Angeissiius leiocarpus gum has been fractionated on a preparative scale, by means of cetavlon, into two components, leiocarpan A and B. Leiocarpan A, the major component, had a uronic acid anhydride content of approximately 31% and a specific rotation of +14.0°.

Autohydrolysis of leiocarpan A affords an essentially arabinose-free degraded gum and a mixture of sugars. The degraded gum was methylated and the products hydrolysed. The following methylated sugars have been identified by their crystallinity or by the formation of crystalline derivatives,

- 2,3,4,6-tetra-O-methyl-D-mannose,
- 3,4,6-tri-O-methyl-D-mannose,
- 3,4-di-O-methyl-D-mannose,
- 2,3,4,6-tetra-O-methyl-D-galactose,
- 2,3,4-tri-O-methyl-D-galactose,
- 2,3-di-O-methyl-D-glucose, and
- 2,3,4-tri-O-methyl-D-xylene,

together with smaller amounts of other sugars.

A sample of acetylated leiocarpan A was reduced with diborane gas produced externally from sodium borohydride and boron trifluoride. The carboxyl-reduced polymer was subjected to a partial acetylation. The following neutral sugars were characterised fully or in part,

- 4-O-α-D-mannopyranosyl-D-glucose,
- 2-O-β-D-glucopyranosyl-D-mannose,
- O-β-D-glucopyranosyl-(1→2)-O-α-D-mannopyranosyl-(1→4)-D-glucose,
- O-α-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-D-mannose,
- O-α-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→2)-O-α-D-
  mannopyranosyl-(1→4)-D-glucose,
- O-β-D-glucopyranosyl-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-β-D-
  glucopyranosyl-(1→2)-D-mannose,
- O-β-D-glucopyranosyl-(1→2)-O-α-D-mannopyranosyl-(1→4)-O-β-D-
  glucopyranosyl-(1→2)-D-glucose,
- 6-O-β-D-galactopyranosyl-D-galactose.

A sample of leiocarpan A was partially hydrolysed with N sulphuric acid. The hydrolysates were separated using DEAE-Sephadex column and thick-paper chromatography. The following acidic sugars were recognised,
2-Ω-(β-D-glucopyranosyluronic acid)-D-mannose,
6-Ω-(β-D-glucopyranosyluronic acid)-D-galactose,
Ω-(β-D-glucopyranosyluronic acid)-1→6-Ω-β-D-galactopyranosyl-
(1→3)-L-arabinose,
Ω-(β-D-glucopyranosyluronic acid)-1→2-Ω-α-D-mannopyranosyl-
(1→4)-Ω-(β-D-glucopyranosyluronic acid)-1→2-D-mannose,
together with smaller amounts of other acidic sugars.

Partial structures for the gum are discussed in the light of these results.

The structural features of the gum have been compared with those of other gums, special reference being made to its relationship with gum ghatti.

2-Ω-β-D-glucopyranosyl-D-mannose has been synthesised by the epimerisation of sophorose.
STRUCTURAL STUDIES ON PLANT GUMS,

WITH SPECIAL REFERENCE TO

MANNOSE-CONTAINING POLYSACCHARIDES

by

JAMES M. McNAB, B.Sc.

Thesis submitted for the degree of Doctor of Philosophy

University of Edinburgh

August, 1965
TO MY PARENTS
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INTRODUCTION
INTRODUCTION

The plant gums have been defined as those substances of plant origin which are obtained as exudations from the bark, leaves or fruit of certain trees or shrubs. Although certain plants will spontaneously produce gums it is usually necessary to stimulate the source artificially by the infliction of mechanical injury. This can be effected by the removal of a branch, by incision of the bark, or after invasion by bacteria or fungi. Thus natural rubber, the terpenoid resins and the polysaccharide exudates from plants, which are viscous and have adhesive properties, have all been called gums at some time.

In this thesis attention will be given exclusively to the carbohydrate exudates which have been shown to have a highly branched molecular structure. They bear a marked similarity to the mucilages, which are considered to be the products of normal plant metabolism, and, when their chemistry is reviewed, they are generally taken together.\(^{1-7}\) This resemblance in structure may be some indication that the gums are also of metabolic origin. Support for this viewpoint is obtained from the observation that the chemical structure of the gum is to some extent related to its botanical origin. Thus different trees of the same species have been found to yield gums with similar general features in chemical structure.\(^{8,9}\)

There has been little agreement as to the origin of the gum exudates. Much thought has been given to this problem and many theories have been put forward but no definite conclusions
have yet been reached.\(^{(1)}\) It does, however, seem probable
that gum production is caused by stimulation within the plant
of some dormant metabolic defence mechanism to combat the
effects of invading micro-organisms and to conserve moisture.
It is of interest to note in this context that pneumococcus
polysaccharides are known to offer protection to the pneumo-
coccus organisms. Many gums are similar to this group of
polysaccharides and it may be that the gums have a similar
function in protecting the plant tissue.

Whatever their origin, there is a rapid mobilisation of
carbohydrate material in the cell sap near the point of injury,
although generation of the gum elsewhere and its transportation
to the damaged site may also occur. Exudation of an aqueous
solution of the gum begins in the form of droplets. These
gradually dry out to give a viscid syrup, which hardens into
yellow or brown nodules composed almost entirely of poly-
saccharides.

The plant gums are high molecular weight compounds: a
value as high as nine and a half millions has been quoted for
Karaya gum from the tree *Sterculia urens*.\(^{(10)}\) The exudate
gums may consist of more than one polysaccharide each of which
can contain two to four neutral sugars and one or two acidic
residues. The most frequently encountered neutral components
are residues of \(\text{D-galactose}, \text{D-mannose}, \text{D-xylose}, \text{L-arabinose}\)
and \(\text{L-rhamnose}\) while \(\text{L-fucose}\) occurs in rarer cases. The
acidity is provided by residues of \(\text{D-glucuronic acid}, 4-\text{O-}
\text{methyl-D-glucuronic acid, and D-galacturonic acid.}\)
The fact that so many different sugar components are present and that each may be involved in more than one type of linkage, makes these natural products among the most complicated chemical systems ever studied. The wide range of physical and chemical properties which they exhibit makes systemisation a difficult task unless a large number of exceptions are considered. The formerly accepted methods of classification were generally based on the behaviour of the gum in water. Thus physical properties such as solubility, viscosity and adhesiveness determined the category into which the gum fell until quite recently. This type of grouping has proved useful especially in industry, but it can only be regarded as a very rough one and chemically very unsatisfactory. Associated protein,\(^{(11)}\) the presence of inorganic ions,\(^{(12)}\) and variations in pH,\(^{(13,14)}\) have all been shown to affect the physical behaviour of such compounds. The presence of \(\text{\(D\)}\)-glucuronic acid in some polymers and \(\text{\(D\)}\)-galacturonic acid in others has been the basis of a second attempt at grouping. However, gums which contain neither of the above acids or both are fairly common and form a large group of exceptions.

The most recent grouping of the plant gums is one founded on similar arrangements of sugar residues in the basal chains. By this method families of structurally-related polysaccharides have been recognised of which the arabinogalactan, acidic galactan and galacturonan are three well-known groups. Such a classification means that certain types of polysaccharides which are not normally considered to be plant gums must be
included in each grouping. Thus the first two groups include polysaccharides which are generally regarded as hemicelluloses while the third group includes pectic substances and mucilages. Some understanding of the relationship between the various polysaccharides within each group may lead to the determination of the precursors of the exudate gums. This is perhaps the most formidable task which confronts the worker in this field.

Some insight into the early stages of biosynthesis\(^{(15)}\) has been obtained and it is now known how the various sugar derivatives are formed and transformed into one another. It has been generally accepted that the gums are formed by an enzymic mechanism rather than by direct chemical polymerisation. It is now believed that complex polysaccharides are built up in a sequential manner.\(^{(16)}\) The building up of the main chain is probably followed by the gradual apposition of the various side-chains. This theory is consistent with the concept of structurally-related families.

The arabinogalactans from coniferous woods are known to form a group of closely related polysaccharides. For example, the polysaccharides from various species of larch\(^{(17-20)}\) and from Scots pine\(^{(21)}\) contain basal chains of 1,3-linked \(\beta-D\)-galactopyranose residues to which other \(\beta-D\)-galactose residues and \(L\)-arabinose residues are attached by \(1\rightarrow 6\) linkages. This type of arrangement of \(D\)-galactose units provides the framework of the Acacia gums, those of the Prunus genus, the gum from Araucaria bidwilli\(^{(22)}\) and the minor component of Khaya senegalensis.\(^{(23)}\) Although no direct biogenetic relationship
between the two groups of polysaccharides has yet been demonstrated, it is possible that arabinogalactans may be the precursors of certain exudate gums and that the later stages of synthesis involve the attachment of \(\delta\)-glucuronic acid and \(L\)-rhamnose residues and further \(L\)-arabinose. It is perhaps significant that some polysaccharides from species of larch contain small amounts of uronic acid residues and recent work on the arabinogalactans from tamarack larch,\(^{(24)}\) mountain larch\(^{(25)}\) and maritime pine,\(^{(26)}\) has illustrated their closer similarity in structure to these exudate gums.

The second family of polysaccharides, the acidic galactan group, is characterised by the presence of \(\delta\)-galactopyranose residues mutually joined by 1\(\rightarrow\)4 linkages together, in some cases, with 1\(\rightarrow\)6 linkages. Many polysaccharide components of woods have been shown to have this basal structure. Birch wood,\(^{(27)}\) Norway spruce compression wood,\(^{(28)}\) and beech tension wood,\(^{(29)}\) all contain \(\alpha\)-1\(\rightarrow\)4 linked \(\delta\)-galactopyranose units while the latter also contains \(\delta\)-galactose residues mutually linked 1\(\rightarrow\)6. There may be some similarity in the mode of biogenesis of these wood polysaccharides and *Combretum leonense* gum which also has these same arrangements of \(\delta\)-galactose units in the basal chains.\(^{(30)}\)

Another similar structural relationship can be observed between pectic acids, constituents of plant cell walls, and the exudate gums of *Khaya*\(^{(23,31)}\) genus and tragacanthic acid\(^{(34)}\) which all contain backbones of \(\alpha\)-1,4-linked \(\delta\)-galacturonic acid residues. That the relationship between the *Khaya* gums
and some pectic acids may be still closer is suggested by observations that several pectic acids contain appreciable amounts of neutral sugar components, particularly L-arabinose, D-galactose and L-rhamnose. Since residues of D-glucuronic acid occur exclusively as end groups in the Khaya, Cochlospermum gossypium, Sterculia urens, and other Sterculia gums, it is possible that these acidic units may be transferred to a pectic acid-like polysaccharide at a late stage in the biosynthesis.

Isolation, Purification and Fractionation

The plant gums are found in most parts of the world but the gums of commerce are generally collected from wild trees in Africa or Asia. Over one hundred species of the genus Acacia produce gums which have commercial applications. Other gums which have found uses in industry come from the Astragalus, Prunus, Sterculia and Anogeissus genera. The marketing of the plant gums under collective names has led to confusion in structural work and it is essential that the botanical origin of a particular gum is fully authenticated before any analytical studies are started.

The natural occurrence of the exudate gums is in the form of salts in contrast to pectin which occurs as the methyl ester. The crude nodules are generally contaminated with mechanical impurities in the shape of bark, insects and terpenoid resins. It is essential that a polysaccharide isolated for structural
study be rigorously purified. Methods used for the isolation and purification of individual polysaccharides vary widely. In favourable cases, however, a water-soluble gum may be isolated by filtration to remove insoluble material and precipitation of the polysaccharide by a large excess of an organic solvent, generally ethanol. When the polysaccharide is not water-soluble, as in the case of acetyl-esterified polymers, the gum is dissolved in alkali and treated as in the previous case. Care must be exercised in the use of alkali, especially at elevated temperatures since degradation may occur. Further purification may be effected by reprecipitation, by treatment with ion-exchange resins, by dialysis or electrodialysis to remove inorganic matter. The highly purified polysaccharide may be obtained in a dry state either by solvent exchange followed by air-drying or by freeze-drying. Disadvantages in these purification procedures have been encountered and it has been reported that water and the organic solvents used have been retained by the carbohydrate material. These solvents have also been known to produce artifacts.

A problem of fundamental importance in the study of the plant gums, and indeed in the whole of polysaccharide chemistry, concerns the assessment of homogeneity of the samples under examination. As in the study of all polymers there are three possible types of heterogeneity. The first of these involves the separation of polysaccharides which contain either different sugar components or the same sugar residues linked in
different ways. The second concerns the separation of those which contain the same component sugars linked in the same way but in different proportions. In the third type a plant gum may be heterogeneous in the sense of being composed of particles, which, although of identical structure, differ in molecular size. The latter kind of heterogeneity is probably fairly common among polysaccharides and is not too serious since it does not prevent the correct elucidation of a structure. The first, however, can cause great practical difficulty and, unless fractionation is completely effective, the results will be of little structural significance. Therefore the primary task in structural studies is the separation of such mixtures of polysaccharides preparatively and showing unambiguously that the products are homogeneous.

Probably the most widely used method of separation has been by fractional precipitation. It involves the addition of non-solvent, generally ethanol, to an aqueous solution of the gum followed by the removal of components of differing solubilities. Co-precipitation and the occlusion of other polysaccharides are two of the principal drawbacks using this technique; also, unless there is a considerable difference in solubility between two components, only a rough separation will be effected and several reprecipitations may be required before a pure fraction is obtained. However, Khaya senegalensis gum has been fractionated in this way(23) into two acidic components. They were shown to be of entirely different structural character, an important factor in obtaining a
separation by this method. The major component, a polymer of high uronic acid content was found to be similar to *Khaya grandifolia* gum,\(^{(31)}\) whereas the minor component had a much lower acid content and was shown to resemble gum arabic\(^{(47)}\) in structure. The two components of *Olibanum* gum\(^{(48)}\) have also been separated from each other in this way. This fractionation technique has met with greater success when applied to mixtures of methylated, acetylated or nitrated polysaccharides.

A similar method is that of fractional precipitation by increasing the concentration of a suitably dissolved salt in a manner analogous to the fractionation of proteins. For example the gelatinising effect of potassium ions has been used for the fractionation of carageenan\(^{(49)}\) and alginic acid.\(^{(50)}\)

Precipitation by a specific complexing agent is another general method and metallic salts have been used widely in this context. Barium hydroxide has been proposed as a procedure for the identification of gums.\(^{(51)}\) Copper (II) salts have been widely used to fractionate mixtures of acidic polysaccharides.\(^{(52,53,54)}\) Fehlings solution is often used and a variation of this method has been described in detail.\(^{(55)}\) In most of these cases an excess of precipitant is added and the insoluble complex is removed by filtration and is decomposed by the addition of an alcoholic solution of acid or by a chelating agent.

Another group of very useful complexing agents are quaternary ammonium salts such as cetyltrimethylammonium bromide (cetavlon) and cetylpyridinium bromide. With acidic
polysaccharides they form salts which are insoluble in water.\(^{56,57}\) Neutral polysaccharides only react as borate complexes provided that they can be formed\(^{17,58}\) or at very high pH, when some hydroxyl groups ionise. Acidic polymers are, therefore, easily separated from neutral polysaccharides by precipitation with either of the above reagents under acid, neutral or mildly alkaline conditions. The precipitates can be destroyed by increasing the acidity or ionic strength.\(^{59}\) Gum tragacanth is one of the many natural products which have been fractionated using cetavlon.\(^{34}\)

Once a mixture of polysaccharides has been fractionated using one or a combination of the above techniques, it is necessary to assess the homogeneity of the individual samples. Unfortunately, however, homogeneity cannot be established directly. Deviations from it may be detected by examining the material with as many as possible of the methods available for such an assessment. The methods most commonly applied are those of ionophoresis, ultracentrifugation, gel filtration and ion-exchange chromatography. Certain of these procedures have also been used to effect small-scale separations preparatively with considerable success.

Adsorption chromatography has had limited applications in polysaccharide fractionation until it was shown that cellulose ion-exchangers such as diethyl-aminoethyl (DEAE) cellulose can be used for the fractionation of acidic as well as neutral polysaccharides\(^{60}\) and mucopolysaccharides.\(^{61,62}\) Acidic polysaccharides are readily adsorbed on DEAE cellulose in
various forms (e.g. borate, phosphate) at pH 6 and can be readily eluted, (a) by increasing the buffer concentration at the same pH or (b) by alkaline solutions of increasing strength or (c) by acidic solutions of increasing strength. The retention of neutral polymers is negligible at this pH. The structure of the polysaccharide strongly influences its adsorption and it is difficult to give a general procedure applicable to every polymer. Nevertheless, high molecular weight components are more readily retained than lower molecular weight material\(^{(63)}\) and increase in acid-content enhances adsorption. The heterogeneous nature of a large number of polymers has been illustrated using this technique.\(^{(47,37,64,65)}\)

Electrophoresis has had limited application in the preparative separation of polysaccharides.\(^{(66,67,68)}\) Zone electrophoresis is well suited to the analysis of acidic polysaccharides, and much of the work in this field has been comprehensively reviewed.\(^{(69)}\) In this manner, gum arabic has been separated from contaminating *Acacia cyanophylla* gum\(^{(70)}\) and the isolation of the glucomannan from sugar maple has been accomplished.\(^{(71)}\) For a long time, a disadvantage of this technique lay in the difficulty in detecting the polysaccharide since the cellulose support reacted with all the known spray reagents. The advent of glass-fibre paper\(^{(68)}\) overcame this difficulty and was taken advantage of by Smith and his collaborators.\(^{(72)}\) He examined several gums in 2M potassium hydroxide solution and he has suggested that several polysaccharides, which were previously considered to be pure
compounds, are heterogeneous. The possibility of polymer degradation by such a strongly alkaline solution cannot be ruled out and the results may be misleading. These undoubtedly interesting observations have never been confirmed by other techniques and the method merits further examination.

Sedimentation analysis using the ultracentrifuge provides a powerful analytical procedure for the study of molecular weight distributions of natural polymers. The method also yields information regarding the molecular weight of individual components.

The application of serological cross-reaction to polysaccharide chemistry has been developed by Heidelberger. It involves the utilisation of certain specific antipneumococcus sera which cause precipitation reactions with polysaccharides to which they are structurally similar. The reason for this phenomenon is that mutual precipitation is caused by characteristic multiple groupings of sugar residues which may be present in more than one type of polysaccharide. Treatment of gum arabic with Type II antipneumococcus serum gave a precipitate, from which a polysaccharide containing only one third to one fifth the amount of rhamnose present in the original gum has been isolated. This immunological specificity has only been employed in a limited number of cases and must have considerable potential, since, as well as providing a method of fractionation, it could yield information on the general structure of the polysaccharide.

There are strong indications from studies such as those
outlined that heterogeneity among the plant polysaccharides may be the rule rather than the exception. The three kinds of heterogeneity which have been recognised are fairly common among the exudate gums and examples of each kind are known. The simplest example of heterogeneity is probably exhibited by *Combretum leonense* gum. Fractionation of the gum was effected to yield components differing in uronic acid content but with no difference in the nature of the sugar units nor in the mode of their attachment. It has also been shown that different nodules of this gum have a significant variation in uronic acid content. The existence of this micro-heterogeneity is not too serious in structural studies and could arise from families of polysaccharides of closely similar structure which differ either in chain length or in molecular weight.
A number of different techniques are employed in the structural investigation of a polysaccharide. The nature and proportions of the sugar constituents are determined by an examination of the total acid hydrolysis products of the polysaccharide in question. Information on the mode of linkage of each sugar unit and, in certain instances, on the ring structure of sugars are obtained from methylation studies. This procedure also yields information on the proportion of non-reducing end groups.

Finally, the order of the sugar units and the linkages are determined by partial hydrolysis studies using mineral acid of varying strength. Additional information may be obtained from periodate oxidation and alkaline or enzymic degradations.

In many cases, information can be more readily obtained from investigations on the modified polysaccharide. In view of the complex nature of many natural polymers, any modification, which involves simplification of the structure, makes studies on the molecule much easier. In this respect, the heating of an acidic polysaccharide in aqueous solution, a process known as autohydrolysis, enables one to obtain a degraded polymer. Structural studies on this modified polysaccharide by the above techniques yield information on the core of the molecule. Information can also be obtained regarding the periphery, by examination of the acid-labile fragments. A typical scheme of analysis is outlined in Figure I.
Figure I

1. Gum Acid $\xrightarrow{10\% \text{ sol.}}$ ca. 30 hrs. $100^\circ$ Degraded Gum I + Arabinobiose
   $\xrightarrow{0.5 \text{ N Acid}}$
   $\xrightarrow{2 \text{ hrs. } 100^\circ}$ Degraded Gum II + neutral oligosaccharides
   $\xrightarrow{1 \text{ N Acid}}$
   $\xrightarrow{3 \text{ hrs. } 100^\circ}$ Monosaccharides + acidic oligosaccharides

2. Gum Acid
   (i) Oxidation
   (ii) KBH$_4$
   (iii) N H$_2$SO$_4$
   Degraded Gum III
   (i) Oxidation
   (ii) KBH$_4$
   (iii) N H$_2$SO$_4$

3. Gum Acid
   (i) Acetylation
   (ii) Diborane Reduction
   (iii) Deacetylation
   Reduced polysaccharide

Scheme showing typical degradations of a plant gum
The chemistry of the mannose-containing plant gums

The first mannose-containing acidic polysaccharides to be subjected to a structural study were obtained from damson and cherry gums. They are both members of the *Prunus* genus and, although they have been fairly widely investigated, the studies have been carried out predominantly by the methylation technique, a process which does not indicate the order of the sugar residues within the molecule. Some oligosaccharides have been isolated as partial acid hydrolysis products, but it is not yet possible to put forward even partial structures for either gum. It has, however, been established that both polysaccharides give rise to the same aldobiouronic acid, \(2-O-\beta-D\)-glucopyranosyluronic acid-\(D\)-mannose,\(^{77,78}\) and that the mannose only forms part of the acid resistant nucleus, remaining with \(D\)-galactose and \(D\)-glucuronic acid in the degraded polymer.

Both polysaccharides contain substantial proportions of \(L\)-arabinose, which is the major residue in the acid-labile periphery. From methylation studies on the parent gums\(^{79,80}\) and on the arabinose-free degraded gums,\(^{81,82}\) it appears that both polysaccharides consist of a framework which is built up with \(1:3\)- and \(1:6\)-linked \(D\)-galactose residues. The presence of branch points has also been established by the isolation of both di-\(O\)-methyl-\(D\)-galactose and di-\(O\)-methyl-\(D\)-mannose residues from the methylated degraded gums.

Further information on the mode of union of the sugar units is forthcoming from a study of the action of periodate on
cherry gum.\(^{(83)}\). Indications are that no galactose units are oxidised but that fifty per cent of the arabinose residues are cleaved. Use of some of the more modern techniques now available would clarify many of the outstanding problems connected with these two polysaccharides. Nevertheless it would appear that they have a similar structure to the \textit{Acacia} gums in general and gum arabic in particular.

\textbf{Gum Ghatti}

Of the mannose-containing plant gums studied, gum ghatti has received the most exhaustive treatment. It is the exudate from the stems of \textit{Anogeissus latifolia}, Wall., a tree which is widely distributed in India. Although it was first investigated by Hanna and Shaw,\(^{(83)}\) the first studies of structural significance were carried out by Aspinall and his collaborators in this laboratory.\(^{(84)}\) Further investigations\(^{(85-88)}\) have elucidated many of the major structural problems and partial structures have now been put forward.

A purified sample of the gum acid had an equivalent weight of 1600 and an uronic acid content of 12 per cent. Analyses for individual sugar residues yielded the following results: \textit{L}-arabinose - 41.1\%, \textit{D}-xylose - 2.7\%, \textit{D}-galactose - 26.7\%, \textit{D}-mannose - 8.3\% and traces (<1\%) of \textit{L}-rhamnose.

Autohydrolysis of the gum acid removed 80\% of the \textit{L}-arabinose residues before any galactose was liberated, and left a degraded gum A, which contained 4\% of \textit{L}-arabinose and
had an equivalent weight of 1000. This treatment completely eliminated the D-xylose and L-rhamnose components from the polysaccharide. Hydrolysis conducted under more severe conditions caused extensive degradation and two partial acid hydrolysies under different conditions produced a wide spectrum of acidic and neutral oligosaccharides. Less drastic conditions have formed the basis for a third partial acid hydrolysis which resulted in the isolation of an acidic trisaccharide. These degradations of the polysaccharide are shown in the scheme in Figure II, and the compositions of the products are given.

Gum ghatti has been subjected to a periodate oxidation and it was found that three moles of formic acid were liberated per equivalent of gum. 80% of the arabinose units are oxidised together with about 33% of the galactose residues. To account for this amount of formic acid, it follows that most of the galactose residues are joined through C1 and C3, the remainder being joined by 1:6 linkages.

Further insight into the structure of gum ghatti has been obtained from methylation studies of the parent polysaccharide and of its degraded derivative A formed on autohydrolysis. The methyl sugars obtained are given in Table I and their approximate molar ratios are indicated.

In order to construct the chemical constitution of the gum from the experimental data outlined on the previous pages, it is convenient to consider in turn the acid-labile periphery, the neutral framework and the acidic part of the gum molecule.
Figure II

Degraded Gum A + Monosaccharides
Arabinose (1 part) Arabinose (4 parts)
Galactose (3 parts) Xylose (1 part)
Mannose (1 part) Rhamnose (trace)
Glucuronic acid (1 part)

↑ autohydrolysis

Gum Ghatti 0.5 N - H₂SO₄ 0.5 N - H₂SO₄
24 hr., 100°C 24 hr., 100°C
Arabinose (5 parts) G.A. β 1→2 Man
Galactose (3 parts) G.A. β 1→6 Gal
Mannose (1 part) G.A. β 1→2 Man
Glucuronic acid (1 part)

Xylose (1 part) G.A. β 1→3 Gal
Rhamnose (trace) II Gal β 1→6; Galβ1→6 Gal (n = 0-2)

↑ Periodate Oxidation

Oxidised gum

Gal β 1→6; Galβ1→3 Ara (n = 0-3)

Scheme showing the hydrolysis pattern of gum ghatti
<table>
<thead>
<tr>
<th>Methyl Sugars</th>
<th>Methylated Degraded Gum A</th>
<th>Methylated Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>-</td>
<td>3.9</td>
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<tr>
<td>2,3-di-O-methyl-L-arabinose</td>
<td>-</td>
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</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
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<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>3,5-di-O-methyl-L-arabinose</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
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<td>traces</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>2.3</td>
<td>0.7</td>
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<tr>
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<tr>
<td>2,4-di-O-methyl-D-galactose</td>
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</tr>
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<td>2-O-methyl-D-galactose</td>
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<td>1.2</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-rhamnose</td>
<td>-</td>
<td>traces</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucuronic acid</td>
<td>+</td>
<td>traces</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-glucuronic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4-O-methyl-D-mannose</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Methyl Sugars obtained from methylated and methylated-degraded gum ghatti
The Acid-Labile Periphery

That about 80% of the arabinose in the gum is present as non-reducing terminal arabinofuranose residues, followed from the observation that about 80% of this sugar was easily removed by autohydrolysis. This was substantiated by methylation studies on the gum acid, when 2,3,5-tri-\(\text{O}\)-methyl-\(\text{L}\)-arabinose was one of the major components of the hydrolysate. The remaining 20% appeared in the hydrolysate of the methylated gum as a mixture of about equal parts of 2,3-, 2,4-, 2,5- and 3,5-di-\(\text{O}\)-methyl-\(\text{L}\)-arabinose. Of these four dimethyl ethers of \(\text{L}\)-arabinose, only the first could arise from residues susceptible to oxidation with periodate. This agrees with the finding that 20% of the \(\text{L}\)-arabinose residues in the gum were not affected by the oxidant. The presence of non-terminal \(\text{L}\)-arabinose residues in the periphery of the molecule was indicated by the isolation of small amounts of arabinobiose on autohydrolysis and of the 2,3- and 3,5-di-\(\text{O}\)-methyl-\(\text{L}\)-arabinose on hydrolysis of the methylated gum. Since the vast majority of the arabinose is present as single, non-reducing end-groups, there must be relatively few parts of the molecule containing contiguous arabinose units.

The methylation studies have shown that C3 of the galactose residues were as a rule substituted in the parent polysaccharide but free in the degraded gum A. This is easily seen from the fact that the major product from methylated degraded gum A was 2,3,4-tri-\(\text{O}\)-methyl-\(\text{D}\)-galactose, whereas less than one third of the methylated galactose residues obtained
from methylated gum ghatti were substituted at C3. A fair proportion of the labile groupings must therefore be attached to this position: the possibility that some may be attached at C4 of galactose cannot, however, be completely excluded. The structural features of the acid-labile periphery of the gum may be represented as follows:

\[
\text{Araf} \xrightarrow{1 \rightarrow 3} \text{Gal and } \text{Araf} \xrightarrow{1 \rightarrow (\text{Ara} \xrightarrow{1 \rightarrow 3} \text{Gal}}
\]

**The Neutral Framework**

The principal features of this part of the molecule have emerged from the products of partial hydrolysis II and these results are substantiated by the methylated sugars isolated from methylated degraded gum A. The bulk of oligosaccharides obtained on partial hydrolysis belong to the homologous series of 1:6\(^{-}\)-linked galactose residues illustrating that the backbone of the molecule must be built up with 1:6\(^{-}\)-linkages. Confirmation of this was forthcoming from the methylation studies on degraded gum A where the predominant sugar residue was 2,3,4-tri-O-methyl-D-galactose. Only small amounts of di-O-methyl-D-galactose residues were detected. The isolation and characterisation of the family of oligosaccharides of the type, Gal \(\xrightarrow{1 \rightarrow 6} \text{Gal} \xrightarrow{1 \rightarrow 3} \text{Ara}\), from partial hydrolysis has illustrated the presence of 3\(^{1}\)-linked arabinose within the galactose chains. Whether these arabinose residues are in the pyranose or furanose form is not yet known since no methylated derivatives of arabinose were isolated from methylated degraded
gum A and both the 2,4- and 2,5-dimethyl ethers were observed in the methylated undegraded polysaccharide. The experimental evidence indicates the following partial structure:

\[ \text{Gal} 1 \rightarrow 6 \text{Gal} 1 \rightarrow 3 \text{Ara} 1 \]

Since the only dimethyl galactose isolated from the methylated degraded gum A was the 2,3-dimethyl ether, the branching points on these galactose chains is probably at C4.

The location of the small amount of 3-\(\text{O}-\beta\)-D-galactopyranosyl-D-galactose units in relation to the other parts of the molecule is not clear but it is possible that this galactobiose could arise from a small degree of branching in these chains. This would also explain the isolation of trace amounts of 2,4-di-\(\text{O}\)-methyl-D-galactose from the methylated degraded gum A.

The Acidic Portion

The identity of the two aldobiouronic acids, which are incorporated in the molecule of gum ghatti, has been established although the way they are attached to the galactose chains is still obscure. However, since the principal branch point in the galactose residues is at C4, it was formerly thought likely that at least some of the aldobiouronic acids were linked directly (I) or indirectly (II) to this position.

\[
- 6 \text{Gal} 1 - \\
\begin{array}{c}
\text{Gal} \\
\text{I} \\
\text{G.A.}
\end{array}
\]

\[
\text{Gal} 1 \rightarrow 2 \text{Man} 1 \rightarrow 4 \text{Gal} 1
\]
or II $\text{G.A.1} \rightarrow 6 \text{Gal} \rightleftharpoons \text{Gal} \rightarrow 6 \text{Gal} \rightarrow 4 \text{Gal} \\
\text{and G.A.1} \rightarrow 2 \text{Man} \rightarrow 6 \text{Gal} \rightarrow 4 \text{Gal}$

If the attachment occurred as in II above, it seemed reasonable to expect that a partial acid hydrolysis would release oligosaccharides containing $1:4^\text{L}$-linked galactose residues. Owing to the great stability of the uranosyl linkage, this may not be obtained from a product with structure I. The fact that such oligosaccharides have not been detected was taken as an indication that the aldobiouronic acids are attached directly to the galactose chains. Present investigations, which will be discussed later, indicate that the D-mannose and D-glucuronic acid residues may make up the main chain of the molecule and that the galactose chains are attached to the D-mannose units at position C3 or C6 either directly or via L-arabinose units.

The isolation, from partial hydrolysis III, of the aldotriouronic acid has furnished evidence for the existence of contiguous mannose units within the gum. Part of the molecule may, in fact, contain blocks of three mannose residues, since evidence for the existence of the polymer homologous aldotetraouronic acid has been observed. These results, however, are a little difficult to explain, since, if three contiguous mannose units are present, within the molecule, a mannobiose might be expected to be present in the products from partial hydrolysis II. In addition, a later examination of the polysaccharide
obtained by degradation by the Smith procedure gave no indication for contiguous mannose residues and further studies will be required before this question is answered. Present investigations again indicate that the suspected aldotriouronic acid may, in fact, be an aldotetraouronic acid. The details and significance of this observation will be discussed later. Therefore, on the basis of the experimental evidence so far outlined, the known structural features of gum ghatti were summarised by the following partial structures:

\[
\begin{align*}
\text{III} & \quad \ldots \ldots 6 \text{D-galp} 1 \rightarrow 6 \text{D-galp} 1 \rightarrow 6 \text{D-galp} 1 \rightarrow 3 \text{L-ara}.
\end{align*}
\]

\[
\begin{align*}
\text{IV} & \quad (\ldots \ldots 4) \text{D-Gp.A.} 1 \rightarrow 6 \text{D-galp} 1.
\end{align*}
\]

\[
\begin{align*}
\text{V} & \quad \ldots \ldots 4 \text{D-Gp.A.} 1 \rightarrow 2 \text{D-man} 1 \rightarrow 2 \text{D-manp}
\end{align*}
\]

\[
\begin{align*}
R & \quad \text{R}
\end{align*}
\]

\[
\begin{align*}
3 & \quad 3
\end{align*}
\]

\[
\begin{align*}
6 & \quad 6
\end{align*}
\]

\[
\begin{align*}
\uparrow & \quad \uparrow
\end{align*}
\]

R is mainly single L-arabinofuranose residues but, in some cases, can also be multiple units of L-arabinose residues or single L-rhamnopyranose residues.

Results from later work confirmed most of the above conclusions. Reduction of the periodate-oxidised gum followed by controlled acid hydrolysis afforded degraded gum B. This degraded polysaccharide has been examined by methylation, partial acid hydrolysis, and periodate oxidation.
and the products obtained are shown in Figure III and Table II.

That one third of the $1:6^L$-linked galactose residues are branch points has already been shown. The isolation of oligo-saccharides up to and including the hexasaccharide, has indicated that at least six contiguous galactose residues are present as branch points in parts of the molecule, and suggests that the branching is statistically irregular. Methylation studies on degraded gum B show that the degradation has brought about the removal of the substituent from C3 of the galactose residues.

No further information has been gathered about the $1:3^L$-linked galactobiose, although its isolation from both degraded gums B and C indicated that it must arise from the interior chains.

Previous studies provided no evidence for the ring size of the arabinose residues which terminate the $1:6^L$-linked galactose chains. The isolation from methylated degraded gum B of 2,4-di-$\alpha$-methyl-$L$-arabinose in greater quantity (3 to 1) than the 2,5-dimethyl ether indicates that the majority of the internal arabinose residues are in the pyranose form.

Not all the peripheral arabinose was removed by the periodate oxidation and the $L$-arabinose end groups present in degraded gum B must have arisen from 2- and/or 3-$\alpha$-substituted residues in the original gum.

Most of the residues at C6 and some of those at C3 have been removed from the mannose units by the action of the periodate. This conclusion follows from the isolation of
Figure III

Gum Acid

(i) Oxidation

(ii) KBH₄

(iii) N₂ - H₂SO₄

Degraded Gum B → 0.5 N₂ - H₂SO₄

0.5 N₂ - H₂SO₄

3x0.5 hr., 100°

D-Gal pβ₁ [β1→6 - D-Gal pβ₁]n → 6-D-Gal

D-Gal pβ₁ [β1→6 - D-Gal pβ₁]n → 3-L-Ara

(n = 0-4)

(n = 0-3)

Gal pβ₁ → 3 Gal

Gal pβ₁ → 3-Gal

Gal pβ₁ → 6-Gal

Gal pβ₁ → 3-Ara

Arap 1→3-Man

Scheme showing the hydrolysis of degraded gum ghatti
### Table II

<table>
<thead>
<tr>
<th>Methyl Sugars</th>
<th>Methylated Degraded Gum B</th>
<th>Methylated Degraded Gum C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-arabinose</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>traces</td>
<td>traces</td>
</tr>
<tr>
<td>2-O-methyl-L-arabinose</td>
<td>traces</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>traces</td>
<td>-</td>
</tr>
<tr>
<td>2,4,6-tri-O-methyl-D-galactose</td>
<td>traces</td>
<td>+</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-galactose</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>2,4-di-O-methyl-D-galactose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2-O-methyl-D-galactose</td>
<td>traces</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-mannose</td>
<td>traces</td>
<td>+++</td>
</tr>
<tr>
<td>2,4,6-tri-O-methyl-D-mannose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>(?) 4,6-di-O-methyl-D-mannose</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4-O-methyl-D-mannose</td>
<td>traces</td>
<td>-</td>
</tr>
</tbody>
</table>

Methyl Sugars obtained from methylated degraded gums B and C
3,4,6-tri-O-methyl- and (?) 4,6-di-O-methyl-D-mannose and only small amounts of 4-O-methyl-D-mannose from methylated degraded gum B.

Degradation of degraded gum B with periodate by Smith's procedure afforded degraded gum C. Partial acid hydrolysis of degraded gum C yielded, among other things, the disaccharide, 3-O-L-arabinopyranosyl-D-mannose. The following, therefore, may be an important structural feature of the gum:

\[
\begin{align*}
\text{4-G.A.} & \rightarrow \text{2-Manp} \quad \text{1}
\end{align*}
\]

As has already been said it may be that the main chain of the molecule consists of D-mannose and D-glucuronic acid residues and that the galactose chains are attached to this backbone via arabinose to C3 of mannose. That no mannobiose has been isolated from any partial hydrolysis studies is peculiar, and an additional investigation of the acidic sugars would be required before further structures can be put forward.

**Anogeissus Leiocarpus Gum**

Another tree of the genus *Anogeissus*, *A. leiocarpus*, is found in northern Nigeria and in eastern parts of the Sudan. The tree was formerly known as *A. schimperi*, and its gum was
first investigated by Mcllroy\(^8\) who showed that hydrolysis of the polysaccharide yielded arabinose, galactose and glucuronic acid. Structural studies were first carried out on the gum in this laboratory by Aspinall and Christensen\(^9\) who examined the products of partial hydrolysis. Further studies on this gum form the major part of this thesis.

Preliminary studies had shown that the gum had an uronic acid anhydride content of $\sim 22\%$ and, on hydrolysis, gave D-xylose (12\%), L-arabinose (32\%), D-galactose (5\%), D-mannose (2\%) and a mixture of acidic oligosaccharides (20\%).

Graded hydrolysis was carried out in three stages as shown on Figure IV, degraded gums A and B being separated from the soluble sugars after the first and second stages. Isolation of many of these products has indicated that the two gums from the Anogeissus genus have many structural features in common, but that there are also significant differences.

Consideration of the acid-labile periphery leads to the conclusion that, as in gum ghatti, the majority of L-arabinose residues are present in the furanose form and are to be found in the outer parts of the molecule. The presence of the adjacent L-arabinose units in the gum has been established by the isolation of 3-O-L-arabinofuranosyl-L-arabinose from autohydrolysis of the gum acid. There is also chromatographic evidence for the presence of other arabinobiose residues at this stage, thus indicating that they must be mutually linked in more than one way.

Like gum ghatti, the polysaccharide from $A.\text{leiocarpus}$
Figure IV

Gum Acid

\[
0.1 \text{ N } \text{H}_2\text{SO}_4 \\
2\times4 \text{ hr.}, 100^\circ
\]

↓

Degraded Gum A + \( \begin{array}{c}
\text{L-Araf} \rightarrow 3\text{-L-Ara} + \text{monosaccharides}
\end{array} \)

\[
0.5 \text{ N } \text{H}_2\text{SO}_4 \\
2\times1 \text{ hr.}, 100^\circ
\]

↓

Degraded Gum B + \( \begin{array}{c}
\text{Ara} \rightarrow 3\text{ Ara}
\end{array} \)

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

\[
\text{D-Galp} \rightarrow 6\text{-D-Galp} \rightarrow 6\text{-D-Gal} (n = 0,1,2 \& 3)
\]

\[
\text{D-Galp} \rightarrow 6\text{-D-Galp} \rightarrow 3\text{-L-Ara} (n = 0,1,2 \& 3)
\]

↓

Monosaccharides and traces of acidic oligosaccharides

\[
\text{Gp A} \rightarrow 6\text{ Gal}
\]

\[
\text{D-Gp A} \rightarrow 2\text{ Man}
\]

higher acid oligosaccharides

Scheme showing graded hydrolysis of \( \text{A. leiocarpus} \)
contains some arabinose in the interior chains, since partial acid hydrolysis affords the same homologous series of oligosaccharides, \( \text{Gal} 1 \rightarrow \text{Gal} 1 \rightarrow \text{Ara} (n = 0 - 3) \). The isolation of this series of oligosaccharides and the other containing only the 1:6-linked galactose units shows that some of the interior chains are similarly constituted. \textit{A. leiocarpus} gum, however, does afford a relatively larger amount of 3-O-\( \beta \)-D-galactosyl-D-galactose on hydrolysis. No evidence is available for the structural environment of this unit, although it is perhaps significant that no oligosaccharides containing contiguous 1:3-linkages could be detected in the hydrolysis.

The gum, although furnishing the same two aldobiouronic acids as \textit{gum ghatti}, only produced the mannose-containing acid in substantial amount. The isolation of a third acid sugar, \( \text{D}-\text{glucurono} \rightarrow \text{D}-\text{galactosylarabinose} \), suggests a further difference in structure between the two gums.

Recently the gum has been fractionated on DEAE-cellulose\(^{91}\) to yield two polysaccharides. Polysaccharide A is of high acid content (ca 30\%) and is the major constituent. Polysaccharide B is of lower uronic anhydride content (ca 10\%). No structural studies were carried out on either of the components and it will be of interest, in this context, to discover the nature of the heterogeneity. It may lie mid-way between the gross heterogeneity exemplified by \textit{Khaya senegalensis} gum\(^{23,31}\) and the micro-heterogeneity encountered with \textit{Combretum leonense} gum.\(^{30}\)
Other Mannose-containing Polysaccharides

Structural studies on *Virgilia oroboides* gum have been carried out in great detail by Stephen and his collaborators. Fragmentation analysis using acidic conditions of increasing strength\(^\text{92,93}\) and methylation studies\(^\text{94,95}\) were the principal methods of investigation employed.

The conclusions arrived at have been very concisely summarised\(^\text{96}\) and an analytical study on the polysaccharide and its carboxyl reduced analogue have been described in detail\(^\text{97}\). There are present in the gum long chains of D-galactose units which are linked \(\beta-1\rightarrow6\), every second sugar carrying a substituent at either C3 or C4. Some unbranched galactose units are \(\beta-1\rightarrow3\) linked and some are multi-branched at position 3, 4 and 6 and 2, 3 and 6; these, however, are minor features and represent only 4% of the total sugar residues present. Some of the side-chains consist of L-arabinose residues (some single, others at least three units long) which are \(\alpha-1,5\)-linked. Terminal arabinose units are present both in the pyranose and furanose forms, a small proportion (<1 mole in 10) being replaced by D-xylose.

Other side chains are acidic, containing D-glucuronic acid which is \(\beta\)-glycosidically linked to C2 of D-mannose or, to a lesser extent, to C6 of D-galactose. Some of the D-glucuronic acid units are substituted by another residue at C4, while others are 4-O-methylated. The mannose units represent further branch points, carrying a sugar substituent at position 3.
A major structural feature which has yet to be determined is the identity of the sugars attached to the main galactose chains at position 3 and 4. The polysaccharide does have structural features which are similar to cherry and damson gums and gum ghatti but does possess apparent differences. The gum from the member of the genus *Virgilia, V. divaricata*, has received little attention and, although it contains mannose, no structural features are known.

*Hakea acicularis* gum contains 7% of D-mannose residues but has only been subjected to a very brief structural study.\(^{(98)}\) Periodate oxidation indicates the presence of a high proportion of 1:6-linked galactose units and acid hydrolysis liberated 2-0-β-D-glucuronosyl-D-mannose. In these respects the gum bears a general resemblance to gum ghatti.

*Albizzia zyggia* gum has been the subject of some structural analysis.\(^{(46)}\) It contains 9% of D-mannose residues and hydrolysis liberates the aldobiouronic acid, 2-0-β-D-glucuronosyl-D-mannose. Investigation of the products of partial acid hydrolysis indicate that the polysaccharide bears a resemblance in structure to gums of the *Acacia* genus. In many respects, however, the polysaccharide tends to be unique, differing generally from most other plant gums.

It is perhaps significant in this context that an extracellular polysaccharide isolated from cultures of *Xanthomona*
oryza yields the aldobiouronic acid 2-O-β-D-glucuronosyl-D-mannose on hydrolysis.(99) Structural deductions, however, will not be possible until further oligosaccharides have been isolated. It is of biogenetic interest to discover whether polysaccharides of bacterial origin have similar structural features to the polysaccharides of plant origin such as the plant gums.
The object of the present investigation

The primary aim of this investigation is the fractionation of *Anogeissus leiocarpus* gum on a preparative scale. The intention is then to carry out structural studies on the major component, polysaccharide A, and to compare the results with the known features of gum ghatti.

The structural analysis will be directed particularly towards the determination of the location of the $D$-mannose residues within the molecule and the relation of those units to other parts of the molecular structure. In the light of these findings, it is also hoped to carry out some further analyses of gum ghatti in an effort to determine the location of the $D$-mannose residues in that molecule.
DISCUSSION
Purification of the gum

The sample of *Anogeissus leiocarpus* gum used in the present investigations was botanically authenticated and was obtained from the Tropical Products Institute.

Solution of the crude gum was effected by stirring a powdered sample in water for forty-eight hours. After filtration through layers of muslin and centrifugation to remove the insoluble impurities, the free gum acid was precipitated by the addition of acidified ethanol. After trituration with ethanol, the air-dried polysaccharide was dissolved in the minimum volume of water and the solution was freeze-dried to yield the gum acid $[\alpha]_D +14.0^\circ$, uronic acid anhydride 22.4% (by decarboxylation).

Examination of the gum for heterogeneity

The purified polysaccharide was placed on a DEAE-cellulose column in the phosphate form. Elution of the column with increasing concentration of phosphate buffer followed by potassium chloride solution, gave rise to two polysaccharide fractions having different uronic acid anhydride contents. Both fractions were eluted from the column with phosphate buffers, the potassium chloride eluate containing no trace of polysaccharide. Both fractions, which were freed from inorganic material by dialysis and treatment with ion exchange resins, were subjected to total acid hydrolysis and gave the same component sugars.

Fraction I, which was the minor constituent, was called leiocarpan B. It had an uronic anhydride content of 11.6% and
specific rotation of $-5.4^\circ$.

Fraction II, which was the major constituent, was called leiocarpan A. The polysaccharide had an uronic anhydride content of 31.8% (carbazole method) and a specific rotation of $+15.2^\circ$. A slightly more detailed study of the hydrolysis products from each polysaccharide has recently been carried out\(^{116}\) and it has been observed that, although the sugar constituents do not differ, the amounts of individual sugars vary considerably. Hydrolysis of leiocarpan B gave appreciably more galactose than the hydrolysis of leiocarpan A whereas the latter yielded a greater amount of the aldobiouronic acid, 2-0-$\beta$-D-glucuronosyl-D-mannose. These preliminary experiments would appear to indicate that the oligosaccharides isolated from the previous partial acid hydrolysis\(^{90}\) are to a greater extent representative of the minor component of the gum, leiocarpan B.

Cetavlon has been used in many cases to resolve mixtures of polysaccharides.\(^{34,117}\) Purified *Anogeissus leiocarpus* gum readily gave a precipitate with cetavlon. The cetavlon solution was added to the gum in five portions. After each addition the precipitated complex was removed at the centrifuge and the polysaccharide regenerated by treatment with sodium chloride solution. The five fractions obtained from this procedure (A1, A2, A3, A4 and A5) were analysed for uronic anhydride content (by decarboxylation) and the results indicated that a partial fractionation had been achieved.

Fractions A1, A2 and A3 had uronic anhydride contents of
30.7%, 31.0% and 30.4%, and specific rotations of +14.0°, +14.3° and +14.0° respectively. Each fraction was examined by chromatography on DEAE-cellulose, the column being developed with phosphate buffers of increasing molarity. Each fraction gave identical elution patterns, one polysaccharide fraction only being obtained in each case. These fractions were eluted from the column with the same phosphate buffer as leiocarpan A in the preliminary experiment. The uronic anhydride contents of these chromatographed fractions were determined by the carbazole method and values of 31.4%, 32.1% and 32.1% were obtained for A1, A2 and A3 respectively. It was, therefore, concluded that fractions A1, A2 and A3 were homogeneous polysaccharides containing only leiocarpan A.

Fractions A4 and A5 had uronic anhydride contents of 24.6% and 16.4% and specific rotations of +8.0° and +8.0° respectively. Chromatography of samples of these fractions on DEAE-cellulose indicated that they were heterogeneous, and that they contained both leiocarpans A and B. These fractions were not examined further.

The mother-liquor from this procedure yielded no further precipitate with cetavlon. By the addition of ethanol, however, a further polysaccharide fraction, A6, was isolated. Fraction A6 had an uronic acid anhydride content of 12.1% (by decarboxylation) and a specific rotation of -4.9°. The fraction was further examined by DEAE-cellulose chromatography. Development of the column in the usual manner yielded one polysaccharide fraction only, which was eluted with the same
phosphate buffer as leiocarpan B in the preliminary experiment. Measurement of the uronic anhydride content of this fraction by the carbazole method gave a value of 11.7%. It seems likely that fraction A6 is homogeneous consisting solely of leiocarpan B.

These results indicate conclusively that the gum from *Anogeissus leiocarpus* is heterogeneous, containing two polysaccharides, leiocarpans A and B, which have different uronic anhydride contents. These two polysaccharides can be separated from each other on a preparative scale by fractional precipitation using cetavlon to give three main fractions. The first of these contained pure leiocarpan A, the third pure leiocarpan B while the second contained both components.
Autohydrolysis Results

An aqueous solution (2%) of purified leiocarpan A acid was heated on a boiling water-bath for twenty-four hours. At this time it had been shown by preliminary experiments that the degraded gum was essentially arabinose-free. Autohydrolyses are generally followed by measuring the change in optical rotation, the degraded polysaccharide in question being arabinose-free, when a constant value is attained. It was discovered, however, in this particular case, that the optical rotation changes were misleading owing to the release of xylose in addition to arabinose. A scheme was devised whereby the arabinose-release and xylose-release were measured. It was observed that most of the arabinose was removed long before the gum was xylose-free, and that it would be possible to obtain an arabinose-free degraded polysaccharide which still contained an appreciable proportion of xylose residues. The reaction was, therefore, stopped when the arabinose release had reached a maximum value.

The hydrolysis fragments were examined chromatographically and a complex mixture of oligosaccharides was observed in addition to xylose, arabinose and galactose. None of these oligosaccharides were obtained in a pure state, but sugars with chromatographic mobilities identical to 3-O-β-L-arabinopyranosyl-L-arabinose and to 3-O-L-arabinofuranosyl-L-arabinose were recognised. Both of these disaccharides were isolated as partial hydrolysis products from the unfractionated gum.

The degraded gum, which was isolated in the usual way, had
an uronic acid anhydride content of 41.5% and specific rotation of -4.8°.

Methylation Results

A sample of the substantially arabinose-free degraded gum, which had been prepared by the autohydrolysis of the gum acid, was methylated with methyl sulphate and sodium hydroxide. The partially methylated polysaccharide was allowed to react with silver carbonate and the silver salt methylated completely by several additions of methyl iodide and silver oxide. The fully methylated polysaccharide (OMe 42.1%) was reduced with lithium aluminium hydride.

The reduced, methylated, degraded leiocarpan A (OMe 40.5%) was hydrolysed with hydrochloric acid and the hydrolysate neutralised by the addition of silver carbonate. The syrup was fractionated by partition chromatography on a cellulose column. Further fractionation was effected by paper chromatography and displacement chromatography on charcoal columns giving the following methylated sugars.

<table>
<thead>
<tr>
<th>Approximate Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-&lt;o-methyl-D-mannose</td>
</tr>
<tr>
<td>2,3,4,6-tetra-&lt;o-methyl-D-galactose</td>
</tr>
<tr>
<td>2,3,4-tri-&lt;o-methyl-D-xylose</td>
</tr>
<tr>
<td>3,4,6-tri-&lt;o-methyl-D-mannose</td>
</tr>
<tr>
<td>2,3,4-tri-&lt;o-methyl-D-galactose</td>
</tr>
<tr>
<td>3,4-di-o-methyl-D-mannose</td>
</tr>
<tr>
<td>2,3-di-o-methyl-D-glucose</td>
</tr>
</tbody>
</table>
These sugars were all fully characterised by the formation of crystalline derivatives. The tri-O-methylxylose, both mannose derivatives, and the di-O-methylglucose crystallised themselves but crystalline derivatives were also formed. The galactose derivatives and the tetra-O-methylmannose were all identified as anilides: the tri-O-methylxylose and di-O-methylmannose as aldonolactones, the tri-O-methylmannose as the aldonamide and the di-O-methylglucose as the phenylhydrazide of the derived aldonic acid.

In addition to the above major components, the following were also detected chromatographically,

- $2,3,4$-tri-O-methyl-$\Delta^6$-arabinose
- $2,4$-di-O-methyl-$\Delta^6$-arabinose
- $2$-O-methyl-$\Delta^6$-arabinose: trace
- $2,3,6$-tri-O-methyl-$\Delta$-galactose
- $2,6$-di-O-methyl-$\Delta$-galactose
- $2,4$-di-O-methyl-$\Delta$-galactose
- $2$-O-methyl-$\Delta$-galactose: trace
- $2,3$-di-O-methyl-$\Delta$-xylose
- $2,4$-di-O-methyl-$\Delta$-xylose
- $2,5,4$-tri-O-methyl-$\Delta$-mannose: trace
- (?) $4,6$-di-O-methyl-$\Delta$-mannose
- $4$-O-methyl-$\Delta$-mannose
- $2,3,4$-tri-O-methyl-$\Delta$-glucose
- $2$-O-methyl-$\Delta$-glucose
- $3$-O-methyl-$\Delta$-glucose

These sugars were identified by paper chromatography and
ionophoresis of the sugars and their derivatives, and gas chromatography of their methyl glycosides. In addition to the above, the presence of a trace amount of 2,3,5-tri-O-methyl-L-arabinose was detected gas chromatographically.

The proportions of the methylated sugars that have been given are very approximate, since many were present as mixtures and several refractionations were necessary before pure sugars were obtained. Many of these processes inevitably involved losses which cannot be estimated.

The yield of fully methylated ethers is lower than would be expected. This low yield is probably caused by the loss of the highly volatile 2,3,4-tri-O-methyl-D-xylose on evaporation.
Reduction of the gum and Partial Acetolysis Studies

A sample of leiocarpan A was acetylated by the procedure of Carson and Maclay\(^{118}\) and the acetylated polymer was reduced with diborane gas. Since previous investigations have shown that the formation of artifacts occurred when the gas was generated \(\text{in situ}^{119}\) the diborane was generated externally and pumped into the reaction mixture for several weeks. The neutral polymer obtained after deacetylation was free from artifacts of reduction.

A sample of the carboxyl-reduced polysaccharide was methylated by the classical procedure. The products from hydrolysis were examined by paper chromatography, and the cleavage products from methanolysis examined by gas-liquid partition chromatography. The results from both of these examinations indicated the presence of the same sugar derivatives as had been obtained from the reduced methylated gum acid. Therefore, it was assumed that no degradation of the molecule had taken place during the reduction and subsequent deacetylation.

It was hoped by the examination of this reduced polymer to gain some insight into the environment of the mannose-residues. Previous investigations had shown that most, if not all, the mannose in the gum was associated with the glucuronic acid residues by a 1→2 linkage. Owing to the stability of the uranosyl bond, it has not been possible to determine to which units the mannose residues are attached. The reduction of the acid to glucose will considerably reduce the strength of this...
bond and, by some method of partial fragmentation, it is hoped to obtain mannose-containing oligosaccharides.

Preliminary experiments using various methods of fragmentation showed that the greatest spectrum of oligosaccharides was obtained by a partial acetolysis of the carboxyl-reduced polysaccharide for sixty hours.

A large-scale partial acetolysis was carried out and the fragments, after deacetylation, were placed on a charcoal-celite column. Elution with water removed most of the monosaccharides. A preliminary fractionation of the oligosaccharides that remained on the column was made by eluting with water containing increasing proportions of ethanol. A final fractionation was accompanied by partition chromatography on thick paper and in one case by an ionophoretic separation. A total of nine chromatographically and ionophoretically pure sugars was obtained and the presence of several more was recognised.

The first component isolated (oligosaccharide A) had a chromatographic mobility comparable to that of a disaccharide. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained mannose and glucose, the latter as the reducing unit. A positive reaction towards triphenyltetrazolium chloride and alkaline degradation to mannose, indicated that position C-2 of the glucose was unsubstituted.

A sample was methylated by the Kuhn procedure and the methylated disaccharide methanolysed. Examination of the cleavage products by gas-liquid partition chromatography
indicated the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose. The disaccharide was, therefore, thought to be 4-O-D-mannopyranosyl-D-glucose.

The sugar was methylated with Haworth and Purdie reagents and hydrolysed. The hydrolysate was separated by partition chromatography on thick paper into two components. The first pure fraction, 2,3,4,6-tetra-O-methyl-D-mannose, was characterised as its crystalline aniline derivative and the second component, 2,3,6-tri-O-methyl-D-glucose was characterised as the crystalline 1,4-di-p-nitrobenzoate. The linkage was, therefore, 1→4.

An α configuration has been assigned to the glycosidic linkage on the basis of the positive rotation (+61°) of the disaccharide and on the fact that the sugar is chromatographically and ionophoretically different from 4-O-β-D-mannopyranosyl-D-glucose ([α]D = +19.7°) isolated from the glucomannan from European larch. (120)

The second component isolated (oligosaccharide B) was also chromatographically comparable to a disaccharide. Hydrolysis
and reduction followed by hydrolysis indicated that the sugar contained glucose and mannose, the latter as the reducing unit. A negative reaction to triphenyltetrazolium chloride and stability to alkali indicated that position C2 of the mannose residue was substituted.

The disaccharide was methylated and methanolysed. The cleavage products were examined by gas-liquid partition chromatography and the methyl glycosides of 2,3,4,6-tetra-\(\text{O}\)-methyl-D-glucose and 3,4,6-tri-\(\text{O}\)-methyl-D-mannose were detected. The linkage is, therefore, 1\(\rightarrow\)2.

The sugar was chromatographically and ionophoretically identical to a synthetic sample of 2-\(\text{O}\)-\(\beta\)-D-glucopyranosyl-D-mannose prepared by the epimerisation of sophorose. In view of this and of the negative rotation (\(-19.8^\circ\)), the \(\beta\) configuration has been assigned to the glycosidic linkage of the disaccharide.

B.

The third component (oligosaccharide C) was shown to be a trisaccharide by determination of chain length. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained mannose and glucose, the latter existing as the
reducing unit and as a non-reducing unit.

The sugar gave a positive reaction to triphenyltetrazolium chloride and was degraded by alkali to oligosaccharide B, 2-\(\beta\)-D-glucopyranosyl-D-mannose. In the absence of oxygen, it is known that alkali degrades some oligosaccharides in a stepwise manner from the reducing end of the molecule. Oligosaccharides which have substituted hydroxyl groups on positions C-3, C-4, or C-6 of the reducing end group are degraded to metasaccharinic, isosaccharinic and lactic acids, respectively, whereas similar substitution on position C-2 inhibits degradation. This fact has been utilised widely in the present section for the detection of the 1→2 linkages in the various sugars.

A partial hydrolysis was carried out on a small sample and oligosaccharides A and B were both recognised as components of the hydrolysate. A partial hydrolysis on the derived glycitol was carried out and oligosaccharide B was the only reducing oligosaccharide present in the hydrolysate.

The sugar was methylated by the Kuhn procedure and the product methanolysed. The cleavage products were examined by gas-liquid partition chromatography and the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-mannose were detected in equal amounts. Similar treatment of the derived glycitol led to the detection of 2,3,4,6-tetra-O-methyl-D-glucose, 3,4,6-tri-O-methyl-D-mannose and 1,2,3,5,6-penta-O-methylsorbitol in equal amounts.
From these results, it is obvious that the trisaccharide is linear and may be assigned the following structure.

C.

The fourth component (oligosaccharide D) was also shown to be trisaccharide by chain length determination. Hydrolysis and reduction followed by hydrolysis showed that the sugar contained glucose and mannose, the latter existing as the reducing and as a non-reducing unit.

A negative reaction to triphenyltetrazolium chloride and stability to the action of dilute alkali indicated that position C-2 of the reducing mannose unit was substituted.

Partial acid hydrolyses were carried out on the sugar and its derived glycitol. Both disaccharides isolated earlier, 2-\(\beta\)-\(\beta\)-glucopyranosyl-\(\beta\)-mannose and 4-\(\alpha\)-\(\beta\)-mannopyranosyl-\(\beta\)-glucose were recognised as products from the sugar but the latter disaccharide was the only reducing sugar recognised from the glycitol.

A sample of the sugar and the derived glycitol were methylated by the Kuhn procedure and the methylated sugars methanolysed. Examination of the cleavage products from the sugar by gas-liquid partition chromatography indicated the
presence of equal quantities of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-mannose. 1,3,4,5,6-penta-O-methylmannitol and the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose were detected in equal amounts as products from the derived methylated, methanolysed glycitol.

On the basis of the above information it is apparent that the trisaccharide is linear and may be assigned the following structure.

D.

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

The fifth component (oligosaccharide E) was shown to be a tetrasaccharide by the determination of the chain length. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained mannose and glucose, the latter existing as the reducing unit as well as a non-reducing unit.

A positive reaction to triphenyltetrazolium chloride indicated that position C-2 of the reducing glucose unit was unsubstituted. Confirmation of this was forthcoming from an examination of the product obtained from degradation by dilute alkali, when the trisaccharide D was the only sugar observed.
Samples of the sugar and the derived glycitol were subjected to partial acid hydrolysis. Oligosaccharides A, B, C and D were all recognised as components of the hydrolysate from the free sugar. The only reducing oligosaccharides present in the hydrolysate of the glycitol were both disaccharides A and B and the trisaccharide D.

Methylations by the Kuhn procedure were carried out on the parent sugar and on the derived glycitol. After methanolysis, the cleavage products of both were examined by gas-liquid partition chromatography. The methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose (1 part), 3,4,6-tri-O-methyl-D-mannose (1 part) and 2,3,6-tri-O-methyl-D-glucose (2 parts) were all recognised as products from the parent sugar. Equal quantities of 1,2,3,5,6-penta-O-methylsorbitol and the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-mannose were all detected as products from the glycitol.

On the basis of all the above results, it is apparent that the tetrasaccharide is linear and can be assigned the following structure.

\[ E. \]
The sixth component to be investigated (oligosaccharide F) was shown to be another tetrasaccharide by chain length determination. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained only glucose and mannose, the latter existing as the reducing unit as well as a non-reducing unit.

A negative reaction to triphenyltetrazolium chloride and stability to the extended action of dilute alkali indicated that position C-2 of the reducing mannose residue was substituted.

Samples of the sugar and its derived glycitol were subjected to partial hydrolysis. Oligosaccharides A, B, C and D were all detected in the hydrolysate of the sugar as well as some unchanged starting material. Examination of the hydrolysate from the glycitol for reducing sugars led to the detection of both disaccharides A and B but only one tri-saccharide C.

Methylations were carried out on the sugar and the derived glycitol by the Kuhn procedure. The cleavage products arising from the methanolysis of both methylated samples were examined by gas-liquid partition chromatography. The methyl glycosides of $2,3,4,6$-tetra-$\beta$-methyl-$D$-glucose (1 part), $3,4,6$-tri-$\beta$-methyl-$D$-mannose (2 parts) and $2,3,6$-tri-$\beta$-methyl-$D$-glucose (1 part) were all recognised as products from the parent sugar. Equal quantities of $1,3,4,5,6$-penta-$\beta$-methylmannitol and the methyl glycosides of $2,3,4,6$-tetra-$\beta$-methyl-$D$-glucose, $3,4,6$-tri-$\beta$-methyl-$D$-mannose and $2,3,6$-tri-$\beta$-methyl-$D$-glucose were
all detected as products from the glycitol.

On the basis of the above information, another linear tetrasaccharide is indicated and it can be assigned the following structure.

F.

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

The seventh component (oligosaccharide G) was shown to be a pentasaccharide. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained mannose and glucose, the latter existing as the reducing unit as well as a non-reducing unit.

A positive reaction to triphenyltetrazolium chloride indicated that position C-2 of the reducing glucose residue was unsubstituted. The oligosaccharide was degraded to the tetrasaccharide F by the extended action of dilute alkali, confirming that position C-2 of the reducing glucose residue was unsubstituted and indicating that position C-2 of the penultimate residue was substituted.

Partial hydrolysis studies on the pentasaccharide were carried out and oligosaccharides A, B, C, D, E and F were all recognised by their chromatographic mobilities. Similar studies on the derived glycitol indicated the presence of all
the above oligosaccharides with the exception of E as reducing sugars.

Methylation of the pentasaccharide by the Kuhn procedure was followed by methanolysis of the derivative. Examination of the cleavage products by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose (1 part), 2,3,6-tri-O-methyl-D-glucose (2 parts) and 3,4,6-tri-O-methyl-D-mannose (2 parts). Similar examination of the cleavage products from the methanolysed, methylated glycitol indicated the presence of 1,2,3,5,6-penta-O-methylsorbitol (1 part) and the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose (1 part), 2,3,6-tri-O-methyl-D-glucose (1 part) and 3,4,6-tri-O-methyl-D-mannose (2 parts).

On the basis of the above results, a linear oligosaccharide is indicated and it has been assigned the following structure.

The eighth component (oligosaccharide H) was shown to be another pentasaccharide. Hydrolysis and reduction followed by hydrolysis indicated that the sugar contained glucose and mannose, the latter existing as the reducing unit as well as being present elsewhere.
A negative reaction to triphenyltetrazolium chloride indicated that the reducing mannose unit was substituted at position C-2. This conclusion was endorsed by the fact that the sugar was stable when treated for extended periods with dilute alkali and that no degradation products were observed.

Partial hydrolysis studies were carried out on the sugar and oligosaccharides A, B, C, D, E and F were all recognised by their chromatographic mobilities. Similar partial hydrolysis on the derived glycitol indicated the presence of all the above oligosaccharides, with the exception of the tetrasaccharide F, as reducing sugars.

It seems from the above evidence that the sugar can be assigned the following structure, in keeping with the previous allotted structures.

H.

Methylation of the sugar and its derived glycitol was effected by the Kuhn procedure and, after methanolysis, the cleavage products were examined by gas-liquid partition chromatography. The products detected from the parent sugar were the methyl glycosides of 2,3,4,6-tetra-O-methyl-β-D-mannose (1 part), 2,3,6-tri-O-methyl-D-glucose (3 parts) and 3,4,6-tri-O-methyl-
D-mannose (2 parts): and from the glycitol, were 1,2,3,5,6-penta-O-methylsorbitol (1 part) and the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose (1 part), 2,3,6-tri-O-methyl-D-glucose (2 parts) and 3,4,6-tri-O-methyl-D-mannose (2 parts).

On the basis of the methylation evidence it is obvious that the sugar is linear and that it is not a penta- but a hexasaccharide and may be assigned the following structure.

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

It is difficult to reconcile the results from the methylation with the other evidence. The stability to dilute alkali is fairly good evidence for the 2-O-substituted mannose reducing residue. The possibility of a mixture of the two sugars cannot be ruled out, but it must be stated that no penta-O-methylmannitol was observed among the methanalysis products from the methylated glycitol.

It can however be said that the sugar does belong to the series of oligosaccharides which have been obtained from the partial acetolysis and which have been seen to conform to a general pattern.

Further higher oligosaccharides have been isolated from these studies but attempts to obtain pure compounds were not
successful. Hydrolysis did show that all the later sugars contained only glucose and mannose and that all gave rise to oligosaccharides A to H on partial hydrolysis. Methylation by the Kuhn procedure and methanolysis, followed by examination of the cleavage products by gas-liquid partition chromatography indicated that the major constituents were the methyl glycosides of 2,3,6-tri-\(\alpha\)-methyl-\(\beta\)-glucose and 3,4,6-tri-\(\beta\)-methyl-\(\beta\)-mannose.

It would, therefore, appear that the characterised sugars A to H are the first members of two series of oligosaccharides, which are linear and increase their length by the addition of 2-\(\alpha\)-substituted mannose residues and 4-\(\alpha\)-substituted glucose residues alternately.

A ninth component (oligosaccharide I) was isolated in very low yield. Hydrolysis and reduction followed by hydrolysis indicated that the sugar was a disaccharide containing galactose only. It was chromatographically and ionophoretically identical to 6-\(\alpha\),\(\beta\)-D-galactopyranosyl-\(\beta\)-galactose.

A sample was methylated by the Kuhn procedure and the fully methylated disaccharide methanolysed. The cleavage products were examined by gas-liquid partition chromatography and the methyl glycosides of 2,3,4,6-tetra-\(\alpha\)-methyl-\(\beta\)-galactose, 2,3,4-tri-\(\beta\)-methyl-\(\beta\)-galactose and 2,3,5-tri-\(\beta\)-methyl-\(\beta\)-galactose were detected.

On the basis of the above evidence the disaccharide has been assigned the following structure.
Two further oligosaccharides were also detected but were not obtained as pure compounds. The estimated amounts of these two were also very low. The first of these sugars (oligosaccharide J) was contaminated with oligosaccharides containing glucose and mannose. Hydrolysis of this mixture gave glucose, mannose and xylose, so oligosaccharide J contained xylose. No xylitol was detected among the hydrolysis products of the derived glycitol, so xylose does not exist as the reducing group. The chromatographic mobility of the sugar was comparable to that of a disaccharide. The sugar is, therefore, either a xylosylmannose or a xylosylglucose and, since the principal branch point in the molecule has been shown to be at position C-6 of mannose, it seems reasonable to suggest that it is 6-\(\beta\)-D-xylosyl-\(\beta\)-mannose and has the following structure.

\[J.\]
The second of these sugars (oligosaccharide K) also had a chromatographic mobility comparable to a disaccharide. Indications were obtained from hydrolysis and reduction followed by hydrolysis that the sugar contained arabinose and galactose, the latter existing as the reducing unit. No structure can be proposed for this component.
Graded Hydrolysis

Graded hydrolysis of the gum acid was carried out with N sulphuric acid at 100° for five hours. Examination of the hydrolysate indicated the presence of xylose, arabinose, mannose, galactose, glucuronic acid and a complex mixture of acidic oligosaccharides.

The complex hydrolysate was placed on a DEAE-Sephadex column in the formate form and the neutral sugars were eluted with water. A preliminary fractionation of the acidic sugars remaining on the column was made by eluting with water containing increasing proportions of formic acid. A final fractionation was accomplished by partition chromatography on thick paper.

A total of twelve acidic sugars were recognised of which eight were chromatographically pure.

The first component (oligosaccharide I) was isolated in high yield. Hydrolysis, reduction with sodium borohydride followed by hydrolysis and methyl ester methyl glycoside formation followed by reduction and hydrolysis indicated that the sugar was a disaccharide containing glucuronic acid and mannose, the latter existing as the reducing unit. A negative reaction to the triphenyltetrazolium chloride spray showed that position C-2 of the mannose residue was substituted, indicating that the disaccharide was 2-0-(glucosyluronic acid)-D-mannose.

Since it is difficult to distinguish between the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid and 3,4,6-tri-O-methyl-D-mannose by gas-liquid partition chromatography
the oligosaccharide could not be methylated as it stood. Instead, the product obtained from the reduction of the derived methyl ester methyl glycoside was methylated by the Kuhn procedure and methanolysed. The cleavage products were examined by gas-liquid partition chromatography and the methyl glycosides of \(2,3,4,6\)-tetra-\(\beta\)-methyl-D-glucose and \(3,4,6\)-tri-\(\beta\)-methyl-D-mannose were detected.

From these results and from the value of the specific rotation (-32.2°), this oligosaccharide is identical to \(2-\beta-(\beta -\beta\)-glucopyranosyluronic acid)-\(\beta\)-mannose isolated from gum ghatti and from the unfractionated \(A.\) leiocarpus gum and has the following structure.

I.

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image}
\end{figure}}
\]

The second component (oligosaccharide II) was isolated in reasonable yield. Hydrolysis, reduction with sodium borohydride followed by hydrolysis and methyl ester methyl glycoside formation followed by reduction and hydrolysis indicated that the sugar was a disaccharide containing glucuronic acid and galactose, the latter existing as the reducing unit.

The sugar was methylated by the Kuhn procedure and the resultant syrup methanolysed. The cleavage products were
examined by gas-liquid partition chromatography and the methyl glycosides of 2,3,4-tri-O-methyl-D-glucuronic acid, 2,3,5-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose were detected.

On the basis of the above evidence and of the specific rotation (+22.1°), the disaccharide has been assigned the following structure.

II.

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

The third component (oligosaccharide III) was isolated in very low yield. Hydrolysis, reduction with sodium borohydride followed by hydrolysis and methyl ester methyl glycoside formation followed by reduction and hydrolysis indicated that the sugar was a trisaccharide containing the sugars glucuronic acid, galactose and arabinose, the arabinose existing as the reducing unit.

A partial hydrolysis was carried out on a small sample and oligosaccharide II, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose was recognised as the main component of the hydrolysate.

A sample of the sugar was methylated by the Kuhn procedure and the derivative methanolysed. The cleavage products were examined by gas-liquid partition chromatography and the methyl
glycosides of 2,3,4-tri-O-methyl-\(\text{D}\)-glucuronic acid, 2,3,4-tri-O-methyl-\(\text{D}\)-galactose, 2,4-di-O-methyl-\(\text{L}\)-arabinose and 2,5-di-O-methyl-\(\text{L}\)-arabinose.

From the results of the methylation studies, it is apparent that the trisaccharide is linear. The glycosidic linkage between the glucuronic acid unit and the galactose unit can be assigned the \(\beta\)-configuration by comparison to oligosaccharide II. In order to accommodate the specific rotation (+9.3\(^\circ\)) obtained, the glycosidic link between the galactose and arabinose units has been assigned the \(\beta\)-configuration to give the following structure.

\[ \text{III.} \]

![Structure III](image)

It is known that 3-O-\(\beta\)-\(\text{D}\)-galactopyranosyl-\(\text{L}\)-arabinose is a structural feature of the polysaccharide and this factor has also been taken into account in assigning the \(\beta\)-configuration. The oligosaccharides IV, V, VI and VII were not isolated as pure sugars and conclusions of structural significance cannot be drawn. They were all isolated in very low yield and are probably of little structural importance.

The fourth pure sugar (oligosaccharide VIII) was isolated in high yield. Reduction of the methyl ester methyl glycosides
followed by hydrolysis gave glucose and mannose. Partial hydrolysis of the sugar afforded oligosaccharide I, 2-O-(glucopyranosyluronic acid)-D-mannose, glucuronic acid and mannose, whilst partial hydrolysis of the glycitol gave oligosaccharide I, the glycitol of oligosaccharide I, glucuronic acid and mannitol. The two disaccharides from the second partial hydrolysis were distinguished from each other by a small difference in chromatographic movement and by their different colour reactions with the periodate Schiff's spray reagent. The aldobiouronic acid was oxidised to a derivative of malondialdehyde and gave a brilliant yellow colour after one hour. The 2-O-(glucopyranosyluronic acid)-mannitol, on the other hand, gave an immediate purple coloration, indicating the rapid formation of formaldehyde. The similarity of the staining reaction with aniline oxalate to that given by oligosaccharide I, and the negative reaction to triphenyltetrazolium chloride supplied further evidence that the reducing mannose residue was 2-O-substituted.

The chain length of the oligosaccharide was determined by the phenol sulphuric acid method on the sugar and its derived glycitol and a value of 3.9 indicated that the sugar was a tetrasaccharide.

The uronic acid anhydride of the sugar was determined by the carbazole method in conjunction with the phenol sulphuric acid method. A value of approximately 50% indicated that the sugar contained two acidic and two neutral units.

On the basis of the above information the oligosaccharide
has been assigned the following structure.

VIII.

The 1→4 linkage between the mannose and glucuronic acid units has been assigned on the basis of the methylation studies on the degraded gum A. A major product from the reduced methylated polysaccharide was 2,3-di-O-methyl-D-glucose showing that the D-glucuronic acid residues in the gum are 4-O-substituted. Support for this has come from the partial acetolysis studies on the carboxyl-reduced polysaccharide, when a disaccharide was isolated and characterised as 4-O-α-D-mannosyl-D-glucose.

This oligosaccharide is believed to be identical to a partial acid hydrolysis product of gum ghatti, which was previously thought to be a trisaccharide. However, more conclusive proof will be required before the issue is finally settled, but examination of the hydrolysis products from the methylated sugar should provide the answer.

The fifth pure sugar (oligosaccharide IX) was isolated in low yield. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and mannose. Hydrolysis of the sugar gave glucuronic acid, mannose and oligosaccharide I.
Hydrolysis of the derived glycitol gave glucuronic acid, mannose, and oligosaccharide I but no mannitol. The similarity of the staining reaction with aniline oxalate to that given by $\alpha$-glucuronic acid indicates that the reducing unit may also be that residue. A positive reaction to triphenyltetrazolium chloride indicated that the sugar was not 2-$\alpha$-substituted.

On this evidence, the sugar has been tentatively assigned the following structure.

IX.

The sixth pure sugar (oligosaccharide X) isolated was similar to oligosaccharide VIII, on preliminary examinations. It may be that it is the polymer-homologous aldohexaouronic acid.

The seventh pure sugar (oligosaccharide XI) isolated was similar to oligosaccharide IX in the preliminary experiments carried out and it may be a polymer-homologous acid.

The eighth and final sugar (oligosaccharide XII) isolated was identical to oligosaccharides VIII and X in the preliminary experiments carried out. No definite conclusions could be drawn from the presence of these last four sugars as unambiguous
structures cannot be assigned to them. There are indications, however, that the polysaccharide consists of glucuronic acid and mannose residues which are alternately linked from these graded hydrolysis products.
Reduction of gum ghatti

A purified sample of gum ghatti was acetylated by the procedure developed by Carson and Maclay and the acetylated polysaccharide was reduced with diborane gas in the same manner as described for leiocarpan A. Hydrolysis of the modified polymer indicated that there were no artifacts of reduction.

Like leiocarpan A, a sample of the carboxyl-reduced polysaccharide was methylated by the classical procedure and the hydrolysis products examined by paper chromatography. The cleavage products from the methanolysis of a sample were examined by gas-liquid partition chromatography. The presence of the same sugars, which were present in samples of reduced methylated gum ghatti, was good indication that no degradation of the molecule had occurred during the reduction and deacetylation.

A small-scale partial acetolysis of the carboxyl-reduced polysaccharide was carried out, and the products obtained were examined by paper chromatography. The presence of galactose-containing oligosaccharides was observed but there were also indications that the same series of oligosaccharides, A to H, isolated from the partial acetolysis of leiocarpan A, were also present.

This observation will justify a large-scale partial acetolysis of the carboxyl-reduced gum and adds some weight to the theory that gum ghatti is structurally related to leiocarpan A.
The synthesis of $2\rightarrow\beta-D$-glucopyranosyl-$D$-mannose

Crystalline sophorose was prepared by the method described by Coxon and Fletcher.\(^{122}\) Kuhn and his coworkers\(^{123}\) have made use of the known stability of oligosaccharides with their reducing groups $2\rightarrow$-substituted to dilute alkali, to synthesise new sugars by epimerisation reactions. In this way they have prepared $2\rightarrow\alpha-L$-fucopyranosyl-$D$-talose from its epimer, $2\rightarrow\alpha-L$-fucopyranosyl-$D$-galactose.

The sophorose ($2\rightarrow\beta-D$-glucopyranosyl-$D$-glucose) was heated for a short time in a solution of 0.1 N sodium carbonate. In this manner, the sugar was epimerised to $2\rightarrow\beta-D$-glucopyranosyl-$D$-mannose which was separated from monosaccharides and unchanged sophorose by charcoal-column chromatography.

The purified epimer was obtained in approximately 20% yield and was chromatographically pure. Its structure was confirmed by small-scale experiments but attempts to fully characterise the sugar by the formation of a crystalline derivative were not successful.
Structural features of Anogeissus leiocarpus A

The present studies on the structure of the major component from the gum Anogeissus leiocarpus have essentially been restricted to the acid-stable basal chains, and no further insight into the nature of the linkages of the peripheral acid-labile units has been obtained.

The isolation of the following oligosaccharides,

A. $4\alpha-D$-mannopyranosyl-$\alpha-D$-glucose,
B. $2\beta-D$-glucopyranosyl-$\beta-D$-mannose,
C. $\beta-D$-glucopyranosyl-$(1\to2)\alpha-D$-mannopyranosyl-$(1\to4)D$-glucose,
D. $\alpha-D$-mannopyranosyl-$(1\to4)\beta-D$-glucopyranosyl-$(1\to2)D$-mannose,
E. $\alpha-D$-mannopyranosyl-$(1\to4)\beta-D$-glucopyranosyl-$(1\to2)\alpha-D$-mannopyranosyl-$(1\to4)D$-glucose,
F. $\beta-D$-glucopyranosyl-$(1\to2)\alpha-D$-mannopyranosyl-$(1\to4)\beta-D$-glucopyranosyl-$(1\to2)D$-mannose, and
G. $\beta-D$-glucopyranosyl-$(1\to2)\alpha-D$-mannopyranosyl-$(1\to4)\beta-D$-glucopyranosyl-$(1\to2)\alpha-D$-mannopyranosyl-$(1\to4)D$-glucose,

as partial acetolysis products from the carboxyl-reduced polysaccharide, is good indication that the basal chains of the molecule are made up of $D$-glucuronic acid and $D$-mannose residues linked to each other in a strictly alternate manner. That the glucuronic acid residues are linked to the C-2 position of the mannose residues and that the mannose residues are, in turn,
linked to C-4 position of glucuronic acid, follows from the partial characterisation of these neutral oligosaccharides. It would, therefore, appear that the main chains of the molecule comprise the following repeating unit,

\[ \ldots \ldots \ldots \quad 4\text{D-G.pA.} \quad 1 \rightarrow 2\text{D-Manp} \quad 1 \ldots \ldots \]

This general picture is confirmed by the graded hydrolysis studies where the major aldobiouronic acid isolated was \( 2\text{-Q-}(\beta \text{-D-glucopyranosyluronic acid})\text{-D-mannose.} \) Evidence was also obtained for the presence of the corresponding aldotetrauronic acid, which was also isolated in high yield. The characterisation of this acid will be a further factor in the assignation of this strictly alternating backbone. There were also indications for the presence of the polymer-homologous hexacuronic acid among the hydrolysis products.

The fact that no disaccharides composed entirely of glucose or of mannose were isolated from the modified polysaccharide is fairly sound proof that contiguous mannose or glucuronic acid units are not present. The isolation of only two trisaccharides and two tetrasccharides furnishes further evidence for the existence of this structure.

The methylation studies on the arabinose-free degraded polysaccharide confirm this general structure and indicate that the glucuronic acid residues exist principally as chain units. The indication of branch points from the detection of very small amounts of monomethylglucoses is probably not significant, and it is possible to account for the isolation of comparatively
small amounts of 2,3,4-tri-\(\text{O}\)-methyl-\(\text{D}\)-glucose by considering other structural features of the gum. The presence of terminal non-reducing glucuronic acid residues in the gum is not in doubt, and they may arise in part from terminal units in these basal chains.

The isolation of 3,4,6-tri-\(\text{O}\)-methyl-\(\text{D}\)-mannose and, in greater amount, of 3,4-di-\(\text{O}\)-methyl-\(\text{D}\)-mannose indicates that the mannose residues exist both as chain units and as branch points. In view of the large quantities of the dimethylether isolated it is obvious that this sugar is the principal branch point in the degraded polymer. Some evidence was obtained for the presence of 4,6-di-\(\text{O}\)-methyl-\(\text{D}\)-mannose, a sugar which would arise from a second type of branch point.

The characterisation of 2,3,4,6-tetra-\(\text{O}\)-methyl-\(\text{D}\)-mannose shows that mannose units also exist as terminal non-reducing groups and a small proportion probably exist as double branch points, since a small but significant quantity of 4-\(\text{O}\)-methyl-\(\text{D}\)-mannose was isolated among the hydrolysate.

The following, therefore, are the principal features of the main chains of the molecule on the basis of the methylation results,

\[
(1) \quad 2\text{D-Manp} \quad \text{1} \quad , \quad (\text{ii}) \quad 2\text{D-Manp} \quad \text{1} \quad \\
(\text{iii}) \quad 4\text{D-G.pA.} \quad \text{1} \\
\]

and the following are minor but significant features,
The xylose residues have been found to exist predominantly, if not entirely, as terminal non-reducing groups in the degraded gum by the isolation of large quantities of 2,3,4-tri-O-methyl-D-xylose from hydrolysis of the methylated degraded gum. Since the main branch point in the basal chains has been shown to be at position 0-6 on the mannose, it may be that xylose is attached through this point. A xylose containing disaccharide was isolated among the acetolysis fragments, but in very low yield. Proof was obtained, however, that it was present at the non-reducing end of the disaccharide and that it was attached to either glucose or mannose. The following is also a structural feature of the degraded gum,

\[(\text{vii}) \quad \text{D-Xylp} \quad \]

Evidence was obtained for the presence of the disaccharide, 6-O-\(\beta\)-D-galactopyranosyl-D-galactose, among the products of partial acetolysis. Small amounts of 2,3,4-tri-O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose were isolated and characterised from the hydrolysate of the methylated degraded polysaccharide, thus indicating that galactose residues exist both as terminal non-reducing groups and also as chain units. The following are, therefore, minor but important
features in the gum,

(viii) $\text{D-Galp} \xrightarrow{1} \text{ and (ix) } 6\text{D-Galp} \xrightarrow{1}$

Higher homologues of this $1\rightarrow 6$ linked galactose series have been isolated as partial hydrolysis products from un-fractionated Anogeissus leiocarpus gum. That none were detected as products of the partial acetolysis, may in part be accounted for by the small proportion of galactose residues present in leiocarpan A and the known lack of stability of the $1\rightarrow 6$ linkage to acetolysis conditions.\(^{124,125,126}\)

No evidence was obtained for the mode of linkage of these galactose chains to the main framework of the gum molecule. These units may be attached to the mannose residues at position C-3 either directly or via arabinopyranose units. That arabinopyranose residues are present within the acid-stable part of the molecule in small amount is confirmed by the isolation of a small quantity of its 2,4-di-$\text{O}$-methylether. Proof that the galactose residues are attached to position C-3 of these arabinose units was obtained by the isolation of the aldotriouronic acid, $\text{O}-(\beta -\text{D-glucopyranosyluronic acid})-(1\rightarrow 6)-\text{O}-(\beta -\text{D-galactopyranosyl})-(1\rightarrow 3)-\text{L-arabinose}$, as one of the products from the partial acid hydrolysis. The isolation of the aldobiouronic acid, $6\text{-O}-(\beta -\text{D-glucopyranosyluronic acid})-\text{D-galactose}$, from the partial hydrolysis indicates that some of the galactose chains are terminated by $\text{D}$-glucuronic acid residues in the gum. This feature would also give rise to the small quantity of 2,3,4-tri-$\text{O}$-methyl-$\text{D}$-glucose which was
Present in the hydrolysate of the methylated degraded gum acid.

The structural features of leiocarpan A may, therefore, be summarised by the following partial structures:

\[
\begin{align*}
\text{(a)} & \quad \ldots \quad 4\text{D-G.pA.} \quad 1 \rightarrow 2\text{D-Manp} \quad 1 \rightarrow 4\text{D-G.pA.} \quad 1 \rightarrow 2\text{D-Manp} \quad 1 \ldots \ldots \\
\text{(b)} & \quad 4\text{D-G.pA.} \quad 1 \rightarrow 6\text{D-Galp} \quad 1 \rightarrow 3\text{L-Arap} \quad 1 \ldots \ldots \\
& \quad \text{and} \\
\text{(c)} & \quad 4\text{D-Galp} \quad 1 \rightarrow 6\text{D-Galp} \quad 1 \ldots \ldots \\
\text{(d)} & \quad \ldots \ldots 2\text{D-Manp} \quad 1 \quad \text{and} \quad \ldots \ldots 2\text{D-Manp} \quad 1 \ldots \ldots \\
\end{align*}
\]
Comparison of the structural features of the major component of Anogeissus leiocarpus gum with those of other gums

A large number of the gum exudates have now been subjected to structural investigations and the grouping of these into families has been suggested. The general structural features of the three principal families has already been discussed and will not be dealt with here.

From these investigations it is rather difficult to accommodate leiocarpan A into any of these groups, and it is quite obviously necessary to put forward the concept of a fourth polysaccharide family, known as the glucuronomannans. The present studies have indicated that this group should comprise polysaccharides with basal chains consisting of alternating units of D-glucuronic acid linked through positions C-1 and C-4 and D-mannose linked through positions C-1 and C-2,

\[ \text{\ldots} \xrightarrow{4D-G.za. 1 \rightarrow 2D-Manp 1} \xrightarrow{4D-G.\alphaA. 1 \rightarrow 2D-Manp 1} \text{\ldots} \]

In this respect the family would bear some resemblance to the gums of the Sterculia genera, which have been shown to consist of units of D-galacturonic acid and L-rhamnose similarly linked.

\[ \text{\ldots} \xrightarrow{4D-Galp A. 1 \rightarrow 2L-Rhap 1} \text{\ldots} \]

However, in this case, evidence has been obtained for the presence of contiguous galacturonic acid units and the existence of a strictly alternating backbone does not seem probable.

The results from this investigation of leiocarpan A may be
compared with those from the earlier work carried out on gum ghatti, which is derived from the botanically related species Anogeissus latifolia. The isolation in high yield of the two polymer-homologous series of oligosaccharides, \((1,n = 0, 1, 2, 3 \text{ and } 4)\) and \((2,n = 0, 1, 2 \text{ and } 3)\), quite reasonably leads to the conclusion that gum ghatti consisted of basal chains of 1-6 linked galactose residues.

1. \[\text{\text{D-Galp}} \underset{1}{\rightarrow} \text{6D-Galp} \underset{n}{\rightarrow} \text{6D-Gal}\]

2. \[\text{\text{D-Galp}} \underset{1}{\rightarrow} \text{6D-Galp} \underset{n}{\rightarrow} \text{3L-Ara}\]

However, in the light of this investigation and in view of the results obtained from the degradation of the periodate-oxidised polysaccharide, it is clearly necessary to reassess this opinion.

It is clear that leiocarpan A contains a substantially higher proportion of D-glucuronic acid and D-mannose, and considerably less D-galactose than gum ghatti. Whereas xylose is only a minor constituent of gum ghatti it is present in substantial proportions in leiocarpan A. Despite these obvious differences in sugar composition, gum ghatti has been shown to have structural features in common with the unfractionated leiocarpan.

The known structural features of gum ghatti have already been outlined and the formulation of the various partial structures has previously been summarised. At the time, it was thought that there existed in the molecule contiguous
mannose units, when the partial characterisation of the believed aldotriouronic acid, \( O-(\beta-D-glucopyranosyluronic acid)-(1\rightarrow2)-O-D-mannopyranosyl-(1\rightarrow2)-D-mannose \) was carried out. A similarity in behaviour of this acid to the aldotetraouronic acid isolated from leiocarpan A definitely exists, and it does not seem too unreasonable to assume they are the same. A further examination of the partial hydrolysis products from gum ghatti would undoubtedly prove interesting in view of these observations.

If, as seems reasonable, the fragment is shown to be the aldotetraouronic acid consisting of alternately linked \( D-glucuronic acid \) and \( D-mannose \) units, the relationship in structure between the two polysaccharides would be strengthened. It would then be possible to suggest that gum ghatti also had basal chains comprising \( D-glucuronic acid \) and \( D-mannose \) in an alternate manner, with the long chains of \( \beta-1\rightarrow6 \) linked galactose units attached to this backbone. Evidence for this mode of attachment has, in fact, been obtained from the periodate oxidation studies on the Smith-degraded gum acid, when \( 3-O-L-arabinopyranosyl-D-mannose \) was isolated as a partial hydrolysis product.

\[ L-Arap \ 1\rightarrow3D-Manp \]

Since some, if not all, the galactose chains are known to terminate with \( L-arabinose \) units by the isolation of the series of oligosaccharides (2) as partial hydrolysis products, structure (3) may provide the key relationship of the galactose
and glucuronomannan chains. The isolation, among the hydrolysis products from the methylated periodate oxidised gum, of 2,4-di-\(\Omega\)-methyl-L-arabinose in larger amount than the 2,5-dimethyl ether (approximate ratio, 3:1), gives an indication that the majority of these interior 3-\(\Omega\)-substituted L-arabinose residues are in the pyranose form. Hence the following partial structure may be of major significance,

\[ \text{By analogy, this partial structure may also prove to be of significance in the structure of leiocarpan A. Internal arabinopyranose residues are known to be present in the gum and some of the mannose residues are known to be 3-\(\Omega\)-substituted. Further investigations will, however, have to be carried out before this interesting point is settled.} \]

\[ \text{Leiocarpan A is a member of the new glucuronomannan family and it seems quite possible that gum ghatti belongs to the same structural group of polysaccharides. A third member may also exist in the form of leiocarpan B. Structural studies on the minor component of } \text{Anogeissus leiocarpus} \text{ gum have not yet been} \]
carried out in great detail, but the work completed on the unfractionated gum and the preliminary experiments on the purified leiocarpan B, indicate that it may be more similar in structure to gum ghatti than to the major component.

At this stage it seems likely that the principal difference between gum ghatti and leiocarpan A lies in the length and the frequency of the 1→6 linked galactose side chains. Another rather obvious difference is the fact that, in leiocarpan A, some of $\beta$-arabinose units have been replaced by D-xylose.

These conclusions will now make it possible to answer some of the queries which have surrounded the structure of gum ghatti. The higher proportion of the aldobiouronic acid, 6-$\beta$-$\beta$-D-glucopyranosyluronic acid)-D-galactose, can be explained if, as seems probable, this unit terminates the majority of the galactose chains. The absence of any traces of mannose-containing disaccharides as hydrolysis products from the parent gum or any of its modified derivatives is not surprising in view of the proposed new structure.

In the light of this structure, it is still not possible to suggest a location for the small proportion of 3-$\beta$-D-galactopyranosyl-D-galactose units (4) in relation to the other structural units in gum ghatti.

$$4. \ D-Galp \ 1\rightarrow 3D-Gal$$

It is interesting to note in this context, although this galactobiose has been shown to be present among the hydrolysis
products of leiocarpan A, it was not recognised among the
products of partial acetolysis from the carboxyl-reduced poly-
saccharide. In view of the observation that the unfractionated
gum afforded relatively larger amounts of this disaccharide than
gum ghatti on partial hydrolysis, it seems reasonable to assume
that this unit plays a more important part in the structure of
leiocarpan B.

The isolation of the arabinose-containing aldotriouronic
acid, as a partial hydrolysis product from leiocarpan A, may be
taken as further evidence that the galactose side-chains in the
latter polysaccharide are considerably shorter than in gum
ghatti. This fragment has never been detected among the
hydrolysis products of this gum.

Little evidence has been obtained for the nature of the
linkages of the acid-labile units or their mode of attachment
to the main backbone of the molecule. The hydrolysis
fragments of the methylated gum are being investigated at
present (116) and some insight should soon be forthcoming on
this part of the molecule of leiocarpan A. A large quantity
of 2,3,5-tri-O-methyl-L-arabinose has been recognised, thus
indicating that terminal non-reducing L-arabinofuranose units
account for a high proportion of the L-arabinose present in the
periphery of the molecule. Dimethyl ethers of L-arabinose are
present but have not yet been obtained in a pure state and
quantitative assessment of their structural significance cannot
yet be made.

It now seems likely that the polysaccharides from the
genus *Anogeissus* differ from the other mannose-containing plant gums in structure. The similarity between these polysaccharides and the gum from *Virgilia oroboides* cannot be as strong as was originally suspected, and it would now appear that the latter gum bears a more striking resemblance to the mannose-containing *Prunus* gums. The products of methanolysis from methylated degraded damson gum have recently been examined by gas-liquid partition chromatography. (127) All the sugars, which had been previously characterised, were recognised and the absence of 2,4-di-β-methyl-β-arabinose was confirmed. This sugar was also absent in the hydrolysate of methylated degraded *Virgilia oroboides* gum and in this respect the two gums are similar. The presence of the internal β-arabinopyranose units may well be the key link between the galactose and the glucuronomannan chains in the three *Anogeissus* gums.

A fourth member of the family Combretaceae, *Combretum leonense* gum, may now have a greater structural similarity to the *Anogeissus* gums than was originally believed. Despite the obvious difference in the nature of the sugar units, a similar acidic fragment, 2-β-(α-D-galactopyranosyluronic acid)-β-rhamnose, has been isolated from graded hydrolysis studies. Although there is no direct evidence to suggest that this aldobiouronic acid arises from a main chain, the possibility is reasonably high. Other features of this polysaccharide, e.g. the galactose chains and the acid-labile periphery, do bear a strong resemblance to those of the *Anogeissus* gums, and *Combretum leonense* gum could have the same general pattern in
molecular structure.

These 1→6 linked galactose chains do appear to play an integral part in the structure of many of the plant gums. In *Virgilia oroboides* gum it has been concluded that these chains represent the backbone of the molecule. However, these chains may be attached to another repeating unit and, it is perhaps significant that the trisaccharide, \( \alpha-\beta-D\)-galactopyranosyl-(1→6)-\( \alpha-\beta-D\)-galactopyranosyl;(1→3)-\( \alpha-D\)-galactose, has been isolated as a partial hydrolysis product from the gum acid.

In the *Acacia* gums, the 1→6 linked galactose units have been unambiguously shown to be attached to basal chains of 1→3 linked galactose residues directly. The same could also occur in the *Prunus* gums but partial fragmentation analyses will have to be carried out before the issue is settled. Whatever the significance of these 1→6 linked \( \alpha-D\)-galactose units, it is evident that they exist as side-chains attached either directly or indirectly to a wide variety of basal chains in many of the exudate gums.

It is apparent from this comparison, that a comprehensive structural review will soon be possible between leiocarpan A and gum ghatti. It will also be of interest to determine the structural features of leiocarpan B and to discover its relationship to the other two *Anogeissus* gums.
EXPERIMENTAL
General Methods

Paper chromatography was carried out on Whatman No. 1 paper and, occasionally, on Whatman No. 4 paper. The following solvent systems were employed to develop the chromatograms:

A. ethyl acetate : pyridine : water (10:4:3),
B. ethyl acetate : pyridine : water (8:2:1),
C. ethyl acetate : acetic acid : formic acid : water (18:3:1:4),
D. ethyl acetate : acetic acid : formic acid : water (18:8:3:9),
E. butanol : pyridine : water : benzene (5:3:3:1, upper layer),
F. butanol : ethanol : water (1:1:1),
G. butanol : ethanol : water (4:1:5, upper layer),
H. methyl ethyl ketone : water : concentrated ammonia (200:17:1),
I. benzene : ethanol : water (169:47:15, upper layer),
J. methyl ethyl ketone : acetic acid : water (9:1:1 saturated with boric acid).

Detection of sugars on chromatograms

A. Reducing sugars were detected by spraying the dried chromatograms with a solution of either aniline oxalate in methylated spirits or p-anisidine hydrochloride in butan-1-ol.
B. Sugar alcohols were detected with the periodate-permanganate
spray reagent. (100)

C. Polyhydroxy-compounds in general, whether reducing or not, were detected with alkaline silver nitrate. The spray was particularly useful for the detection of small quantities.

D. Detection of sugars with C2 at the reducing end unsubstituted was effected by spraying the chromatogram with the triphenyltetrazolium chloride spray. (102)

E. Hydroxylamine hydrochloride and ferric chloride (103) were used to detect sugar residues to which ester groupings were attached.

The following abbreviations have been used to describe the chromatographic mobilities of the residues.

\[ R_g = \frac{\text{distance travelled by the component}}{\text{distance travelled by } 2,3,4,6\text{-tetra-O-methyl-D-glucose in solvent system G}} \]

\[ R_{gal} \] refers to the distance travelled by the sugar compared with that of galactose.

Preparative paper chromatographic separations were carried out on Whatman 3 mm filter sheets, the paper having been first extracted with water in a Soxhlet extractor. The positions of the components were located by cutting off narrow side-strips which were developed with spray reagent A. The appropriate parts of the filter sheets were then cut out and the sugar residues eluted with water.

Ionophoresis (104) was carried out qualitatively on Whatman
No. 1 paper in borate buffer (pH, 10) at a potential of 350 volts for six hours. In this context the Mg value is the ratio of the distance travelled by the component to that travelled by glucose. Preparative ionophoresis was carried out on Whatman 3 mm. paper in the same buffer at a potential of 1,000 volts for 1½ hours. The sugars were located and extracted from the papers as above. Spraying was carried out using the usual aniline oxalate spray to which 10% glacial acetic acid had been added.

Column Chromatography

Charcoal/celite columns were packed as a slurry of charcoal:celite (1:1). The activated charcoal was prewashed by decantation with three portions of boiling water and the celite was washed three times (once overnight) with 6 N-hydrochloric acid followed by water until free from chloride ions. The columns were thoroughly washed with water before applying the sugar mixtures.

Cellulose columns were packed as a slurry in acetone. The contents were washed with an acetone:water gradient (2 l.), water and finally with the solvents to be used.

DEAE-Cellulose columns were prepared as follows. The powder was washed alternatively with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide solution. The cellulose was washed free from alkali with water and packed as a slurry, air pressure being applied to the top of the column. The DEAE-cellulose was then generated in the phosphate form by elution
with 0.5 M sodium dihydrogen phosphate buffer (pH 6, 1 l.).
The support was then equilibrated with 0.005 M sodium di-
hydrogen phosphate buffer (pH 6, 1 l.).

DEAE-Sephadex columns were packed as a slurry with the
DEAE-Sephadex in the formate form. The powder (30 g.) was
first allowed to swell overnight in water (250 mls.). It was
then washed with 0.5 M hydrochloric acid followed by distilled
water until free from chloride ions and then with 0.5 M sodium
hydroxide solution followed by distilled water until free from
base. This procedure was repeated with further portions of
acid and alkali. Finally the DEAE-Sephadex was generated in
the formate form by stirring with 15% formic acid for fifteen
minutes four times. The column was then packed and free acid
was removed by washing with water.

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

Small scale hydrolyses were carried out on 2-10 mg. of
material in 0.5 to 2 ml. of N sulphuric acid in a sealed tube
at 100° for periods varying from four hours for neutral oligo-
saccharides to 18 hours for acidic polysaccharides. Neutrali-
sation was effected by adjusting to pH 5 by the addition of
saturated barium hydroxide and subsequently to pH 7 on addition
of barium carbonate in the case of neutral sugar residues.
With acidic components, the neutralisation was effected with
Amberlite LA-1 resin. The basic solutions were then treated
with Amberlite IR 120 (H) resin and concentrated to a syrup which was then examined by paper chromatography. Solvent systems A, B, E and F were used for the detection of neutral components and solvent systems C and D for acidic components.

Cations were removed from sugar solutions with Amberlite IR 120 (H) resin and anions were removed with Duolite A-4 or Amberlite LA-1 liquid resin.

Optical rotations were measured at $18^\circ \pm 2^\circ$ in aqueous solution unless otherwise stated.

Hydrolysis of methylated polysaccharides were carried out on $0.05 - 4$ g. of material in $5.0$ to $400$ ml. of $2 \, \text{M}$ hydrochloric acid. The solution was allowed to stand at room temperature for $48$ hours and heated overnight at $40^\circ$. The temperature was gradually raised to $100^\circ$ over a period of $6$ hours and the heating maintained until the optical rotation was constant. The solution was neutralised with silver carbonate: excess silver was precipitated with hydrogen sulphide and the filtered solution evaporated to a syrup. The syrups obtained were examined in solvent systems G, H and I.

Demethyllations$^{(105)}$ were carried out by dissolving the methylated sugars in anhydrous dichloromethane and treating the solution with boron trichloride at $-70^\circ$ for thirty minutes. The solution was then allowed to evaporate at room temperature.
The resulting syrup was exhaustively evaporated (6 times) with methanol to remove borate ions and finally examined in solvent system B.

Borohydride reductions\[^{(106)}\] were carried out by the addition of sodium borohydride to an aqueous solution of the sugar and allowing the solution to stand overnight. Excess borohydride was destroyed by the addition of Amberlite IR 120 (H) resin. The solution was evaporated to dryness and boric acid was removed with methanol as above.

Methoxyl contents were estimated by the semi-micro Zeisel method.\[^{(107)}\]

Acetyl contents were estimated by the method of Weissenberger.\[^{(107)}\]

Uronic acid anhydride contents (U.A.A.) were determined by decarboxylation\[^{(108)}\] or by the carbazole colorometric method.\[^{(109)}\]

Total sugar content of solutions were determined using the phenol sulphuric acid colorometric method\[^{(110)}\] generally. In certain cases a variation of the p-aminobenzoic acid method was employed, sulphosalicylic acid being substituted for the more usual oxalic acid.\[^{(111)}\]

Small scale periodate oxidation of methylated sugars was carried out by the method of Lemieux and Bauer.\[^{(112)}\] The sugar (1-2 mg.) was dissolved in 0.5 M sodium metaperiodate solution (0.2 ml.) and kept at 0° for one hour. The excess of
periodate was destroyed by the addition of ethylene glycol (1 drop) and the solution made alkaline to phenolphthalein with 0.5 M sodium hydroxide solution. The solution was taken to dryness and extracted with acetone. After reducing the volume of the solution, the syrup was chromatographically examined in solvents G and H.

Alkaline degradations. Small scale alkaline degradations were carried out by dissolving the sugar (5 mg.) in a solution of calcium hydroxide (1 ml.), bubbling in nitrogen to expel all traces of oxygen from the vessel and allowing the solution to stand at room temperature for ten to fifteen days in the stoppered flask. At the end of this time, the solution was treated with Amberlite IR 120 (H) resin, evaporated to dryness and examined chromatographically in the appropriate solvent.

Methyl glycosides and methyl ester methyl glycosides were prepared by treating the sugar in a sealed tube at 100° with dry methanolic hydrogen chloride (4%). The solution was neutralised with silver carbonate, filtered and evaporated to dryness. Six hours were sufficient for methylated neutral oligosaccharides but longer reaction times (up to eighteen hours) were allowed for the methanolysis of methylated polysaccharides and acidic oligosaccharides.

Small-scale methylations were carried out by the Kuhn procedure. The sugar (0.5 - 2.0 mg.) was dissolved in
dimethyl formamide (0.2 ml.) and methyl iodide (0.2 - 0.4 ml.) and silver oxide (0.5 - 2 mg.) added. The mixture was shaken at room temperature in the dark for eighteen to twenty-four hours. The silver salts were filtered off and the solution evaporated to small volume. The dimethyl formamide was removed as an azeotrope by repeated evaporation with re-distilled toluene. The resultant syrup was dried in a vacuum desiccator and methanolysed. The products were examined by gas liquid partition chromatography outlined as follows.

Gas-liquid partition chromatography\(^{(114)}\) was carried out on a "Pye Argon Chromatograph". The stationary liquid phase was supported on acid washed celite (80-100 mesh) and consisted of,

a) 10\% by weight of butan-1,4-diol succinate polyester.
b) 10\% by weight of polyphenyl ether (m-bis(m-phenoxyphenoxy) benzene).
c) A 1:1 mixture of 20\% by weight butan-1,4-diol succinate polyester and 20\% by weight Apiezon M.
d) 3\% by weight of neopentyl glycol adipate.

Operating temperatures used were 175\(^{\circ}\) for (a), 200\(^{\circ}\) for (b), 175\(^{\circ}\) for (c) and 150\(^{\circ}\) and 175\(^{\circ}\) for (d).

The retention times (\(T\)) of the methyl ethers methylglyco
d-sides are relative to that of methyl-2,3,4,6-tetra-O-methyl-
D-glucopyranoside.

For the determination of the degree of polymerisation of an oligosaccharide,\(^{(114)}\) a portion of the sugar was reduced
with sodium borohydride, essentially as described by Peat, Whelan and Roberts, \(^{115}\) and subsequently hydrolysed with concentrated sulphuric acid in the presence of phenol. \(^{110}\) Another sample of the oligosaccharide was treated similarly without prior reduction. The absorbance of the two solutions were measured spectrophotometrically and the average of the reducing power quotient from four measurements was used for calculating the chain length.

**Lactones** of aldonic acids of methylated sugars were prepared as follows. The sugar (10-100 mg.) was dissolved in water (2-3 ml.), bromine (5-20 drops) added, and the mixture kept in the dark for three days. The excess bromine was removed by aeration and the solution neutralised with silver carbonate, filtered and the filtrate evaporated to dryness. The sugar was extracted with acetone, filtered and evaporated to dryness. The lactone crystallised and was recrystallised from the given solvent.

**Aldonamides** were prepared from the corresponding lactones which had been dried in a vacuum desiccator. The lactone (10-100 mg.) was dissolved in dry methanolic ammonia (1-5 ml.) and allowed to stand at 0\(^\circ\) for two days. The excess solvent was evaporated and the amide crystallised. The crystalline derivative was recrystallised to constant melting point from the given solvent.
Phenylhydrazides of aldonic acids were also prepared from the lactone which had been prepared in the usual manner and had been dried in vacuo. The lactone (50 mg.) was dissolved in dry ether (10 ml.). The solution was then heated under reflux for thirty minutes when redistilled phenylhydrazine (30 mg.) in ether (0.5 ml.) was added. After four hours the solution was concentrated to a dark-brown syrup which was heated on a boiling water-bath for three hours. The syrup was then triturated with ether and the resulting crystals recrystallised from ethanol/ether mixtures.

The p-nitrobenzoate derivatives of methylated sugars were prepared as follows. The sugar (15 mg.) was dissolved in dry pyridine and p-nitrobenzoyl chloride (50 mg.), which had been recrystallised from pyridine, added. The solution was heated for thirty minutes at 70°. After allowing the solution to stand at room temperature for eighteen hours, a saturated solution of sodium bicarbonate was added dropwise until no further effervescence occurred. Water (5 ml.) was added and the product extracted with chloroform (3 x 15 ml.). The chloroform extracts were dried over anhydrous sodium sulphate and evaporated to small volume. Addition of light petroleum (b.p. 60-80°) at 0° induced crystallisation of the derivative. The derived p-nitrobenzoate was recrystallised from methanol.

Aniline derivatives of the methylated sugars were prepared by refluxing the sugar with ethanolic redistilled aniline
(equimolecular proportions) for one to six hours in the dark. Evaporation of the solvent gave the derived anilide which crystallised and was recrystallised from the given solvent.

The solvents were purified as outlined below.

- Butan-1-ol was refluxed for two hours with potassium hydroxide (1% w/v) and distilled.
- Light petroleum was shaken overnight with concentrated sulphuric acid (10% v/v), washed free from acid with distilled water and itself distilled.
- Pyridine was refluxed for three hours with potassium hydroxide (1% w/v) and distilled.
- Methyl iodide was distilled over silver oxide (1% w/v).
- Aniline was distilled in the dark in an atmosphere of carbon dioxide.
- Tetrahydrofuran, diglyme and dimethoxyethane were allowed to stand over sodium wire. They were filtered off as required and distilled over lithium aluminium hydride.
- Dimethyl sulphate was redistilled and allowed to stand over potassium carbonate until it was no longer acid to congo red paper.
Isolation and Purification of Anogeissus leiocarpus gum

The crude gum was obtained as pale yellow, glassy lumps contaminated with bark and other extraneous material. The gum nodules (20 g.) were crushed into small pieces and dissolved in water (2 l.) with stirring. Complete solution was attained after two days. The larger pieces of bark were removed by filtration through muslin and the remaining wood particles were removed by centrifugation through a "Sharples centrifuge". The filtrate was poured slowly with stirring into a solution of ethanol (6 l.) and concentrated hydrochloric acid (40 ml.). The precipitated polysaccharide was allowed to settle and the supernatant liquid decanted off. The gum acid was washed with ethanol until free from chloride ions, dissolved in water (1,500 ml.) and freeze dried. Yield = 15.6 g. $[\alpha]_D = +14.0^\circ$ ($c = 0.70$). Uronic acid anhydride = 22.7% (by decarboxylation).

A further quantity (40 g.) of the gum was isolated in exactly the same manner. Yield = 32.4 g. $[\alpha]_D = +14.0^\circ$ ($c = 0.82$). Uronic acid anhydride = 22.1% (by decarboxylation).

DEAE-cellulose chromatography of the purified gum acid

The gum acid (84.6 mg.) was dissolved in water (10 ml.) and the solution pipetted on to a DEAE-cellulose column (30 g., 35 x 3.5 cm.) which had been prepared in the usual way. The gum was allowed to soak into the column overnight. The column was then eluted successively with
Fractions (10 ml.) were collected every half-hour and the amount of polysaccharide present in the fractions was determined by the phenol-sulphuric acid method, using a standard curve based on the polysaccharide. The curve obtained by plotting the polysaccharide concentration of each fraction against the fraction number is shown on graph I. Two fractions were obtained.

**Fraction I** (tubes 85-101)

This fraction was eluted with the 0.25 M phosphate buffer and had an uronic acid anhydride content of 11.6% (carbazole method).

**Fraction II** (tubes 133-150)

This fraction was eluted with the 0.5 M phosphate buffer and had an uronic acid anhydride content of 31.8% (carbazole method).

The acid-contents were estimated by the carbazole method, using a calibration curve based on D-glucuronic acid.

Fraction I is the minor component of the gum and will now be known as leiocarpan B. Fraction II, the major polysaccharide component, will be known as leiocarpan A.

Both sugar solutions were dialysed against tap water for three days, stirred with mixed resins, evaporated to a small
volume and freeze dried.

Leiocarpan A (32.3 mg.) had specific rotation, 
\[ [\alpha]_D = +15.2^\circ (C = 1.06). \]
Chromatographic examination of the products of acid hydrolysis (M-acid, 12 hr.) in solvents A, B and C indicated the presence of xylose, arabinose, mannose, galactose and glucuronic acid.

Leiocarpan B (9.7 mg.) had specific rotation, 
\[ [\alpha]_D = -5.4^\circ (C = 0.97). \]
The same sugars present in leiocarpan A were identified chromatographically from hydrolysis of this fraction.

Fractionation of the complex gum acid using cetavlon

The complex gum (20 g.) was dissolved in water (2 l.). A saturated aqueous solution of cetyltrimethylammonium bromide (50 ml.) was added with stirring. The solution immediately became cloudy and the sticky precipitate which separated out was removed at the centrifuge and washed with water until free from adhering cetavlon. The complex was then decomposed by the addition of excess aqueous sodium chloride solution (10%) to an aqueous suspension and with stirring for one hour, by which time solution was complete. The gradual addition of ethanol (3 volumes) gave a polysaccharide (fraction I), which was removed at the centrifuge; washed with ethanol until free from chloride ions, dissolved in water and freeze-dried.

A summary of the whole procedure is outlined in the following table. The uronic acid anhydride contents were determined by the decarboxylation method and are generally the
average of three determinations. The optical rotations were measured in water at concentrations slightly below unity.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Cetavlon added (ml.)</th>
<th>U.A.A.</th>
<th>$[\alpha]_D$</th>
<th>Weight (in g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>50</td>
<td>30.7%</td>
<td>+ 14.0°</td>
<td>4.7</td>
</tr>
<tr>
<td>A2</td>
<td>50</td>
<td>31.0%</td>
<td>+ 14.3°</td>
<td>4.7</td>
</tr>
<tr>
<td>A3</td>
<td>50</td>
<td>30.4%</td>
<td>+ 14.0°</td>
<td>2.3</td>
</tr>
<tr>
<td>A4</td>
<td>50</td>
<td>24.6%</td>
<td>+ 8.0°</td>
<td>2.6</td>
</tr>
<tr>
<td>A5</td>
<td>100</td>
<td>16.4%</td>
<td>+ 8.0°</td>
<td>3.8</td>
</tr>
<tr>
<td>A6</td>
<td>residue</td>
<td>12.1%</td>
<td>- 4.9°</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Total recovery = 19.4 g.

**DEAE-cellulose chromatography of the fractions**

Each of the six fractions isolated by the cetavlon precipitation were chromatographed on DEAE-cellulose columns. The columns were eluted successively with

a) 0.25 M sodium dihydrogen phosphate buffer pH 6 (500 ml.)

b) 0.50 M sodium dihydrogen phosphate buffer pH 6 (500 ml.)

c) 1 M potassium chloride solution (500 ml.)

**Fractions A1, A2 and A3 (11.7 g.)**

These fractions gave identical elution patterns. Graph II was obtained from fraction A2 and is typical of the three fractions. The uronic acid anhydride content of these fractions was determined by the carbazole method:

- Fraction 1 = 31.4%
- Fraction 2 = 32.1%
- Fraction 3 = 32.1%
Fractions A4 and A5 (6.4 g.)

These fractions gave elution patterns similar to that obtained with the complex gum and represented on graph I. These fractions were not examined further.

Fraction A6 (1.3 g.)

This fraction gave an elution pattern as depicted on graph III. Measurement of the uronic acid content by the carbazole method gave a value of 11.7%.

Autohydrolysis of Purified leiocarpan A

Trial Experiment I

A solution of the gum acid (2 g.) in water (100 ml.) was heated on a boiling water-bath for sixty hours. Samples (5 ml.) were withdrawn at various times and their specific rotations were determined.

<table>
<thead>
<tr>
<th>Time (in hrs.)</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>20</th>
<th>24</th>
<th>30</th>
<th>48</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\alpha])_D</td>
<td>+14.0°</td>
<td>+20.2°</td>
<td>+22.0°</td>
<td>+28.1°</td>
<td>+28.4°</td>
<td>+30.0°</td>
<td>+34.0°</td>
<td>+34.0°</td>
</tr>
</tbody>
</table>

After measuring the optical rotations, the samples were treated with Amberlite resin IR 4B (OH), concentrated and poured into ethanol (4 volumes) to precipitate any polysaccharide present, which was removed at the centrifuge and was washed with ethanol. The supernatant and ethanol washings were concentrated and examined chromatographically in solvents A, B and C. The table below shows the sugars present and a
rough idea of the amounts (visual examination).

<table>
<thead>
<tr>
<th>Time (in hrs.)</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Oligosaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>+</td>
<td>tr.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>tr.</td>
</tr>
<tr>
<td>12</td>
<td>++++</td>
<td>+</td>
<td>tr.</td>
<td>tr.</td>
</tr>
<tr>
<td>20</td>
<td>++++</td>
<td>+</td>
<td>tr.</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>++++++</td>
<td>+</td>
<td>tr.</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>++++++</td>
<td>++</td>
<td>tr.</td>
<td>+</td>
</tr>
<tr>
<td>48</td>
<td>++++++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>60</td>
<td>++++++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Trial Experiment II**

A solution of the gum acid (2 g.) in water (100 ml.) was heated on a boiling water-bath for sixty hours. Samples (1 ml.) were withdrawn at various intervals and each sample was treated in the same manner as in trial experiment I. The resultant syrups were then fractionated into their individual components by chromatography on Whatman 3 MM paper in solvent system B.

Side strips were cut and developed in the usual manner, the individual sugars being eluted from the appropriate areas with water. The solutions were made up to 250 ml. in standard flasks, and the concentrations of the two pentoses, arabinose and xylose, were determined by the p-aminobenzoic acid colorometric method, using standard graphs based on the particular sugar. Corrections were estimated for the cutting of side-
The following table summarises the results obtained and graph IV illustrates the release-pattern.

<table>
<thead>
<tr>
<th>Time (in hrs.)</th>
<th>Arabinose Conc. (μg/ml.)</th>
<th>Xylose Conc. (μg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>360.0</td>
<td>140.0</td>
</tr>
<tr>
<td>8</td>
<td>520.0</td>
<td>160.0</td>
</tr>
<tr>
<td>20</td>
<td>680.0</td>
<td>175.0</td>
</tr>
<tr>
<td>24</td>
<td>720.0</td>
<td>180.0</td>
</tr>
<tr>
<td>32</td>
<td>720.0</td>
<td>300.0</td>
</tr>
<tr>
<td>37</td>
<td>720.0</td>
<td>340.0</td>
</tr>
<tr>
<td>48</td>
<td>720.0</td>
<td>380.0</td>
</tr>
<tr>
<td>60</td>
<td>720.0</td>
<td>400.0</td>
</tr>
</tbody>
</table>

**Large Scale autohydrolysis**

The gum acid (20 g.) in water (1,000 ml.) was heated on a boiling water-bath until the rotation was equal to approximately +28.4°. This value was obtained after twenty-four hours, as had been indicated by the trial experiments. At this stage the degraded polysaccharide should be essentially arabinose-free.

The solution was filtered to remove the small insoluble residue, and concentrated to smaller volume. The concentrated solution was then poured into ethanol (4 volumes) acidified with hydrochloric acid. The precipitated polysaccharide was centrifuged off, washed free from chloride ions with ethanol and
dissolved in water (200 ml.). The gum acid was reprecipitated by the addition of ethanol (800 ml.), washed with ethanol and ether, dissolved in water (800 ml.) and freeze-dried (8.6 g.). \([\alpha_d^\text{I} = -4.8^\circ (\bar{C} = 0.90)\). Uronic acid anhydride = 41.5\% (by decarboxylation).

The supernatant and all the ethanol washings were deionised in the usual manner and concentrated to a syrup (9.7 g.) which was found to be a mixture of arabinose, xylose, galactose and other higher oligosaccharides. An attempt to separate the mono- and disaccharides by charcoal column chromatography gave no further information. Oligosaccharides having \(R_{\text{gal}} = 0.82\) and 1.20 were, however, recognised but others too were present.
Methylation of degraded leiocarpan A

The degraded gum (8 g.) was dissolved in water (50 ml.) and treated with methyl sulphate (65 ml.) and 30% (w/v) aqueous sodium hydroxide (130 ml.) below 5° in an atmosphere of nitrogen. The reagents were added dropwise over a period of eight hours with vigorous stirring. Further similar quantities of the reagents were added on four successive days and secondary octyl alcohol was added occasionally to control frothing. The final mixture was heated on a boiling water-bath for an hour. On cooling the precipitated sodium sulphate was filtered off and washed with water. The filtrate and washings were dialysed against running water until free from sulphate ions. The partially methylated polysaccharide was isolated by concentration of the aqueous solution and freeze-drying (6.8 g.; OMe, 30.1%).

The partially methylated gum acid was dissolved in water (100 ml.) and the solution was deionised by passage through a column of Amberlite IR 120 (H⁺) resin. The silver salt of the polysaccharide was prepared by the addition of silver carbonate to pH 7 to the deionised solution, and was isolated by concentration of the filtrate and freeze-drying (7.0 g.).

The silver salt was dissolved in the minimum amount of dry methanol (25 ml.). Methyl iodide (100 ml.) was added and the mixture refluxed for eighteen hours. Silver oxide (10 g.) was added to the solution at intervals. The mixture was filtered and the residue extracted with boiling chloroform in a Soxhlet
extractor. The filtrate and chloroform extractions were combined, concentrated to small volume and poured into a large excess of light petroleum (b.p. 60-80\(^\circ\)). The precipitated, methylated polysaccharide was filtered off and dried in vacuo. Two additional methylations were carried out by the Purdie procedure in exactly the same way. One further methylation failed to increase the methoxyl content significantly.

The product was the fully methylated degraded leiocarpan A (6.0 g.). Found: OMe, 42.1\%. \([\alpha]_D = -12^\circ\) (\(c = 0.96\) in chloroform).

Reduction of methylated, degraded leiocarpan A

The methylated polysaccharide (5.25 g.) was dissolved in tetrahydrofuran (250 ml.) and lithium aluminium hydride (5.25 g.) in tetrahydrofuran (250 ml.) was added slowly. After standing at room temperature for thirty minutes, the mixture was stirred under reflux for three hours. The excess lithium aluminium hydride was destroyed by the simultaneous addition of ethyl acetate and water (1:1). The resultant mixture was brought to pH 4 by the addition of 4 N-sulphuric acid and extracted with chloroform (5 x 100 ml.).

The chloroform extracts were dried over anhydrous sodium sulphate and concentrated to a small volume. The reduced, methylated, degraded polysaccharide was precipitated by the addition of a large excess of light petroleum (b.p. 60\(^\circ\) - 80\(^\circ\)). The precipitated polysaccharide was filtered and dried in vacuo (3.75 g.). Found: OMe, 40.5\%. \([\alpha]_D = -14.2^\circ\) (\(c = 1.12\)
in chloroform).

Samples of the reduced methylated polysaccharide were methanolyzed and the resultant syrup examined by gas-liquid partition chromatography on systems (a) and (b). A wide-spectrum of methyl glycosides was observed. Another sample was hydrolysed and examined by paper chromatography in solvents G, H and I. The presence of a complex mixture of methylated sugars was again indicated.

Hydrolysis of the reduced methylated degraded leiocarpan A

The reduced methylated polysaccharide (3.6 g.) was suspended in 2 M-hydrochloric acid (150 ml.) for two days at room temperature and at 40-50° for a further day. The resulting solution was diluted to 300 ml., heated on a boiling water-bath for twelve hours (constant rotation), cooled and neutralised with silver carbonate. The precipitated silver salts were removed at the centrifuge and washed with water. The supernatant and washings were concentrated to 100 ml. and hydrogen sulphide gas allowed to bubble through the solution. The precipitated silver sulphide was removed at the centrifuge and washed with water. The supernatant and washings were concentrated to a thick syrup (3.2 g.) which was placed on a cellulose column (80 x 3.5 cm.) and the cellulose was eluted successively with the following solvents:

(a) light petroleum (b.p. 100-120°) : butan-1-ol (80:20, saturated with water);
(b) light petroleum (b.p. 100-120°) : butan-1-ol (70:30,
saturated with water);
(c) light petroleum (b.p. 100-120°) : butan-1-ol (50:50, saturated with water);
(d) butan-1-ol saturated with water;
(e) water.

Twenty fractions were collected in the usual manner and were examined chromatographically in various solvent systems. The results are tabulated below. The $R_g$ values refer to solvent G and represent the major constituent of each fraction, unless specifically stated otherwise.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>Weight (in mg.)</th>
<th>$R_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 52</td>
<td>76.1</td>
<td>1.01</td>
</tr>
<tr>
<td>2</td>
<td>53 - 67</td>
<td>30.3</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>68 - 84</td>
<td>105.7</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>85 - 123</td>
<td>49.2</td>
<td>0.98</td>
</tr>
<tr>
<td>5</td>
<td>124 - 142</td>
<td>18.8</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>143 - 190</td>
<td>54.2</td>
<td>0.90</td>
</tr>
<tr>
<td>7</td>
<td>191 - 228</td>
<td>218.1</td>
<td>0.87</td>
</tr>
<tr>
<td>8</td>
<td>229 - 250</td>
<td>23.7</td>
<td>0.85</td>
</tr>
<tr>
<td>9</td>
<td>251 - 333</td>
<td>148.4</td>
<td>0.87</td>
</tr>
<tr>
<td>10</td>
<td>334 - 370</td>
<td>10.9</td>
<td>0.86</td>
</tr>
<tr>
<td>11</td>
<td>371 - 577</td>
<td>37.1</td>
<td>0.75</td>
</tr>
<tr>
<td>12</td>
<td>578 - 631</td>
<td>48.6</td>
<td>0.62</td>
</tr>
<tr>
<td>13</td>
<td>632 - 730</td>
<td>236.1</td>
<td>0.60</td>
</tr>
<tr>
<td>14</td>
<td>731 - 750</td>
<td>33.8</td>
<td>0.60</td>
</tr>
<tr>
<td>15</td>
<td>751 - 847</td>
<td>533.7</td>
<td>0.55</td>
</tr>
<tr>
<td>16</td>
<td>848 - 1,212</td>
<td>313.3</td>
<td>0.52</td>
</tr>
<tr>
<td>17</td>
<td>1,213 - 1,393</td>
<td>162.0</td>
<td>0.50</td>
</tr>
<tr>
<td>18</td>
<td>1,394 - 1,495</td>
<td>679.8</td>
<td>0.50</td>
</tr>
<tr>
<td>19</td>
<td>1,496 - 1,539</td>
<td>165.0</td>
<td>0.32</td>
</tr>
<tr>
<td>20</td>
<td>1,540 - 1,700</td>
<td>21.9</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Examination of the Fractions

Fraction 1 (76.1 mg.)

Paper chromatography of this fraction in solvents G and I indicated the presence of tetra-O-methylmannose, with a trace of a sugar, $R_g = 0.60$ (solvent G). Methanolysis of a sample and examination of the products by gas-liquid partition chromatography on columns (a) and (b) indicated the presence of the methyl glycosides of the following sugars:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Retention times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-(D)-mannose</td>
<td>1.45</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-(D)-galactose</td>
<td>1.80</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-(D)-galactose</td>
<td>7.5</td>
</tr>
</tbody>
</table>

The major component of the mixture was the tri-O-methyl-D-galactose.

The fraction (71.2 mg.) was hydrolysed with $N$-sulphuric acid for eighteen hours and the neutralised sugar solution was examined chromatographically in solvents G and I. Components corresponding to tetra-O-methylmannose ($R_g = 1.00$), tetra-O-methylgalactose ($R_g = 0.92$) and 2,3,4-tri-O-methyl-D-galactose ($R_g = 0.77$) were observed.

The syrup was separated into three fractions on thick paper using solvent G.

Subfraction 1a

The syrup (5.2 mg.), $R_g = 1.00$ was chromatographically
pure and indistinguishable from 2,3,4,6-tetra-O-methyl-D-mannose. Demethylation gave mannose only.

Subfraction 1b

The syrup (5.6 mg.), \( R_g = 0.92 \) was chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose. Demethylation gave galactose only.

Subfraction 1c

The syrup (46.1 mg.) had \( R_g = 0.76 \). Gas-liquid partition chromatography of the methyl glycosides and paper chromatography of the sugars in three solvents, G, H and I, showed the fraction to be pure and identical to 2,3,4-tri-O-methyl-D-galactose. The sugar was characterised by conversion to the aniline derivative m.p. 166-167° and mixed m.p. 168-169° (with an authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine of m.p. 167°).

Fraction 2 (30.3 mg.)

Gas chromatographic examination of methyl glycosides from this fraction indicated the presence of tetra-O-methylmannose, 2,3,5-tri-O-methyl-L-arabinose (trace) and 2,3,4-tri-O-methyl-D-xylose. Paper chromatography in solvent I indicated the presence of 2,3,4,6-tetra-O-methyl-D-mannose (\( R_g = 1.04 \), solvent I) and 2,3,4-tri-O-methyl-D-xylose (\( R_g = 0.92 \), solvent I).

This fraction was combined with fraction 4 with which it was identical.
Fraction 3 (105.7 mg.)

This fraction was examined by paper chromatography and gas-liquid partition chromatography, and was shown to have a similar composition to fraction 2.

The syrup was dissolved in boiling ether and the solution left at room temperature for four hours and at 0°C overnight. The white crystals which formed were filtered off and recrystallised from ether, m.p. 90-91°C, \([\alpha]_D^o = +60\rightarrow +19^\circ\) (const.) in three hours (\(C = 0.96\)).

The sugar was characterised by conversion to the lactone m.p. = 54°C and mixed m.p. 54°C (with an authentic sample of 2,3,4-tri-O-methyl-\(\beta\)-xylopyranosyl lactone of m.p. 56°C).

The supernatant ether and washings were combined and added to fraction 4.

Fraction 4 (49.2 mg.)

This fraction was shown by paper chromatography in solvents G, H and I to consist of 2,3,4,6-tetra-O-methyl-\(\beta\)-mannose and 2,3,4-tri-O-methyl-\(\beta\)-xylose.

The combined syrups (110.2 mg.) were applied to a charcoal-celite column (25 x 2.5 cm.) and the column was eluted with water containing a gradient of methyl ethyl ketone (0→5%, 2 l.) to give the following fractionation,
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tubes</th>
<th>Weight (in mg.)</th>
<th>Sugars present</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>160-185</td>
<td>24.0</td>
<td>2,3,4-tri-O-methyl-D-xylose</td>
</tr>
<tr>
<td>4b</td>
<td>186-208</td>
<td>49.8</td>
<td>2,3,4-tri-O-methyl-D-xylose + 2,3,4,6-tetra-O-methyl-D-mannose (tr.)</td>
</tr>
<tr>
<td>4c</td>
<td>209-260</td>
<td>29.3</td>
<td>2,3,4,6-tetra-O-methyl-D-mannose</td>
</tr>
</tbody>
</table>

**Subfraction 4a**

This fraction had \([\alpha]_D = +42^\circ \rightarrow +16^\circ (\epsilon = 0.62)\). Paper chromatography in solvents G and I indicated the presence of 2,3,4-tri-O-methyl-D-xylose. Demethylation and examination of the products in solvent B indicated the presence of xylose only. Gas-liquid partition chromatography of the methyl glycosides indicated the presence of the methyl glycosides of the following sugars:

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>0.49</td>
</tr>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>0.71</td>
</tr>
</tbody>
</table>

**Subfraction 4b**

This fraction had \([\alpha]_D = +60^\circ \rightarrow +24^\circ (\epsilon = 0.99)\). Paper chromatography in solvents G and I and gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of both 2,3,4-tri-O-methyl-D-xylose and 2,3,4,6-tetra-
O-methyl-D-mannose (trace). Demethylation followed by chromatographic examination in solvent B indicated the presence of xylose and mannose (trace).

**Subfraction 4c**

This fraction had \([\alpha]_D = +26^\circ\) (\(c = 1.21\) in chloroform). It was chromatographically pure in solvents G, H and I and identical to 2,3,4,6-tetra-O-methyl-D-mannose.

The sugar was characterised by conversion to the aniline derivative m.p. and mixed m.p. 143-144\(^\circ\) (with an authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-mannosylamine, m.p. 144\(^\circ\)).

**Fraction 5 (18.5 mg.)**

This fraction was chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose in solvents G and I. The sugar was characterised by conversion to the aniline derivative, which, after recrystallisation from ethanol, had m.p. 196-197\(^\circ\) and mixed m.p. 195-197\(^\circ\) (with an authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 197\(^\circ\)).

**Fraction 6 (54.2 mg.)**

Chromatographic examination in solvent G indicated the presence of two components, \(R_g = 0.92\) and 0.88. Gas-liquid chromatography of the derived methyl glycosides indicated the presence of the following sugars,
### Sugars

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Time (T)</th>
<th>Column (a)</th>
<th>Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.78</td>
<td>1.49</td>
<td>1.59</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucose</td>
<td>2.61</td>
<td>3.70</td>
<td>1.33</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>3.08</td>
<td></td>
<td>1.65</td>
</tr>
</tbody>
</table>

The syrup was separated into two fractions by thick paper ionophoresis, fraction (a) which was ionophoretically mobile and fraction (b) which was ionophoretically immobile.

**Subfraction 6a (30.2 mg.)**

This fraction was chromatographically and ionophoretically pure and identical to 3,4,6-tri-O-methyl-D-mannose. Demethylation and chromatographic examination in solvent B indicated the presence of mannose only.

The syrup crystallised and was recrystallised from ether to give white crystals m.p. 105-106° and mixed m.p. 104-106° (with an authentic sample of 3,4,6-tri-O-methyl-D-mannose m.p. 106°).

**Subfraction 6b (11.1 mg.)**

This fraction indicated the presence of components \( R_g = 0.88 \) and 0.92 in solvent G. Demethylation indicated, by chromatographic examination in solvent B, the presence of galactose, glucose and mannose (trace). Gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of the following sugars,
Retention Times (T)

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Column (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>1.78</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucose</td>
<td>2.60 3.70</td>
</tr>
<tr>
<td>(?) 2,3,4-tri-O-methyl-D-mannose</td>
<td>3.10</td>
</tr>
</tbody>
</table>

These sugars were not examined further.

Fraction 7 (218.1 mg.)

This fraction was shown by chromatography in solvents G and H to consist of at least two components, Rf, 0.89 (pink) and 0.86 (brown). The colours in brackets are stain reactions with the aniline oxalate spray.

The syrup was dissolved in a minimum amount of acetone. The solution was heated under reflux and light petroleum (b.p. 80-100°) added to the boiling solution until a permanent turbidity was observed. The solution was left at room temperature overnight and at 0° for a further day. The colourless crystals, which were deposited, were filtered off and recrystallised from dry ether. The thrice-recrystallised crystals had m.p. and mixed m.p. 104-106° (with an authentic sample of 3,4,6-tri-O-methyl-D-mannose, m.p. 106°).

The sugar was finally characterised by conversion to the crystalline aldonamide, which, after recrystallisation from acetone, had m.p. and mixed m.p. 139-140° (with an authentic sample of 3,4,6-tri-O-methyl-D-mannonamide, m.p. 141°).
The supernatant and ether washings were combined and added to the similar part obtained from fraction 9.

**Fraction 8 (23.7 mg.)**

The presence of 2,3,4-tri-\(\text{O}\)-methyl-\(L\)-arabinose and 3,4,6-tri-\(\text{O}\)-methyl-\(D\)-mannose was indicated by chromatography in solvent I. The derived methyl glycosides were examined by gas-liquid partition chromatography on column (a). The presence of the following sugars were indicated,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times ((T))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-(\text{O})-methyl-(L)-arabinose</td>
<td>1.06</td>
</tr>
<tr>
<td>(?) 3,4-di-(\text{O})-methyl-(D)-xylose</td>
<td>1.34 and 1.64</td>
</tr>
<tr>
<td>2,3,4,6-tetra-(\text{O})-methyl-(D)-galactose</td>
<td>1.81</td>
</tr>
<tr>
<td>3,4,6-tri-(\text{O})-methyl-(D)-mannose</td>
<td>3.10</td>
</tr>
<tr>
<td>(?) 2,4,6-tri-(\text{O})-methyl-(D)-mannose</td>
<td>3.84</td>
</tr>
</tbody>
</table>

Demethylation and chromatographic examination of the products in solvent B indicated the presence of mannose, arabinose with traces of galactose and xylose.

**Fraction 9 (148.4 mg.)**

Chromatographic examination of this fraction in solvents G and H indicated the presence of 3,4,6-tri-\(\text{O}\)-methyl-\(D\)-mannose (main) with traces of other sugars. The methyl glycosides were formed and examination by gas-liquid partition chromatography indicated the presence of the sugars also present in
The syrup was treated with acetone and petrol-ether in a similar manner to fraction 7. The resultant crystals had m.p. and mixed m.p. 104° - 106° (with an authentic sample of 3,4,6-tri-0-methyl-D-mannose, m.p. 106°). \([\alpha]_D = +20 \rightarrow +9°\) (constant after 90 minutes, \(c = 1.31\)).

The supernatant and washings were combined with those of fraction 7, and were separated on thick paper by the application of ionophoresis into two fractions.

**Subfraction 9a (125.7 mg.)**

This fraction was chromatographically and ionophoretically pure and identical to 3,4,6-tri-0-methyl-D-mannose. The syrup crystallised and was recrystallised from dry ether, m.p. and mixed m.p. 104-106° (with an authentic sample of 3,4,6-tri-0-methyl-D-mannose, m.p. 106°). \([\alpha]_D = +21 \rightarrow +9°\) (constant after 90 minutes, \(c = 1.10\)).

**Subfraction 9b (21.1 mg.)**

This fraction was examined chromatographically in solvent G and showed the presence of components indistinguishable from 2,3,4,6-tetra-0-methyl-D-galactose \((R_g = 0.92)\) and 2,3,4-tri-0-methyl-D-glucose \((R_g = 0.87)\). Gas-liquid partition chromatography of the derived methyl glycosides on columns (a) and (b) indicated the presence of the following sugars,
<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
<th>Column (a)</th>
<th>Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-O-methyl-β-D-galactose</td>
<td>1.80 1.52 1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-β-D-glucose</td>
<td>2.61 3.70 1.35 1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>3.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A sample (5 mg.) was demethylated and the products examined in solvent B, indicating the presence of glucose with traces of galactose, mannose and xylose.

A sample (8 mg.) was reduced with sodium borohydride. The derived glycitol was then oxidised with sodium metaperiodate, according to the method of Lemieux and Bauer. Examination of the products in solvents G and I indicated the presence of 2,3,4-tri-O-methyl-β-D-xylose. Gas-liquid partition chromatography of the derived glycosides indicated the presence of the same sugar as the principal component.

This fraction was not examined further.

**Fraction 10 (10.9 mg.)**

This fraction was shown by paper chromatography in solvent G to consist of 3,4,6-tri-O-methyl-β-D-mannose (Rg = 0.86) and 2,3-di-O-methyl-β-D-xylose (Rg = 0.82). Examination of the methyl glycosides by gas-liquid partition chromatography indicated the presence of the following sugars,
<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
<th>Column (a)</th>
<th>Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-L-arabinose</td>
<td>1.04</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>1.32</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-xylose</td>
<td>1.77</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>3.09</td>
<td>1.68</td>
<td></td>
</tr>
</tbody>
</table>

Demethylation of a sample (5 mg.) and examination of the products in solvent B indicated the presence of mannose and xylose with a trace of arabinose.

This fraction was not examined further.

**Fraction II (37.1 mg.)**

Paper chromatographic examination indicated the presence of 2,3-di-O-methyl-D-xylose ($R_g = 0.80$) and 2,3,6-tri-O-methyl-D-galactose ($R_g = 0.75$) in solvent G. Demethylation of a sample (5 mg.) and examination of the products in solvent B indicated the presence of galactose and xylose with traces of mannose and arabinose. The syrup (32 mg.) was applied to a charcoal-celite column (12 x 1 cm.) and eluted with water containing (a) 0.5% methyl ethyl ketone (500 ml.), (b) 1% methyl ethyl ketone (500 ml.) and (c) 2.5% methyl ethyl ketone (500 ml.) to give three fractions.

**Subfraction IIa (12.4 mg.)**

This fraction was chromatographically pure and identical to 2,4-di-O-methyl-D-xylose. Gas-liquid partition chromatographic examination of the derived methyl glycosides indicated
the presence of the following sugar,

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Retention Times (T)</th>
<th>Column (a)</th>
<th>Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-di-O-methyl-D-xylose</td>
<td></td>
<td>1.48</td>
<td>1.99</td>
</tr>
</tbody>
</table>

Demethylation of a sample (3 mg.) indicated the presence of only xylose.

**Subfraction 11b (8.1 mg.)**

Demethylation of a sample (4 mg.) indicated the presence of xylose and mannose. Paper and gas-liquid partition chromatography indicated the presence of 2,4-di-O-methyl-D-xylose and 3,4,6-tri-O-methyl-D-mannose.

**Subfraction 11c (5.7 mg.)**

Paper chromatographic examination in solvent G showed the presence of 2,3,6-tri-O-methyl-D-galactose. Gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,6-tri-O-methyl-D-galactose and traces of 2,4-di-O-methyl-D-xylose and 3,4,6-tri-O-methyl-D-mannose.

**Fraction 12 (48.6 mg.)**

The fraction was separated into two fractions, mobile and immobile, by thick paper ionophoresis.

**Subfraction 12a (20.2 mg.)**

This fraction was chromatographically pure and identical to 3,4-di-O-methyl-D-mannose (Rg = 0.60) in solvents G and H. The syrup was dissolved in the minimum quantity of ethyl acetate at 80°, allowed to sit at room temperature for one day
and at 0° for a further three days. The crystals were filtered off and recrystallised from ethyl acetate/ether to give colourless plates, m.p. 105-107° and mixed m.p. 106-107° (with an authentic sample of 3,4-di-O-methyl-D-mannose, m.p. 107°).

Subfraction 12b (12.0 mg.)

Chromatographic examination in solvents G and H indicated the presence of 2,3,4-tri-O-methyl-D-galactose (R_g = 0.78) and 2,4-di-O-methyl-D-xylose (R_g = 0.72). Demethylation of a sample and chromatographic examination of the products in solvent B indicated the presence of galactose and xylose. The methyl glycosides were examined by gas-liquid partition chromatography and the presence of the following sugars were indicated.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T), Column (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-di-O-methyl-D-xylose</td>
<td>1.20 1.64</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-galactose</td>
<td>3.00 3.66 3.79 4.29</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>6.90</td>
</tr>
</tbody>
</table>

This fraction was not examined further.

Fraction 13 (236.1 mg.)

This fraction was chromatographically pure (R_g = 0.59) and identical to 3,4-di-O-methyl-D-mannose. The syrup was dissolved in the minimum amount of ethyl acetate and allowed to reflux when ether was added until the presence of a permanent
turbidity was observed.

The solution, on standing at room temperature for three weeks, deposited small crystals which were filtered off. The colourless crystals were recrystallised three times from ethyl acetate and ether (3:1) and had m.p. and mixed m.p. 106-107\(^\circ\) (with an authentic sample of 3,4-di-O-methyl-D-mannose, m.p. 107\(^\circ\)).

**Fraction 14 (33.8 mg.)**

Examination of the products by paper chromatography in solvent \(H\) indicated the presence of at least two sugars, the principal component being identical to 3,4-di-O-methyl-D-mannose. Ionophoretic examination indicated the presence of two components which were identical to 3,4-di-O-methyl-D-mannose (\(M_g = 0.30\)) and 2,3-di-O-methyl-D-glucose (\(M_g = 0.12\)). The syrup was separated into two fractions by ionophoresis on thick paper.

**Subfraction 14a (19.8 mg.)**

This fraction was chromatographically and ionophoretically pure and identical to 3,4-di-O-methyl-D-mannose. The sugar was completely characterised by conversion to the lactone of the derived aldonic acid. The lactone after recrystallisation from moist ether had m.p. and mixed m.p. = 157\(^\circ\) (with an authentic sample of 3,4-di-O-methyl-D-mannolactone, m.p. 157\(^\circ\)).

**Subfraction 14b (6.8 mg.)**

This fraction was ionophoretically pure (\(M_g = 0.12\)) and
identical to 2,3-di-O-methyl-D-glucose. Paper chromatography in solvent H indicated the presence of another component moving slightly faster in that solvent. Demethylation of a sample and examination of the products in solvent B, indicated the presence of glucose and arabinose (traces). The derived methyl glycosides were examined by gas-liquid partition chromatography and the presence of the following sugars were indicated,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>2.26</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-glucose</td>
<td>11.4</td>
</tr>
</tbody>
</table>

This fraction was not examined further.

**Fraction 15 (533.7 mg.)**

The fraction was shown by paper chromatography in solvent H to be a mixture of at least four sugars of which the main constituent was identical to 2,3-di-O-methyl-D-glucose. The syrup was dissolved in water (5 ml.) and applied to a charcoal-celite column (30 x 3 cm.). Fractions (10 ml.) were collected every twenty minutes, while the column was eluted with water containing a gradient of methyl ethyl ketone (0→2.5%, 2 l.). Four fractions were collected.

**Subfraction 15a (150.7 mg.)**

This fraction was examined chromatographically in solvent H and the presence of three sugars was indicated, one of which
was identical to 3,4-di-O-methyl-D-mannose. One of the others was slightly faster moving than the above sugar and was probably 2,4-di-O-methyl-L-arabinose. The other sugar was chromatographically slower moving and gave a characteristic dark-brown stain with the aniline oxalate spray. The fraction was separated into mobile and immobile components by thick paper ionophoresis.

Subfraction 15a 1 (7.2 mg.)

This fraction was chromatographically and ionophoretically pure and identical to 2,4-di-O-methyl-L-arabinose. Demethylation of a sample and examination of the products indicated the presence of arabinose only. Finally gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of 2,4-di-O-methyl-L-arabinose.

Subfraction 15a 2 (89.6 mg.)

This fraction contained two components by chromatography in solvent M. Demethylation of a sample and chromatographic examination of the products in solvent B indicated the presence of mannose only. Gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of the following sugars,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times, Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>unknown</td>
<td>1.44</td>
</tr>
<tr>
<td>3,4-di-O-methyl-D-mannose</td>
<td>2.17</td>
</tr>
<tr>
<td>(?) 4,6-di-O-methyl-D-mannose</td>
<td>2.77</td>
</tr>
</tbody>
</table>

This fraction was not examined further.
Subfraction 15b (12.8 mg.)

This fraction was chromatographically \((R_g = 0.60)\) and ionophoretically \((M_g = 0.30)\) pure and identical to 3,4-di-O-methyl-\(D\)-mannose. Demethylation of a sample and chromatographic examination of the products in solvent B indicated the presence of mannose only. The fraction was not examined further.

Subfraction 15c (10.3 mg.)

This fraction was chromatographically and ionophoretically pure and identical to 2,3-di-O-methyl-\(D\)-glucose. It was combined with subfraction 15d.

Subfraction 15d (274.2 mg.)

This fraction was eluted from the column at the end of the gradient and with a further volume \(1\ l.\) of aqueous methyl ethyl ketone \(5\%\). Chromatography in solvent H indicated the presence of 2,3-di-O-methyl-\(D\)-glucose with a trace of 3,4-di-O-methyl-\(D\)-mannose. The syrup was fractionated into mobile and immobile components by thick paper ionophoresis.

Subfraction 15d-1 (12.7 mg.)

This fraction was chromatographically and ionophoretically pure and indistinguishable from 3,4-di-O-methyl-\(D\)-mannose.

Subfraction 15d-2 (176.8 mg.)

This fraction was chromatographically \((R_g = 0.57)\) and ionophoretically \((M_g = 0.12)\) pure and indistinguishable from 2,3-di-O-methyl-\(D\)-glucose. Demethylation and examination of the products in solvent B indicated the presence of glucose only. Periodate oxidation gave the same products as authentic
samples of 2,3-di-0-methyl-galactose and 2,3-di-0-methyl-glucose.

The sugar was finally characterised by preparation of the phenylhydrazide of the aldonic acid. The lactone of the aldonic acid was prepared first and the phenylhydrazine prepared from it. The crystalline derivative after recrystallisation from ethyl alcohol had m.p. and mixed m.p. 173-175° (with an authentic sample of the phenylhydrazide m.p. 174-175°).

**Fraction 16** (313.3 mg.)

This fraction was examined by chromatography in solvents G and H and shown to be a complex mixture of methylated sugars. It was dissolved in water (5 ml.) and applied to a small charcoal column (25 x 2.5 cm.). Fractions (10 ml.) were collected every twenty minutes, during which time the column was eluted with water containing a gradient of methyl ethyl ketone (0 — 2.5%, 4 l.). Five fractions were obtained in the usual manner.

**Subfraction 16a** (17.8 mg.)

This fraction was chromatographically identical to subfraction 15a, the presence of three sugars being indicated. Demethylation and examination of the products in solvent B indicated the presence of mannose and a trace of arabinose. The syrup was separated into a mobile and immobile fraction by ionophoresis on thick paper.
Subfraction 16a 1 (5.1 mg.)

This fraction was chromatographically pure in solvents G and H and identical to 2,4-di-O-methyl-L-arabinose. Demethylation gave arabinose only and examination of the derived methyl glycosides by gas-liquid partition chromatography indicated the presence of 2,4-di-O-methyl-L-arabinose only.

Subfraction 16a 2 (4.3 mg.)

This fraction was examined chromatographically in solvent H, and the presence of two sugars was indicated, 3,4-di-O-methyl-D-mannose and an unknown. Demethylation and chromatographic examination of the products in solvent B indicated the presence of mannose only.

Subfraction 16b (103 mg.)

This fraction was examined chromatographically in solvent H and consisted of four components. It was further fractionated by thick paper in solvent H to yield two simpler fractions.

Subfraction 16b 1 (46.2 mg.)

This fraction was examined chromatographically in solvent H and three sugars were observed. The principal constituent was identical to 2,3-di-O-methyl-D-glucose and the other two were similar to 3,4-di-O-methyl-D-mannose and 2,6-di-O-methyl-D-galactose. Demethylation and subsequent examination of the products in solvent B indicated the presence of glucose with traces of mannose and galactose. Periodate oxidation of a sample of the sugars gave a complex mixture of products and no conclusions were possible.
Subfraction 16b 2 (34.0 mg.)

This fraction was shown to contain an unknown sugar as major component. The constituent moved slightly slower than 3,4-di-\(\mathrm{O}\)-methyl-D-mannose in solvent H. The sample also contained smaller amounts of sugars chromatographically identical to 2,3-di-\(\mathrm{O}\)-methyl-D-glucose and 2,6-di-\(\mathrm{O}\)-methyl-D-galactose. Demethylation of a sample and subsequent examination of the products in solvent B, indicated the presence of mannose with trace quantities of glucose and galactose. Gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of the following sugars,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times ((T))</th>
<th>Column (a)</th>
<th>Column (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-di-(\mathrm{O})-methyl-D-mannose</td>
<td></td>
<td>7.5</td>
<td>2.26</td>
</tr>
<tr>
<td>2,3-di-(\mathrm{O})-methyl-D-glucose</td>
<td></td>
<td>10.9</td>
<td>14.6</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>2.26</td>
<td>3.22</td>
</tr>
</tbody>
</table>

The fraction was not examined further.

Subfraction 16c (42.2 m.g.)

The fraction was examined chromatographically in solvents G and H. The major component had a mobility identical to 2,3-di-\(\mathrm{O}\)-methyl-glucose. Other sugars present had mobilities identical to 3,4-di-\(\mathrm{O}\)-methyl-D-mannose (trace), 2,4- and 2,6-di-\(\mathrm{O}\)-methyl-D-galactose. There was also present an unknown sugar slightly slower moving than 3,4-di-\(\mathrm{O}\)-methyl-mannose in solvent H. Demethylation of a sample (6 mg.) and chromatographic examination of the products in solvent B indicated the
presence of glucose, mannose and galactose. The fraction was not examined further.

Subfraction 16d (101.1 mg.)

This fraction was chromatographically pure and identical to 2,3-di-O-methyl-D-glucose. Periodate oxidation gave the same products as authentic 2,3-di-O-methyl-D-glucose. The sugar was ionophoretically homogeneous ($M_g = 0.12$), identical to 2,3-di-O-methyl-D-glucose. $\left\{\left[\alpha\right]_D = +13.0 \rightarrow +17.4^\circ\right\}$ (equil.). $C = 0.35.$

The syrup partially crystallised on standing and was re-crystallised from ethyl alcohol to give colourless plates m.p. 109-110°.

Gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of the following sugar only,

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-glucose</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Subfraction 16e (11.4 mg.)

This fraction consisted of 2,3-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose in solvents G and H. Gas-liquid partition chromatography indicated the presence of a complex mixture of methyl glycosides, among which 2,3-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose could be recognised. Demethylation of a sample (5 mg.) and chromatographic examination of the products in solvent B indicated the presence of glucose with traces of xylose, mannose and galactose.
Fraction 17 (162.0 mg.)

This fraction was identical in contents with fraction 18 with which it was combined.

Fraction 18 (679.8 mg.)

Chromatographic examination of this fraction in solvents G and H indicated an exceedingly complex mixture of sugars. The syrup (350 mg.) was dissolved in water (10 ml.) and applied to a charcoal-celite column (30 x 3 cm.). The column was eluted with a gradient of aqueous methyl ethyl ketone (0—→3%, 4 l.) and fractions (10 ml.) collected every twenty minutes. Four major fractions were collected and examined further.

Subfraction 18a (11.3 mg.)

Chromatographic examination of this fraction in solvents A and H indicated the presence of sugars which had identical mobilities to those of mannose, 4-0-methyl-mannose and 3-0-methyl-glucose. Ionophoretic examination of the fraction indicated the presence of sugars which were identical to D-mannose ($M_g = 1.0$), 4-0-methyl-D-mannose ($M_g = 0.53$) and 3-0-methyl-D-glucose ($M_g = 0.83$).

A sample (5 mg.) of the sugars was heated with methanolic hydrogen chloride and the resultant methyl glycosides were demethylated. Chromatographic examination of the products in solvent B indicated the presence of glucose and mannose.

Subfraction 18b (46.6 mg.)

The syrup was examined in solvent H and separated into three fractions on thick paper in that solvent.
Subfraction 18b 1 (5.1 mg.)

The sugar was chromatographically and ionophoretically pure and identical to 2-0-methyl-\(\beta\)-arabinose. Demethylation of the derived methyl glycoside indicated the presence of arabinose only in solvent B. Gas-liquid partition chromatography of the derived methyl glycoside indicated the presence of 2-0-methyl-\(\beta\)-arabinose.

Subfraction 18b 2 (8.6 mg.)

The principal component of this fraction was 4-0-methyl-\(\beta\)-mannose with a trace of 3-0-methyl-\(\alpha\)-glucose. Chromatography in solvent H and ionophoresis confirmed the conclusion.

Subfraction 18b 3 (26.3 mg.)

Chromatographic examination of this fraction in solvent H indicated the presence of three components. Ionophoretic examination also indicated three components which were indistinguishable from the authentic sugars, 2-0-methyl-\(\alpha\)-galactose \(M_g = 0.34\), 3-0-methyl-\(\beta\)-glucose \(M_g = 0.83\) and a trace of 2-0-methyl-\(\beta\)-glucose \(M_g = 0.23\). A sample (5 mg.) was heated with methanolic hydrogen chloride and the methyl glycosides were demethylated in the usual manner. Chromatographic examination of the products indicated the presence of glucose and galactose.

This fraction was not examined further.

Subfraction 18c (77.9 mg.)

Chromatography in solvent H and ionophoretic examination showed the presence of sugars with mobilities identical to the following authentic methylated monosaccharides, 2-0-methyl-
galactose, 2-0-methyl-glucose, 4-0-methyl-mannose, 2,4-di-0-methyl-galactose, 2,3-di-0-methyl-glucose and 3,4-di-0-methyl-mannose. A trace of the unknown sugar present in fraction 16 was also present. Demethylation of the derived methyl glycosides and chromatographic examination of the products indicated the presence of glucose and traces of galactose and mannose. No further examination was carried out on this fraction.

**Subfraction 18d (166.0 mg.)**

This fraction was chromatographically and ionophoretically pure and identical to 2,3-di-0-methyl-D-glucose. \[ [\alpha]_D = +13.0^\circ, +17.3^\circ \text{ (equil.)} \] \[ \alpha = 1.23. \] Periodate oxidation gave the same products as were obtained from an authentic sample of 2,3-di-0-methyl-D-glucose and of 2,3-di-0-methyl-D-galactose. Demethylation of a sample and chromatographic examination of the products indicated the presence of glucose only.

The sugar was finally characterised by conversion to its aniline derivative, which after recrystallisation from ethyl acetate-ether had m.p. and mixed m.p. 131-132° (with an authentic sample of 2,3-di-0-methyl-N-phenyl-D-glucosylamine m.p. 134°).

**Fraction 19 (165.0 mg.)**

This fraction was examined chromatographically in solvent H and was shown to consist of 2,3-di-0-methyl-glucose, 4-0-methyl-mannose, 2- and 3-0-methyl-glucoses, 2-0-methyl-galactose, glucose and mannose. It was not examined further.
as the principal component (visual examination) was the dimethyl ether.

**Fraction 20 (21.9 mg.)**

This fraction was examined chromatographically in solvents A and B and the constituents were identical to 4-O-methyl-D-mannose, glucose and mannose. It was not examined further.
Preparation of the Acetate of Leiocarpan A

The freeze-dried gum acid (22 g.) was shaken with freshly distilled formamide (900 ml.) for eighteen hours and a small quantity of insoluble material was removed at the centrifuge and discarded. Pyridine (500 ml.) was then added dropwise to the clear solution over a period of eight hours. The pyridine had been freshly distilled over potassium hydroxide. The reaction mixture was stirred vigorously throughout the addition and again shaken for 18 hours. Freshly distilled acetic anhydride (350 ml.) was then added dropwise over a period of 3 hours and the mixture again shaken overnight. Finally the dark-brown solution was poured into ice-cooled hydrochloric acid (2% w/v, 4 l.) with vigorous stirring.

The precipitated gum acetate was removed at the centrifuge and washed with hydrochloric acid (0.2% w/v) followed by water (6 x 2 l.). The grey, sticky acetate was extracted with chloroform (6 x 200 ml.). The extracts were combined, dried over anhydrous sodium sulphate and evaporated to a smaller volume (ca. 120 ml.) under reduced pressure. The solution (120 ml.) was then poured into light petroleum (b.p. 60-80°, 1,200 ml.) and the white gum acetate was filtered at the pump and dried in vacuo at 40°. Yield = 22.8 g. Acetyl content = 37.4%.

The acetylated gum acid was subjected to another acetylation by the above procedure. Complete acetylation was assumed, since the acetyl content was not raised by a
significant amount. Yield = 20.2 g. Acetyl content = 37.6%.

All acetylated polysaccharides used in the course of these experiments were prepared in the above manner by the method of Carson and Maclay.

**Trial reductions of leiocarpan A acetate with diborane**

**Experiment I.** Externally generated diborane (prepared by the action of boron trifluoride diethyletherate on sodium boro-hydride in diglyme) was bubbled through a solution of the gum acetate (0.5 g.) in diglyme (30 ml.) with vigorous stirring, for three days. There was no apparent change in the reaction mixture for two days, after which localised gelling was observed. After the third day the mixture was shaken vigorously for 18 hours.

The mixture was then poured into water (200 ml.) and the neutral solution stirred for an hour. All the material dissolved to give a homogeneous solution, which was evaporated to a small volume (ca. 50 ml.). The solution was poured into methanol (200 ml.) and the precipitated polysaccharide was removed by centrifugation and washed several times with methanol to remove all traces of boric acid.

The white solid was then dissolved in 5% ammonia (200 ml.) and heated at 60° for one hour. The excess ammonia was removed using a rotary film evaporator and the solution was reduced in volume (ca. 20 ml.) and poured into ethanol (100 ml.). The precipitate was dissolved in water (5 ml.) and
freeze dried (0.218 g.).

Uronic acid anhydride (i) = 13.1% (by decarboxylation);
(ii) = 12.8% (by carbazole).

Hydrolysis of a sample and chromatographic examination of
the products in solvents A, B and C, indicated the presence of
arabinose, xylose, mannose, glucose, galactose and slow-moving
acidic sugars.

Experiment II. The acetate (0.5 g.) was dissolved in 1,2-di-
methoxyethane (30 ml.) and treated with diborane as in experiment I. The acetate appeared to be more readily soluble in
this solvent. The partially reduced polysaccharide was
dissolved in water and freeze dried (0.267 g.).

Uronic acid anhydride (i) = 11.8% (by decarboxylation);
(ii) = 11.1% (by carbazole).

Reduction of Leiocarpan A acetate with diborane (large scale)

The gum acetate (18 g.) was dissolved in 1,2-dimethoxy-
ethane (1,000 ml.) and allowed to stir under anhydrous
conditions for 48 hours, by which time solution was complete.

Diborane gas was generated externally. Sodium boro-
hydride (25 g.) was mixed to a slurry in dry 1,2-dimethoxy-
ethane and the diborane generated by the addition of aliquot
portions of boron trifluoride etherate. The evolved gas was
passed through two traps, the first of which contained sodium
borohydride in dry 1,2-dimethoxyethane and the second was
empty, before allowing it to enter the reaction flask.
Small additions of the boron trifluoride etherate were made every hour for eight to twelve hours per day with vigorous stirring being maintained at all times. Local gelling was observed in the solution after three days. At this stage, the reaction mixture had become increasingly lighter in colour.

The addition of the gas was continued for a further seven days when an aliquot portion was removed and worked up as described in experiment I. Sample I was examined in solvents A, B and C after hydrolysis and still showed the presence of acidic sugars.

Uronic acid anhydride (i) = 8.4% (by decarboxylation);
(ii) = 8.0% (by carbazole).

The reaction was continued for another seven days and another aliquot portion was removed and worked up as above to give sample II which gave the same sugars as sample I on hydrolysis.

Uronic acid anhydride (i) = 4.6% (by decarboxylation);
(ii) = 3.8% (by carbazole).

Passage of diborane was continued for seven more days and the dispersion was allowed to stand overnight at room temperature when it was poured into water (5 l.). The neutral solution was stirred until all the solid material had dissolved to give a homogeneous solution, which was evaporated under reduced pressure to a small volume (ca. 40 ml.). The viscous solution was poured into methanol (200 ml.), stirred for thirty minutes and the solid material removed at the centrifuge. This material was washed with methanol (5 x 50 ml.) to remove
all traces of boric acid. The white solid was then dissolved in 5% ammonia (1,200 ml.) and heated at 60° for one hour. The excess ammonia was removed on a rotary film evaporator and the solution reduced in volume (ca. 60 ml.) when it was poured into ethanol (300 ml.). The precipitated, carboxyl-reduced polysaccharide was removed by centrifugation, dissolved in water (500 ml.) and freeze-dried (8.4 g.). \[ \alpha \] = +15.3° (c = 0.52).

Uronic acid anhydride (i) = 3.9% (by decarboxylation);
(ii) = 1.6% (by carbazole).

Hydrolysis of a sample (10 mg.) and a paper chromatographic examination of the products in solvents A, B and C indicated the presence of arabinose, xylose, mannose, glucose and galactose. Only the smallest trace of acidic material was observed (Rgal = 0.42 in solvent C).

Methylation of Carboxyl-reduced leiocarpan A

Carboxyl-reduced polysaccharide (100 mg.) was dissolved in water (3 ml.) with a stream of nitrogen passing through the solution and the reaction flask surrounded by an ice-bath. Methyl sulphate (1.5 ml.) and sodium hydroxide solution (30%, 3 ml.) were added dropwise and with stirring over a period of six hours. Five further additions of these reagents were made on five successive days.

Twenty-four hours after the final addition of the reagents had been made the solution was heated on a boiling water-bath
for one hour, allowed to cool and the pH was adjusted to four with dilute sulphuric acid. Sodium sulphate was precipitated by the addition of methylated spirits (8 volumes). The precipitate was filtered off and washed with methylated spirits. The supernatant and washings were combined and the solution concentrated. The partially-methylated polysaccharide was extracted with chloroform (4 x 30 ml.). The chloroform extracts were combined, dried over anhydrous sodium sulphate, filtered and the filtrate concentrated. The pale yellow syrup was dried in vacuo overnight.

The partially-methylated polysaccharide was dissolved in methyl iodide (5 ml.) and stirred at its boiling point for thirty minutes. Silver oxide (5 x 20 mg.) was added every hour for five hours. The reaction mixture was stirred under reflux for eighteen hours.

The insoluble residue was filtered off and continuously extracted with chloroform in a Soxhlet extractor for eighteen hours. The filtrate and chloroform extract were combined, dried over anhydrous sodium sulphate and concentrated to a syrup.

The above Purdie methylation was repeated. The syrup was dissolved in chloroform (1 ml.) and poured into light petroleum (b.p. 60-80°, 50 ml.). The precipitate was filtered off, washed with petrol-ether and air-dried to give methylated carboxyl-reduced leiocarpan A (48.5 mg.), with methoxyl content 42.7%. A further Purdie methylation failed to raise the methoxyl content.
A sample was methanolysed and the cleavage products examined by gas-liquid partition chromatography. The methyl glycosides of the following were detected,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>0.46 0.55</td>
</tr>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>0.55 0.71</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-arabinose</td>
<td>1.04</td>
</tr>
<tr>
<td>2,3-di-O-methyl-L-arabinose</td>
<td>1.54</td>
</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>2.34</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>1.85</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>3.05</td>
</tr>
<tr>
<td>3,4-di-O-methyl-D-mannose</td>
<td>7.4</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-glucose</td>
<td>3.46 4.69</td>
</tr>
</tbody>
</table>

A sample was also hydrolysed with dilute mineral acid and the hydrolysate examined in solvent systems G, H and I. The presence of the following methylated sugars was indicated,

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Estimate of amount present</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>++</td>
</tr>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>+++</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>+</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-glucose</td>
<td>+++</td>
</tr>
<tr>
<td>3,4,6-tri-O-methyl-D-mannose</td>
<td>+</td>
</tr>
<tr>
<td>3,4-di-O-methyl-D-mannose</td>
<td>++</td>
</tr>
<tr>
<td>4-O-methyl-D-mannose</td>
<td>+</td>
</tr>
<tr>
<td>(?) di-O-methyl-D-glucose</td>
<td>+</td>
</tr>
</tbody>
</table>
Other sugars were also present but were not identified.

**Trial acid hydrolysis of carboxyl-reduced leiocarpan A**

The carboxyl-reduced polysaccharide (100 mg.) was heated on a boiling water-bath in aqueous sulphuric acid (0.5 N, 10 ml.). Samples (1 ml.) were removed after 1, 2, 3, 4, 6, 10, 12 and 24 hours. The degraded polysaccharide was precipitated by pouring the samples into ethanol (5 ml.). The supernatant solutions were neutralised with Amberlite LA 1 and IR 120 resins, concentrated and examined chromatographically in solvent system A, B and E.

Sugars which did not correspond to standard monosaccharides appeared strongest on chromatograms of the hydrolysate removed after four hours. These oligosaccharides were in poor yield in comparison to the amounts of monosaccharides present.

**Trial acetylation and partial acetolysis of carboxyl-reduced leiocarpan A**

The carboxyl-reduced polysaccharide was insoluble in the acetolysis mixture. In order to achieve solubility the polysaccharide was first acetylated by the method of Carson and Maclay. The freeze-dried, reduced gum (300 mg.) was dissolved in formamide (20 ml.). Pyridine (15 ml.) and acetic anhydride (10 ml.) were added dropwise on successive days and the reaction carried out as described in detail earlier.

The acetylated carboxyl-reduced gum (520.5 mg.) was added
to the acetolysis mixture (acetic acid, 5 ml.; acetic anhydride, 5 ml.; sulphuric acid, 0.5 ml.) at 0°. The mixture was allowed to come to room temperature. Dissolution was immediate and a dark, red-brown solution was obtained.

Samples (1 ml.) were removed after 24, 36, 48, 60, 72, 84, 96 and 108 hours and each processed in the following manner. The sample was poured into water (20 ml.) and the milky suspension brought to pH 4 by the addition of solid sodium bicarbonate. The mixture was extracted with chloroform (3 x 15 ml.) and the chloroform extracts dried over anhydrous sodium sulphate, filtered and the filtrate concentrated to a syrup.

The syrup was dissolved in dry methanol (2 ml.) and sufficient barium methoxide (0.5 M) was added at 0°, with shaking, until the solution was alkaline to phenolphthalein. A flocculent precipitate formed and the mixture was left at 0° for twenty-four hours. The mixture was then poured into water (20 ml.) and stirred until solution was complete. The clear solution was then deionised with Amberlite IR 120 (H) resin, filtered and the filtrate concentrated to a syrup. Chromatographic examination of the syrup in solvents A, B and E indicated the presence of oligosaccharides in all samples but in maximum quantity after 60 hours.

The relative amount of oligosaccharides produced by the partial acetolysis was far in excess of those produced by the partial acid hydrolysis and it was decided to carry out a larger scale partial acetolysis.
Acetylation and Partial Acetolysis of the carboxyl-reduced gum

The carboxyl-reduced polysaccharide (7.5 g.) was dissolved in formamide (400 ml.) and allowed to shake overnight. The clear, brown solution was acetylated by the dropwise addition of pyridine (250 ml.) and acetic anhydride (200 ml.) on successive days according to the method of Carson and Maclay and as described earlier.

The reaction yielded a greyish-white acetate (11.2 g.) which was dried in vacuo overnight.

The reduced gum acetate was added slowly to the acetolysis mixture (acetic acid, 250 ml.; acetic anhydride, 250 ml.; sulphuric acid, 25 ml.) at 0°C with vigorous shaking. It was allowed to come to room temperature where it was left for sixty hours.

The partial acetolysis products were then precipitated by pouring the reaction mixture into ice-cooled water (4 l.) and the mixture was brought to pH 4 by the addition of solid sodium bicarbonate. The aqueous suspension was extracted with chloroform (5 x 750 ml.). The chloroform extracts were thoroughly washed with a solution of saturated sodium bicarbonate (3 x 250 ml.) and water (4 x 250 ml.), dried over anhydrous sodium sulphate, filtered and concentrated to a syrup.

This syrup was dissolved in dry methanol (50 ml.) and sufficient barium methoxide was added at 0°C until the solution was alkaline to phenolphthalein. The mixture was left at 0°C for twenty-four hours when it was poured into water (1,500 ml.).
A small insoluble residue was filtered off and discarded. Barium ions were then removed by precipitation as the insoluble sulphate by the gradual addition of dilute sulphuric acid to pH 7, and the barium sulphate was removed at the centrifuge and washed with water (2 x 250 ml.). The supernatant and washings were combined, deionised by passage through columns of Amberlite IR 120 (H) and IR 4B (OH) resins and finally concentrated to a syrup (5.1 g.).

Preliminary separation of the oligosaccharides

The above syrup (5.1 g.) was dissolved in water (20 ml.) and allowed to run on to a charcoal-celite column (50 x 5 cm., 600 g.). The column was first eluted with water (2 l.) until most of the monosaccharides had been removed. A further elution with water (2 l.) was intended to remove all traces of monosaccharides. The column was then eluted with water containing increasing proportions of ethanol as outlined in the following table,

<table>
<thead>
<tr>
<th>Eluant</th>
<th>Tube numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) water (4 l.)</td>
<td></td>
</tr>
<tr>
<td>(ii) 0→5% ethanol gradient (4 l.)</td>
<td>1 → 200</td>
</tr>
<tr>
<td>(iii) 5% ethanol (2 l.)</td>
<td>201 → 300</td>
</tr>
<tr>
<td>(iv) 5→7.5% ethanol gradient (4 l.)</td>
<td>301 → 500</td>
</tr>
<tr>
<td>(v) 7.5% ethanol (2 l.)</td>
<td>501 → 600</td>
</tr>
<tr>
<td>(vi) 7.5→12.5% ethanol (4 l.)</td>
<td>601 → 800</td>
</tr>
<tr>
<td>(vii) 12.5→25% ethanol (4 l.)</td>
<td>801 → 1,000</td>
</tr>
</tbody>
</table>
The charcoal was finally eluted with water containing 20% methyl ethyl ketone (2 l.).

Fractions (20 ml.) were collected every thirty minutes on an automatic turntable. Every third tube was evaporated to dryness and examined chromatographically in solvent A. These fractions were combined according to their contents and treated with Amberlite IR 45 (OH) resin. Fifteen fractions in all were obtained and the following table summarises the entire procedure.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube numbers</th>
<th>Weight (in mg.)</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>water</td>
<td>1,786.3</td>
<td>monosaccharides</td>
</tr>
<tr>
<td>2</td>
<td>water</td>
<td>194.0</td>
<td>monosaccharides</td>
</tr>
<tr>
<td>3</td>
<td>1 - 80</td>
<td>20.1</td>
<td>monosaccharides</td>
</tr>
<tr>
<td>4</td>
<td>81 - 155</td>
<td>104.5</td>
<td>oligosaccharides A, C and I</td>
</tr>
<tr>
<td>5</td>
<td>156 - 167</td>
<td>31.8</td>
<td>oligosaccharides A, B and C and others</td>
</tr>
<tr>
<td>6</td>
<td>168 - 220</td>
<td>125.4</td>
<td>oligosaccharides A, B and C and others</td>
</tr>
<tr>
<td>7</td>
<td>221 - 269</td>
<td>85.4</td>
<td>oligosaccharides B and C</td>
</tr>
<tr>
<td>8</td>
<td>270 - 304</td>
<td>38.5</td>
<td>oligosaccharides B, C and D</td>
</tr>
<tr>
<td>9</td>
<td>305 - 407</td>
<td>17.2</td>
<td>oligosaccharides C and D</td>
</tr>
<tr>
<td>10</td>
<td>408 - 427</td>
<td>52.0</td>
<td>oligosaccharides C and D</td>
</tr>
<tr>
<td>11</td>
<td>428 - 581</td>
<td>121.3</td>
<td>oligosaccharides D and E</td>
</tr>
<tr>
<td>12</td>
<td>582 - 769</td>
<td>334.7</td>
<td>oligosaccharides D and E</td>
</tr>
<tr>
<td>13</td>
<td>770 - 833</td>
<td>215.6</td>
<td>oligosaccharides F, G and H</td>
</tr>
<tr>
<td>14</td>
<td>834 - 1000</td>
<td>772.7</td>
<td>higher oligosaccharides</td>
</tr>
<tr>
<td>15</td>
<td>20% methyl ethyl ketone</td>
<td>610.5</td>
<td>higher oligosaccharides</td>
</tr>
</tbody>
</table>
Examination of these fractions and identification of the oligosaccharides

All $R_{gal}$ values in this section were observed in solvent system A. In a very few cases, solvents E and F were employed, but this will be clearly stated.

Unless otherwise stated, the cleavage products resulting from the methanolysis of the methylated and the methylated reduced oligosaccharides, were examined by gas-liquid partition chromatography on columns (a) and (c). The following table shows the relative retention times of the various sugars which were encountered most frequently.

<table>
<thead>
<tr>
<th>Sugar derivative</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3,4,6-tetra-$O$-methyl-D-mannose</td>
<td>1.40</td>
</tr>
<tr>
<td>2,3,4,6-tetra-$O$-methyl-D-glucose</td>
<td>1.00 1.42</td>
</tr>
<tr>
<td>3,4,6-tri-$O$-methyl-D-mannose</td>
<td>3.05</td>
</tr>
<tr>
<td>2,3,6-tri-$O$-methyl-D-glucose</td>
<td>3.47 4.70</td>
</tr>
<tr>
<td>1,3,4,5,6-penta-$O$-methyl-mannitol</td>
<td>2.72</td>
</tr>
<tr>
<td>1,2,3,5,6-penta-$O$-methyl-sorbitol</td>
<td>2.43</td>
</tr>
</tbody>
</table>

Column (a) was particularly satisfactory in distinguishing between the two penta-$O$-methyl alcohols, whereas column (c) distinguished between the two tetra-$O$-methyl hexoses.

Fractions 1, 2 and 3 (1.9 g.)

Chromatographic examination of these fractions indicated
the presence of sugars with mobilities identical to standard monosaccharides. In solvents A, B, C and E evidence was obtained for the presence of arabinose, xylose, mannose, glucose and galactose. These fractions were not examined further.

**Fraction 4 (104.5 mg.)**

Paper chromatography in solvents A and E indicated that the fraction was a mixture of at least two sugars. The components were separated into two bands on Whatman 3 MM paper in solvent system A.

**Subfraction 4a. Oligosaccharide A (39.1 mg.)**

\[ R_{gal} = 0.63, M_g = 0.37, \ [\alpha]_D^{+61.0} = 0.96 \]

Total hydrolysis of a sample (2 mg.) with mineral acid for four hours and chromatographic examination of the products in solvent B showed the presence of mannose and glucose. Reduction of a sample (3 mg.) with sodium borohydride to the corresponding glycitol, followed by hydrolysis and chromatographic examination of the products in solvent J indicated the presence of mannose and sorbitol.

A positive reaction towards the triphenyltetrazolium chloride spray indicated that position C2 of the glucose unit was not substituted.

A lime-water degradation of a sample (5 mg.) and chromatographic examination of the products after five days showed the presence of mannose only.

A small scale methylation of a sample (2 mg.) was carried
out. The resultant syrup was methanolysed and the products examined by gas-liquid partition chromatography. The methyl glycosides of 2,3,4,6-tetra-\(\beta\)-methyl-\(\alpha\)mannose and 2,3,6-tri-\(\beta\)-methyl-\(\alpha\)glucose were observed.

The disaccharide was chromatographically and ionophoretically different from 4-\(\alpha\)-\(\beta\)-mannosyl-\(\alpha\)glucose.

**Methylation of Oligosaccharide A**

The syrup (30 mg.) was dissolved in water (1 ml.) and methylated by the dropwise addition of methyl sulphate (1 ml.) and 30% sodium hydroxide (2 ml.). Vigorous stirring was maintained throughout. The reaction flask was surrounded by an ice-bath and the reaction carried out in an atmosphere of nitrogen. Five further additions of methyl sulphate (1 ml.) and 30% sodium hydroxide solution (2 ml.) were made over a period of two hours on five successive days. Twenty-four hours after the final addition, the solution was heated at 100° for one hour to destroy excess dimethyl sulphate. The solution was acidified (pH 4) by the addition of dilute sulphuric acid and extracted continuously with boiling chloroform (200 ml.) for fourteen hours. The chloroform extract was dried over anhydrous sodium sulphate and evaporated to a syrup (32.6 mg.). An aliquot portion (1 mg.) was hydrolysed with dilute sulphuric acid and the products examined by paper chromatography in solvent G. The presence of one slow-moving sugar (trace) indicated incomplete methylation.

The syrup (31.6 mg.) was dissolved in methyl iodide (2 ml.) and silver oxide (15 mg.) added at hourly intervals in
The mixture was refluxed with stirring for fourteen hours. The silver salts were removed by filtration and extracted continuously for four hours with boiling chloroform. The filtrate and extractions were combined and reduced in volume to a syrup (30.9 mg.).

The syrup was hydrolysed with sulphuric acid (4 N) in the usual manner. The products were applied to Whatman number 1 paper and the paper eluted with solvent H. Two fractions were obtained by elution of the appropriate portions with water and concentration of the resultant solutions.

**Fraction I** (11.2 mg.)

This fraction was chromatographically pure and identical to 2,3,4,6-tetra-\(\text{O}\)-methyl-\(\text{D}\)-mannose \((R_g, 1.0\) in solvent G). The sugar was characterised by conversion to the aniline derivative, which, after recrystallisation from alcohol, had m.p. and mixed m.p. 140° (with an authentic sample of 2,3,4,6-tetra-\(\text{O}\)-methyl-\(\text{N}\)-phenyl-\(\text{D}\)-mannosylamine, m.p. 143-144°).

**Fraction II** (13.8 mg.)

This fraction was chromatographically pure and identical to 2,3,6-tri-\(\text{O}\)-methyl-\(\text{D}\)-glucose \((R_g = 0.87\) in solvent G). The sugar was characterised by conversion to the di-\(\text{p}\)-nitrobenzoate, which after one recrystallisation from methanol had m.p. 187° and mixed m.p. 187° (with an authentic sample of the 1,4-di-\(\text{p}\)-nitrobenzoate, m.p. 189-190°).

**Subfraction 4b** (30.2 mg.). \(R_{gal} = 0.26\)

Total hydrolysis of a sample (2 mg.) and examination of the products in solvent B showed the presence of glucose,
mannose and galactose. Reduction of a sample (3 mg.) followed by hydrolysis and chromatographic examination of the products in solvents A and J showed the presence of mannose, glucose, galactose, sorbitol and galactitol.

Electrophoretic examination of sample indicated the existence of two components. The syrup (21.1 mg.) was therefore separated into two bands by electrophoresis on thick paper. Subfraction 4b 1 (4.2 mg.). R_{gal} = 0.26 \quad M_g = 0.49

This oligosaccharide was chromatographically and ionophoretically identical to oligosaccharide C isolated from fraction 10 and it was combined with it.

Subfraction 4b 2 (6.9 mg.). Oligosaccharide I

R_{gal} = 0.24 \quad M_g = 0.82

Hydrolysis of a sample (2 mg.) and examination of the products in solvents A and B showed galactose only. Reduction of a sample (3 mg.) followed by hydrolysis and examination of the products in solvents A and J indicated the presence of galactose and galactitol. The sugar was chromatographically and ionophoretically pure and indistinguishable from 6-\text{O-}^{\text{D}}\text{D}-galactopyranosyl-D-galactose. A small scale methylation of a sample (1 mg.) was carried out and the resultant syrup methanolysed. The cleavage products were examined by gas-liquid partition chromatography. The methyl glycosides of the following sugars were detected,
<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
<th>Column (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-(\beta)-methyl-(\alpha)-galactose</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>2,3,5-tri-(\beta)-methyl-(\alpha)-galactose</td>
<td>4.45</td>
<td></td>
</tr>
<tr>
<td>2,3,4-tri-(\beta)-methyl-(\alpha)-galactose</td>
<td>7.35</td>
<td></td>
</tr>
</tbody>
</table>

Fraction 5 (31.8 mg.)

This fraction was a mixture of at least four oligosaccharides with \(R_{\text{gal}}\) values corresponding to oligosaccharides A (\(R_{\text{gal}} = 0.63\)), B (\(R_{\text{gal}} = 0.72\)) and C (\(R_{\text{gal}} = 0.27\)). One further component of the mixture (\(R_{\text{gal}} = 0.52\)) gave a pink staining reaction with the aniline oxalate spray. Hydrolysis of a sample (5 mg.) and chromatographic examination of the products in solvents A and B indicated the presence of glucose, mannose, xylose (trace) and galactose (trace).

In view of the small quantity of the fraction and its complex nature, it was not examined further.

Fraction 6 (125.4 mg.)

This fraction was also a very complex mixture and sugars corresponding to oligosaccharides A (\(R_{\text{gal}} = 0.63\)), B (\(R_{\text{gal}} = 0.72\)) and C (\(R_{\text{gal}} = 0.27\)) were observed. In addition to these sugars, two components which gave pink staining reactions with aniline oxalate were also present, oligosaccharide J (\(R_{\text{gal}} = 0.52\)), which was also present in fraction 6, and oligosaccharide K (\(R_{\text{gal}} = 0.58\)).
Hydrolysis of a sample (5 mg.) and examination of the products in solvents A and B indicated the presence of glucose, mannose, galactose, arabinose and xylose.

Reduction of another sample (10 mg.) and examination of the products in solvents A and B indicated the presence of glucose, mannose, arabinose with a trace of xylose.

A small-scale methylation of the complex mixture and subsequent examination of the derived methyl glycosides gave a complicated gas chromatogram. Peaks corresponding to tetra-0-methylmannose and tetra-0-methylglucose, 2,3,6-tri-0-methyl-D-glucose and 3,4,6-tri-0-methyl-D-mannose were the only ones recognised.

The fraction was not examined further.

**Fraction 7 (85.4 mg.)**

Chromatographic examination of this fraction in solvents A and B indicated the presence of only two sugars. They were fractionated into individual components on Whatman 3 MM paper in solvent system A.

**Subfraction 7a. Oligosaccharide B (35.6 mg.)**

\[ R_{gal} = 0.72, M_g = 0.61, [\alpha]_D = -19.8^\circ (c = 1.90) \]

Total hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent A showed the presence of glucose and mannose. Reduction of a sample (3 mg.), followed by hydrolysis of the derived glycitol and examination of the products in solvent J indicated the presence of glucose and mannitol.
The sugar gave a negative reaction to the triphenyltetrazolium chloride spray, indicating that position C2 of the mannose unit was substituted.

A lime-water degradation of a sample (5 mg.) effected no change, the original disaccharide being the only product of the reaction.

A small-scale methylation of a sample (2 mg.) was carried out. The resultant syrup was methanolysed and the cleavage products examined by gas-liquid partition chromatography. The methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-mannose were observed.

Subfraction 7b (22.8 mg.) \( R_{gal} = 0.28 \) \( M_g = 0.49 \)

This oligosaccharide was chromatographically and ionophoretically pure and identical to oligosaccharide C isolated from fraction 10 and it was combined with it.

Fraction 8 (38.5 mg.)

Chromatographic examination of this fraction in solvents A and E indicated the presence of at least three sugars with the following \( R_{gal} \) values, (i) 0.72, (ii) 0.37 and (iii) 0.26. Hydrolysis of a sample (4 mg.) gave mannose and glucose only.

In view of the small quantity present and the fact that oligosaccharides with identical \( R_{gal} \) values have been dealt with elsewhere, this fraction was not examined further.

Fraction 9 (17.2 mg.) and Fraction 10 (52.0 mg.)

These fractions were combined and separated into two sub-
fractions on Whatman 3 MM paper in solvent system A.

Subfraction 10a (18.4 mg.)  \( R_{gal} = 0.37 \)  \( M_g = 0.41 \)

This fraction was chromatographically and ionophoretically pure and was identical to oligosaccharide D isolated from fraction 11 and it was combined with it.

Subfraction 10b.  Oligosaccharide C (29.9 mg.)

\( R_{gal} = 0.28, M_g = 0.49, [\alpha]_D = +35.8^o (\epsilon = 1.06) \)

Hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent B indicated the presence of glucose and mannose. Reduction of a sample (3 mg.), followed by hydrolysis and chromatographic examination of the products in solvent J indicated the presence of glucose, mannose and sorbitol.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid method, using a standard curve based on mannose, on the sugar and its derived glycitol. A ratio of three to two indicated that the sugar was a trisaccharide.

A positive reaction to the triphenyltetrazolium chloride spray illustrated that position C2 of the reducing glucose unit was unsubstituted.

Partial acid hydrolysis of a sample (3 mg.) with 0.5 M sulphuric acid (1 ml.) for 0.5 hour at 100\(^o\) and chromatographic examination in solvent A indicated the presence of glucose, mannose and sugars with chromatographic mobilities identical to the two disaccharides, oligosaccharides A and B. A similar partial acid hydrolysis on the derived glycitol, yielded mannose and glucose and a sugar with chromatographic mobility
identical to the disaccharide B only.

A lime-water degradation of a sample (5 mg.) and chromatographic examination of the product in solvent A indicated that the trisaccharide had been degraded to the disaccharide, oligosaccharide B.

Methylation of a sample (2 mg.) was carried out and the resultant syrup methanolysed. Examination of the cleavage products by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-\(\text{O}-\)methyl-\(\text{D}\)-glucose (1 part), 2,3,6-tri-\(\text{O}-\)methyl-\(\text{D}\)-glucose (1 part) and 3,4,6-tri-\(\text{O}-\)methyl-\(\text{D}\)-mannose (1 part).

A similar methylation of the derived glycitol was also carried out and the methyl glycosides of the following methylated sugars were recognised, 2,3,4,6-tetra-\(\text{O}-\)methyl-\(\text{D}\)-glucose (1 part), 3,4,6-tri-\(\text{O}-\)methyl-\(\text{D}\)-mannose (1 part) and 1,2,3,5,6-penta-\(\text{O}-\)methyl-sorbitol (1 part). The relative proportions of the various sugar derivatives are indicated in brackets. They were estimated by visual inspection of the peak areas only.

**Fraction II (121.3 mg.)**

This fraction contained glucose as the major component and sugars with chromatographic mobilities identical to oligosaccharides C and D. In view of their suspected small quantity, this fraction was not examined further.
Fraction 12 (234.7 mg.)

Paper chromatographic examination of this fraction in solvents A and F indicated the presence of two components. Separation was effected on Whatman 3 MM paper in solvent A.

Subfraction 12a. Oligosaccharide D (117.7 mg.)

\[ \text{R}_{\text{gal}} = 0.37, \ M_g = 0.41, \ [\alpha]_D = +23.8^\circ \ (\varphi = 0.88) \]

Hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent B showed the presence of mannose and glucose. Reduction of a sample (3 mg.) followed by hydrolysis and chromatographic examination of the products in solvent J indicated the presence of glucose, mannose and mannitol.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid colorometric method on the sugar and its derived glycitol. A ratio of three to two indicated that the sugar was a trisaccharide.

A negative reaction to the triphenyltetrazolium chloride spray indicated that position C2 of the reducing mannose unit was substituted.

A partial acid hydrolysis was carried out on the sugar and its derived alcohol. A chromatographic examination of the products from the sugar indicated the presence of both disaccharides, A and B, whereas the alcohol yielded only the disaccharide, A. In both cases, mannose and glucose were also reaction products and, in the first case, unchanged starting material was also present.

A lime-water degradation of a sample (5 mg.) was carried
out but examination of the products in solvent system A and E indicated that no degradation had occurred, the only sugar present being the starting material.

A small-scale methylation of a sample (2 mg.) was carried out. Methanolysis and examination of the cleavage products by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-$O$-methyl-$D$-mannose (1 part), 2,3,6-tri-$O$-methyl-$D$-glucose (1 part) and 3,4,6-tri-$O$-methyl-$D$-mannose (1 part).

The derived glycitol was also methylated and methanolysed. Methyl glycosides corresponding to the following sugars were recognised by their retention times, 2,3,4,6-tetra-$O$-methyl-$D$-mannose (1 part), 2,3,6-tri-$O$-methyl-$D$-glucose (1 part) and 1,3,4,5,6-penta-$O$-methyl-mannitol (1 part).

Subfraction 12b. Oligosaccharide $E$ (43.5 mg.)

$R_{gal} = 0.16, \ M_g = 0.37, \ [\alpha]_D = +20.0^\circ (c = 1.11)$

Hydrolysis of a sample (2 mg.) gave mannose and glucose only. Reduction of a sample (3 mg.) followed by hydrolysis and chromatographic examination of the products in solvent $J$ indicated the presence of glucose, mannose and sorbitol.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid method on the sugar and its derived glycitol. A ratio of four to three indicated that the sugar was a tetrasaccharide.

A positive reaction to the triphenyltetrazolium spray indicated that position C2 of the reducing glucose unit was unsubstituted.
A partial acid hydrolysis of a sample (4 mg.) was carried out and chromatographic examination of the products in solvent A indicated the presence of both disaccharides A and B, both trisaccharides C and D and unchanged starting material.

A similar partial acid hydrolysis of the derived glycitol and chromatographic examination of the products in solvent A indicated the presence of both disaccharides but only one trisaccharide, D.

A lime-water degradation of a sample (5 mg.) and subsequent chromatographic examination of reaction products in solvent A indicated the presence of the trisaccharide, D, only.

A small-scale methylation of a sample (3 mg.), methanolysis and subsequent examination of the cleavage products by gas-liquid partition chromatography, indicated the presence of the methyl glycosides of 2,3,4,6-tetra-\(\alpha\)-methyl-D-mannose (1 part), 2,3,6-tri-\(\alpha\)-methyl-D-glucose (2 parts) and 3,4,6-tri-\(\alpha\)-methyl-D-mannose (1 part).

Similar treatment of the derived glycitol indicated the presence of the methyl glycosides of 2,3,4,6-tetra-\(\alpha\)-methyl-D-mannose (1 part), 2,3,6-tri-\(\alpha\)-methyl-D-glucose (1 part) and 3,4,6-tri-\(\alpha\)-methyl-D-mannose (1 part), and 1,2,3,5,6-penta-\(\alpha\)-methyl-sorbitol (1 part).

Fraction 13 (115.6 mg.)

Chromatographic examination of a sample of this fraction in solvent systems A, E and F indicated the presence of three components. The syrup was separated into the individual
sugars by chromatography on Whatman 3 MM filter sheets in solvent A.

Subfraction 13a. Oligosaccharide F (34.0 mg.)

\[ R_{gal} = 0.23, \ M_g = 0.30, \ [\alpha]_D^{23} = 11.7^\circ (c = 0.86) \]

Hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent B showed the presence of glucose and mannose. Reduction of a sample (3 mg.) followed by hydrolysis and subsequent examination of the products in solvent J indicated the presence of glucose, mannose and mannitol.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid method on the sugar and its derived glycitol. A ratio of four to three indicated that the sugar was a tetrasaccharide.

A negative reaction to the triphenyltetrazolium chloride spray showed that position C2 of the reducing mannose unit was substituted.

A partial acid hydrolysis was carried out on a sample (4 mg.). Chromatographic examination of the products in solvent A indicated the presence of sugars with \( R_{gal} \) values corresponding to both disaccharides A and B, both trisaccharides C and D, and unchanged starting material.

A similar partial acid hydrolysis of the derived glycitol and chromatographic examination of the products in solvent A indicated the presence of sugars with \( R_{gal} \) values corresponding to both disaccharides A and B, and one trisaccharide, C.

A lime-water degradation of a sample (5 mg.) was carried
Chromatographic examination of the reaction products in solvent A indicated that no degradation had taken place and that unchanged starting material was the only sugar present.

A small sample (2 mg.) was methylated. Subsequent methanolysis and examination of the cleavage products by gas-liquid partition chromatography revealed the presence of the methyl glycosides of 2,3,4,6-tetra-\(\text{\textbeta}\)-methyl-D-glucose (1 part), 2,3,6-tri-\(\text{\textbeta}\)-methyl-D-glucose (1 part), and 3,4,6-tri-\(\text{\textbeta}\)-methyl-D-mannose (2 parts).

The derived glycitol (2 mg.) was also methylated and methanolysed. Examination of the cleavage products by gas-liquid partition chromatography revealed the presence of the methyl glycosides of 2,3,4,6-tetra-\(\text{\textbeta}\)-methyl-D-glucose (1 part), 3,4,6-tri-\(\text{\textbeta}\)-methyl-D-mannose (1 part) and 2,3,6-tri-\(\text{\textbeta}\)-methyl-D-glucose (1 part) and 1,3,4,5,6-penta-\(\text{\textbeta}\)-methyl-mannitol (1 part).

Subfraction 13b. Oligosaccharide G (30.6 mg.)

\[ R_{\text{gal}} = 0.11, \quad M_g = 0.24, \quad [\alpha]_D = +25.7^\circ \quad (c = 1.01) \]

Hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent A indicated the presence of glucose and mannose. Reduction of a sample (3 mg.), hydrolysis and chromatographic examination of the products in solvent system J indicated the presence of glucose, mannose and sorbitol.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid method on the sugar and its derived glycitol. A ratio of approximately five to four indicated that the sugar was probably a pentasaccharide.

A positive reaction to the triphenyltetrazolium chloride
spray indicated that position C2 of the reducing glucose unit was unsubstituted.

Partial acid hydrolysis of the sugar and its derived alcohol were carried out. The products were examined chromatographically in solvents A and E. The sugar gave products with chromatographic mobilities identical to both tetrasaccharides, E and F, both trisaccharides, C and D, both disaccharides, A and B, in addition to unchanged starting material. The derived glycitol gave products with chromatographic mobilities identical to the two disaccharides, the two trisaccharides but only one tetrasaccharide, F.

Chromatographic examination of the products obtained from a lime-water degradation of a sample (5 mg.) in solvent system A, indicated the presence of the tetrasaccharide, oligosaccharide F.

A sample (2 mg.) of the sugar was methylated and methanolyzed. Subsequent examination of the cleavage products by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-glucose (1 part), 2,3,6-tri-0-methyl-D-glucose (2 parts) and 3,4,6-tri-0-methyl-D-mannose (2 parts).

A sample (2 mg.) of the derived sugar alcohol was also methylated. Methanolysis and examination of the cleavage products by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-0-methyl-D-glucose (1 part), 2,3,6-tri-0-methyl-D-glucose (1 part) and 3,4,6-tri-0-methyl-D-mannose (2 parts) and 1,2,3,5,6-penta-0-methyl-sorbitol (1 part).
Subfraction 13e. Oligosaccharide H (33.9 mg.)

\[ R_{gal} = 0.07, \ M_g = 0.27, \ [\alpha]_D = +16.9^\circ \ (C = 0.77) \]

Hydrolysis of a sample (2 mg.) and chromatographic examination of the products in solvent B indicated the presence of glucose and mannose. Hydrolysis of the derived glycitol (2 mg.) indicated the presence of glucose, mannose and mannitol on examination in solvent J.

The chain-length of the oligosaccharide was determined by the phenol-sulphuric acid method on the sugar and its derived glycitol. A ratio of five to four implied a pentasaccharide.

A negative reaction to the triphenyltetrazolium spray indicated that position C2 of the reducing mannose unit was unsubstituted.

Partial acid hydrolysis of both the sugar and its derived glycitol were carried out and the products examined chromatographically in solvents A and E. The parent sugar gave rise to products with chromatographic mobilities identical to the two tetrasaccharides E and F, the two trisaccharides C and D, the two disaccharides A and B, in addition to unchanged starting material. The products obtained from the glycitol had chromatographic mobilities corresponding to both disaccharides A and B, both trisaccharides C and D, but to one tetrasaccharide, E, only.

The products obtained from a lime-water degradation of the oligosaccharide were examined in solvents A and F. The original material appeared to be undegraded.

Methylations were carried out on samples (2 mg.) of the
sugar and the glycitol. Methanolysis and examination of the cleavage products from (a) the methylated sugar by gas-liquid partition chromatography indicated the presence of the methyl glycosides of 2,3,4,6-tetra-\(\Omega\)-methyl-\(\underline{D}\)-mannose (1 part), 2,3,6-tri-\(\Omega\)-methyl-\(\underline{D}\)-glucose (3 parts) and 3,4,6-tri-\(\Omega\)-methyl-\(\underline{D}\)-mannose (2 parts); and (b) the methylated sugar alcohol indicated the presence of the methyl glycosides of 2,3,4,6-tetra-\(\Omega\)-methyl-\(\underline{D}\)-mannose (1 part), 2,3,6-tri-\(\Omega\)-methyl-\(\underline{D}\)-glucose (2 parts) and 3,4,6-tri-\(\Omega\)-methyl-\(\underline{D}\)-mannose (2 parts) and 1,2,3,5,6-penta-\(\Omega\)-methyl-sorbitol (1 part).

Fraction 14 (772.7 mg.)

This fraction was examined by paper chromatography in solvents A and F and the presence of oligosaccharides G and H was recognised. However, in addition to these sugars, the presence of higher molecular weight material was indicated. An attempt at fractionation on a Dowex 50W x 2 resin column in the \(\text{Li}^+\) form was unsuccessful and pure sugars were not obtained. A sample of the mixture was hydrolysed and the products were examined in solvents A and B. The presence of mannose and glucose only was indicated. Reduction followed by hydrolysis and examination of the products in solvent J indicated the presence of glucose, mannose, sorbitol and mannitol.

A partial acid hydrolysis was carried out and the products examined in solvents A and F. Sugars with chromatographic mobilities identical to oligosaccharides A to H were recognised.

Samples of the sugars and the reduced sugars were
methylated by the Kuhn procedure. After methanolysis, the cleavage products were examined by gas-liquid partition chromatography. The major components from the methanolysis of both were the methyl glycosides of 2,3,6-tri-\(\alpha\)-methyl-D-glucose and 3,4,6-tri-\(\alpha\)-methyl-D-mannose. In each case, the methyl glycosides of both tetramethylmannose and tetramethylglucose were present and 1,2,3,5,6-pentamethylsorbitol and 1,3,4,5,6-pentamethylmannitol were recognised as products from the methylated glycitols. No dimethyl sugars were detected.

This fraction was not examined further.

**Fraction 15 (610.5 mg.)**

This fraction was similar to fraction 14 and was not studied further.
Partial hydrolysis of the gum acid

**Trial experiment**

Gum acid (300 mg.) was dissolved in water (5 ml.) and 2 N sulphuric acid (5 ml.) was added. The solution was heated on a boiling water bath for ten hours. Aliquot portions (1 ml.) were removed at hourly intervals and poured into ethanol (5 ml.).

The precipitated polysaccharide, if any was present, was removed at the centrifuge and each solution was neutralised with Amberlite LA 1 liquid resin and Amberlite IR 120 (H) resin. The neutral solutions were then concentrated to syrups and examined chromatographically in solvents A, C and D.

Visual inspection of the chromatograms indicated that the best yield of acidic oligosaccharides could be obtained by hydrolysing the polysaccharide with N sulphuric acid for five hours. It was hoped to obtain reasonably high yields of the longer chain acidic sugars by this relatively mild treatment.

**Large scale hydrolysis**

Gum acid (20 g.) was dissolved in water (500 ml.) and the solution heated on a water bath at 100°. To the hot solution was added sulphuric acid (6 N, 100 ml.) and the solution was stirred at 100° for five hours. The hydrolysate was allowed to cool and poured into ethanol (2 l.). The small amount (< 500 mg.) of polysaccharide was removed by centrifugation, washed with 75% ethanol and discarded.
The supernatant and washings were combined and reduced in volume (500 ml.). The acidic solution was neutralised by the addition of saturated barium hydroxide solution and solid barium carbonate. The precipitate was removed at the centrifuge and washed with water (3 x 200 ml.). The water washings and supernatant were combined, concentrated, deionised with Amberlite IR 120 (H) resin and further concentrated to a syrup (15.3 g.).

Chromatographic examination of a sample of the syrup in solvents A, C and D indicated the presence of a large amount of the monosaccharides, xylose, arabinose and galactose with lesser amounts of mannose and glucuronic acid. Also present was a complex mixture of oligosaccharides, the majority of which were acidic.

Separation of the acidic oligosaccharides

A DEAE-sephadex column (20 x 3 cm., 30 g.) was prepared in the formate form. The syrup (15.3 g.) was dissolved in water (40 ml.) and the acidic solution was placed on the column in five batches, each batch being washed on to the column with water (100 ml.) before the next batch was applied.

The column was allowed to stand for four hours and the neutral sugars eluted from the column with water until the eluate gave a negative reaction to the phenol sulphuric acid colorometric test. The acidic sugars which remained on the column were eluted with a gradient of water containing increasing amounts of formic acid (0 to 1.5%, 4 l.).
Fractions (20 ml.) were collected and the contents of every third tube were concentrated and examined in solvent C. The similar fractions were bulked, concentrated to a small volume (40 ml.) and extracted in a liquid-liquid extractor for eighteen hours with ether. The formic acid-free solutions were filtered and concentrated to dryness.

The column was then eluted with batches of 2%, 5% and 15% formic acid solutions. The washing with each solution was continued until the eluate gave a negative reaction towards the phenol sulphuric acid colorometric test.

The following fractions were obtained.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>Weight</th>
<th>Components</th>
<th>Components R&lt;sub&gt;gal&lt;/sub&gt; (solvent C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>water washings</td>
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<td>neutral</td>
<td>neutral</td>
</tr>
<tr>
<td>B</td>
<td>1 - 146</td>
<td>889.5 mg.</td>
<td>0.40, 0.21</td>
<td>0.10, 0.04</td>
</tr>
<tr>
<td>C</td>
<td>147 - 176</td>
<td>309.7 mg.</td>
<td>1.10, 0.40</td>
<td>0.21</td>
</tr>
<tr>
<td>D</td>
<td>177 - 276</td>
<td>106.4 mg.</td>
<td>0.40, 0.10</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>277 - 400</td>
<td>399.4 mg.</td>
<td>0.06 + others</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2% formic acid</td>
<td>731.6 mg.</td>
<td>0.06 + others</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>5% formic acid</td>
<td>2.110 g.</td>
<td>immobile</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>15% formic acid</td>
<td>1.290 g.</td>
<td>immobile</td>
<td></td>
</tr>
</tbody>
</table>

Total weight recovered, 13.666 g.

89.1%

Fraction B (889.5 mg.)

Chromatography of this fraction in solvent systems C and D
indicated the presence of at least six sugars. This fraction was separated into three subfractions on Whatman 3 MM paper in solvent system C. The slowest of these subfractions was further separated into two fractions by thick paper chromatography in solvent D to give four final fractions.

**Subfraction B1.** This fraction (404.5 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.40 \) in solvent C, and was known as oligosaccharide I.

**Subfraction B2.** This fraction (115.7 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.23 \) in solvent C, and was known as oligosaccharide II.

**Subfraction B3.** This fraction (22.1 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.11 \) in solvent C, and was known as oligosaccharide III.

**Subfraction B4.** This fraction (80.9 mg.) was examined chromatographically in solvents C and D and consisted of at least three oligosaccharides IV, V, VI.

**Fraction C (309.7 mg.)**

Chromatography of this fraction in solvents C and D indicated the presence of four sugars. Like the previous fraction, this syrup was separated into four subfractions on Whatman 3 MM paper in solvent C.

**Subfraction C1.** This fraction (14.9 mg.) was chromatographically pure, \( R_{\text{gal}} = 1.1 \) in solvent C, and identical to an authentic sample of D-glucuronic acid. It was not examined further.
Subfraction C2. This fraction (198.5 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.40 \) in solvent C, and identical to oligosaccharide I, with which it was combined.

Subfraction C3. This fraction (12.0 mg.) was examined chromatographically in solvents C and D and was found to consist of two components, one of which was identical to oligosaccharide I. The other, oligosaccharide VII, had \( R_{\text{gal}} = 0.32 \) in solvent C.

Subfraction C4. This fraction (8.9 mg.) was chromatographically pure and identical to oligosaccharide II \( (R_{\text{gal}} = 0.23 \) in solvent C) with which it was combined.

Fraction D (106.4 mg.)

Chromatography of this fraction in solvents C and D indicated the presence of three components. The syrup was separated into three subfractions on Whatman 3 MM paper in solvent C.

Subfraction D1. This fraction (50.0 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.40 \) in solvent C, and identical to oligosaccharide I, with which it was combined.

Subfraction D2. This fraction (7.5 mg.) was examined chromatographically in solvents C and D. It was found to contain two sugars, identical to oligosaccharides I and VII. In this respect it was identical to subfraction C3 with which it was combined.

Subfraction D3. This fraction (17.9 mg.) was chromatographically pure, \( R_{\text{gal}} = 0.34 \) in solvent D, and was known as oligosaccharide VIII.
Fraction E (399.4 mg.)

Chromatography of this fraction in solvents C and D indicated the presence of five sugars. It was separated on Whatman 3 MM paper in solvent D into five subfractions.

Subfraction E1. This fraction (145.3 mg.) was chromatographically pure, $R_{gal} = 0.34$ in solvent D, and identical to oligosaccharide VIII, with which it was combined.

Subfraction E2. This fraction (27.4 mg.) was chromatographically pure, $R_{gal} = 0.10$ in solvent D, and was known as oligosaccharide X.

Subfraction E3. This fraction (20.7 mg.) was chromatographically pure, $R_{gal} = 0.07$ in solvent D, and was known as oligosaccharide XI.

Subfraction E4. This fraction (27.0 mg.) was chromatographically pure, $R_{gal} = 0.04$ in solvent D, and was known as oligosaccharide XII.

Subfraction E5. This fraction (41.2 mg.) was immobile in both solvents C and D and was not examined further.

Fraction F (731.6 mg.)

Chromatography of this fraction in solvents C and D indicated the presence of at least five sugars. It was separated on Whatman 3 MM paper in solvent D into three subfractions.

Subfraction F1. This fraction (41.5 mg.) was chromatographically pure, $R_{gal} = 0.44$ in solvent D, and will be known as oligosaccharide IX.
Subfraction F2. This fraction (193.8 mg.) was chromatographically pure, $R_{\text{gal}} = 0.34$ in solvent D, and identical to oligosaccharide VIII, with which it was combined.

Subfraction F3. This fraction (228.8 mg.) was examined in solvent D and found to contain oligosaccharides X to XII which were isolated from fraction E.

Fraction G (2.1 g.) and Fraction H (1.29 g.)

These fractions were examined chromatographically in solvent D and only very slow moving components were observed. They were obviously fairly high molecular weight fragments and were not examined further.

Oligosaccharide I (652.0 mg.) $[\alpha]_D = -32.2^o$ ($c = 2.7$)

The sugar was chromatographically pure in solvent C ($R_{\text{gal}} = 0.40$) and in solvent D ($R_{\text{gal}} = 0.75$) and was identical to 2-$\Omega$-(D-glucosyluronic acid)-D-mannose in this respect.

A sample of the sugar was hydrolysed with $m$ sulphuric acid for four hours. Chromatography of the hydrolysate in solvents A and C showed the presence of glucuronic acid, glucurone and mannose. Reduction of the sugar with sodium borohydride and examination of the products of hydrolysis in solvents A, C and J indicated the presence of glucuronic acid, glucurone and mannitol.

The sugar (5 mg.) was heated with 4% methanolic hydrogen chloride for six hours and the product was reduced with sodium borohydride. The reduced sugar was hydrolysed with $m$ sulphuric
acid for four hours. Chromatography of the hydrolysate in solvent A indicated the presence of glucose and mannose.

A negative reaction towards the triphenyltetrazolium chloride spray indicated that position C-2 of the mannose unit was substituted.

A sample (10 mg.) of the sugar was dissolved in dry 1% methanolic hydrogen chloride and allowed to stand at room temperature for sixteen hours. The solution was then heated in a boiling water-bath for one hour. The solution was neutralised with silver carbonate and the resultant methyl ester methyl glycoside dissolved in water (2 ml.) and treated overnight with sodium borohydride (10 mg.).

The neutral sugar was then methylated by the Kuhn procedure and the methylated disaccharide methanolysed. Examination of the cleavage products by gas-liquid partition chromatography on system (a) gave the following sugars.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-tetra-&lt;sup&gt;3&lt;/sup&gt;-methyl-D-glucose</td>
<td>1.00 1.42</td>
</tr>
<tr>
<td>3,4,6-tri-&lt;sup&gt;3&lt;/sup&gt;-methyl-D-mannose</td>
<td>3.05</td>
</tr>
</tbody>
</table>

Oligosaccharide II (133.7 mg.) \([\alpha]_D^0 = +22.1 (C = 1.4)\)

The sugar was chromatographically homogeneous in two solvents, solvent C \((R_{gal} = 0.23)\) and solvent D \((R_{gal} = 0.55)\).

A sample of the sugar (3 mg.) was hydrolysed and examination of the products in solvents A and C indicated the presence
of glucuronic acid, glucurone and galactose. Another sample (3 mg.) of the sugar was reduced with borohydride and the derived glycitol hydrolysed. Examination of the products in solvents A, C and J indicated the presence of glucuronic acid, glucurone and galactitol.

A sample (5 mg.) of the sugar was converted into the methyl ester methyl glycoside, reduced with sodium borohydride and hydrolysed. Chromatographic examination of the hydrolysate in solvent B showed glucose and galactose.

A sample (2 mg.) of the sugar was methylated by the Kuhn procedure and the product methanolysed. Gas-liquid partition chromatography of the derived methyl glycosides indicated the presence of the following sugars on column (a).

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-D-glucuronic acid</td>
<td>2.41 3.11</td>
</tr>
<tr>
<td>2,3,5-tri-O-methyl-D-galactose</td>
<td>4.36</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>7.38</td>
</tr>
</tbody>
</table>

Attempts to crystallise the sugar from aqueous ethanol were unsuccessful.

Oligosaccharide III (22.1 mg.) \[\alpha\]_D \text{= +9.3° (C \text{= 1.1})}

The sugar was chromatographically pure in solvent C (R_gal \text{= 0.11}) and in solvent D (R_gal \text{= 0.42}), and gave a pink staining reaction with aniline oxalate.

A sample of the sugar was hydrolysed and the products
examined chromatographically in solvents B and C. The presence of arabinose, galactose, glucuronic acid and 6-\text{-}(\text{glucosyluronic acid})-D-galactose. Reduction of the derived methyl ester methyl glycoside with sodium borohydride followed by hydrolysis gave arabinose, galactose and glucose in approximately equal proportions. Hydrolysis of the derived glycitol, which was prepared by borohydride reduction of the acid, gave 6-\text{-}(\text{glucosyluronic acid})-D-galactose, galactose (trace), glucuronic acid (trace) and arabitol. These products were examined in solvents B, C, D and J.

A sample (5 mg.) was methylated by the Kuhn procedure and the resultant syrup methanolysed. The cleavage products were examined by gas-liquid partition chromatography on systems (a) and (d), and the presence of the following sugars was indicated.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>1.88</td>
</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>2.17</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-glucuronic acid</td>
<td>2.44</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>7.39</td>
</tr>
</tbody>
</table>

Oligosaccharides IV, V and VI (80.9 mg.)

These three sugars were never obtained in a pure state owing to their very similar chromatographic mobilities. Oligosaccharide IV had $R_{gal} = 0.37$ in solvent D, oligosaccharide V had $R_{gal} = 0.32$ in solvent D and oligosaccharide VI, which
gave a pink staining reaction with aniline oxalate, had \( R_{gal} = 0.26 \) in solvent D. Total hydrolysis of the complex mixture indicated the presence of glucuronic acid, glucurone, galactose, arabinose, mannose (trace) and the aldobiouronic acid, 6-\Omega-(glucosyluronic acid)-D-galactose. The mixture was not examined further.

**Oligosaccharide VII** (19.5 mg.)

This sugar was not chromatographically pure and the presence of about equal quantities of 2-\Omega-(glucosyluronic acid)-D-mannose was indicated. It had \( R_{gal} = 0.32 \) (in solvent C) and could not be separated from oligosaccharide I in solvent D. Hydrolysis of the mixture gave glucuronic acid, glucurone and mannose only. In view of the small amount, the sugar was not examined further.

**Oligosaccharide VIII** (357.0 mg.)

This sugar was chromatographically pure (\( R_{gal} = 0.34 \) in solvent D) and gave the same characteristic orange-brown fluorescence in ultraviolet light as oligosaccharide I, when stained with aniline oxalate. The sugar gave no colour reaction with the triphenyltetrazolium chloride spray.

Reduction of the methyl ester methyl glycosides with sodium borohydride afforded both glucose and mannose in approximately equal proportions. Hydrolysis of the sugar (3 mg.) and examination of the products in solvents A, C and D indicated the presence of glucuronic acid, glucurone, mannose
and 2-Ω-(glucosyluronic acid)-mannose.

A sample of the sugar (5 mg.) was reduced with sodium borohydride and the glycitol hydrolysed. Examination of the products in solvent systems A, C, D and J indicated the presence of glucuronic acid (trace), mannose (trace), 2-Ω-(glucosyluronic acid)-mannose and mannitol. The hydrolysate was again examined in solvent J, the chromatogram being irrigated with the solvent for an extended period (6 days). The chromatogram was then stained with the periodate-Schiff's spray reagent. This spray reacts with different derived aldehydes in different ways and the various sugars can be distinguished by the colours formed. For example, if the sugar is oxidised to formaldehyde, an immediate purple coloration is obtained; if the sugar is oxidised to a derivative of malondialdehyde, a yellow coloration appears after an hour. All other aldehydes give dark-blue stains after periods of an hour.

The sample in question, when sprayed, indicated (a) the presence of a sugar which gave an immediate purple coloration and which had a chromatographic mobility identical to 2-Ω-(glucosyluronic acid)-mannitol, which, itself, was stained purple and (b) the presence of a slower-moving sugar which gave a yellow coloration and which had a chromatographic mobility identical to 2-Ω-(glucosyluronic acid)-D-mannose, which was stained yellow as well.

Determination of the degree of polymerisation of the sugar was carried out in the usual manner. Measurement of the sugar
content, by the phenol sulphuric acid method, of the oligosaccharide and its derived glycitol indicated that the sugar was a tetrasaccharide, a value of 3.9 being obtained.

Determination of the uronic acid anhydride content by the carbazole method and the total sugar content by the phenol sulphuric acid method showed that the ratio of glucuronic acid to total sugar content was 1:2.

Oligosaccharide IX (41.5 mg.)

This sugar was chromatographically pure ($R_{gal} = 0.44$ in solvent D) and, when stained with aniline oxalate, gave the same characteristic dull-red fluorescence in ultraviolet light as glucuronic acid. The sugar gave a pink coloration when sprayed with triphenyltetrazolium chloride.

Hydrolysis of a sample (3 mg.) and examination of the products in solvents A, C and D indicated the presence of glucuronic acid, glucurone and mannose in addition to 2-O-(glucosyluronic acid)-D-mannose. Reduction of the derived methyl ester methyl glycoside with sodium borohydride followed by hydrolysis afforded glucose and mannose. Hydrolysis of the derived glycitol furnished glucuronic acid, mannose, 2-O-(glucosyluronic acid)-D-mannose but no mannitol.

No further examination was carried out.

Oligosaccharide X (27.4 mg.)

This fraction was chromatographically pure ($R_{gal} = 0.10$ in solvent D) and, when stained with aniline oxalate gave the same
characteristic orange-brown fluorescence in ultraviolet light as oligosaccharides I and VIII. The sugar gave no coloration with the triphenyltetrazolium chloride spray.

Hydrolysis of a sample (3 mg.) and examination of the products in solvents A, C and D indicated the presence of glucuronic acid, mannose and oligosaccharides I and VIII.

Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis afforded glucose and mannose. Mannitol was one of the products observed from the hydrolysis of the derived glycitol.

No further examination was carried out.

Oligosaccharide XI (20.7 mg.)

This sugar was chromatographically pure (R_gal = 0.07) in solvent D and, when stained with aniline oxalate, gave the same characteristic dull-red fluorescence in ultraviolet light as glucuronic acid. The sugar also gave a pink coloration when sprayed with triphenyltetrazolium chloride.

Hydrolysis of a sample (3 mg.) and examination of the products in solvents A, C and D indicated the presence of glucuronic acid, glucurone, mannose and 2-O-(glucosyluronic acid)-D-mannose. No mannitol was observed in the products from the hydrolysis of the derived glycitol.

Reduction of the methyl ester methyl glycosides with sodium borohydride afforded glucose and mannose only.
Oligosaccharide XII (27.0 mg.)

This sugar was chromatographically pure ($R_{gal} = 0.04$ in solvent D) and, when stained with aniline oxalate, gave the same characteristic orange-brown fluorescence as oligosaccharides I, VIII and X, when viewed in ultraviolet light. The sugar gave no coloration with the triphenyltetrazolium chloride spray.

Hydrolysis of a sample (5 mg.) and examination of the products in solvents A, C and D indicated the presence of glucuronic acid, glucurone, mannose and oligosaccharides I and VIII.

Reduction of the methyl ester methyl glycosides with sodium borohydride followed by hydrolysis afforded only glucose and mannose. Finally, mannitol was observed among the products of hydrolysis of the derived glycitol.

The oligosaccharide was not examined further.
Synthesis of sophorose

Sophorose (2-\(\beta\)-D-glucopyranosyl-D-glucose) was synthesised by the condensation of methyl 4,6-\(\alpha\)-benzylidene-\(\alpha\)-D-glucoside with tetra-\(\alpha\)-acetyl-\(\alpha\)-D-glucopyranosyl bromide as described by Coxon and Fletcher.\(^1\) The condensation gave a sophorose derivative from which the substituent groups were removed (through successive acetolysis and deacetylation) to give crystalline sophorose (6.1 g., 24.8%), m.p. 184-187°. (\(R_\text{gal} = 0.67\) in solvent A.)

Epimerisation of sophorose

Sophorose (5 g.) was dissolved in water (500 ml.) and heated on a boiling water-bath. Sodium carbonate solution (500 ml., 0.1 M) was similarly heated and then added very quickly to the sophorose solution. The solution was then heated for a further five minutes and after cooling was de-ionised with Amberlite IR 120 (H) and Amberlite IR 45 (OH) resins. The solution was concentrated to a syrup (4.8 g.) and examined chromatographically in solvent A. The presence of mannose, glucose, sophorose and an unknown sugar (\(R_\text{gal} = 0.72\)) was observed.

The syrup (4.8 g.) was dissolved in water (10 ml.) and applied to a squat charcoal column (300 g.). The column was eluted with water (5 l.) to remove the monosaccharides and the eluate discarded.

Elution of the column with water containing increasing
proportions of ethanol in a gradient manner was carried out and fractions (20 ml.) were collected every half-hour. Every third tube was evaporated to dryness and examined chromatographically in solvent A. Similar fractions were combined and evaporated to dryness. The following table outlines the complete procedure.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Eluant</th>
<th>Tube Nos.</th>
<th>Weight (in g.)</th>
<th>Contents</th>
<th>( R_{gal} ) (solvent A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0→5% ethanol</td>
<td>1→368</td>
<td>1.10</td>
<td>mannose, glucose</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5→7.5% ethanol</td>
<td>369→397</td>
<td>1.90</td>
<td>( R_{gal} = 0.66 )</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>5→7.5% ethanol</td>
<td>398→500</td>
<td>0.95</td>
<td>( R_{gal} = 0.66, 0.73 )</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>7.5% ethanol</td>
<td>501→600</td>
<td>0.76</td>
<td>( R_{gal} = 0.73 )</td>
<td></td>
</tr>
</tbody>
</table>

Fraction III was separated into two fractions on Whatman 3 MM paper in solvent A. The faster moving sugar (\( R_{gal} = 0.73 \)) was combined with fraction IV.

2-\( \alpha \)-\( \beta \)-D-glucopyranosyl-D-mannose \[ \left[ \alpha \right]_D = -21.3^\circ \]

Fraction IV (0.98 g.) was chromatographically pure and identical to the suspected 2-\( \alpha \)-\( \beta \)-D-glucosyl-D-mannose, isolated from the products of partial acetolysis of the carboxyl-reduced leiocarpan A. Hydrolysis of a small sample and chromatographic examination of the products in solvent A indicated the presence of glucose and mannose in approximately equal amounts. Hydrolysis of the derived glycitol gave glucose and mannitol.

Attempts to crystallise the oligosaccharide from aqueous ethanol and aqueous acetone were not successful.
The derived octa-acetate was prepared but attempts to crystallise the syrup were also unsuccessful.

Further attempts were made to fully characterise the sugar by forming the glycitol and its acetate, but crystallisation could not be induced in either case.

A final attempt was made to form a crystalline derivative and the phenylhydrazone was prepared. Unfortunately this derivative would not crystallise either.
Reduction of *gum ghatti*

Purified *gum ghatti* (45 g.) was dissolved in formamide (2,000 ml.) by shaking overnight. To the clear solution with constant stirring, pyridine (1,000 ml.) was added dropwise over a period of eight hours. The solution was again shaken overnight. Acetic anhydride (700 ml.) was then added dropwise over a period of two hours and the solution again shaken overnight.

The acetate of the polysaccharide was isolated in exactly the same manner as described for leiocarpan A. Yield = 52.3 g. (Found, Acetyl, 39.0%)

The acetate (25 g.) was dissolved in 1,2-dimethoxyethane (1,000 ml.) and reduced with diborane in the same manner as described for the reduction of leiocarpan A. After four weeks the reduced polysaccharide was isolated (12.2 g.).

Uronic acid anhydride content (1) = 3.1% (by decarboxylation)

(2) = 0.9% (by carbazole)

A small sample (2 mg.) was hydrolysed and the hydrolysate examined in solvents A, C and D. The presence of arabinose, glucose and galactose was indicated and a trace of a slow moving acidic sugar only.

Methylation of carboxyl-reduced *gum ghatti*

A sample of the carboxyl-reduced polysaccharide (200 mg.) was methylated in the same manner as was described for the carboxyl-reduced leiocarpan A. The methylated polysaccharide
(OMe, 40.7%, $[\alpha]_D = -69.2^\circ$ ($c = 0.94$ in chloroform)) was isolated in the usual manner and a sample methanolysed with 4% methanolic hydrochloric acid for sixteen hours. The cleavage products were examined by gas-liquid partition chromatography. The methyl glycosides of the following were detected:

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Retention Times (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column (a)</td>
</tr>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>0.54</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-arabinose</td>
<td>1.05</td>
</tr>
<tr>
<td>3,5-di-O-methyl-L-arabinose</td>
<td>1.05</td>
</tr>
<tr>
<td>2,3-di-O-methyl-L-arabinose</td>
<td>-</td>
</tr>
<tr>
<td>2,4-di-O-methyl-L-arabinose</td>
<td>2.29</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>1.85</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>7.2</td>
</tr>
<tr>
<td>2,4-di-O-methyl-D-galactose</td>
<td>16.8</td>
</tr>
<tr>
<td>2,3,6-tri-O-methyl-D-glucose</td>
<td>3.42</td>
</tr>
</tbody>
</table>

A sample was hydrolysed with mineral acid in the usual manner and the hydrolysate examined in solvents G, H and I. The following sugars were recognised by their chromatographic mobilities:

- 2,3,5-tri-O-methyl-L-arabinose,
- 2,5-di-O-methyl-L-arabinose,
- 2,3,4,6-tetra-O-methyl-D-galactose,
- 2,3,4-tri-O-methyl-D-galactose,
- 2,4-di-O-methyl-D-galactose,
2-O-methyl-D-galactose (trace),
3,4-di-O-methyl-D-mannose (trace),
4-O-methyl-D-mannose.

**Acetolysis of carboxyl-reduced gum ghatti**

The carboxyl-reduced polysaccharide (300 mg.) was acetylated by the method described by Carson and Maclay. The acetate (512.0 mg.) was added to the acetolysis mixture (acetic acid, 5 ml.; acetic anhydride, 5 ml.; sulphuric acid, 0.5 ml.) slowly and with shaking at 0°. The acetate dissolved at once and was allowed to come to room temperature. Samples (2 ml.) were removed after 48 hrs., 60 hrs. and 72 hrs. Each sample was poured into water (20 ml.) and solid sodium bicarbonate added to pH 4. The solution was then extracted with chloroform (3 x 15 ml.) and the extracts dried over anhydrous sodium sulphate, filtered and concentrated to a syrup. The syrup was dissolved in dry methanol (2 ml.) and barium methoxide (0.5 N) added until the solution was alkaline to phenolphthalein. The turbid solution was allowed to stand at 0° for twenty-four hours and was poured into water (20 ml.). The clear aqueous solution was deionised with Amberlite IR 120 (H) resin and concentrated to a syrup.

Each of the syrups was examined chromatographically in solvent A, when the highest yield of oligosaccharides was observed in the sample removed after 48 hours. The following sugars were recognised by their chromatographic mobilities,
3-0-[\beta-L-arabinopyranosyl-L-arabinose],
2-0-[\beta-D-glucopyranosyl-D-mannose],
3-0-[\beta-D-galactopyranosyl-L-arabinose],
3-0-[\beta-D-galactopyranosyl-D-galactose],
6-0-[\beta-D-galactopyranosyl-D-galactose],

and oligosaccharides C, D, E and F which were isolated from the
partial acetolysis of leiocarpan A.
I would like to take this opportunity to thank Professor Sir Edmund Hirst, C.B.E., F.R.S., for providing the facilities for carrying out this research. I should also like to express my sincere thanks to Dr. G.O. Aspinall for his enthusiastic supervision of this work and for the invaluable advice which he has given at all times; to Carol for her great help in compiling this thesis; to my colleagues in this department for many helpful discussions; and, finally, to the University of Edinburgh for the award of a Post-Graduate Studentship.
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