increase in the viscosity of the mixture. Their use in thickening of paints and in sizing of textiles and paper are among the oldest uses known.

A large number of plants exude gum in smaller or larger quantities. The origin of gums and mechanism of their formation is not yet clearly understood. That the gums are produced at the site of injury or infection of the plants, suggests that they are produced by some kind of protective mechanism in order to seal
off the injured site from further attack by micro-organism and
to prevent loss of moisture. The close structural relation-
ship of some of the exudate gums to other types of plant
polysaccharides such as cell-wall polysaccharides, has been
suggested to indicate that similar biosynthetic pathways are
followed in their formation\(^9\).

The large number of plant gums studied so far have been
found to be composed of two to five neutral sugar residues and
one or two acidic sugar residues. The neutral sugars commonly
encountered are \(\text{D-}\)galactose, \(\text{D-}\)mannose, \(\text{L-}\)rhamnose, \(\text{L-}\)arabinose
and \(\text{L-}\)fucose and the acidic sugars are \(\text{D-}\)galacturonic acid and
\(\text{D-}\)glucuronic acid (sometimes as its \(\text{L-}\)-methyl ether). These
different sugars are present in the branched chain molecules of
plant gums in a variety of glycosidic linkages. It is also not
uncommon to find more than one polysaccharide having widely
different structures to be constituents of the same gum. These
observations make it clear that the plant gums constitute a very
complex class of organic compounds.

The concept of homogeneity or heterogeneity of polysaccharides
is relatively recent. In the past many polysaccharides were
considered homogeneous due to the inability to effect
fractionation by the methods available. As new methods have been
developed for fractionation, so also new techniques are now
available by which it is possible to examine homogeneity of
polysaccharide preparations in greater detail.
Although most plant gums consist almost entirely of polysaccharide material, there are instances of more than one kind of polysaccharide being present in the same gum. Structural investigation on a material which may contain more than one kind of polymer is of limited value. An elaborate purification treatment, therefore, must forego any structural study.

The heterogeneity of plant gums may be regarded as extending from the micro-heterogeneity as observed in Combretum leonense gum (10), to the gross heterogeneity exhibited by Khaya senegalensis gum (11) and gum tragacanth (12). In between these two extreme kinds of heterogeneity is the case of Anogeissus leiocarpus gum (13, 14), from which two polysaccharide components were obtained which are composed of the same sugar units linked in the same way but in widely different proportions.

The large scale fractionation of plant gums involves either fractional precipitation from aqueous solution by the addition of a non-solvent, or precipitation with reagents which form insoluble salts of acidic polysaccharides. It is sometimes found that the gum is insoluble in water, particularly in case of partly acetylated gums such as those from Khaya and Sterculia genera, and in these cases dissolution can be effected by treatment with dilute alkali resulting in concomitant deacetylation. The possibility of alkaline degradation (15) occurring during such treatment should not be overlooked. The polysaccharide may be precipitated out of solution by the addition of acidified ethanol or acetone. Further purification can be achieved by repeating
the same treatment. In cases where the gum consists essentially of a single polysaccharide, a homogeneous product can be obtained by this treatment alone, as was the case with cholla gum\(^{(16)}\) and many gums of the \textit{Prunus} family\(^{(17-19)}\). However, where the gum consists of a mixture of different polysaccharides, this treatment would not give a satisfactory separation unless the components have widely different solubilities. Precipitation with salt forming reagents provides a complementary method of fractionation in these cases.

Acidic polysaccharides can be separated from neutral polysaccharides by precipitation as calcium or barium salts. Copper salts such as cupric acetate\(^{(20)}\) has been found to effect precipitation of acidic polysaccharides and by careful control of the amount of reagent added it is possible to effect fractionation of polysaccharides containing varying amounts of uronic acids. In our present investigation this method has been made use of, which will be described in a later section. Precipitation with long-chain quaternary ammonium bases is another very useful method for fractionation of acidic polysaccharides. Most commonly used reagents are cetyltrimethyl-ammonium bromide (Cetavlon) and cetyl-pyridinium chloride or bromide. Scott\(^{(21)}\) has summarised the use of these reagents. Gum tragacanth\(^{(12)}\) and \textit{Anogeissus leiocarpus} gum\(^{(13)}\) have been fractionated using cetavlon.

Chromatography on ion-exchange columns provides a promising method for finer resolution of polysaccharide mixtures. Although this method has not yet been applied to large scale
fractionation of plant gums, the new ion-exchange materials such as diethylaminoethyl (DEAE)-cellulose\(^{(22)}\) and DEAE-sephadex, which provide high resolution of polysaccharides and flexibility of use in different forms and with different eluants, may, in time, prove very useful for the fractionation of such complex polymers. The major polysaccharide component from *Khaya senegalensis* gum was obtained in a homogeneous state by DEAE-cellulose chromatography\(^{(23)}\). Bouveng\(^{(24)}\) successfully fractionated polysaccharides in pollen by this method using potassium acetate solution as eluant. The low capacity of DEAE-cellulose and the observed leakage of cellulosic or xylose containing material from such column are some of the limitations of this method. The excellent resolution of polysaccharides obtained by this method, however, makes it a valuable analytical tool for assessing the homogeneity of polysaccharide preparations.

DEAE-sephadex on the other hand has a higher capacity than DEAE-cellulose and as such is of more interest for large scale fractionation. Its use for the fractionation of plant gums has not been reported, but encouraging results are forthcoming. A citrus pectin has been successfully fractionated in this laboratory\(^{(25)}\) on DEAE-sephadex A-50 in the formate form. It has been observed that the resin can absorb a tenth of its weight of citrus pectin.

Whereas the methods mentioned above are used for the fractionation of complex polysaccharides, it is essential to assess the homogeneity of polysaccharides prepared in this way.
As mentioned before, the ion-exchange chromatography on DEAE-cellulose provides a means for such assessment. Free-boundary electrophoresis is another important method for the assessment of homogeneity. Since plant gums are acidic polysaccharides, they will migrate in solution under the influence of an applied electric field. Using appropriate buffer solutions it is possible to achieve satisfactory resolution of mixtures of polysaccharides having different acidity. The methods of ultracentrifugation (26) and gel filtration on the other hand provide information about the molecular size distribution in a polymer, and as such may be used for assessing the molecular heterogeneity of plant gums.
METHODS OF STRUCTURAL INVESTIGATION

Once a reasonably pure and homogeneous polysaccharide has been prepared by a method or a combination of methods described in the previous section, structural investigation may be carried out on this material. Estimation of uronic anhydride content in the polysaccharide can be carried out by decarboxylation\(^{(27)}\) or by carbazole-sulphuric acid colorimetric method\(^{(28)}\). Information as to the nature and proportion of different sugar residues present in the molecule may be obtained by total acid hydrolysis followed by chromatography\(^{(29,30)}\) and identification of sugars as crystalline sugars or sugar derivatives. In cases of acidic polysaccharides where total hydrolysis is not possible without causing decomposition, a complete quantitative estimation cannot be carried out directly.

These preliminary investigations have to be followed by various fragmentation analyses to indicate the sequence of sugar residues, the ring size of the individual sugars, the proportion of glycosidic linkages and the points of branching in the molecule. These studies fall into one or other of the following methods.

Partial acid hydrolysis

This is variously called as graded hydrolysis, controlled acid hydrolysis or linkage analysis and is based on the fact that hydrolysis of a polysaccharide under mild conditions results in the formation of oligosaccharides containing two, three or more sugar units, characterisation
of which provides information regarding the sequence of sugar residues in the molecule. The stability of glycosidic linkages towards acids varies considerably according to the class of sugars, their ring form, and the type of linkage. Advantage may be taken of the greater stability of pyranosides as opposed to furanosides and the relative resistance of glycosiduronic acid linkages to acid hydrolysis.

A very mild form of partial hydrolysis is possible in case of acidic polysaccharides having labile structural units at the periphery of the molecule. Heating an aqueous solution of the polysaccharide itself results in the cleavage of such labile units giving rise to mono- and oligosaccharides, characterisation of which provide information about the periphery of the molecule. This process called "Autohydrolysis" also gives rise to a relatively simpler degraded polymer, structural studies on which provide information about the inner core of the molecule. Whereas the partial acid hydrolysis refers to hydrolysis at low pH values, it has recently been reported\(^{31}\) that poly- and oligouronides have a high rate of hydrolysis at pH values above 2, relative to neutral polysaccharides under identical conditions. Applying this technique, Lindahl\(^{32}\) has isolated three neutral oligosaccharides from heparin as shown below:
UA-GlcNAc-GlcUA-Gal-Gal-Xyl-Serine

\[ \text{HCl} \quad \text{Citrate buffer} \quad \text{pH 3} \]

GlcUA → Gal

\{ Gal→Gal

Gal→Xyl

Gal→Gal→Xyl \}

[Where UA = uronic acid, GlcNAc = N-acetylglucosamine, GlcUA = glucuronic acid, Gal = galactose and Xyl = Xylose]

A complementary method of fragmentation is that of partial acetolysis, in which the polysaccharide or its acetylated derivative is treated with acetic anhydride in the presence of concentrated sulphuric acid as catalyst. The cleavage in this case seems to occur in a slightly different fashion than in the case of aqueous acid hydrolysis. For example the formation of oligosaccharides containing 6-deoxyhexosidic linkages is favoured in this case as opposed to the latter. Thus a new range of oligosaccharides can be obtained by this method.

Advantage has also been taken of changes in the rates of cleavage of different glycosidic bonds consequent upon structural modification of polysaccharides such as reduction of \( ^{33} \) or oxidation to \( ^{34} \) hexuronic acids.

Methylation studies

The classical method of methylation \( ^{35,36} \) followed by complete hydrolysis and identification of methylated sugars as crystalline sugars or sugar derivatives still remains a powerful tool for structural investigation in carbohydrate chemistry. The ever-increasing modifications \( ^{37-40} \) in the process leading
to simpler and micro-methods of methylation, together with
the development of the technique of gas-liquid chromatography\(^{(41)}\) for the identification of methylated and partially methylated
sugars have made it possible to work on a micro scale. The
results of methylation study provide information regarding the
mode of linkage of the individual sugar units, their ring form,
the points of branching in a molecule and the nature of end
groups.

**Periodate oxidation studies**

Oxidation with periodate ion followed by analysis of the
oxidation products often gives useful information about the
carbohydrate structure. The measurement of consumption of
periodate and the amount of formic acid released would indicate
the number of \(\alpha\)-glycol groups and the involvement of \(\alpha\)-triole
groups respectively. In the case of mono- and oligosaccharides
these values can be used to distinguish between alternative
structures. In case of polysaccharides, however, the main
product of oxidation is the polyaldehyde. If the polysaccha-
ride contains periodate resistant residues in the molecule,
the polyaldehyde will contain these intact residues. The
polyaldehyde thus obtained is usually reduced with borohydride
into polyalcohol, a procedure known as Smith degradation\(^{(42)}\).
The polyalcohol in contrast to the polyaldehyde is hydrolysed
with great ease. Non-oxidised residues attached to adjacent
oxidised units are then split off by mild acid treatment as
glycosides of erythritol or glycerol. Adjacent non-oxidised
residues will give rise to oligosaccharides identification of which
provides evidence for the presence of such structural features.
CHEMISTRY OF PLANT GUMS OF THE GENUS KHAYA

The gums from two species of plants of this genus namely those from Khaya grandifoliola (previously reported as K. grandifolia) and Khaya senegalensis have been investigated previously. The gum from the latter species was fractionated into two polysaccharide components (polysaccharide A and polysaccharide B). Results of these investigations will be reviewed here.

Khaya grandifoliola gum

The gum exudate of Khaya grandifoliola, a West African mahogany tree, was first examined by McLlroy [43] who obtained evidence for the presence of residues of galactose, rhamnose and galacturonic acid. The same sample of gum was further examined in this laboratory [44]. The gum was deacetylated with Na-sodium hydroxide solution and precipitated with acidified acetone. After reprecipitation from aqueous solution with ethanol the polysaccharide had an uronic anhydride content of 47%. The gum has been shown to be composed of residues of D-galactose, L-rhamnose, D-galacturonic acid, and 4-O-methyl-D-glucuronic acid, with traces of L-arabinose.

Partial hydrolysis of the polysaccharide with Na-sulphuric acid at 100° for 6 hr. gave rise to L-rhamnose (3.7%), L-arabinose (0.2%), and D-galactose (18.0%), together with incompletely hydrolysed acidic oligosaccharides (52% of the total gum taken). The acidic oligosaccharides were fractionated by stepwise elution from a column of an anion-
exchange resin with aqueous acetic acid. Two aldobiouronic acids and an aldotriouronic acid were identified as 2-\(\text{O}-(\xi\text{-galactopyranosyluronic acid})\)-H-rhamnose (I), 4-\(\text{O}-(4-\text{O}-\text{methyl-}\xi\text{-glucopyranosyluronic acid})\)-H-galactose (II), and 4-(\xi\text{-galactopyranosyluronic acid})-2-\(\text{O}-(\xi\text{-rhamnopyranosyl})\)-H-galactose (III) respectively. In addition some incompletely identified acidic oligosaccharides were obtained. The high proportion of acid residues in one of these fractions (equivalent weight 300) suggested that the gum may contain adjacent galacturonic acid residues.

\[
\begin{align*}
\xi\text{-GalpA} & \rightarrow 2 \xi\text{-Rha} \quad \text{.... (I)} \\
4-\text{Me} \xi\text{-GpA} & \rightarrow 4 \xi\text{-Gal} \quad \text{.... (II)} \\
\xi\text{-GalpA} & \rightarrow 2 \xi\text{-Rhap} \rightarrow 4 \xi\text{-Gal} \quad \text{.... (III)}
\end{align*}
\]

Hydrolysis of the methylated gum followed by identification of the methyl sugars showed presence of residues of 2,3,4,6-tetra- and 2,3,6-tri-\(\text{O}-\text{methyl-}\xi\text{-galactose}, \xi\text{-methyl-}\xi\text{-rhamnose, 2,3,4-tri-}\text{O}-\text{methyl-}\xi\text{-glucuronic acid and 2,3-di-}\text{O}-\text{methyl-}\xi\text{-galacturonic acid.}

These results indicated the presence in the gum of \xi\text{-galactose and 4-}\text{O}-\text{methyl-}\xi\text{-glucuronic acid end groups. The rhamnose residues provide the only branching points in the molecule and 1,4-linked \xi\text{-galacturonic acid residues and 1,2-linked \xi\text{-rhamnose residues occur in the main chains. The isolation of the trisaccharide (III) from the partial hydrolysis products showed presence of 1,4-linked galactose units in the main chain. The aldobiouronic acid (II) shows that the 4-}\text{O}-\text{methyl-}\xi\text{-glucuronic acid end groups are linked to the main chain}
through 1,4-linked  \( \beta \)-galactose residues probably as a two-residue side chain. On the basis of these results, the following partial structure was put forward for the gum.

\[
-4\text{GalpA} \rightarrow 2\text{Rhap} \rightarrow 4\text{Galp} \rightarrow 4\text{GalpA} \rightarrow 2\text{Rhap} \rightarrow 4\text{GalpA} \rightarrow \text{GalpA} \\
\]

\[
\text{Galp} \\
4\text{MeGpA}
\]

([GalpA =  \( \beta \)-galactopyranosyluronic acid, Galp =  \( \beta \)-galactopyranose, Rhap =  \( \alpha \)-rhamnopyranose and 4-MeGpA = 4-O-methyl- \( \beta \)-glucopyranosyluronic acid])

**Khaya Senegalensis gum (Polysaccharide A)**

The gum was partly acetylated in the natural state and was deacetylated by treatment with 1N sodium hydroxide solution. The deacetylated gum was found to contain two polysaccharide components. The major component (polysaccharide A) was precipitated out of solution by the addition of acidified ethanol. After several reprecipitations the isolated polysaccharide had  \([\alpha]_D +140^0\) and uronic anhydride content 55%. Structural investigation was carried out on this material. A second polysaccharide component (polysaccharide B) was isolated from the supernatant liquors as its insoluble calcium salt. Later, when structural investigation on the polysaccharide A was almost complete, the gum and its fractions were examined by glass-fibre paper ionophoresis in 2N-sodium hydroxide solution. By this criterion, the unfractionated gum contained two polysaccharides, the
polysaccharide B with a higher mobility was homogeneous and the polysaccharide A was still contaminated by a small proportion (ca 5%) of the polysaccharide B.

Partial acid hydrolysis of the polysaccharide A gave rise to two aldobiouronic acids which were identified as 2-O-(galactopyranosyluronic acid)-L-rhamnose (I) and 4-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-galactose (II). Hydrolysis of the methylated polysaccharide followed by identification of the methyl sugars showed presence of residues of 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri- and 2,4-di-O-methyl-D-galactose, 2,3,4-tri- and 3,4-di-O-methyl-D-glucuronic acid; 2,3,4-tri-, 3,4-di, and 3-O-methyl-L-rhamnose and 2,3-di-O-methyl-D-galacturonic acid. In addition, smaller amounts of 2,3,5-tri-O-methyl-L-arabinose; 2,3- and 2,6-di-O-methyl-D-galactose and L-rhamnose were obtained.

These results showed the close structural relationship between this polysaccharide and the polysaccharide of Khaya grandifoliola gum. Both have 1,4-linked galacturonic acid and 1,2-linked rhamnose residues in the main chain, where rhamnose units alone provide the branching points. Both contain end groups of galactose and 4-O-methylglucuronic acid. The observed structural features of the polysaccharide A from the deacetylated Khaya senegalensis gum are represented in the partial structure (III).
In addition the presence of smaller amounts of other methylated sugars may be represented by the following partial structures (IV - IX).

\[
\begin{align*}
\text{L-Araf}^1 & \quad \text{(IV)} & \text{L-Rhap}^1 & \quad \text{(V)} \\
-2\text-L-Rhap^1 & \quad \text{(VI)} & -6\text-D-Galp}^1 & \quad \text{(VII)} \\
-6\text-D-Galp}^1 & \quad \text{(VIII)} & -4\text-Me D-Gp}^2 & \quad \text{Al}^3 \quad \text{(IX)} \\
\end{align*}
\]

In view of the fact that the polysaccharide A was found to be contaminated by polysaccharide B (ca 5%), it can be argued that some of these minor structural features are due to the latter polysaccharide.

Khaya Senegalensis gum (Polysaccharide B)\(^{(46)}\)

The polysaccharide had \([\alpha]_D + 11^\circ\) and uronic anhydride content of ca 21%, and gave galactose, arabinose and acidic sugars on hydrolysis. Partial acid hydrolysis gave rise to three aldobiouronic acids, which were identified as 6-\text-O-(\(\beta\)-\text-D-glucopyranosyluronic acid)-\text-D-galactose (I), 6-\text-O-(4-\text-O-methyl-\(\beta\)-\text-D-glucopyranosyluronic acid)-\text-D-galactose (II), and 4-\text-O-(4-\text-O-methyl-\(\lambda\)-\text-D-glucopyranosyluronic acid)-\text-D-galactose (III) respectively.

The results of methylation analysis indicated that the polysaccharide is highly branched and contains inner chains of \text-D-galactopyranose residues to which are attached a variety of side chains terminated by residues of \text-L-arabinofuranose, \text-D-galactopyranose and \text-D-glucuronic acid (in part as the 4-methyl ether). In order to obtain further information about the
relative disposition of the different types of linkages in the interior chains, the following experiments were carried out:

**Polysaccharide B**

- Carboxyl Reduction
- Carboxyl-reduced polysaccharide B
- Smith degradation
- Degraded galactan B
- Smith degradation
- Degraded galactan B''
- Partial acid hydrolysis

\[ \text{D-Galp} \rightarrow \text{3D-Gal} \]

These results indicated that the inner core of the polysaccharide molecule consists of a linear 1,3-linked galactan.

To obtain evidence regarding the attachment of arabinofuranose residues in the polysaccharide, it was subjected to autohydrolysis which resulted in the release of the majority of the arabinose residues together with a small proportion of galactose residues, and an arabinose-free degraded polysaccharide was obtained.

The degraded polysaccharide B and the degraded galactans B' and B'' were methylated and the cleavage products from the various methylated polymers were identified. The following Table shows the relative proportions of different methyl sugars obtained from the various methylated degraded polysaccharides compared with those from the methylated polysaccharide B.
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<tr>
<td>2,3,5-Me$_3$-arabinose</td>
<td>+++</td>
<td>tr</td>
<td>tr</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4,6-Me$_4$-galactose</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me$_3$-galactose</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
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<td>2,3,6-Me$_3$-galactose</td>
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<tr>
<td>2,4-Me$_2$-galactose</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,6-Me$_2$-galactose</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-Me galactose</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4-Me$_3$-glucuronic acid</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3,4-Me$_2$-glucuronic acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
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The results of methylation studies clearly indicated a decrease in the relative proportion of 2,4-di-O-methylgalactose and an increase in that of 2,4,6-tri-O-methylgalactose as cleavage products from the methylated degraded polysaccharide B compared to those from the methylated polysaccharide B, thus showing the probable attachment of the majority of the L$_6$-arabinofuranose residues to position 6 of the 1,3-linked galactose residues in the main chain. On the basis of these results the following partial structures were put forward for the polysaccharide B from deacetylated *Khaya senegalensis* gum.
3Galpl \rightarrow 3Galpl \rightarrow 3Galpl \rightarrow 3Galpl \rightarrow 3Galpl \rightarrow 3Galpl

\text{Araf} \quad \text{Galp} \quad \text{Araf}

\begin{align*}
P-\text{GpAl} \xrightarrow{\beta} 6 \ P-\text{Galpl} \quad \text{(I)}
4-\text{MeP-} \text{GpAl} \xrightarrow{\beta} 6 \ P-\text{Galpl} \quad \text{(II)}
4-\text{MeP-} \text{GpAl} \xrightarrow{\alpha} 4 \ P-\text{Galpl} \quad \text{(III)}
\end{align*}
In continuation of the previous work done in this laboratory on gums from the two species of the genus Khaya, we wanted to examine the structural features of the gum from a third species, namely Khaya ivorensis gum. Structural studies have been carried out on the major polysaccharide component (polysaccharide A) of this gum. An attempt has also been made to obtain polysaccharide A from the gum of the related species, Khaya senegalensis, in a homogeneous state and to obtain additional evidence for the structure of this polysaccharide.
DISCUSSION
Purification and Fractionation

The sample of gum under examination was collected at the Agricultural Research Station, Kade, Ghana, and was botanically authenticated. In the natural state the polysaccharides in the gum were partly acetylated and the gum was insoluble in water. It had to be treated with Na-sodium hydroxide solution to effect dissolution with concomitant deacetylation. Sodium borohydride (1% w/w of the gum) was added at this stage to prevent alkaline degradation taking place from the reducing end of the polysaccharide molecule. The gum acid which was obtained by precipitation from aqueous solution with non-solvent contained a major polysaccharide component (polysaccharide A), which was obtained in an essentially homogeneous state by precipitation from aqueous solution with cupric acetate. The polysaccharide A thus obtained had $[\alpha]_D + 138^\circ$ and uronic anhydride content 38% (by decarboxylation). Structural investigation was carried out on this material. Later in the course of investigations on Khaya senegalensis gum it was found that the polysaccharide A obtained from this gum contained a small proportion of a highly acidic component (polysaccharide C, uronic anhydride content Ca 70%). A re-examination of the polysaccharide A from Khaya ivorensis gum indicated the possibility of the presence of a small proportion (Ca 5%) of such a component.

Structural investigation

Hydrolysis of the polysaccharide A with $\text{H}_2\text{SO}_4$-sulphuric acid
at 100° for 16 hr. gave galactose, arabinose, rhamnose, galacturonic acid and 4-0-methylglucuronic acid together with incompletely hydrolysed acidic oligosaccharides. Partial acid hydrolysis of the polysaccharide with H₂SO₄ at 100° for 7 hr. gave neutral monosaccharides, acidic mono- and oligosaccharides and an acid resistant degraded polysaccharide (12% of the total polysaccharide taken). The degraded polymer precipitated out of solution during hydrolysis. The high uronic anhydride content (76%) of this material and the release of only galacturonic acid on drastic hydrolysis showed it to be essentially a degraded galacturonan.

The soluble sugars from the partial hydrolysate were fractionated on DEAE-sephadex A-25. Elution with water removed the neutral sugars. The acidic sugars were fractionated by gradient elution with water containing formic acid. The individual fractions were further fractionated, where necessary, by chromatography on filter sheets. Six acidic sugars were thus obtained in sufficient quantities for further investigation. The acidic sugars I and II were identified as 4-0-methyl-D-glucuronic acid (I) and D-galacturonic acid (II) respectively.

\[ \begin{align*}
\text{(I)} & \quad \begin{array}{c}
\text{COOH} \\
\text{MeO} \\
\text{OH} \\
\text{H,OH}
\end{array} \\
\text{(II)} & \quad \begin{array}{c}
\text{COOH} \\
\text{HO} \\
\text{OH} \\
\text{H,OH}
\end{array}
\end{align*} \]
Acidic Sugar III was found to be composed of galacturonic acid and rhamnose residues with the latter at the reducing end by hydrolysis, borohydride reduction and hydrolysis and reduction of the methyl ester methyl glycoside followed by hydrolysis. The sugar was identified as 2-((\alpha-D-galactopyranosyluronic acid))-L-rhamnose by conversion into the crystalline methyl glycoside pentamethyl ether dihydrate.

Acidic Sugar IV gave $4-\Omega$-methylglucuronic acid and galactose on hydrolysis, and $4-\Omega$-methylglucuronic acid and galactitol on reduction with sodium borohydride followed by hydrolysis. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave $4-\Omega$-methylglucose and galactose. The identity of the methyl ether was indicated by paper chromatography of the periodate oxidation products. The methanolysis products from the methylated oligosaccharide were identified by gas-liquid chromatography as methyl glycosides of 2,3,4-tri-$\Omega$-methylglucuronic acid and 2,3,6-tri-$\Omega$-methylgalactose. The latter sugar was identified as 2,3,6-tri-$\Omega$-methyl-$\alpha$-galactose by gas chromatography of the derived 2,3,6-tri-$\Omega$-methyl-$\alpha$-galactonolactone. The high
positive rotation \([\alpha_d^+ + 42^\circ]\) of the oligosaccharide indicated an \(\alpha\)-linkage.

Acidic Sugar V was found to be composed of only galacturonic acid units by hydrolysis, and reduction of the methyl ester methyl glycoside followed by hydrolysis. Methanolysis of the methylated carboxyl-reduced oligosaccharide followed by gas-liquid chromatography showed presence of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-\(\alpha\)-methylgalactose. The oligosaccharide had the same chromatographic mobility as a sample of oligosaccharide having the structure 4-\(\alpha\)-\(\alpha\)-galactopyranosyluronic acid)-\(\alpha\)-galacturonic acid. The high positive rotation \([\alpha_d^+ + 120^\circ]\) of the oligosaccharide also indicated an \(\alpha\)-linkage.
Acidic Sugar VI gave galacturonic acid and rhamnose on hydrolysis and galacturonic acid, rhamnose and rhamnitol on borohydride reduction and hydrolysis. The oligosaccharide had the same chromatographic mobility as a tetrasaccharide having the structure \( \alpha-(D\text{-galactopyranosyluronic acid})\text{-1\textendash}2\text{-}\alpha-(L\text{-rhamnopyranosyl})\text{-1\textendash}4\text{-}\alpha-(D\text{-galactopyranosyluronic acid})\text{-1\textendash}2\text{\beta-rhamnose} \), obtained from soybean hull pectin \(^{47}\). The structure of the oligosaccharide was determined as follows:

Partial hydrolysis of the oligosaccharide glycitol gave products with the same chromatographic mobility as galacturonosyl 1→2 rhamnose and galacturonosyl 1→2 rhamnitol. The ratio of galacturonic acid to rhamnose in the oligosaccharide and the oligosaccharide glycitol was found to be 1:1 and 2:1 respectively. Methanolyis of the methylated oligosaccharide glycitol followed by gas chromatography showed presence of 1,3,4,5-tetra-\( \alpha \)-methylrhamnitol and methyl glycosides of 2,3,4-tri-\( \alpha \)-methylglucuronic acid, 2,3-di-\( \alpha \)-methylgalacturonic acid and 3,4-di-\( \alpha \)-methylrhamnose. The structure of the oligosaccharide is therefore,
The polysaccharide A was methylated by Haworth and Purdie methods. A portion of the methylated polysaccharide was methanolyzed and examination of the products by gas-liquid chromatography showed presence of methyl glycosides of 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri-, and 2,4-di-β-methylgalactose; 3,4-di- and 3-β-methylrhamnose, 2,3,5-tri-β-methylarabinose; 2,3,4-tri-β-methyl-glucuronic acid and 2,3-di-β-methylgalacturonic acid.

The methylated polysaccharide was reduced with lithium aluminium hydride in tetrahydrofuran and the resulting reduced methylated polysaccharide was hydrolysed with H₂-sulphuric acid. The hydrolysate was fractionated on a cellulose column and further fractionated by chromatography on filter sheets or on charcoal-celite columns. The methylated sugars were identified as crystalline sugars and/or as crystalline derivatives. The approximate relative proportions of the various methylated sugars are shown in the following Table:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Relative proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-β-methyl-β-galactose</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,5-tri-β-methyl-β-arabinose</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4-tri-α-methyl-β-glucose</td>
<td>++</td>
</tr>
<tr>
<td>3,4-di-β-methyl-β-glucose</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-tri-β-methyl-β-galactose</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,4-tri-β-methyl-β-galactose</td>
<td>+</td>
</tr>
<tr>
<td>3-β-methyl-β-rhamnose</td>
<td>+++</td>
</tr>
<tr>
<td>2,3-di-β-methyl-β-galactose</td>
<td>+++</td>
</tr>
<tr>
<td>2,4-di-β-methyl-β-galactose</td>
<td>+</td>
</tr>
<tr>
<td>β-rhamnose</td>
<td>++</td>
</tr>
</tbody>
</table>

[+++ = 10-15%, ++ = 6-8%, and + = 2-5% of the total]
(In addition smaller amounts of 2,6-di- and 2- and 3-\(\beta\)-methyl-D-galactose and 3,4-di-\(\beta\)-methyl-D-glucose were obtained. The structural significance of these sugars cannot be assessed at present). Since the sugars 2,3-di-\(\beta\)-methylgalactose and 2,3,4-tri-\(\beta\)-methylglucose were not present among the cleavage products from the methylated polysaccharide, but were obtained from the reduced methylated polysaccharide, they must have been produced by reduction of the corresponding uronic acids.

The results of methylation analysis together with those of the partial acid hydrolysis indicates the presence in the polysaccharide A of a main chain consisting of 1,4-linked \(\beta\)-galacturonic acid and 1,2-linked \(\beta\)-rhamnose residues. Some regions in the main chain contain consecutive galacturonic acid residues as indicated by the isolation of the galacturonobiose (V) and the degraded galacturonan from partial hydrolysis products. There are also regions of alternating galacturonic acid and rhamnose residues as indicated by the isolation of the acidic tetra-saccharide (VI). The rhamnose residues provide the only branching points in the molecule. These residues carry substituents either at position 4 or both at positions 3 and 4, while there are a small proportion of rhamnose residues which are unsubstituted. The 4-\(\beta\)-methyl-glucuronic acid residues are present as non-reducing end groups attached to position 4 of \(\beta\)-galactose residues as in the acidic sugar 4-\(\beta\)-methylglucuronosylgalactose (IV). A substantial proportion of \(\beta\)-galactopyranose residues are also present as end groups. The relatively high proportion of these end groups suggests that they are attached as single or double unit side chains to
rhamnose residues which provide the only branching points in the molecule. The main structural features of the polysaccharide A from deacetylated *Khaya ivorensis* gum may, therefore, be represented by the partial structure (I)

\[
\begin{align*}
-\text{4GalpA} & \rightarrow \text{4GalpA} \rightarrow 2\text{Rhap} \rightarrow \text{4GalpA} \rightarrow 2\text{Rhap} \rightarrow 4\text{GalpA} \\
\text{Galp} & \quad \text{Galp} \\
& \quad \quad \text{4MeGpA}
\end{align*}
\]

(I)

[GalpA = D-galactopyranosyluronic acid, Galp = D-galactopyranose, Rhap = L-rhamnopyranose and 4MeGpA = 4-O-methyl-D-glucopyranosyluronic acid]

The presence of other methylated sugars may be represented by the partial structures II - IV, although their exact mode of attachment in the polysaccharide cannot be fully assessed.

\[
\begin{align*}
-\text{6Galp} & \rightarrow \ldots \quad \text{(II)} \\
\text{L-Arap} & \rightarrow \ldots \quad \text{(IV)}
\end{align*}
\]

These results show the close structural relationship of the polysaccharide A from deacetylated *Khaya ivorensis* gum with the corresponding polysaccharides from *Khaya senegalensis* and *Khaya grandifoliola* gums. All the three polysaccharides contain some of the same structural features although differing in details. For example all of them contain a main chain essentially the same as shown in partial structure (I). The sugars represented in...
partial structures (II - IV) have not been obtained from the polysaccharide of *Khaya grandifoliola* gum and although these sugars were obtained from the polysaccharide A from *Khaya senegalensis* gum, their structural significance was doubtful due to the contamination of the polysaccharide A by polysaccharide B. In fact these sugars have later been obtained from the polysaccharide B(46) from *Khaya senegalensis* gum. In the present case of *Khaya ivorensis* gum, however, the virtual absence of the polysaccharide B from polysaccharide A (Fig. II) makes it imperative to consider these sugars as integral parts of the polysaccharide A.

The main structural features of the polysaccharide A may also be compared with the structurally related polysaccharides from the gums of the genus *Sterculia* namely *Sterculia urens*(48), *S. setigera*(49) and *S. caudata*(50) and with that of *Cochlospermum gossypium* gum(51). All the polysaccharides contain the four sugar residues:—galactose, rhamnose, galacturonic acid and glucuronic acid (in some cases as its 4-methyl ether) where galacturonic acid and rhamnose residues are present in the main chain. The glucuronic acid (or its 4-methyl ether) residues are present only in the side chain as non-reducing end groups. An important distinction between the polysaccharide A from *Khaya ivorensis* gum and the polysaccharides from the gums of the genus *Sterculia* lies in the fact that whereas in the former polysaccharide the 4-0-methylglucuronic acid end groups are attached to galactose residues which in turn are probably attached to the rhamnose residues in the main chain (Partial Structure V), in the
latter polysaccharides the glucuronic acid end groups are directly linked to galacturonic acid residues in the main chain (Partial Structure VI).

\[ \text{\textbullet \textbullet \textbullet \textbullet \textbullet} \quad 4\text{GalpAl} \rightarrow 2\text{Rhap1} \quad \text{(V)} \]

\[ \begin{array}{c}
\circ \circ \circ \circ \\
\text{Galp} \\
\circ \circ \circ \circ \\
\text{MeGpA} \\
\circ \circ \circ \circ \\
\end{array} \]

\[ \text{\textbullet \textbullet \textbullet \textbullet \textbullet} \quad 3 \quad 1 \\
\text{GpA} \]

The polysaccharides from the gums of Khaya and Sterculia genera are also related to the pectins such as those from lemon peel\(^{[52]}\), lucern (alfalfa)\(^{[53]}\), and the bark of Ambilis fir\(^{[54]}\), in having main chains consisting of blocks of similarly linked \(\alpha\)-galacturonic acid residues interposed by \(\beta\)-rhamnose residues. They differ, however, in the proportion of the two sugars mentioned and also in the nature of side chains.
KHAYA SENEGALENSIS GUM

Purification and fractionation

The sample of gum was the same as was used in previous investigations. Two polysaccharide components (polysaccharide A and polysaccharide B) have been isolated before from the deacetylated Khaya senegalensis gum and structural investigations on them have been carried out (11, 46). It was observed, however, that the polysaccharide A was contaminated with a small proportion of polysaccharide B. We have now re-examined the fractionation of the gum in an attempt to prepare a homogeneous polysaccharide A. It was observed that ca 80% of the gum was soluble in water and since this is the mildest treatment that can be used for the isolation of the gum acid, the aqueous solution of the gum was examined by boundary electrophoresis (picture 3), which indicated the heterogeneous nature of the polysaccharides in the gum. No distinct separation of components was however obtained. Treatment of the gum with dilute ammonia effected partial deacetylation and boundary electrophoresis (picture 4) of the resulting product gave a slightly different picture in that partial separation of components was obtained and the presence of three or four components was observed. Treatment of the gum with \( \text{NaOH} \) solution effected complete deacetylation and the solution after removal of sodium ions was examined by boundary electrophoresis (picture 5). The presence of three distinct components was indicated, of which the major component (ca 50% of the total) had the mobility intermediate between the two minor
components. The sharp difference between the electrophoretic pattern of the aqueous solution of the gum and that of the alkali deacetylated gum cannot be explained at present. The possibility that this may be due to degradation of the polysaccharide in the gum during alkaline treatment has been minimised by treatment of the gum with alkali in the presence of sodium borohydride to prevent the peeling type of alkaline degradation and also by treatment of the gum with alkali in an atmosphere of nitrogen to avoid any oxidative degradation. In both instances similar electrophoretic patterns as shown in picture 5, were obtained.

Attempts were however made to obtain a homogeneous polysaccharide from the native gum, by fractional precipitation from aqueous solution with ethanol and also by fractional precipitation from aqueous solution with copper acetate. Only very limited fractionation was achieved in this way. Fractionation was therefore carried out on the deacetylated Khaya senegalensis gum. It was observed that precipitation from aqueous solution with copper acetate in this case gives a fairly homogeneous product, and the polysaccharide obtained after two subsequent precipitations with this reagent was the best that could be obtained i.e. no further fractionation was effected by repeating the same treatment. The polysaccharide prepared in this way was examined by boundary electrophoresis (picture 7), and also by DEAE-cellulose chromatography (Fig. III), which indicated the presence in the polysaccharide of three components, of which the major component (polysaccharide A) constituted ca 85% of the total and had uronic anhydride content 53%, a minor component
(polysaccharide B, ca 5%) had uronic anhydride content 18% and a third component (polysaccharide C) had uronic anhydride content 70% and constituted ca 10% of the total polysaccharide. Attempts to precipitate the highly acidic component (polysaccharide C) preferentially, were not successful, in all cases it being co-precipitated with the polysaccharide A.

The physical constants for this polysaccharide preparation i.e. uronic anhydride content 55% and $\left[ \alpha \right]_D + 140^\circ$, were comparable to those reported for the polysaccharide A used in the previous investigation\(^{(11)}\). Structural investigation was therefore carried out on this material which will be referred to as polysaccharide A.
Structural investigations

The previous investigations on the polysaccharide A from deacetylated *Khaya senegalensis* gum indicated the presence in the polysaccharide of residues of D-galactose, L-rhamnose, D-galacturonic acid and L-2-O-methyl-D-glucuronic acid. Two aldobiouronic acids isolated from the partial acid hydrolysis of the polysaccharide were identified as 2-O-(D-galactopyranosyluronic acid)-L-rhamnose and L-2-O-(L-2-O-methyl-D-glucopyranosyluronic acid)-D-galactose. These results indicated the presence in the polysaccharide of 1,4-linked galacturonic acid and 1,2-linked rhamnose residues. No direct evidence regarding the relative disposition of these sugar residues was however available.

Since partial acetolysis of polysaccharides is known to give rise to a new range of oligosaccharides, particularly the oligosaccharides containing intact 6-deoxy hexosidic linkages, additional evidence has now been sought regarding the sequence of sugar residues in the polysaccharide A, through partial acetolysis of the carboxyl-reduced polysaccharide.

The carboxyl-reduced polysaccharide A has been prepared by reduction of the acetylated glycol ester of polysaccharide A with lithium borohydride in tetrahydrofuran (72). The acetolysis of the carboxyl-reduced polysaccharide A gave rise to mono- and oligosaccharide acetates, which after deacetylation were fractionated on a charcoal-celite column by gradient elution with water containing increasing concentration of ethanol. The individual fractions were further fractionated by chromatography on filter sheets where necessary. A number of oligosaccharides were thus
obtained of which the following seven have been identified.

Oligosaccharide I gave rhamnose and galactose on hydrolysis and rhamnose and galactitol on borohydride reduction and hydrolysis. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of 2,3,4-tri-O-methylrhamnose and 2,3,6-tri-O-methylgalactose. The oligosaccharide is therefore, 4-O-(\(\beta\)-rhamnopyranosyl)-\(\beta\)-galactose (I).

\[ \text{I} \quad \overbrace{\text{L-Rhap}} \longrightarrow \text{4 \(\beta\)-Gal} \]

Oligosaccharide II gave only galactose on hydrolysis and had the same chromatographic mobility as a sample of 4-O-(\(\beta\)-galactopyranosyl)-\(\beta\)-galactose. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-O-methyl-galactose. The specific rotation \([\alpha]_D + 130^\circ\) of the oligosaccharide indicated an \(\alpha\)-linkage. The oligosaccharide is therefore, 4-O-(\(\alpha\)-\(\beta\)-galactopyranosyl)-\(\beta\)-galactose (II).

\[ \text{II} \]

![Diagram of oligosaccharides](image-url)
Oligosaccharide III gave galactose and rhamnose on hydrolysis and galactose and rhamnitol on borohydride reduction followed by hydrolysis. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of 2,3,4,6-tetra-α-methylgalactose and 3,4-di-α-methylrhamnose. The high positive rotation, [α]_D + 100º of the oligosaccharide indicated an α-linkage. The oligosaccharide is therefore, 2-α-(α-D-galactopyranosyl)-L-rhamnose (III).

\[ \text{III} \]

Oligosaccharide IV gave galactose and rhamnose on hydrolysis. The ratio of reducing sugar in the oligosaccharide and the oligosaccharide glycitol was found to be 3:4:2. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-α-methylgalactose and 3,4,6-di-α-methylrhamnose. Methanolysis of the methylated oligosaccharide glycitol gave 1,2,3,5,6-penta-α-methylgalactitol and methyl glycosides of 2,3,4,6-tetra-α-methylgalactose and 3,4,6-di-α-methylrhamnose. The oligosaccharide is therefore a trisaccharide.
having the structure, $\beta$-Gal $(\beta$-galactopyranosyl) $1\rightarrow$ 2-$\alpha$-Gal $\rightarrow$ l-Rha $\rightarrow$ 4-$\beta$-Galactose (IV).

\[
\beta$-Gal $\rightarrow$ 2-$\alpha$-Rha $\rightarrow$ 4-$\beta$-Gal
\]

(IV)

Oligosaccharide V gave only galactose on hydrolysis. The ratio of reducing sugar present in the oligosaccharide and the oligosaccharide glycitol determined after hydrolysis was found to be $3.3 : 2$ showing the oligosaccharide to be a trisaccharide. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-$\beta$-methylgalactose. Methanolysis of the methylated oligosaccharide glycitol gave 1,2,3,5,6-penta-$\beta$-methylgalactitol and methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-$\beta$-methylgalactose. The oligosaccharide is therefore, $\beta$-Gal $(\beta$-galactopyranosyl) $1\rightarrow$ 4-$\beta$-Gal $(\beta$-galactopyranosyl) $1\rightarrow$ 4-$\beta$-galactose (V). The specific rotation $[\alpha]_D + 104^\circ$, of the oligosaccharide indicated $\alpha$-linkage.
Oligosaccharide VI gave \( \beta-\text{D}-\text{methylglucose} \) and galactose on hydrolysis and \( \beta-\text{D}-\text{methylglucose} \) and galactitol on borohydride reduction followed by hydrolysis. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography showed the presence of methyl glycosides of \( 2,3,4,6 \)-tetra-\( \beta-\text{D}-\text{methylglucose} \) and \( 2,3,6 \)-tri-\( \beta-\text{D}-\text{methylgalactose} \). Similarly methanolysis of the methylated oligosaccharide glycitol gave \( 1,2,3,5,6 \)-penta-\( \beta-\text{D}-\text{methylgalactitol} \) and methyl glycosides of \( 2,3,4,6 \)-tetra-\( \beta-\text{D}-\text{methylglucose} \). The high positive rotation, \([\alpha]_D^{+} +114^\circ\) of the oligosaccharide indicated an \( \alpha \)-linkage. The oligosaccharide is therefore, \( \beta-\text{D}-\text{D(-4-D-methyl-\( \alpha \)-D-glucopyranosyl)-D-galactose} \) (VI).

\[ \text{(VI)} \]

Oligosaccharide VII gave galactose and rhamnose on hydrolysis and galactose, rhamnose and rhamnitol on borohydride reduction followed by hydrolysis. The ratio of total reducing sugar to rhamnose in the oligosaccharide and the oligosaccharide glycitol was found to be \( 2:2 : 1 \) and \( 3:2 : 1 \) respectively showing the oligosaccharide to be a tetrasaccharide having two rhamnose residues. Methanolysis
of the methylated oligosaccharide glycitol followed by gas-liquid chromatography showed the presence of 1,3,4,5-tetra-O-methylrhamnitol and methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-O-methylgalactose and 3,4-di-O-methylrhamnose. The tetrasaccharide therefore has one of the following structures:

$$\text{D-Galp} \rightarrow 2 \text{L-Rhap} \rightarrow 4 \text{D-Galp} \rightarrow 2 \text{L-Rha}$$

or

$$\text{D-Galp} \rightarrow 4 \text{D-Galp} \rightarrow 2 \text{L-Rhap} \rightarrow 2 \text{L-Rha}$$

(VII)

In the light of these results together with those from the previous investigation on this polysaccharide, a detailed structure can be put forward for the main chain of the polysaccharide A from Khaya senegalensis gum. Previous studies indicated the presence in the polysaccharide of residues of D-galacturonic acid linked to the 2 position of L-rhamnose residues. The isolation of the oligosaccharides rhamnoseyl 1→4 galactose (I), galactosyl 1→2 rhamnose (III), and galactosyl 1→2 rhamnosyl 1→4 galactose (IV), together probably with the tetrasaccharide (VII), from the partial acetolysis of the carboxyl-reduced polysaccharide A, now clearly indicates the presence of galacturonic acid and rhamnose residues linked 1,2 and rhamnose units linked to galacturonic acid 1,4 and that there are some regions in the polysaccharide molecule containing alternating galacturonic acid and rhamnose residues. Similarly the oligosaccharides galactosyl 1→4 galactose (II) and the galactotriose (V) represents some regions in the main chain
containing consecutive 1,4-linked galacturonic acid residues. The oligosaccharide 4-O-methylglucosyl 1→4 galactose (VI) however, represents a feature of the side chain where 4-O-methylglucuronic acid end groups are attached to the galactose residues which in turn are probably linked to the rhamnose residues in the main chain. It is also known that the polysaccharide A contains a substantial proportion of 2-galactopyranose end groups. On the basis of these results the following partial structure may be put forward to represent the main structural features of the polysaccharide A from deacetylated Khaya senegalensis gum.

\[
\text{GalpAl} \rightarrow 4\text{GalpAl} \rightarrow 2\text{Rhap} \rightarrow 4\text{GalpAl} \rightarrow 2\text{Rhap} \rightarrow 4\text{GalpAl} - \]

It is now obvious that the main structural features of the polysaccharide A from Khaya senegalensis gum are very much similar to those of the polysaccharide A from Khaya ivorensis gum. Both polysaccharides contain blocks of similarly linked galacturonic acid residues interposed by rhamnose residues in the main chain and both contain non-reducing end groups of galactopyranose residues and 4-O-methylglucuronic acid residues linked to galactose. The two polysaccharides differ however in some of the minor structural features, for example the uronic anhydride contents of the two polysaccharides (55% for the polysaccharide A from...
Khaya senegalensis gum and 38% for the polysaccharide A from Khaya ivorensis gum), are sufficiently different to indicate the variation in the frequency of occurrence of the uronic acid residues in the two polymers.
Carboxyl-reduced *Khaya senegalensis* gum

It has been discussed earlier that the observed presence of three distinct components in the deacetylated *Khaya senegalensis* gum in contrast to apparently a single component in the native gum, may have been due to degradation of a single polysaccharide during alkaline treatment. In order to avoid treatment of the gum with alkali an alternative method was sought for the fractionation of the gum, in which the native gum was converted into the carboxyl-reduced gum through its acetylated glycol ester. The carboxyl-reduced gum was fractionated by fractional precipitation with ethanol into two fractions (I and II) which will be referred to as carboxyl-reduced polysaccharide A' and carboxyl-reduced polysaccharide B' respectively, because of their similarity to the corresponding polysaccharides obtained from the deacetylated *Khaya senegalensis* gum as discussed below.

\[
\begin{align*}
\text{Gum} & \xrightarrow{\text{N}_2\text{-NaOH}} \text{Deacetylated gum} \\
\text{Carboxyl-reduced} & \xrightarrow{\text{Methylated}} \text{Carboxyl-reduced} \\
\text{Polysac. A'} & \text{Polysac. B'} \\
\text{Methylated} & \text{Methylated} \\
\text{carboxyl-reduced} & \text{carboxyl-reduced} \\
\text{Polysac. A'} & \text{Polysac. B'} \\
[\alpha]_D + 70^\circ & [\alpha]_D - 10^\circ \\
[\alpha]_D + 76^\circ
\end{align*}
\]

The similarity between the methylated carboxyl-reduced polysaccharides A and A' shown by their specific rotations, were further substantiated
by the isolation of similar cleavage products from the two methylated polymers. Methylated carboxyl-reduced polysaccharide B on the other hand showed the presence of a relatively large proportion of sugars such as 2,3,5-tri-0-methylarabinose, 2,3,4-tri-0-methylgalactose and 2,4-di-0-methylgalactose, which have been obtained from the polysaccharide B from the deacetylated Khaya senegalensis gum (46). These results indicate the possibility that the native gum contains two main polysaccharide components.
GENERAL METHODS

Paper Chromatography.

Whatman No.1 and Whatman No.4 papers were used for qualitative chromatography. Preparative separations were carried out on Whatman No. 3MM filter sheets, which were pre-washed with boiling water in a Soxhlet extractor. In all cases descending technique was employed and the following solvent systems (v/v) were used:


E. Methyl ethyl ketone : acetic acid : water (9 : 1 : 1), saturated with boric acid.


H. Methyl ethyl ketone : water : ammonia (200 : 17 : 1).


$R_{\text{GalA}}$ values refer to the rate of movement of sugars relative to D-galacturonic acid on Whatman No.4 paper in solvent A.

$R_{\text{Gal}}$ values refer to the rate of movement relative to D-galactose on Whatman No.1 paper in solvent B.

And $R_G$ values of methylated sugars refer to the rate of
movement relative to 2,3,4,6-tetra-\text{-}O\text{-}methyl-\text{-}\text{D}\text{-}glucose on Whatman No.1 paper in solvent F.

The chromatograms after being developed were air dried and the sugars were located by spraying with a suitable spray reagent $^{(55-58)}$.

Paper ionophoresis $^{(59)}$ was carried out qualitatively on Whatman No.1 paper in borate buffer $(\text{pH} 10)$ at a potential of 350 volts for six hours. The sugars were located by spraying the air dried ionophoretogram with $\text{p}$-anisidine hydrochloride [1\% solution in butan-1-ol : acetic acid $(9:1)$].

Column chromatography was carried out on cellulose, DEAE-cellulose, DEAE-sephadex and charcoal-celite. The columns were prepared as follows:

**Cellulose column** $^{(60)}$

Whatman standard grade cellulose powder was washed thoroughly with distilled water followed by butan-1-ol. The cellulose was then packed in a column $(3.5 \times 80 \text{ cm.})$ as a slurry in butan-1-ol and was washed with the starting eluant.

**DEAE-cellulose columns**

Diethylaminoethylcellulose was washed alternately with $0.5\text{M}$-hydrochloric acid and $0.5\text{M}$-sodium hydroxide, and finally washed free of alkali with distilled water. The exchanger was generated in the phosphate form by stirring in $0.5\text{M}$-sodium dihydrogen phosphate (buffered at pH 6 by the addition of sodium hydroxide) and packed into a column as a slurry in this buffer. The exchanger was equilibrated with $0.05\text{M}$-sodium dihydrogen
phosphate (at the same pH).

**DEAE-Sephadex columns**

Diethylaminoethylsephadex (A-25 and A50) was allowed to swell in water and fine particles were removed by decantation. The resin was stirred in 0.5M-sodium hydroxide solution for half an hour and then washed free of alkali with distilled water. The exchanger was taken to the desired form by treatment with a solution of acid containing the desired counter ion (aqueous 15% formic acid for formate form, aqueous 20% acetic acid for acetate form). After leaving in the acid for half an hour the resin was washed free of acid with water. The exchanger was packed in a column as a slurry in water and was equilibrated with the starting eluant before use.

**Charcoal-celite columns**

The charcoal was washed several times with boiling water and the fine particles were removed by decantation. It was then washed with cold water. The celite was washed with 6M-hydrochloric acid and then washed free of acid with distilled water. The mixture of charcoal and celite (1:1) was packed in a column as a slurry in water, and was washed thoroughly with the eluants to be used followed by distilled water.

**Gas-liquid chromatography**

Qualitative separation and identification of methyl glycosides of methylated sugars were carried out on a "Fye Argon chromatograph" using various liquid phases supported on acid washed celite (80-100 Mesh). The following liquid phases
were used:

(a) 10% by weight of polyphenyl ether \( m \)-bis-\( m \)-phenoxyphenoxy)-benzene, at 200\(^\circ\).

(b) 11% by weight of diethylene glycol succinate polyester, at 175\(^\circ\).

(c) 15% by weight of butan-1,4-diol succinate polyester, at 175\(^\circ\).

(d) 3% by weight of neopentylglycol adipate polyester at 150\(^\circ\).

(e) 5% by weight of XE-60, at 125\(^\circ\).

(f) 3% by weight of ECN3S, at 175\(^\circ\).

(g) 15% by weight of polyethylene glycol adipate, at 175\(^\circ\).

Retention times (\( T \)) of methyl glycosides of methylated sugars are relative to that of 2,3,4,6-tetra-O-methyl-\( \beta \)-D-glucopyranoside.

Methyl glycosides and methyl ester methyl glycosides were prepared by heating with methanolic 4% hydrogen chloride in a sealed tube at 100\(^\circ\) for 3 hr. in case of mono-and oligosaccharides and for 16 hr. in case of methylated polysaccharides.

Free boundary electrophoreeses were carried out on a Beckmann model H Electrophoresis/Diffusion instrument using either phosphate buffer (pH 6)\(^\text{(63)}\) or pyridine-acetate buffer (pH 5.5)\(^\text{(64)}\). The polysaccharide (80 - 100 mg.) in the buffer (15 - 20 ml.) was dialysed overnight against a large volume of the buffer before electrophoresis was performed.
Optical rotations were measured at 18 ± 2° in aqueous solution (unless otherwise stated) using the D-line of sodium light.

Melting points were measured by using a Kofler hot-stage microscope (unless stated otherwise).

Small scale methylations (Kuhn's method)(65)

The sugar (1 - 5 mg.) in N, N-dimethylformamide (0.2 ml.) was treated with methyl iodide (0.2 ml.) and silver oxide (20 - 30 mg.). The mixture was shaken in the dark for 48 hr. at room temperature, diluted with chloroform (2 ml.) and filtered. The chloroform solutions were concentrated and the dimethylformamide removed by azeotropic distillation with toluene under reduced pressure.

Demethylations(66)

The methylated sugar (1 - 10 mg.) was dissolved or suspended in methylene chloride (1 - 2 ml.) and cooled in acetone/cardice (-80°). Boron trichloride (1 - 2 ml.), cooled in the same way was added to it and the mixture was kept at -80° for 30 minutes. The reaction mixture was allowed to warm up to room temperature (20°) and left for a further 16 hr. The boron trichloride was allowed to evaporate off in a fume cupboard and the solution was evaporated to dryness. The boric acid was removed by repeated evaporations with methanol.

Small scale periodate oxidations were carried out by the method of Lemieux and Bauer(67) in which the sugar (1 - 2 mg.) in 0.5M-sodium metaperiodate solution (0.2 ml.) was kept at 0° for one hour. Excess periodate was destroyed by the addition of
ethylene glycol (1 drop) and the solution warmed up to room temperature. The solution was made alkaline to phenolphthalein with 0.5 N-sodium hydroxide, and then evaporated to dryness. The reaction products were extracted with acetone.

*Borohydride reduction*(68)

The sugar (2 - 5 mg.) in water (1 ml.) was treated with sodium borohydride (5 - 10 mg.) and the mixture was allowed to stand overnight at room temperature. Excess borohydride was destroyed and the sodium ions were removed with Amberlite resin IR-120(H) and the solution was evaporated to dryness. The boric acid was removed by repeated evaporation with methanol.

**Hydrolysis** was carried out on samples (1 - 10 mg.) with N-sulphuric acid (1 - 2 ml.) in a sealed tube at 100º for 4 hr. in case of neutral oligosaccharides and for 16 hr. in the case of acidic polysaccharides. The cooled solutions were neutralised with barium carbonate, filtered, and the barium ions were removed with Amberlite resin IR-120(E).

**Methoxyl contents** were determined by semi-micro Zeisel method(69), using a saturated solution of potassium antimony tartrate as the scrubbing reagent.

**Uronic anhydride** contents were determined either by the decarboxylation method(27), in which the sample is refluxed with 19% hydrochloric acid and the amount of carbon dioxide liberated is measured or by the carbazole-sulphuric acid colorimetric method(28).
Estimation of sugars by phenol-sulphuric acid method

To the aqueous sugar solution (1 ml.) containing 10 - 70 μg. of sugar was added aqueous 5% phenol (1 ml.) and concentrated sulphuric acid (5 ml.). The mixture was allowed to stand for 10 minutes and then thoroughly mixed. It was allowed to stand for a further 20 minutes before measuring absorbance at 490 μm for hexoses and 480 μm for pentoses and uronic acids.

Estimation of rhamnose by L-cysteine-sulphuric acid method

To the solution of rhamnose (1 ml.) containing 10 - 100 μg. of the sugar was added under cooling in iced water, 86% by volume sulphuric acid (5 ml.). After 2 minutes the mixture was shaken well and heated in a boiling-water bath for 3 minutes. After cooling in tap water, a 3% solution of L-cysteine hydrochloride monohydrate (0.1 ml.) was added and the sample was vigorously shaken. The optical density was measured at 400 μm after leaving the solution for 2 hr. at room temperature.

Aniline derivatives of methylated sugars were prepared by refluxing the sugar (10 - 50 mg.) in ethanol (2 - 5 ml.) with aniline (equimolar proportion) in the dark for 0.5 hr. The solvent was evaporated off and the aniline derivatives were allowed to crystallise. The derivatives were recrystallised from appropriate solvents.

Aldonolactones were prepared by treating the methylated sugar (5 - 50 mg.) in water (2 - 5 ml.) with bromine (2 - 5 drops) in the dark for two days. Excess bromine was removed by aeration,
and the solution was neutralised with silver carbonate. Silver ions were removed with Amberlite resin IR-120(H), and the solution was evaporated to dryness. The aldonolactone was crystallised from appropriate solvent.

**Aldonamides**

The aldonolactone prepared as above was dried in a desiccator and then treated with methanolic 10% ammonia (w/v) and allowed to stand at 0°C for two days. The solvent was removed and the aldonamide was allowed to crystallise. It was recrystallised from appropriate solvent.

**Purification of organic solvents:**

**N, N-dimethylformamide** was distilled under reduced pressure (below 60°C) and stored over molecular sieve type 4a.

**Methanol** was dried over methyl magnesium iodide and distilled.

**Tetrahydrofuran** was kept over sodium wire for 48 hr. and then distilled over lithium aluminium hydride.

**Methyl iodide** was refluxed for 0.5 hr. over silver oxide and then distilled.

**Aniline** was distilled under reduced pressure and stored in the dark.

**Formamide** was dried over anhydrous magnesium sulphate and then distilled under reduced pressure (105°C).

**Pyridine** was refluxed with potassium hydroxide for 0.5 hr. and then distilled.
Light petroleum was shaken overnight with concentrated sulphuric acid (10% v/v), washed free of acid with distilled water and then distilled.

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

Dichloromethane was dried over anhydrous calcium chloride and then distilled. The fraction boiling at 39.5 - 41° was collected.
KHAYA IVORENSIS GUM

Purification of the gum

The gum was obtained as clusters of nodules, yellow to dark brown in colour. The nodules were hand picked and powdered in a mortar and pestle type grinding machine. The powdered gum (30 g.) was stirred in $\frac{1}{2}$-sodium hydroxide solution (3 l.) containing sodium borohydride (0.3 g.) for two days, when a viscous solution was obtained. The solution was acidified with 6 $\frac{1}{2}$-hydrochloric acid (510 ml.) and the polysaccharide was precipitated by pouring the solution slowly with stirring into acetone (6 l.). The precipitated polysaccharide was removed by centrifugation, dissolved again in water (2 l.) and reprecipitated by pouring into ethanol (4 l.). The precipitate was separated by centrifugation, washed with ethanol and ether and dried in a vacuum desiccator (yield 21 g.). The purified polysaccharide was examined by boundary electrophoresis in pyridine acetate buffer (pH 5.5) as shown in picture 1. Another portion (100 mg.) of the purified polysaccharide in water (10 ml.) was pipetted on to the top of a DEAE-cellulose column (2.5 x 40 cm.; phosphate form) and allowed to stand overnight. The column was then successively eluted with the following solvents.

\[
\begin{align*}
(a) & \quad 0.1 \, \text{M} \ (500 \, \text{ml.}) \quad \text{of sodium} \\
(b) & \quad 0.2 \, \text{M} \ (500 \, \text{ml.}) \quad \text{dihydrogen phosphate} \\
(c) & \quad 0.3 \, \text{M} \ (500 \, \text{ml.}) \quad \text{buffered at} \\
(d) & \quad 0.5 \, \text{M} \ (1000 \, \text{ml.}) \quad \text{pH 6.}
\end{align*}
\]
**PICTURE 1.** Boundary electrophoresis of deacetylated *Khaya ivorensis* gum in phosphate buffer pH 6, (165V; 13 mA)

**PICTURE 2.** Boundary electrophoresis of polysaccharide A from *Khaya ivorensis* gum in phosphate buffer pH 6, (165V; 13 mA)
Fractions were collected in tubes and the contents of every third tube were examined by phenol-sulphuric acid method. A graph of the optical density (at 490 mu) against tube number and eluant is shown in Figure I. It showed the presence in the polysaccharide of three components, of which the major fraction (Ca 60% of the total) was eluted with 0.5 M phosphate-buffer.

**Fractionation of the gum acid.**

The gum acid (20 g.) was dissolved in water (2 l.) and aqueous 7% cupric acetate solution was added to it slowly with stirring until a precipitate (I) appeared (15 ml.), which was removed at the centrifuge. To the supernatant was added further (10 ml.) reagent and the precipitate (II) thus obtained was removed by centrifugation. On adding further (10 ml.) reagent to the supernatant a gel was formed which was broken by adding more (10 ml.) copper acetate solution with vigorous stirring. The precipitate (III) was removed at the centrifuge. No further precipitate appeared on adding excess (30 ml.) reagent to the supernatant. A precipitate (IV) was, however, obtained on adding ethanol to this solution.

The precipitated copper salts (I - IV) were decomposed with 5% hydrochloric acid in acetone and washed free of acid with ethanol, finally washed with ether and dried under vacuum. Analyses were carried out on these polysaccharides I - IV as shown in the following Table.
**Figure I**

**DEAE-CELLULOSE CHROMATOGRAPHY**

**OF DEACETYLATED KHAYA IVORENSIS' GUM**

**Optical Density (490 m\(\mu\))**

**Tube Number and Buffer Concentration**

- 0.1 M
- 0.2 M
- 0.3 M

- 10
- 20
- 30
- 40
- 50
- 60
- 70
- 80
- 90
- 100
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Weight (g.)</th>
<th>$[\alpha]_D$</th>
<th>Uronic Anhydride content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.5</td>
<td>+135°</td>
<td>39%</td>
</tr>
<tr>
<td>II</td>
<td>3.5</td>
<td>+139°</td>
<td>38%</td>
</tr>
<tr>
<td>III</td>
<td>8.6</td>
<td>+137°</td>
<td>40%</td>
</tr>
<tr>
<td>IV</td>
<td>0.8</td>
<td>+138°</td>
<td>25%</td>
</tr>
</tbody>
</table>

Fraction I - III were combined and fractionated again with copper acetate solution as described above and fraction Ia - IVa were obtained. The corresponding values for optical rotation and uronic anhydride content are shown below:

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Weight (g.)</th>
<th>$[\alpha]_D$</th>
<th>Uronic Anhydride content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>2.0</td>
<td>+139°</td>
<td>35%</td>
</tr>
<tr>
<td>IIa</td>
<td>3.5</td>
<td>+137°</td>
<td>36%</td>
</tr>
<tr>
<td>IIIa</td>
<td>7.0</td>
<td>+139°</td>
<td>39%</td>
</tr>
<tr>
<td>IVa</td>
<td>0.2</td>
<td>+31°</td>
<td>26%</td>
</tr>
</tbody>
</table>

Fractions Ia - IIIa were combined to give polysaccharide A (12.5 g.).

**Assessment of homogeneity of polysaccharide A**

The polysaccharide (100 mg.) in water (15 ml.) was decactionised with Amberlite resin IR-120(H) and the solution was pipetted on to the top of a DEAE-cellulose column (2.5 x 40 cm., phosphate form). After leaving overnight the column was developed successively with the following eluants:
0.2 M (500 ml.) of sodium dihydrogen phosphate buffered at pH 6.
0.3 M (500 ml.) phosphate buffered
0.5 M (1000 ml.)

The elution pattern was followed by phenol-sulphuric acid method. A plot of the optical density (at 490 μm) against tube number and buffer concentration is shown in Figure II. Only one peak was obtained corresponding to the buffer concentration of 0.5 M phosphate. Polysaccharide A was also tested for homogeneity by free boundary electrophoresis in phosphate buffer (pH 6) and was found to be essentially homogeneous, as shown in picture 2.

[In subsequent fractionations of the gum acid, fractions I - III were precipitated in one bulk and the polysaccharide A was obtained after two successive bulk precipitation with copper acetate]
FIGURE II

DEAE-CELLULOSE CHROMATOGRAPHY OF POLYSACCHARIDE 'A'
FROM KHAYA IVORENSIS GUM
PARTIAL HYDROLYSIS STUDIES

Polysaccharide A (10 g.) in H₂SO₄ (500 ml.) was heated on a boiling-water bath for seven hours during which time a precipitate appeared, which was separated by centrifugation and hydrolysed again in the same way. The remaining precipitate (1.2 g.) was removed at the centrifuge, washed with ethanol and dried. The degraded polysaccharide had \([\alpha]_D + 268^\circ (c 1.0 \text{ in } 0.1 \text{ N-sodium hydroxide}),\) (Found uronic anhydride 76%), and gave only galacturonic acid on drastic hydrolysis. The combined supernatant liquids were neutralised with barium hydroxide and barium carbonate, barium ions were removed from the filtrate with Amberlite Resin IR-120(H) and the solution was concentrated to a syrup (7.4 g.).

The syrup was absorbed on DEAE-sephadex A-25 (3 x 50 cm.; formate form). Elution with water removed the neutral sugars (2.63 g.). The column was then eluted with a gradient of water containing formic acid (0 - 1.5%) and subsequently with 1.5%, 2% and 5% formic acid respectively. The fractions were examined by paper chromatography, similar fractions were combined, extracted overnight with boiling chloroform and concentrated to a syrup. The following fractions were obtained:
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Tube No.</th>
<th>Wt. (mg.)</th>
<th>$R_{GaLA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-30</td>
<td>0.036</td>
<td>0.57, 0.70</td>
</tr>
<tr>
<td>2</td>
<td>31-40</td>
<td>1.043</td>
<td>0.082, 0.19, 0.57, 0.70</td>
</tr>
<tr>
<td>3</td>
<td>41-57</td>
<td>1.356</td>
<td>0.10, 0.20, 0.57, 0.70, 1.00</td>
</tr>
<tr>
<td>4</td>
<td>58-68</td>
<td>0.150</td>
<td>0.00, 3.03</td>
</tr>
<tr>
<td>5</td>
<td>69-92</td>
<td>0.163</td>
<td>0.082, 0.19</td>
</tr>
<tr>
<td>6</td>
<td>93-106</td>
<td>0.073</td>
<td>0.15, 0.72, 1.00</td>
</tr>
<tr>
<td>7</td>
<td>107-124</td>
<td>0.238</td>
<td>0.15, 0.00</td>
</tr>
<tr>
<td>8</td>
<td>125-160</td>
<td>0.040</td>
<td>0.38, 0.18, 0.29, 0.47</td>
</tr>
<tr>
<td>9</td>
<td>161-190</td>
<td>0.050</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>2% Formic acid</td>
<td>0.180</td>
<td>0.023, 0.12</td>
</tr>
<tr>
<td>11</td>
<td>5% Formic acid</td>
<td>0.048</td>
<td>0.09, 0.25, 0.80, 0.00</td>
</tr>
</tbody>
</table>

Total recovery 81%.

The above mentioned fractions were further separated by chromatography on filter sheets using solvent A or C in appropriate instances. The following acidic sugars were obtained in sufficient quantities for further investigation.

**Acidic Sugar I**

This sugar (0.090 g.), $R_{GaLA}$ 3.03, remained unchanged on hydrolysis and had the same chromatographic mobility as 4-O-methylglucuronic acid. A sample (5 mg.) of the sugar was methylated by Kuhn's method, and the methylglycoside of the
methylated sugar was examined by gas-liquid chromatography on column c. The presence of the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid (\( T = 2 \cdot 43, 3 \cdot 14 \)) was indicated. Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis gave a product with the same chromatographic mobility as 4-O-methylglucose. A portion of this sugar was oxidised with periodate and the resulting product was examined by paper chromatography along with a similarly treated sample of 4-O-methyl-\( \beta \)-mannose. The formation of similar products from both samples provided further evidence that the reduced sugar was 4-O-methyl-\( \beta \)-glucose and hence that the acidic sugar was 4-O-methyl-\( \beta \)-glucuronic acid.

**Acidic Sugar II**

This sugar (0.508 g.) had \( R_{Gal} \) 1.00 and remained unchanged on hydrolysis. Reduction of the methyl ester methyl glycoside with sodium borohydride followed by hydrolysis gave a product with the same chromatographic mobility as \( \beta \)-galactose.

**Acidic Sugar III**

This fraction (0.591 g.), \( R_{Gal} \) 0.70, had \([\alpha]_D + 86^\circ\) (c 1.04) and gave galacturonic acid and rhamnose on hydrolysis. Reduction with sodium borohydride followed by hydrolysis gave galacturonic acid and rhamnitol. Reduction of the methyl ester methyl glycoside with sodium borohydride followed by hydrolysis gave galactose and rhamnose.

A sample (5 mg.) of the sugar was methylated by Kuhn's method. The methylated derivative was methanolysed and the
product was examined by gas-liquid chromatography on column c, showing the presence of methyl glycosides of 2,3,4-tri-O-methyl-galacturonic acid ($T = 7.01$) and 3,4,6-di-O-methylrhamnose ($T = 0.98$).

**Haworth methylation of acidic Sugar III**

The oligosaccharide (100 mg.) in water (2 ml.) was treated with methyl sulphate (1 ml.) and 30% sodium hydroxide solution (2 ml.) at $0^\circ$ under an atmosphere of nitrogen. Five further additions of reagents were made on five successive days. Twenty-four hours after the final addition of reagents, the reaction mixture was heated at $100^\circ$ for one hour. The solution was acidified to pH 4 with dilute sulphuric acid, poured into eight volumes of methylated spirits and the precipitated sodium sulphate was centrifuged out. The supernatant was taken to pH 8, reduced in volume (20 ml.), acidified to pH 4 again and was extracted with chloroform (5 x 20 ml.). The combined extract was dried, concentrated to a small volume (10 ml.) and the methylated oligosaccharide was precipitated from light petroleum (b.p. 100 - 120°). The methylated sugar was crystallised from chloroform-light petroleum (b.p. 100 - 120°) and was identified as methyl glycoside of 2-O-(L-D-galactopyranosyluronic acid)-L-rhamnose pentamethyl ether dihydrate by mp. and mixed m.p. 69° (Kofler hot stage) and 110 - 111° (open capillary), $[\alpha]^D_D + 76^\circ$ ($c = 1.06$ in CHCl$_3$) and X-ray powder photograph.
Acidic Sugar IV

This sugar (0.215 g.), $R_{GalA}$ 0.57, had $[\alpha]_D + 42^\circ$ (c 0.95) and gave $\alpha$-0-methylglucuronic acid and galactose on hydrolysis. Borohydride reduction followed by hydrolysis gave $\alpha$-0-methylglucuronic acid and galactitol. Reduction of the methyl ester methyl glycoside followed by hydrolysis gave $\alpha$-0-methylglucose and galactose. A portion of the mixture on oxidation with periodate gave products similar to those obtained from similarly treated $\alpha$-0-methyl-$\alpha$-glucose.

A sample (100 mg.) of the oligosaccharide was methylated by Haworth's method as described for acidic sugar III. A portion of the methylated sugar was methanolysed and the resulting methyl glycosides were examined by gas-liquid chromatography on column e, showing the presence of methyl glycosides of 2,3,4-tri-$\alpha$-methylglucuronic acid ($T = 2.47, 3.29$) and 2,3,6-tri-$\alpha$-methylgalactose ($T = 2.58, 3.88$).

The remaining portion of the methylated oligosaccharide (50 mg.) in tetrahydrofuran (2 ml.) was treated with lithium aluminium hydride (50 mg.) in tetrahydrofuran (2 ml.). The mixture was allowed to stand at room temperature for 0.5 hr. and was then heated under reflux for 2 hr. The excess hydride was destroyed by careful addition of water. The solution was taken to pH 4 with dilute sulphuric acid and filtered. The filtrate was reduced to a small volume (20 ml.) and was extracted with chloroform (5 x 20 ml.). The chloroform extract was taken to dryness. A portion of the resulting reduced methylated disaccharide was methanolysed and examined by gas-liquid
chromatography on column c. Methyl glycosides of 2,3,6-
tri-\(\beta\)-methylgalactose \(T = 3.10, 4.46\) and 2,3,\(\beta\)-tri-\(\beta\)-methyl-
glucose \(T = 2.53, 3.59\) were detected.

The remaining portion of the reduced methylated oligo-
saccharide was hydrolysed with \(\text{H}_2\text{SO}_4\) sulphuric acid and the
hydrolysate was examined by paper chromatography in solvents
\(P, G\) and \(H\). The above-mentioned sugars only were detected.
The two methylated sugars were separated by chromatography on
filter sheets using solvent \(H\). 2,3,\(\beta\)-tri-\(\beta\)-methyl-\(\beta\)-glucose
was identified by gas-liquid chromatography of the derived
methyl glycosides, and the 2,3,6-\(\beta\)-methyl-\(\beta\)-galactose by gas-
liquid chromatography of the derived 2,3,6-\(\beta\)-methyl-\(\beta\)-
galactonolactone. The acidic sugar IV is therefore \(\beta\)-\(\beta\)-(\(\beta\)-methyl-
\(\beta\)-glucopyranosyluronic acid)-\(\beta\)-galactose.

**Acidic Sugar V**

This sugar (0.160 g.), \(R_{\text{GalA}} 0.15\), had \([\alpha]_D + 120^\circ\)
\((g 0.60\) and gave only galacturonic acid on hydrolysis. The
oligosaccharide had the same chromatographic mobility as 1,\(\beta\)-
galacturonobiose in both solvents \(A\) and \(C\). Reduction of the
methyl ester methyl glycoside with sodium borohydride followed
by hydrolysis gave galactose only.

A portion (50 mg.) of the oligosaccharide was treated with
methanolic 4\% hydrogen chloride at room temperature for six
hours. The resulting ester glycoside was reduced with sodium
borohydride and was then methylated by Haworth's method. The
methylated reduced oligosaccharide was methanolysed and
examination of the product by gas-liquid chromatography on column c showed the presence of methyl glycosides of 2,3,4,6-tetra-O-methylgalactose ($\ell = 1.76$) and 2,3,6-tri-O-methylgalactose ($\ell = 3.18, 4.62$). The aldobiouronic acid was therefore 4-O-\((\alpha-D-galactopyranosyluronic acid)\)-D-galacturonic acid.

**Acidic Sugar VI**

This sugar (0.051 g.) had $R_{\text{GalA}} 0.082$ and gave galacturonic acid and rhamnose on hydrolysis. Reduction with sodium borohydride followed by hydrolysis gave galacturonic acid, rhamnose and rhamnitol. Reduction of the methyl ester methyl glycoside with sodium borohydride followed by hydrolysis gave galactose and rhamnose.

The ratios of galacturonic acid and rhamnose in the oligosaccharide and in the oligosaccharide glycitol formed on reduction with sodium borohydride, were determined by the carbazole-sulphuric acid colorimetric method\(^{(28)}\) for galacturonic acid and \(\lambda\)-cysteine-sulphuric acid colorimetric method\(^{(71)}\) for rhamnose. The observed ratios were 1 : 0.92 and 2.2 : 1 respectively.

A sample (5 mg.) of the oligosaccharide was reduced with sodium borohydride and partial hydrolysis of a portion of the reduced oligosaccharide with 0.5 $N$-sulphuric acid at 100° for 2 hr. gave products with the same chromatographic mobility as galacturonosyl 1→2 rhamnose and galacturonosyl 1→2 rhamnitol. The remaining portion of the reduced oligosaccharide was methylated by Kuhn's method and methanolysis of the methylated
derivative followed by gas-liquid chromatography on column c showed presence of \(1,3,4,5\)-tetra-\(\alpha\)-methylrhamnitol \((T = 1.11)\)
and methyl glycosides of \(3,4\)-di-\(\alpha\)-methylrhamnose \((T = 1.01)\),
\(2,3\)-di-\(\alpha\)-methylgalacturonic acid \((T = 5.28)\) and \(2,3,4\)-tri-\(\alpha\)-methylglucuronic acid \((T = 7.12)\).

The oligosaccharide had the same chromatographic mobility as a sample of a tetrasaccharide having the structure, \(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)\(1\rightarrow2\)-\(\alpha\)-(\(L\)-rhamnopyranosyl)\(1\rightarrow4\)-\(\alpha\)-(\(\beta\)-galactopyranosyluronic acid)\(1\rightarrow2\) \(L\)-rhamnose.
METHYLATION STUDIES

Methylation of Polysaccharide A.

The polysaccharide (15g.) in water (300 ml.) was neutralised with 30% sodium hydroxide solution to give a clear solution. The solution was taken in a three-necked flask (5 l.) placed on an ice bath. Methyl sulphate (150 ml.) and 30% sodium hydroxide solution (300 ml.) were then added dropwise with stirring over a period of eight hours in an atmosphere of nitrogen. The alkali was added cautiously during the first six hours to avoid gel formation. Six further additions of the reagents were made on six successive days. The bath temperature was maintained at 5-10° during the first three days and then allowed to rise to room temperature. Twenty-four hours after the final addition of reagents the reaction mixture was heated to 80° on a water bath for one hour. The cooled solution was neutralised with dilute sulphuric acid and allowed to stand overnight. The precipitated sodium sulphate was filtered off and the filtrate was dialysed until free of sulphate ions, concentrated to a small volume (300 ml.), treated with Amberlite resin IR-120(H) to remove cations and freeze dried to give methylated polysaccharide (7.5 g.). [Found: OMe, 28.8%]. A solution of the partially methylated polysaccharide (7.5 g.) in water (250 ml.) was neutralised with silver carbonate, and the filtered solution was freeze dried to give the silver salt of the partially methylated polysaccharide A (8 g.).
The pistol-dried silver salt (7.9 g.) was refluxed with methyl iodide (150 ml.) and silver oxide (15 g.) was added in five portions over a period of five hours, and refluxing was continued for a total of nine hours. The cooled reaction mixture was centrifuged, the solid was extracted with hot chloroform in a soxhlet extractor and the combined extract and supernatant was evaporated to dryness. The methylated polysaccharide (4.5 g.) in chloroform (20 ml.) was precipitated from light petroleum (b.p. 60 - 80°), [Found: OMe, 34.8%]. Three further similar methylationa gave methylated polysaccharide (3.6 g.), [a]D + 72° (g 1.2 in CHCl₃), [Found: OMe, 40.4%, not raised on further methylation].

A sample (10 mg.) of the methylated polysaccharide was methanolsed with methanolic 4% hydrogen chloride and the resulting methyl glycosides were examined by gas-liquid chromatography. The following methylated sugars were detected:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T in columns (a)</th>
<th>(b)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-Me₃ arabinose</td>
<td>0.49</td>
<td>0.60, 0.78</td>
<td>0.55, 0.71</td>
</tr>
<tr>
<td>3,4-Me₂ rhamnose</td>
<td>0.65</td>
<td>1.11</td>
<td>1.00</td>
</tr>
<tr>
<td>3-Ne rhamnose</td>
<td>1.03</td>
<td>4.49</td>
<td>3.49</td>
</tr>
<tr>
<td>2,3,4,6-Ne₄ galactose</td>
<td>(1.65)</td>
<td>1.89</td>
<td>1.74</td>
</tr>
<tr>
<td>2,3,6-Ne₃ galactose</td>
<td>(1.65), 2.58</td>
<td>4.96, 5.43</td>
<td>4.50, (3.10)</td>
</tr>
<tr>
<td>2,3,4-Ne₃ galactose</td>
<td>2.99</td>
<td>-</td>
<td>7.12</td>
</tr>
<tr>
<td>2,3,4-Ne₃ glucuronic acid</td>
<td>-</td>
<td>2.70, (3.54)</td>
<td>2.41, (3.10)</td>
</tr>
<tr>
<td>2,3-Ne₂ galacturonic acid</td>
<td>2.26, 6.31</td>
<td>6.64</td>
<td>5.12</td>
</tr>
<tr>
<td>2,4-Ne₂ galactose</td>
<td>3.87, 4.44</td>
<td>-</td>
<td>16.8, 19.2</td>
</tr>
</tbody>
</table>
Separation of neutral and acidic sugars

The methylated polysaccharide (200 mg.) was refluxed in methanolic 4% hydrogen chloride (10 ml.) for 18 hr. The cooled solution was neutralised with silver carbonate, centrifuged, and concentrated to a syrup. The syrup was heated with saturated aqueous barium hydroxide (10 ml.) at 60° for two hours. The cooled solution was passed through a column of Amberlite resin IR-120(H) to remove barium ions, concentrated to a small volume (5 ml.) and placed on top of a column of DEAE-sephadex A-25 (10 x 2 cm., formate form). The neutral methylated sugar glycosides were eluted with water (250 ml.) and the eluate was concentrated to give fraction I. The acidic methylated sugar glycosides were eluted from the column with aqueous 1% formic acid (300 ml.), and the eluate was concentrated to give fraction II.

Fraction I

The syrup was hydrolysed with N-sulphuric acid and the hydrolysate was examined by paper chromatography in solvents F and H. The following methylated sugars were detected: 2,3,4,6-tetra-, 2,3,4- and 2,3,6-tri-, and 2,4-, and 2,6-di-methyl-galactose; 2,3,5-tri-methylarabinose; 3-methylrhamnose; rhamnose; and traces of 2- and 3-methylgalactose.

Fraction II

The syrup was refluxed with methanolic 4% hydrogen chloride (10 ml.) for 4 hr. The cooled solution was neutralised with
silver carbonate, filtered and concentrated to a syrup. The syrup in water (10 ml.) was treated with sodium borohydride (100 mg.) for 18 hr., excess borohydride was destroyed and sodium ions were removed with Amberlite resin IR-120(H), filtered, and the filtrate was worked up as usual. The resulting product was hydrolysed with H-sulphuric acid and paper chromatography of the hydrolysate indicated that the reduction was incomplete. The hydrolysate was therefore converted into the methyl ester methyl glycosides and reduced with lithium aluminium hydride in tetrahydrofuran. The product was hydrolysed and examination of the hydrolysate by paper chromatography in solvents F and H showed the presence of 2,3,4-tri-\(\text{D}\)-methylglucose; 2,3,4,6-tetra-\(\text{D}\)-methylgalactose, 3-\(\text{D}\)-methylrhamnose, rhamnose, and traces of 2- and 3-\(\text{D}\)-methylgalactose.

**Preparation of reduced methylated polysaccharide A**

The methylated polysaccharide (3.0 g.) in tetrahydrofuran (100 ml.) was treated with lithium aluminium hydride (3.0 g.) in tetrahydrofuran (100 ml.). The mixture was allowed to stand for 0.5 hr. at room temperature and then refluxed for 2 hr. A further quantity (1.0 g.) of lithium aluminium hydride in tetrahydrofuran (25 ml.) was added and the mixture was heated under reflux for another 0.5 hr. Excess hydride was destroyed by the addition of water to the cooled solution and the mixture was then acidified with dilute sulphuric acid to pH 4, and filtered. The filtrate was reduced in volume and extracted with chloroform (5 x 100 ml.). The combined organic extracts
were dried, concentrated and the reduced methylated polysaccharide (2.6 g.) was precipitated by pouring the chloroform solution into excess (20 vol.) light petroleum (b.p. 60 – 80°). The reduced methylated polysaccharide had $[\alpha]_D + 52^\circ$ (c 0.92 in CHCl$_3$), [Found: OMe, 34.6%].

A sample of the reduced methylated polysaccharide (10 mg.) was methanolysed with methanolic 4% hydrogen chloride and the methyl glycosides of methylated sugars detected by gas-liquid chromatography of the methanolysate are shown in the following Table:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>T in columns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(b)</td>
</tr>
<tr>
<td>2,3,5-\text{Me}_3 arabinose</td>
<td>0.60, 0.77</td>
</tr>
<tr>
<td>3,4-\text{Me}_2 rhamnose</td>
<td>1.07</td>
</tr>
<tr>
<td>2,3,4,6-\text{Me}_4 galactose</td>
<td>1.83</td>
</tr>
<tr>
<td>2,3,4-\text{Me}_3 glucose</td>
<td>2.82</td>
</tr>
<tr>
<td>2,3,6-\text{Me}_3 galactose</td>
<td>3.58, 4.83, 5.37</td>
</tr>
<tr>
<td>3-\text{Me} rhamnose</td>
<td>4.36, 6.87</td>
</tr>
<tr>
<td>2,3,4-\text{Me}_3 galactose</td>
<td>8.6</td>
</tr>
<tr>
<td>2,3-\text{Me}_2 galactose</td>
<td>–</td>
</tr>
<tr>
<td>2,4-\text{Me}_2 galactose</td>
<td>–</td>
</tr>
<tr>
<td>2,6-\text{Me}_2 galactose</td>
<td>–</td>
</tr>
</tbody>
</table>

Hydrolysis of reduced methylated polysaccharide A

Concentrated sulphuric acid (5 ml.) was added slowly with stirring to the reduced methylated polysaccharide (2.5 g.) in water (85 ml.). The mixture was kept at room temperature (20°)
for two days, at 40° for one day, at 60° for two days and at 80° for one day. The solution was then heated on a boiling-water bath for 8 hr. The cooled solution was neutralised with barium hydroxide and barium carbonate, centrifuged, and the barium ions were removed with Amberlite resin IR-120(H). The resulting solution was concentrated to a syrup (3·0 g.).

The syrup (3·0 g.) in butan-l-ol (10 ml.) was absorbed on top of a cellulose column (3·5 x 60 cm.). Elution of the column was carried out successively with the following solvents:
(a) Light petroleum (b.p. 100-120°): butan-l-ol (7:3) saturated with water.
(b) Light petroleum (b.p. 100-120°): butan-l-ol (1:1) saturated with water.
and (c) Butan-l-ol half saturated with water.

The fractions collected were examined by paper chromatography in solvent F, similar fractions were combined and concentrated to a syrup. The syrup was dissolved in water, passed through a bed of charcoal and evaporated to dryness.

The following Table summarises the results of preliminary examination of the various fractions.

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>weight (mg.)</th>
<th>[α]D</th>
<th>Rg</th>
<th>Probable identity</th>
<th>Other evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>+0·5°</td>
<td>1·01 0·94</td>
<td>2,3,5-Me3 arabinose 2,3,4-Me4 galactose 2,3,4-Me3 glucose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>+100°</td>
<td>0·95</td>
<td>2,3,4,6-Me4 galactose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>Fr.No.</td>
<td>weight (mg.)</td>
<td>$[\alpha]_D$</td>
<td>$R_G$</td>
<td>Probable identity</td>
<td>Other evidence</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------</td>
<td>-------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>3</td>
<td>3148</td>
<td>+76$^\circ$</td>
<td>0.94</td>
<td>$2,3,4,6$-$\text{Me}_4$ galactose $2,3,4$-$\text{Me}_3$ glucose $3,4$-$\text{Me}_2$ rhamnose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>+67$^\circ$</td>
<td>0.93</td>
<td>$2,3,4$-$\text{Me}_3$ glucose $3,4$-$\text{Me}_2$ rhamnose (tr)</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>-</td>
<td>0.92</td>
<td>$3,4$-$\text{Me}_2$ rhamnose $2,3,6$-$\text{Me}_3$ galactose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>6</td>
<td>279</td>
<td>+102$^\circ$</td>
<td>0.81</td>
<td>$2,3,6$-$\text{Me}_3$ galactose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>-</td>
<td>0.81</td>
<td>$2,3,6$-$\text{Me}_3$ galactose $2,3,4$-$\text{Me}_3$ galactose</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>+84$^\circ$</td>
<td>0.78</td>
<td>$2,3,4$-$\text{Me}_3$ galactose Unknown (tr)</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>-</td>
<td>0.76</td>
<td>$2,3,4$-$\text{Me}_3$ galactose $3$-$\text{Me}$ rhamnose Unknown (tr)</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>10</td>
<td>238</td>
<td>+47$^\circ$</td>
<td>0.69</td>
<td>$3$-$\text{Me}$ rhamnose $3,4$-$\text{Me}_2$ glucose</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>-</td>
<td>0.70</td>
<td>$3$-$\text{Me}$ rhamnose $0.59$ $2,3,4$-$\text{Me}_2$ glucose $2,6$-$\text{Me}_2$ galactose</td>
<td>g.l.c. G, H</td>
</tr>
<tr>
<td>12</td>
<td>147</td>
<td>+97$^\circ$</td>
<td>0.58</td>
<td>$2,3$-$\text{Me}_2$ galactose</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>13</td>
<td>143</td>
<td>+99$^\circ$</td>
<td>0.59</td>
<td>$2,3$-$\text{Me}_2$ galactose Unknown</td>
<td>g.l.c. H</td>
</tr>
<tr>
<td>Fr.No.</td>
<td>weight (mg.)</td>
<td>$[\eta]_D$</td>
<td>$R_G$</td>
<td>Probable identity</td>
<td>Other evidence</td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
<td>-------------</td>
<td>-------</td>
<td>-------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>14</td>
<td>52</td>
<td>-</td>
<td>0.59</td>
<td>2,3-Me$_2$ galactose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
<td>2,4-Me$_2$ galactose</td>
<td>H</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>-</td>
<td>0.57</td>
<td>2,4-Me$_2$ galactose</td>
<td>g.l.c.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
<td>rhamnose</td>
<td>B, H</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>-</td>
<td>0.57</td>
<td>2,4-Me$_2$ galactose</td>
<td>B, H</td>
</tr>
<tr>
<td>17</td>
<td>101</td>
<td>+14°</td>
<td>0.45</td>
<td>rhamnose</td>
<td>B, H</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>-</td>
<td>0.38</td>
<td>2-Me galactose</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
<td>rhamnose</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>-</td>
<td>0.38</td>
<td>2-Me galactose</td>
<td>G, H</td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>-</td>
<td>0.38</td>
<td>2-Me galactose</td>
<td>B, H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
<td>3-Me galactose</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td>-</td>
<td>0.32</td>
<td>3-Me galactose</td>
<td>H</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>galactose</td>
<td>B</td>
</tr>
</tbody>
</table>

[The abbreviations used are as follows:

g.l.c. = gas-liquid chromatography of the derived methyl glycosides;


and tr = trace quantity]

**Fraction 1**

The sugar mixture was separated by chromatography on filter sheets using solvent G into three subfractions, which were examined by gas-liquid chromatography of the derived methyl glycosides. Subfraction 1a (35 mg.), 2,3,5-tri-O-methylarabinose;
lb (10 mg.), 2,3,5-tri-α-methylarabinose and 2,3,4,6-tetra-α-

methylgalactose; and lc (8 mg.), 2,3,4-tri-α-methylglucose.

Fraction 1a had [α]D = -20° (c 0.70 in CHCl₃) and was identified

as 2,3,5-tri-α-methyl-L-arabinose by conversion into 2,3,5-tri-α-
methyl-L-arabonamide (recrystallised from ethyl acetate) m.p. and

mixed m.p. 134 - 135°.

Fraction 2

The sugar was characterised as 2,3,4,6-tetra-α-methyl-D-
galactose by conversion into the aniline derivative, (recrystall-

ised from ethyl acetate) m.p. and mixed m.p. 195 - 196°.

Fraction 3

A portion (100 mg.) of the mixture was subjected to paper

ionophoresis in borate buffer (pH 10) and two subfractions were

obtained which were examined by gas-liquid chromatography of

their methyl glycosides. Subfraction 3a (20 mg.), 3,4-di-α-
methylrhamnose. The sugar was crystallised from light

petroleum (b.p. 60 - 80°) to give 3,4-di-α-methyl-L-rhamnose m.p.

and mixed m.p. 91 - 92°. Subfraction 3b (60 mg.) was found to

be a mixture of 2,3,4,6-tetra-α-methylgalactose and 2,3,4-tri-

α-methylglucose.

Fraction 4

The main component of this fraction was characterised as

2,3,4-tri-α-methyl-D-glucose by conversion into the aniline

derivative (recrystallised from ether-light petroleum b.p. 60 -

80°), m.p. and mixed m.p. 146°.
Fraction 6

The sugar was characterised as 2,3,6-tri-O-methyl-D-galactose by conversion into 2,3,6-tri-O-methyl-D-galactonolactone (crystallised from benzene-light petroleum b.p. 60 - 80°C), m.p. and mixed m.p. 98 - 99°C.

Fraction 8

Demethylation with boron trichloride gave only galactose. The main component of this fraction was identified as 2,3,4-tri-O-methyl-D-galactose by conversion into the aniline derivative (recrystallised from ethyl acetate), m.p. and mixed m.p. 167 - 168°C.

Fraction 10

Demethylation gave rhamnose and glucose. The major component of this fraction was crystallised from acetone-light petroleum (b.p. 60 - 80°C) and was recrystallised from the same solvent. The sugar (150 mg.) was characterised as 3-O-methyl-L-rhamnose by m.p. and mixed m.p. 115 - 116°C.

A portion of the supernatant was oxidised with periodate and the methyl glycosides of the products on examination by gas-liquid chromatography showed the presence of 2,3-di-O-methylarabinose as one of the products.

Fraction 11

The mixture was separated by chromatography on filter sheets using solvent H into two subfractions. Fraction 11a (6 mg.), was found to be 2,6-di-O-methylgalactose by gas-liquid
chromatography of the derived methyl glycosides. Periodate oxidation of the sugar followed by paper chromatography gave the characteristic yellow stain ($R_f$ 0.24) of methoxymalondialdehyde with aniline oxalate. Fraction 11b (10 mg.) was found to be a mixture of 3-0-methylrhamnose and 3,4-di-0-methylglucose by gas-liquid chromatography of the derived methyl glycosides. Demethylation gave glucose and rhamnose. Periodate oxidation of the mixture followed by gas-liquid chromatography of the derived methyl glycosides showed the presence of methyl glycosides of 2,3-di-0-methylarabinose as one of the products.

Fraction 12

The sugar was identified as 2,3-di-0-methyl-$D$-galactose by conversion into the aniline derivative (recrystallised from benzene-ethanol), m.p. and mixed m.p. 154°, and into 2,3-di-0-methyl-$D$-galactonamide (recrystallised from ethanol-acetone), m.p. and mixed m.p. 135 - 136°.

Fraction 13

The two components of this fraction were separated by chromatography on charcoal-celite (2.5 x 20 cm.), by gradient elution with water containing 0 - 2% methyl ethyl ketone. Fraction 13a (108 mg.) had $[\alpha]_D + 96^\circ$ (c 1.08 in CHCl$_3$) and was found to be 2,3-di-0-methylgalactose by gas-liquid chromatography of the methyl glycosides. The sugar was characterised by preparing the aniline derivative, m.p. and mixed m.p. 153 - 154°.

Fraction 13b (10 mg.) gave only galactose on demethylation.
Gas-liquid chromatography of the derived methyl glycosides on column f showed only one peak ($T = 1.5$). The sugar was distinguishable from both 2,3- and 2,4-di-$\alpha$-methylgalactose by paper chromatography in solvent $H$, and had mobility intermediate between the two sugars.

**Fraction 15**

The sugar was recrystallised from acetone-water to give 2,4-di-$\alpha$-methyl-$\beta$-galactose monohydrate, m.p. and mixed m.p. 96 - 97$^\circ$ and was further characterised by conversion into the aniline derivative (recrystallised from ethyl acetate), m.p. and mixed m.p. 214$^\circ$.

**Fraction 17**

The sugar was crystallised from acetone-water and recrystallised from the same solvent to give $L$-rhamnose monohydrate, m.p. and mixed m.p. 88 - 89$^\circ$.

**Fraction 19**

Demethylation of the sugar gave galactose and periodate oxidation followed by paper chromatography showed the presence of methoxymalondialdehyde ($R_g 0.22$).

**Fraction 20**

The two sugars were separated by chromatography on filter sheets using solvent $H'$ by multiple development. Fraction 20a (10 mg.) was crystallised from acetone-water to give 2-$\alpha$-methyl-$\beta$-galactose, m.p. and mixed m.p. 144 - 145$^\circ$. 
Fraction 20b (10 mg.) gave only galactose on demethylation. Periodate oxidation of the sugar gave products similar to those obtained from 3-O-methylgalactose under identical conditions.
KHAYA SENEGALENSIS GUM

Purification and fractionation

The sample of gum under investigation was in the form of nodules yellow to dark brown in colour. The nodules were ground to a powder. About 80% of the gum was soluble in water. The aqueous solution of the gum was examined by boundary electrophoresis as shown in picture 3. The gum was partly acetylated (-OAc 2.7%) in the natural state and treatment with dilute ammonia effected only partial deacetylation (-OAc 1.5%). The solution thus obtained was examined by boundary electrophoresis as shown in picture 4. The gum was completely deacetylated with Na-sodium hydroxide solution, sodium ions were removed with Amberlite resin IR-120(H) and the solution was examined by boundary electrophoresis as shown in picture 5. The large scale fractionation of the gum was carried out as follows:

The powdered gum (50 g.) was stirred in water (2 l.) for two hours and then treated with a solution of sodium hydroxide (100 g.) in water (500 ml.). The mixture was stirred for another two hours, after which time the solution was acidified with 6 N-hydrochloric acid (340 ml.), centrifuged and the gum acid was precipitated by pouring the solution into ethanol (2 vol.). The precipitate was removed at the centrifuge, redissolved in water (1 l.) and reprecipitated from ethanol (2 l.). The gum acid (40 g.) was washed with ethanol and ether and dried in a vacuum desiccator. A boundary electrophoretic pattern of the gum acid is shown in picture 6.

An aqueous 7% cupric acetate was added slowly with stirring.
PICTURE 3. Boundary electrophoresis of aqueous solution of Khaya senegalensis gum in pyridine-acetate buffer pH 5.5, (185 V; 13 mA)

PICTURE 4. Boundary electrophoresis of dilute ammonia treated Khaya senegalensis gum in pyridine-acetate buffer pH 5.5, (180 V; 13 mA)
**PICTURE 5.** Boundary electrophoresis of deacetylated *Khaya senegalensis* gum in pyridine-acetate buffer pH 5.5, (180 V; 13 mA)

**PICTURE 6.** Boundary electrophoresis of the gum acid from *Khaya senegalensis* gum in pyridine-acetate buffer pH 5.5, (170 V; 13 mA)
to the gum acid (40 g.) in water (4 l.) until a bulk precipitate was obtained (35 ml.). The whole solution turned into a gel, which was broken by further addition of cupric acetate solution (15 ml.) with vigorous stirring. The precipitate thus obtained was removed by centrifugation, the copper salt was decomposed by treatment with 5% hydrochloric acid in acetone, washed free of acid with ethanol and finally washed with ether and dried. The polysaccharide (30 g.) was precipitated from aqueous solution with cupric acetate once again as described above and the polysaccharide (22 g.) thus obtained had [\( \alpha \)]\textsubscript{D} + 140° and uronic anhydride content 55% (carbazole method).

The polysaccharide (100 mg.) in water (10 ml.) was pipetted on to the top of a column of DEAE-cellulose (2.5 x 30 cm., phosphate form) and was allowed to stand overnight. The column was successively eluted with the following solvents:

\[
\begin{align*}
0.2 \text{ M} & \quad (500 \text{ ml.}) \\
0.3 \text{ M} & \quad (500 \text{ ml.}) \\
0.4 \text{ M} & \quad (750 \text{ ml.}) \\
0.5 \text{ M} & \quad (500 \text{ ml.})
\end{align*}
\]

The elution pattern was followed by phenol-sulphuric acid method. A plot of the optical density (at 490 m\(\lambda\)) against tube number and buffer concentration is shown in Figure III. The presence of three components was indicated of which the major component (polysaccharide A), eluted with 0.4 M phosphate, constituted ca 85% of the total polysaccharide taken. A minor component (polysaccharide B, ca 50%) was eluted with 0.3 M phosphate, and a third component (polysaccharide C) was eluted with 0.5 M
FIGURE III
DEAE-CELLULOSE CHROMATOGRAPHY
OF POLYSACCHARIDE 'A' FROM
KHAYA SENEGALENSIS GUM
phosphate and constituted ca 10% of the total polysaccharide taken.

The fractions containing polysaccharides A, B and C were dialysed free of phosphate, concentrated, and were analysed for uronic anhydride content and sugars given on hydrolysis. Paper chromatography of the hydrolysates indicated the presence of galactose, arabinose, and rhamnose in each fraction together with acidic sugars. The uronic anhydride contents determined on the basis of phenol-sulphuric acid and carbazole-sulphuric acid colorimetric methods were 53%, 18% and 70% respectively for the polysaccharides A, B and C.

Attempts to purify this material further were not successful and structural investigation was carried out on this polysaccharide which will be referred to as polysaccharide A. The boundary electrophoretic pattern of this polysaccharide is shown in picture 7.
PICTURE 7. Boundary electrophoresis of the polysaccharide A from deacetylated Khaya senegalensis gum in pyridine-acetate buffer pH 5.5, (165 V; 13 mA)
PREPARATION OF CARBOXYL-REDUCED POLYSACCHARIDE 'A'

Preparation of glycol ester

Polysaccharide A (20 g.) in water (800 ml.) was treated with ethylene oxide (200 ml.) and the mixture (pH 2·5) was kept in a stoppered flask at room temperature (18°) for several days with occasional shaking. After six days the pH of the solution rose to 6·1 and remained constant at that pH. The excess ethylene oxide was removed by evaporation and the glycol ester of the polysaccharide A was precipitated by pouring the solution into ethanol (3 vol.). The precipitate (21 g.) was washed with ethanol and ether and was dried in a desiccator.

Acetylation of the glycol ester

The glycol ester (21 g.) was stirred in formamide (500 ml.) for 0·5 hr. and pyridine (500 ml.) was added slowly with stirring over a period of 1 hr. The clear solution thus obtained was treated with acetic anhydride (400 ml.), added dropwise with stirring over a period of 6 hr. The mixture was stirred overnight at room temperature and the resulting brown solution was poured into excess (10 vol.) iced-water containing hydrochloric acid (2% v/v). The precipitate thus obtained was removed at the centrifuge, washed free of pyridine with iced water and dried in a desiccator over anhydrous calcium chloride. The acetylated glycol ester (18 g.) was dissolved in chloroform and precipitated with light petroleum (b.p. 60-80°, 20 vol.).
Carboxyl-reduction of the acetylated glycol ester

The acetylated glycol ester (18 g.) in tetrahydrofuran (500 ml.) was refluxed with lithium borohydride (18 g.) in tetrahydrofuran (500 ml.) for 16 hr. The excess borohydride was destroyed by adding water dropwise to the cooled solution. The solution was acidified to pH 4 with dilute sulphuric acid and dialysed free of borate ions. The carboxyl-reduced polysaccharide (8 g.), $[\alpha]_D + 120^0$ (c 1.0), was precipitated by pouring the concentrated solution into ethanol (3 vol.).
ACETOLYSIS STUDIES

The carboxyl-reduced polysaccharide A (6 g.) was dispersed in acetic anhydride (125 ml.) and concentrated sulphuric acid (5 ml.) was added dropwise with stirring over a period of 0.5 hr. at 3°. The mixture was kept at 3° for another 0.5 hr. and was then allowed to rise to room temperature (20°). The clear solution thus obtained was stirred for 24 hr. at room temperature and then poured into iced-water (250 ml.). A precipitate was obtained which was removed by centrifugation and exhaustively extracted with chloroform. The supernatant was extracted with chloroform (5 x 200 ml.) and the combined chloroform extracts were reduced in volume (100 ml.), washed free of acid with aqueous sodium bicarbonate, and was finally dried and concentrated to a syrup (9.2 g.).

The syrup (9.2 g.) in chloroform (15 ml.) was treated with a saturated solution of barium methoxide in methanol (100 ml.) and the mixture was kept at 0° for three days with occasional shaking. The mixture was then poured into water (300 ml.), and a small amount of insoluble material was removed by centrifugation. The supernatant was neutralised with dilute sulphuric acid, centrifuged, de-ionised with IR-120(H) and IR-45(G), and finally concentrated to a syrup (3.8 g.).

The syrup (3.8 g.) in water (50 ml.) was absorbed on top of a charcoal-celite column (5 x 40 cm.). Elution with water followed by a gradient of water containing ethanol (0-5%) removed the monosaccharides (560 mg.). Oligosaccharides were eluted
from the column by gradient elution with water containing increasing concentration of ethanol.

The fractions obtained are shown in the following Table.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Weight (mg.)</th>
<th>( R_{\text{Gal}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5% Ethanol</td>
<td>31</td>
<td>0.74, 1.38</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>36</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>37</td>
<td>0.47, 1.15</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>34</td>
<td>0.47 (tr), 1.15</td>
</tr>
<tr>
<td>5</td>
<td>5-10% Ethanol</td>
<td>20</td>
<td>0.45, 1.14</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>18</td>
<td>0.33, 0.45, 1.15 (tr)</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>21</td>
<td>0.33, 0.50 (tr)</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>136</td>
<td>0.20, 0.68, 0.88, 1.01</td>
</tr>
<tr>
<td>9</td>
<td>10% Ethanol</td>
<td>57</td>
<td>0.51, 1.15</td>
</tr>
<tr>
<td>10</td>
<td>10-15% Ethanol</td>
<td>51</td>
<td>0.50, 0.00</td>
</tr>
</tbody>
</table>

**Fraction 1.**

The two components of this fraction were separated by chromatography on filter sheets using solvent B. Fraction 1a (10 mg.) had \( R_{\text{Gal}} \) 0.76 and gave galactose and rhamnose on hydrolysis. Borohydride reduction followed by hydrolysis gave rhamnose and galactitol. A sample (2 mg.) of the oligosaccharide was methylated by Kuhn’s method, and the methylated sugar was methanolyzed and examined by gas-liquid chromatography on column d. The methyl glycosides of \( 2,3,6\text{-tri-O-methylgalactose} \) (\( R = 2.82, 3.61, 4.12 \)) and \( 2,3,4\text{-tri-O-methylrhamnose} \)
(\(R = 0.45\)) were detected. The oligosaccharide is therefore \(4-\Omega-(\text{L-rhamnopyranosyl})-\text{D-galactose} (I)\).

Fraction 1b (8 mg.) had \(R_{\text{Gal}} 1.40\) and remained unchanged on hydrolysis. It was not further examined.

**Fraction 2.**

The sugar (21 mg.) was purified by chromatography in solvent B. It had \(R_{\text{Gal}} 0.46, [\alpha]_D + 130^\circ (c 0.84)\) and gave only galactose on hydrolysis. Methanolysis of the methylated oligosaccharide followed by gas-liquid chromatography on column \(g\) showed the presence of methyl glycosides of \(2,3,4,6\)-tetra-\(\text{D}-\text{methylgalactose} (T = 1.76)\) and \(2,3,6\)-tri-\(\text{D}-\text{methylgalactose} (T = 3.03, 3.66, 4.01, 4.47)\). The oligosaccharide had the same chromatographic mobility as a sample of a disaccharide having the structure, \(4-\Omega-(\text{D}-\text{galactopyranosyl})-\text{D-galactose} (II)\).

**Fraction 3.**

The two components of this fraction were separated by chromatography on filter sheets using solvent B. Fraction 3a (8 mg.) had \(R_{\text{Gal}} 0.51\) and gave glucose and galactose on hydrolysis. Methanolysis of the methylated sugar followed by gas-liquid chromatography on column \(g\) showed the presence of methyl glycosides of \(2,3,4,6\)-tetra-\(\text{D}-\text{methylglucose} (T = 1.00, 1.45)\), \(2,3,4,6\)-tetra-\(\text{D}-\text{methylglucose} (T = 1.77)\) and \(2,3,6\)-tri-\(\text{D}-\text{methylgalactose} (T = 3.04, 3.58, 4.08, 4.50)\). The sugar was therefore thought to be a mixture of \(4-\Omega-(\text{D}-\text{glucopyranosyl})-\text{D-galactose}, \) and \(4-\Omega-(\text{D}-\text{galactopyranosyl})-\text{D-galactose}\).

Fraction 3b (14 mg.) had \(R_{\text{Gal}} 1.16, [\alpha]_D + 100^\circ (c 0.70)\)
and gave galactose and rhamnose on hydrolysis. Reduction with sodium borohydride followed by hydrolysis gave galactose and rhamnitol. A sample (2 mg.) of the oligosaccharide was methylated by Kuhn's method. Methanolation of the methylated sugar followed by gas-liquid chromatography on column g showed the presence of methyl glycosides of 2,3,4,6-tetra-O-methylgalactose (T = 1.78) and 3,4-di-O-methylrhamnose (T = 0.98).

The oligosaccharide is therefore, 2-O-(α-D-galactopyranosyl)-β-rhamnose (III).

**Fraction 4.**

The main component of this fraction was separated by chromatography on filter sheets using solvent B. The sugar (15 mg.) had R_{Gal} 1.14 and gave galactose and rhamnose on hydrolysis. Methylation by Kuhn's method followed by methanolation and gas-liquid chromatography on column d showed the presence of methyl glycosides of 2,3,4,6-tetra-O-methylgalactose (T = 1.76) and 3,4-di-O-methylrhamnose (T = 0.86). The sugar is therefore the same as oligosaccharide III.

**Fraction 7.**

The main component of this fraction was separated by chromatography on filter sheets using solvent B. The sugar (10 mg.) was chromatographically and ionophoretically pure, R_{Gal} 0.35, and gave galactose and rhamnose on hydrolysis. A portion (2 mg.) of the oligosaccharide was methylated by Kuhn's method. Methanolation of the methylated sugar followed by gas-liquid chromatography on column g showed the presence of methyl
glycosides of 2,3,4,6-tetra-O-methylgalactose \((T = 1.79)\), 2,3,6-
tri-O-methylgalactose \((T = 3.05, 3.60, 4.10, 4.51)\), and 3,4-di-O-
methylrhamnose \((T = 0.97)\).

Another portion \((2 \text{ mg.})\) of the oligosaccharide was reduced
with sodium borohydride and the glycitol was methylated by Kuhn's
method. Methanolyis of the methylated glycitol followed by gas-
liquid chromatography on column \(g\) showed the presence of 1,2,3,5,6-
penta-O-methylgalactitol \((T = 2.72)\) and methyl glycosides of 2,3,4,
6-tetra-O-methylgalactose \((T = 1.78)\) and 3,4-di-O-methylrhamnose
\((T = 0.98)\).

The ratio of total sugar in the oligosaccharide and the
oligosaccharide glycitol determined by phenol-sulphuric acid
method was 3.4 : 2. The oligosaccharide is therefore a tri-
saccharide having the structure, \(\beta-(\beta\text{-galactopyranosyl})_3\rightarrow 2-\beta-
(\beta\text{-rhamnopyranosyl})_1\rightarrow 4 \beta\text{-galactose (IV).}

Fraction 8.

Four subfractions were obtained from this fraction by
chromatography on filter sheets using solvent \(B\). Subfraction
\(8a\) \((19 \text{ mg.})\), \(R_{\text{Gal}} 0.17\), had \([\alpha]_D + 104^\circ\) \((c 0.50)\) and gave only
galactose on hydrolysis. Methylation by Kuhn's method followed
by methanolysis and gas-liquid chromatography on column \(g\) showed
the presence of methyl glycosides of 2,3,4,6-tetra-O-methyl-
galactose \((T = 1.78)\) and 2,3,6-tri-O-methylgalactose \((T = 3.05,\)
3.68, 4.08, 4.46). A sample \((3 \text{ mg.})\) of the oligosaccharide was
reduced with sodium borohydride and the resulting glycitol was
methylated by Kuhn's method. Methanolyis of the methylated
glycitol followed by gas-liquid chromatography on column \(g\) showed
the presence of 1,2,3,5,6-penta-\(\beta\)-methylgalactitol (\(T = 2.74\))
and methyl glycosides of 2,3,4,6-tetra-\(\beta\)-methylgalactose
(\(T = 1.79\)) and 2,3,6-tri-\(\beta\)-methylgalactose (\(T = 3.05, 3.68, 4.09, 4.53\)). The ratio of total sugar in the oligosaccharide
and the oligosaccharide glycitol determined by phenol-sulphuric
acid method was 3.3:2. The oligosaccharide is therefore a
disaccharide having the structure, \(\alpha-(D\)-galactopyranosyl)\(\beta\)-galactopyranosyl)\(\beta\)-galactose (V).

Fraction 8b (6 mg.) was found to be a mixture by
chromatography in solvent B and F, and gave mainly galactose
with a trace of glucose on hydrolysis. It was not further
examined.

Fraction 8c (30 mg.), \(R_{Gal} 0.85\), had \([\alpha]_D +114^o\)
(\(c 0.75\)) and gave 4-\(\alpha\)-methylglucose and galactose on hydrolysis.
Reduction with sodium borohydride followed by hydrolysis gave
4-\(\alpha\)-methylglucose and galactitol. A portion of the oligo-
saccharide glycitol was methylated by Kuhn’s method and
methanolysis of the methylated derivative followed by gas-
liquid chromatography on column g showed the presence of 1,2,3,5,6-
penta-\(\alpha\)-methylgalactitol (\(T = 2.72\)), and methyl glycosides of
2,3,4,6-tetra-\(\beta\)-methylglucose (\(T = 1.00, 1.42\)). Methanolysis
of the methylated oligosaccharide followed by gas-liquid
chromatography on column g showed the presence of methyl
glycosides of 2,3,4,6-tetra-\(\beta\)-methylglucose (\(T = 1.00, 1.45\))
and 2,3,6-tri-\(\beta\)-methylgalactose (\(T = 3.11, 3.71, 4.10, 4.56\)).
The oligosaccharide is therefore, 4-\(\alpha\)-(4-\(\alpha\)-methyl-\(D\)-glucopyran-
osyl)-\(\beta\)-galactose (VI).
Fraction 8d (11 mg.) was found to be a mixture and gave galactose, rhamnose, glucose and 4-O-methylglucose on hydrolysis. Methylation by Kuhn's method followed by methanolysis and gas-liquid chromatography on column g showed the presence of methyl glycosides of 2,3,4-tri-O-methylrhamnose ($T = 0.45$), 3,4-di-O-methylrhamnose ($T = 0.98$), 2,3,4,6-tetra-O-methylglucose ($T = 1.00, 1.45$), 2,3,4,6-tetra-O-methylgalactose ($T = 1.78$), and unknown ($T = 2.97, 3.49, 4.03, 4.48, 5.14$ and $6.81$). It was not further examined.

Fraction 9.

The two components of this fraction were separated by chromatography on filter sheets using solvent B. Fraction 9a (14 mg.), $R_{\text{Gal}} 0.50$ and showed the presence of a single component on paper ionophoresis. Hydrolysis of the sugar gave galactose and rhamnose. Reduction with sodium borohydride followed by hydrolysis gave galactose, rhamnose and rhamnitol. A sample (2 mg.) of the oligosaccharide glycitol was methylated by Kuhn's method and methanolysis of the product followed by gas-liquid chromatography on column d showed the presence of 1,3,4,5-tetra-O-methylrhamnitol ($T = 1.11$) and methyl glycosides of 2,3,4,6-tetra-O-methylgalactose ($T = 1.78$), 2,3,6-tri-O-methylgalactose ($T = 2.78, 3.55, 3.96$) and 3,4-di-O-methylrhamnose ($T = 0.88$). The ratios of total sugar to rhamnose in the oligosaccharide and the oligosaccharide glycitol as determined by phenol-sulphuric acid and L-cysteine-sulphuric acid colorimetric methods were $2:1$ and $3:2:1$ respectively, showing the oligosaccharide to be a tetrasaccharide having two rhamnose units.
The oligosaccharide therefore has one of the following structures:

\[
\text{Gal}^1 \rightarrow \text{2Rhap}^1 \rightarrow \text{4Gal}^1 \rightarrow \text{2Rha}
\]

\[
\text{Gal}^1 \rightarrow \text{4Gal}^1 \rightarrow \text{2Rhap}^1 \rightarrow \text{2Rha}
\]

Fraction 9b (6 mg.) had \( R_{\text{Gal}} 1.01 \) and gave galactose, \( 4-O\) -methylglucose and a trace of rhamnose on hydrolysis. It was not further examined.

**Fraction 10**

The mobile component of this fraction was separated by chromatography on filter sheets using solvent B. The sugar (19 mg.) had \( R_{\text{Gal}} 0.50 \) and gave galactose, \( 4-O\) -methylglucose and rhamnose on hydrolysis. Paper ionophoresis of the sugar, however, showed the presence of two components, of which the major component had the same ionophoretic mobility as fraction 9a.
Methylation of carboxyl-reduced polysaccharide A

Carboxyl-reduced polysaccharide A (250 mg.) was stirred in dimethylsulphoxide (15 ml.) for 0.5 hr. at room temperature, when a clear solution was obtained. The solution was treated with methysulphinyl anion (4 ml.) which was prepared as follows:

Sodium hydride (1.5 g.) was washed with light petroleum (3 x 30 ml.) and was then dried in a desiccator. The hydride was then stirred in dimethylsulphoxide (15 ml.) at 50° in an atmosphere of nitrogen for 1 hr. A greenish solution was obtained which was cooled to room temperature.

The methylation mixture was stirred at room temperature (20°) for 5 hr. and methyl iodide (1 ml.) was then added dropwise with stirring over a period of 10 minutes, and stirring was continued for another 2 hr. The resulting solution was dialysed overnight and then evaporated to dryness. The solid was extracted with boiling chloroform, the chloroform extract was dried and the methylated polysaccharide was precipitated by pouring the chloroform solution into light petroleum (b.p. 60 - 80°, 20 vol.). The methylated carboxyl-reduced polysaccharide A (150 mg.) had $[\alpha]_D^\circ + 76^\circ$ (c 1.5 in CHCl$_3$), [Found: OMe, 43.2%].

A portion (10 mg.) of the methylated derivative was methanolysed and the resulting methyl glycosides were examined by gas-liquid chromatography. The methyl glycosides of the following methylated sugars were detected:

- 2,3,4,6-tetra-, 2,3,6- and 2,3,4-tri-, and 2,4-di-$\alpha$-methylgalactose;
- 2,3,4,6-tetra-$\alpha$-methylglucose;
- 2,3,5-tri-$\alpha$-methylarabinose;
- and 3,4-di- and 3-$\alpha$-methylrhamnose.
PREPARATION OF CARBOXYL-REDUCED KHAYA SENEGALENSIS GUM

Khaya senegalensis gum (6 g.) was stirred in water (400 ml.) for six hours. The solution was centrifuged and the supernatant was decationised with Amberlite resin IR-120(H). Ethylene oxide (100 ml.) was added to this solution and the mixture (pH 2.5) was kept at room temperature for several days with occasional shaking. After six days the pH of the solution rose to 6.9 and remained constant at that pH. The glycol ester of the gum was recovered, acetylated, and reduced with lithium borohydride as described for polysaccharide A. The solution containing the carboxyl-reduced gum was dialysed free of borate ions and the polysaccharide was precipitated by pouring the aqueous solution into ethanol (2 vol.). The precipitate (0.7 g.) was removed at the centrifuge, washed with ethanol and ether and dried. (The aqueous solution of this polysaccharide was translucent and hence optical rotation could not be measured).

The supernatant was reduced in volume and poured into excess (4 vol.) ethanol. A second precipitate (0.4 g.), \([\alpha]_D + 42^\circ (c 1.0)\) was thus obtained. The two fractions will be referred to as carboxyl-reduced gum fractions I and II respectively.

A portion (250 mg.) from each of the above two fractions was methylated by the sodium hydride-dimethylsulphoxide-methyliodide method described earlier. Methylated carboxyl-reduced gum fraction I (120 mg.), had \([\alpha]_D + 70^\circ (c 0.50 \text{ in CHCl}_3)\), [Found: OMe, 42.6%] and methylated fraction II (100 mg.), had \([\alpha]_D - 10^\circ (c 0.50 \text{ in CHCl}_3)\), [Found: OMe, 39.8%].
A portion (15 mg.) from each of the carboxyl-reduced methylated gum fractions I and II were methanolysed with methanolic 4% hydrogen chloride and the resulting methyl glycosides were examined by gas-liquid chromatography. The approximate relative proportions (based on the peak areas in the gas-liquid chromatograms) of the various methylated sugars obtained from the methylated carboxyl-reduced gum fractions I and II and those from the methylated carboxyl-reduced polysaccharide A are shown in the following Table.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Methylated Polysaccharide 'A'</th>
<th>Methylated gum fractions (I)</th>
<th>Methylated gum fractions (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Me₄ galactose</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4,6-Me₄ glucose</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me₃ galactose</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-Me₃ galactose</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3,4-Me₂ rhamnose</td>
<td>+</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>3-Me rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,3,5-Me₃ arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,4-Me₂ galactose</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY

2. B.N. Patel, Drug Cosmet. Ind., 1964, 95(3), 337.

65. See reference 37.


Khaya ivorensis gum has been fractionated, after deacetylation, to give the major polysaccharide component (Polysaccharide A) in an essentially homogeneous state. Partial acid hydrolysis of the polysaccharide A gave, in addition to neutral monosaccharides, a number of acidic mono- and oligosaccharides. The acidic sugars were identified as:

- 4-0-methyl-D-glucuronic acid
- galacturonic acid
- 2-0-(D-galactopyranosyluronic acid)-D-rhamnose
- 4-0-(D-galactopyranosyluronic acid)-D-galacturonic acid
- 4-0-(4-O-methyl-D-glucopyranosyluronic acid)-D-galactose
- and 4-0-(D-galactopyranosyluronic acid)1-2-D-(D-rhamnopyranosyl)1-D-
- 4-0-(D-galactopyranosyluronic acid)1-2-D-rhamnose

Hydrolysis of the reduced methylated polysaccharide A followed by identification of the methyl sugars showed the presence of residues of 2,3,4,6-tetra- and 2,3,6-tri-, and 2,4-di-O-methyl-D-galactose, 2,3,5-tri-O-methyl-L-arabinose, 3,4-di- and 3,6-di-O-methyl-L-rhamnose, D-rhamnose, and 2,3,4-tri-O-methyl-D-glucose, and in smaller amounts 2,6-di- and 2- and 3-di-O-methyl-D-galactose and 3,4-di-O-methyl-D-glucose.

The structural significance of these results is discussed.

An attempt has been made to obtain the major polysaccharide component (Polysaccharide A) from deacetylated Khaya senegalensis gum in a homogeneous state. Partial acetylation of the carboxyl-reduced polysaccharide A gave, in addition to monosaccharides, a number of oligosaccharides, of which the following have been identified:

- 4-0-(D-rhamnopyranosyl)-D-galactose
- 4-0-(D-galactopyranosyl)-D-galactose
- 2-0-(D-galactopyranosyl)-D-rhamnose
- 4-0-(D-galactopyranosyl)1-2-0-(D-rhamnopyranosyl)1-D-
- 4-0-(D-galactopyranosyl)1-2-0-(D-galactopyranosyl)1-D-galactose
- and 4-0-(4-O-methyl-D-glucopyranosyl)-D-galactose

The structural features of the polysaccharide A from deacetylated Khaya senegalensis gum are discussed on the basis of these and previous results.