THE MOLECULAR STRUCTURE OF PLANT GUMS, WITH SPECIAL REFERENCE TO GUMS OF THE GENUS KHAYA

- by -

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INTRODUCTION

Many trees, of a wide variety of species, respond to injury by exuding yellowish viscous fluids, which harden on exposure to the atmosphere, producing glassy nodules. These are the plant gums (1), which are among the most complex polysaccharides known. In molecular structure, they resemble the mucilages and the bacterial polysaccharides; in fact, there are no general structural differences between the gums and the mucilages(2-8). The only distinction lies in their mode of origin, since the mucilages are isolated only by the extraction of seeds or other plant material, in which they apparently serve as food stores or as moisture reservoirs. It is therefore necessary, for the purpose of this thesis, to give a restricting definition of plant gums as uronic acid containing polysaccharide exudates. This definition also excludes resinous exudates of terpenoid structure, and non-exuded neutral polysaccharides which are known colloquially as gums, e.g. carob seed gum, which is a galactomannan (9).

The origin of the gums is still uncertain. They are commonly, although not exclusively, produced in hot, dry climates, and healthy trees tend to exude less gum than those in poor condition. For this reason, it has been suggested that they are the result of infection, and a few gums, including

chagual (10) and honey locust gums (11), are in fact known to be pathological products. On the other hand, some gums, such as gum tragacanth, are exuded copiously immediately after incision of the bark, and are obviously natural products of the plant's metabolism. It also seems unlikely that gums produced on a commercial basis are the products of infection. In general, it is probable that a tree exudes gum in order to seal off the injured part, and to prevent the spread of infection. The similarity of the gums and the bacterial polysaccharides, and the cross-reactions which can take place between gums and some Pneumococcus sera (12), may be significant in this context, and the complexity of structure of the gum polysaccharides may be connected with the necessity for dealing with a variety of attacking bacteria.

The commercial use of gums is almost as old as civilization. The Egyptians used them in embalming, and for the last few hundred years they have been common ingredients of medicines and of 'aids to beauty'. Today, they are still used in the fields of pharmaceuticals and cosmetics, but a wide range of manufacturing processes also employs them as emulsifiers, adhesives, thickeners, binding materials, etc. Being harmless and tasteless, they find many uses in the food industry.

In view of their commercial importance, the scientific

study of gums is worthwhile, and of course is also important for its intrinsic biochemical interest. But perhaps the most important reason for carrying out investigations lies in the resemblance to bacterial polysaccharides. It may be possible, by drawing analogies, to gain insight into the structure and formation of the latter, and thus to proceed to a fuller understanding of the bacteria themselves.

A typical gum possesses a highly branched structure, containing anything from two to four different neutral sugar residues, and a uronic acid residue; each may exist in more than one type of linkage. Several gums recently investigated are further complicated by the fact that they contain two different uronic acid residues. The most common neutral sugars are D-galactose, D-mannose, L-arabinose, D-xylose and L-rhamnose, but L-fucose and D-tagatose have also been observed. The uronic acids of gums are D-glucuronic acid, 4-O-methyl-D-glucuronic acid and D-galacturonic acid.

In the natural state, many gums exist as neutral salts of such cations as calcium and magnesium. Some, e.g. the *Sterculia* gums and *Cochlospermum gossypium*, are acetylated, and give off a distinct odour of acetic acid. Varying molecular weights have been quoted, ranging from 2 - 300,000 for gum arabic, to 9,500,000 for Karaya gum (13). The usual means of measurement is by sedimentation techniques. Because of the

structural complexity of the molecules concerned, chemical methods of molecular weight determination are in general unsatisfactory.

STRUCTURAL INVESTIGATION OF PLANT GUMS

A complication in structural investigations is the difficulty of exact identification of gums. In commerce, a confusion of nomenclature exists, since gums have tended to be grouped according to their physical properties rather than their source. For instance, although true gum arabic is the exudate of _Acacia senegal_, the commercial product may be derived from any one of a number of trees of the Acacia genus, or may be a mixture. Again, the name Karaya, correctly applied to _Sterculia urens_ gum, is often also applied, because of the similarity in properties, to _Cochlospermum gossypium_ gum, the correct name of which is Kutira gum. Both of these gums, and others, are also known as Indian, or India gum. Therefore, samples to be investigated must be obtained from as trustworthy a source as possible.

PURIFICATION AND FRACTIONATION

Since gums in general contain many impurities, both low molecular weight products and mechanical impurities such as adhering bark and dust, some treatment is necessary in order to isolate the pure polysaccharides. The powdered gum is usually dissolved in water, or if necessary in sodium hydroxide. Considerable swelling often accompanies this process.

Insoluble extraneous matter may then be filtered off, and the gum polysaccharide is precipitated from acidified ethanol or methanol (14), or from acetic acid (15), leaving low molecular weight material in solution. Further purification may be effected if necessary, by several reprecipitations, or by dialysis.

The precipitated polysaccharide material cannot be assumed to be homogeneous, since evidence of heterogeneity has been noted in the case of several gums. The main fractionation techniques available for the examination of polysaccharides include fractional precipitation, complex formation, various chromatographic methods, and electrophoresis.

Fractional precipitation is the simplest method. By the addition of small quantities of a precipitant such as ethanol to the aqueous solution of a gum, and immediate removal of any precipitate formed, components of different solubility properties may be separated. Co-precipitation may occur, and may necessitate several reprecipitations of each component. The gum component of Olibanum (16), and <u>Khaya</u> <u>senegalensis</u> gum (17) have been fractionated in this manner, the former into a neutral and an acidic component, and the latter into two acidic polysaccharides of high and low uronic acid content. Gum tragacanth, which consists of an acidic polysaccharide, an arabogalactan and a glycoside, was fractionated (49) by differ-

ences in the solubilities of the methylated components.

The ability of some polysaccharides to form insoluble complexes is also used in fractionation. Copper salts, such as cupric acetate (18), may form polysaccharide complexes which are insoluble in water or in aqueous ethanol. Long chain quaternary ammonium salts, such as 'Cetavlon', or cetyltrimethylammonium bromide, give precipitates with many acidic polysaccharides (19,20), and may be used to separate them (21); the method may also be extended to the separation of neutral polysaccharides, since these may give insoluble Cetavlon-boric acid-polysaccharide complexes (22).

Several chromatographic methods of fractionation have been developed, although their use has so far been restricted to a few polysaccharides of high uronic acid content. Mucopolysaccharides have been separated by Gardell (23) on a cellulose column, by gradient elution with ethanol containing increasing quantities of dilute aqueous barium acetate solution. Berenson et al. (24) have also carried out a separation using a partition technique between an aqueous phase and an organic phase containing a fatty amine, the support being a siliconized celite column. More recently, investigations carried out on the adsorption of polysaccharides by ion exchange cellulose have yielded promising results (25).

Other techniques, while they cannot bring about separations

on a preparative scale, may be used in order to detect heterogeneity. Immunological procedures have shown, for example, that gum arabic is non-homogeneous (26), and are of growing use.

Electrophoresis as a diagnostic test of homogeneity (27,28) was formerly of restricted use because of the absorption of the polysaccharides on the paper used; however, this difficulty has now been overcome by the use of an inert support such as glass fibre paper (29,30). Using this technique, Lewis and Smith (31) examined a number of gums, and as well as confirming heterogeneity in gum tragacanth and in gum arabic, they found two components in gum ghatti, *Acacia pycnantha* and *Acacia arabicum*, which had previously been thought to be homogeneous.

In view of the evidence of widespread heterogeneity in gums, the question arises of whether a definite structure, characteristic of the species, can be assigned to any gum. Torto (33) and Williams (34) have compared randomly selected nodules of *Fagara xanthoxyloides* and *Brachychiton diversifolium* gums respectively, and have found no significant variation in structure; on the other hand, Anderson, Hirst and King (35), in a detailed examination of several nodules of *Combretum leonense* gum, have found differences significant above experimental error, including a variation of 6% in uronic acid content. Hough and Pridham (36) have observed that the gums from the fruit

and from the bark of the Victoria plum tree, although electrophoretically identical, vary slightly in the proportions of the component sugar residues, and that periodate oxidation studies on the two gums give non-identical results. Gum arabic is known to vary in its rhamnose content (26).

It seems likely that, apart from the few gums such as gum tragacanth, which contain widely differing polysaccharides, the majority of gums may consist of 'families' of polysaccharides. These may, for example, possess a backbone of constant structure, to which variable side chains are attached. The extent of the variation is probably slight within each species, however, and increases considerably for the genus taken as a whole.

HYDROLYSIS OF THE GUM

In order to obtain a general picture of the polysaccharide, and to ascertain its state of purity, some preliminary measurements are carried out; these include measurement of optical rotation, neutralisation equivalent, methoxyl value, extent of acetylation, and nitrogen and ash contents. Uronic anhydride content is also measured, either by decarboxylation by heating with hydrochloric acid (37,38), or by a spectrophotometric method (39), which involves the formation of coloured derivatives of the methyl esters of the uronic acid groups.

The next step is the identification of the component sugars,

and in order to isolate these, the glycosidic links of the polysaccharide must be broken. The general hydrolysing agent is dilute hydrochloric or sulphuric acid, although some polysaccharides have been depolymerized enzymatically.

The stability of glycosidic links to acids varies considerably according to the class of sugar, and according to the ring form. Furanosides are in general much more acid-labile than pyranosides; in fact, the acidity of the hot aqueous solution of a gum may be sufficient to strip off arabinose end-groups, which usually exist in the furanose form. The process, which is known as autohydrolysis, is a very useful one, since the degraded polysaccharide produced has a simpler structure than the original gum, and is more easily identified (40).

At the other end of the scale are the uronic acids; glycuronosyl links, especially those involving galacturonic acid, are so stable to acids that the conditions necessary for hydrolysis inevitably result in a certain amount of degradation. The usual procedure, therefore, in the preliminary examination of a gum, is to hydrolyse the polysaccharide with normal acid, at 100°. Under these conditions, all except the glycuronosyl links are broken, and a mixture of neutral monosaccharides and acidic oligosaccharides is obtained. The latter are usually disaccharides, with the uronic acid residue glycosidically linked to a non-reducing group of a neutral sugar residue, and

and are known as aldobiouronic acids. The neutral and acidic components may be separated by means of anion exchange resins.

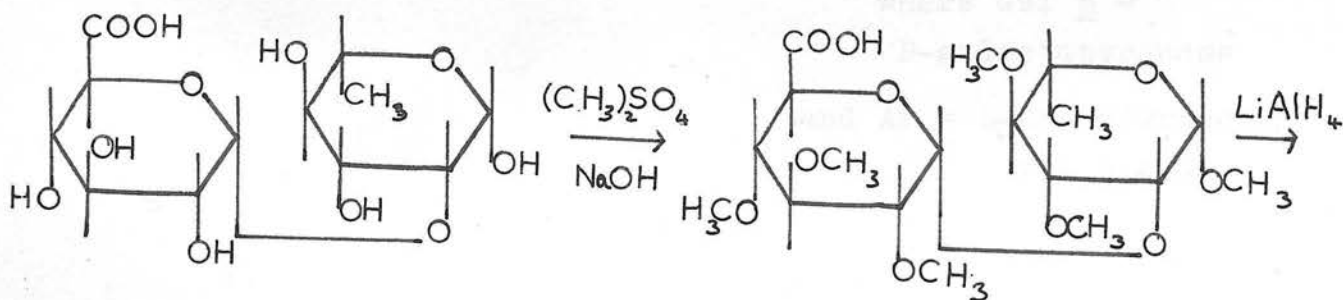
For the neutral sugars, as indeed for all sugar derivatives, chromatography on cellulose provides the ideal method of isolation and identification. The components of the mixture may be quickly and easily identified by paper chromatography (41), at the expense of less than a milligram of material. For an estimation of the proportions in which these components exist, a quantitative separation may be achieved on thick paper (42). The sugars separate as bands, which can be located by cutting off and spraying narrow side strips. After elution from the paper, each component is estimated by some micromethod, e.g. by means of the Somogyi copper reagent (43). Less accurate methods, liable to errors of 10%, involve the comparison of colour intensities of spots produced on chromatograms after spraying with a suitable reagent (44), or of the solutions obtained on elution of the sprayed papers (45). It must be remembered, however, that these proportions do not take into account the neutral sugars linked to uronic acid residues.

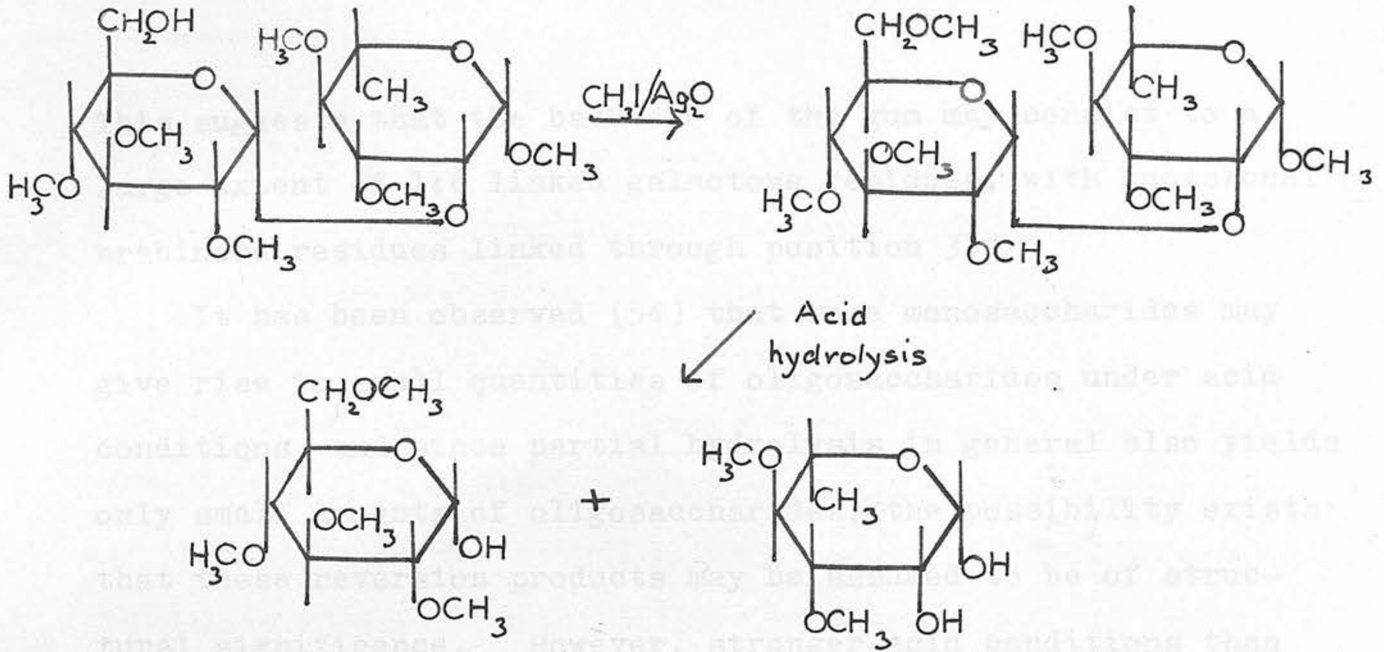
Final identification of the component monosaccharides, including differentiation between sugars of the D- and L-series, depends on the isolation and identification of crystalline derivatives. In order to obtain the relatively large quantities of sugars required for the preparation of these derivatives, the

mixture must be fractionated on a column of powdered cellulose (46,47).

Many gums give rise to only one aldobiouronic acid on hydrolysis, but in others the acidic fraction of the hydrolysate is a mixture, which it is desirable to fractionate. Fractionation has been achieved both by stepwise or gradient elution from weakly basic anion exchange resin columns, using increasing acid concentration (48), and by partition chromatography on cellulose columns, using acidic solvent (50).

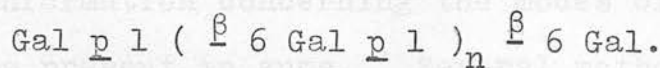
Each aldobiouronic acid may be identified by complete methylation (51), and reduction with lithium aluminium hydride (52) to the corresponding partially methylated disaccharide. This is easily hydrolysed, and the positions of the free hydroxyl groups on the partially methylated sugars obtained indicate the nature of the glycosidic link in the original acid. Remethylation of the disaccharide may also be carried out before hydrolysis. For example, an aldobiouronic acid from <u>Brachychiton</u> <u>diversifolium</u> (34) on methylation, reduction, remethylation and hydrolysis gave 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-L-rhamnose, and therefore had the structure 2-O-α-D-glucuronosyl-L-rhamnose.





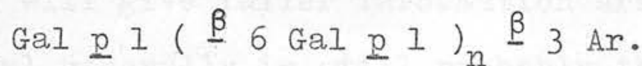
Some gums, provided that they do not contain too high a proportion of uronic acid residues, may be partially hydrolysed under carefully controlled conditions, using very dilute acid, to give neutral oligosaccharides. These may be separated on charcoal-celite columns, and identified by methylation and hydrolysis, and give useful information concerning gum structure.

Gum ghatti, when subjected to graded hydrolysis (53), gave the first three members (n = 0,1 and 2) of the homologous series of oligosaccharides:-



$$\text{Gal } \underline{p}\ 1\ (\ \overset{\beta}{-}\ 6\ \text{Gal } \underline{p}\ 1\ )_n\ \overset{\beta}{-}\ 6\ \text{Gal.}$$

and also the first four members of the series:-



$$\text{Gal } \underline{p}\ 1\ (\ \overset{\beta}{-}\ 6\ \text{Gal } \underline{p}\ 1\ )_n\ \overset{\beta}{-}\ 3\ \text{Ar.}$$

where Gal p = D-galactopyranose

and Ar = L-arabofuranose residues.

This suggests that the backbone of the gum may consist to a large extent of 1:6 linked galactose residues, with occasional arabinose residues linked through position 3.

It has been observed (54) that some monosaccharides may give rise to small quantities of oligosaccharides under acid conditions, and since partial hydrolysis in general also yields only small amounts of oligosaccharides, the possibility exists that these reversion products may be assumed to be of structural significance. However, stronger acid conditions than are usually used are probably necessary for appreciable amounts of reversion, and in any case, it is likely that an equilibrium is eventually reached between monosaccharides and reversion products, which therefore do not disappear on prolonged heating, and can thus be distinguished from true hydrolysis products.

METHYLATION STUDIES

The results of hydrolysis can furnish at best only fragmentary information concerning the modes of linkage of the sugar residues present in gums. Several methods of investigation which will give fuller information are available, but the most useful generally is still probably the classical method of methylation, followed by hydrolysis and identification of the hydrolysis products. On methylation, all the hydroxyl groups of the polysaccharide which are not involved in

glycosidic linkages are protected; hence, the positions of the free hydroxyl groups in the partially methylated sugars obtained on hydrolysis indicate the points of linkage. Moreover, it can be seen from the number of free hydroxyl groups whether each fragment is derived from the end group of a chain, a member of a straight chain, or a branch point. The limitation of the method lies, of course, in its inability to give any indication of the relative positions of the component monosaccharide residues in the polysaccharide chain. It must therefore be used in conjunction with partial hydrolysis and periodate oxidation studies.

Methylation is usually carried out initially by Haworth's procedure (51), using dimethyl sulphate and sodium hydroxide solution. This gives a partially methylated product, which is soluble in organic solvents, and can be fully methylated by Purdie's method (55), in which the methylating agents are methyl iodide and silver oxide. In a few cases, where these techniques have been unsuccessful, other methods, using thallium hydroxide and methyl iodide (56), or methyl iodide with sodium in liquid ammonia (57), have been employed. The latter method has been adapted to the micro-scale (58); here measured quantities of the alkali metal are added from a glass capillary to a closed system containing the methyl iodide and liquid ammonia. Using this technique, as little as 2 milligrammes of poly-

saccharide may be methylated at a time.

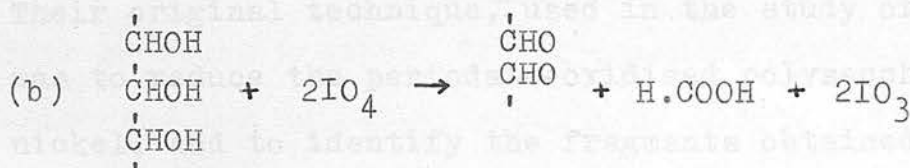
Fully methylated polysaccharides cannot be directly hydrolysed, since they are insoluble in hot inorganic acids. This difficulty may be overcome either by preliminary treatment with cold acid in order to lower the chain length (59), or by boiling with a methanolic solution of hydrochloric acid (60); in both cases hydrolysis is afterwards completed by treatment with hot dilute mineral acid. Alternatively, the methylated polysaccharide may be hydrolysed with hot 90% formic acid (61), and afterwards boiled with dilute sulphuric acid for a short time in order to hydrolyse formyl esters.

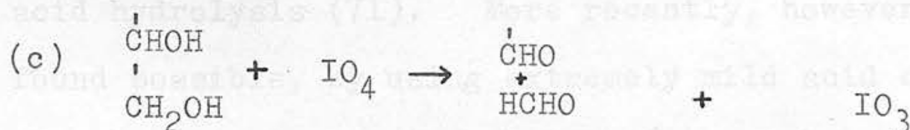
The products of hydrolysis are usually separated on cellulose columns (62), as in the case of non-methylated sugars, but celite (63) and charcoal-celite (64) columns have also been used. The rates of movement of methylated sugars on paper are fairly characteristic (65), and can help considerably in identification. Further information may be obtained from electrophoresis in borate buffer solution (66,67); sugars not otherwise easily distinguishable may be separated provided only one possesses the adjacent free hydroxyl groups which enable it to form a boric acid complex.

STRUCTURAL MODIFICATIONS

In order to obtain further structural information, the gum

being examined may be degraded, or otherwise modified in some way. In this field, sodium metaperiodate is a useful reagent, since the periodate ion will oxidise hydroxyl groups on adjacent carbon atoms, under mild aqueous conditions (68). If more than two contiguous hydroxyl groups are present, formic acid is produced. Primary alcohol end groups give rise to formaldehyde.



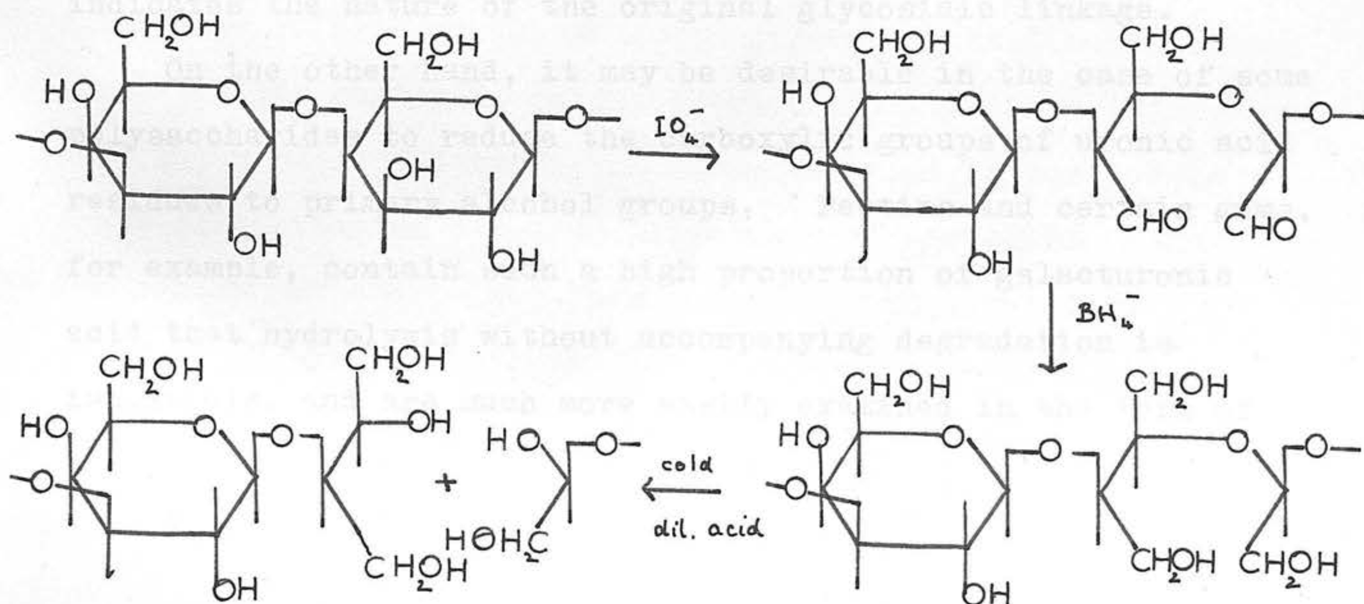


The uptake of periodate and the amount of formic acid and formaldehyde produced may be measured, and any sugar residues unattacked by the periodate may be estimated. From the results, it is possible to deduce much information concerning polysaccharide structure.

Variations of the method have been developed, which are also useful. In Barry's method of stepwise degradation (69), the oxidised polysaccharide is treated with phenylhydrazine acetate. This enables all the oxidised residues to be

progressively removed in the form of osazones, under mild conditions, leaving periodate-resistant portions of the molecule intact. For example, in the case of gum arabic, the side chains are completely removed by this type of degradation. The backbone of the molecule is resistant to further oxidation; the galactose units of which it is composed are therefore linked through the 1:3' positions (70).

An alternative procedure for the examination of polysaccharides has been developed by Smith and his co-workers. Their original technique, used in the study of amylopectin, was to reduce the periodate-oxidised polysaccharide with Raney nickel, and to identify the fragments obtained on ordinary acid hydrolysis (71). More recently, however, it has been found possible, by using extremely mild acid conditions e.g. normal acid at room temperature for one hour, to cleave only the acetal links in the degraded residues, leaving the glycosidic links intact.



Thus, as in the Barry degradation, the periodate-resistant portions of the molecule may be isolated. Further information may be obtained by the isolation of the low molecular weight fragments produced where adjacent sugar residues are attacked by periodate. This method has also been used in the structural investigations on gum arabic (72).

Another technique which may prove useful in the elucidation of gum structures is that of catalytic oxidation. By this means, it is possible to oxidise selectively the primary alcohol groups in a polysaccharide to carboxylic groups (73). This provides a method for determining the nature of linkages which involve acid-labile sugar residues, such as arabinofuranose end groups. Under these circumstances, it is impossible to isolate the disaccharides concerned by partial hydrolysis. The corresponding aldobiouronic acid, however, is easily isolated from the oxidised polysaccharide, since the glycuronosyl link is acid-resistant, and its identification indicates the nature of the original glycosidic linkage.

On the other hand, it may be desirable in the case of some polysaccharides to reduce the carboxylic groups of uronic acid residues to primary alcohol groups. Pectins and certain gums, for example, contain such a high proportion of galacturonic acid that hydrolysis without accompanying degradation is impossible, and are much more easily examined in the form of

the fully reduced polysaccharides, provided these can be obtained. Reduction has been carried out by first treating the polysaccharide with ethylene oxide, and afterwards reducing the glycol ester with potassium borohydride (74). Alternatively, if a polysaccharide derivative soluble in ether-type solvents can be formed, this may be reduced by diborane, generated _in situ_ in such solvents from boron trifluoride and sodium borohydride (75). Mesquitic acid acetate, for example, has been almost completely reduced in this way.

IMMUNOLOGICAL STUDIES.

The application of immunological techniques to the structural investigations of plant gums is relatively recent, but seems likely to become increasingly important in the near future. Its basis lies in the cross-reactions which can take place between bacterial antisera and polysaccharides which are specific to them. It has long been known that mutual precipitation occurs between any antipneumococcus or anti-salmonella serum and the bacterial polysaccharide isolated from that particular bacterial type, and moreover, that in some cases cross-precipitation can take place between bacterial types. The reason for the latter phenomenon, it was later shown, is that mutual precipitation is due to characteristic multiple groupings of sugar residues, which may be present in more than

one type of bacterial polysaccharide (76). Several of these polysaccharides have been subjected to structural investigations, which indicate that the most generally occurring sugar residues are those of galactose, rhamnose, glucose, galacturonic acid and glucuronic acid. They thus show a marked resemblance to the plant gums, and Heidelberger and his co-workers have in fact shown (77) that many gums give co-precipitation reactions with antipneumococcus sera. It is known in only a few cases what particular reactive groups are responsible for precipitation: in the case of pneumococcus Type I, for example, the reactivity is probably due to galacturonic acid residues, in Type II, to glucuronic acid end groups, glucose branch points, and 1,3-linked rhamnose residues, and in Type VIII, to multiple units of cellibiose and of cellobiuronic acid. However, as this field of knowledge is expanded, it will become increasingly easy to predict structural features of a polysaccharide of unknown constitution, if it is known with which bacterial antisera it will undergo mutual precipitation.

THE MOLECULAR STRUCTURE OF PLANT GUMS

A considerable number of gums have been subjected to preliminary investigations; those of which the component sugars are known are listed in Tables I, II, and III (pp. 29-33). The classification depends upon which uronic acid is present. Relatively few have been examined in any great detail, but the results which have so far been obtained suggest that it may also be useful to sub-divide gums into two categories, according to their uronic acid content. To a first approximation, gums within each category show general structural similarities.

The first, and much wider, class consists of exudates containing up to about 20% uronic acid, and includes the numerous gums of the Acacia and Prunus genera. In virtually every case, it is glucuronic acid, or a monomethyl derivative, which is responsible for the acidity.

In gums of the second category, on the other hand, the uronic acid content is usually at least 40%. The acidity is more often than not due to the presence of galacturonic acid residues, although not necessarily wholly so; two different uronic acid residues have been found in a single gum. Most exist in nature in a partially acetylated form. In physical properties, and to some extent in structure, they show resemblances to the pectins (32).

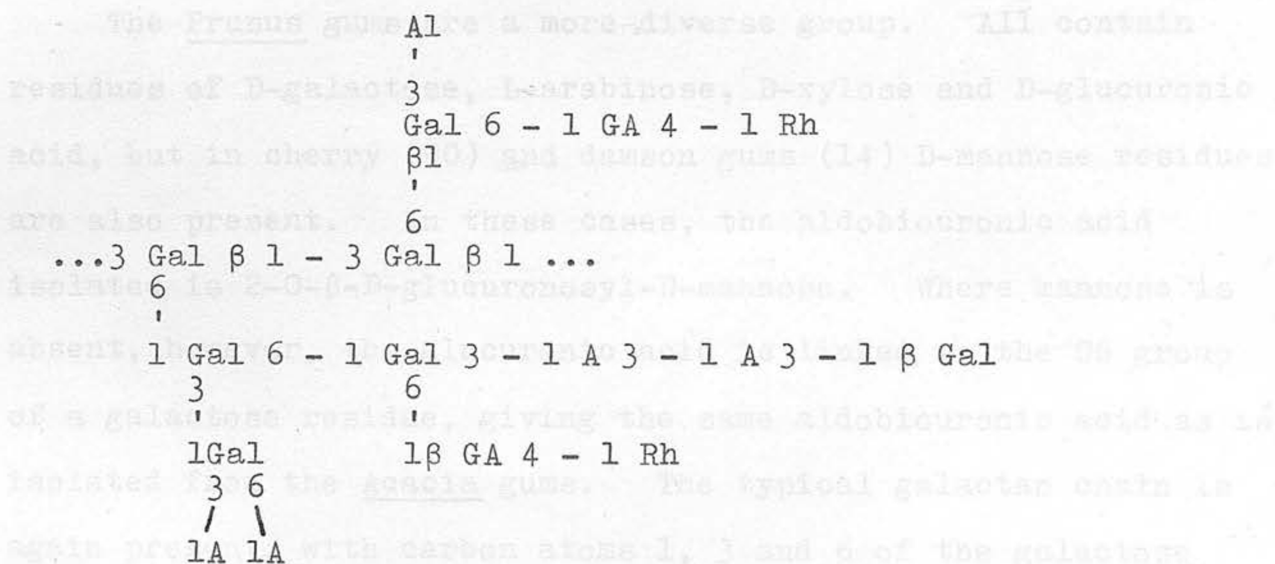
GUMS CONTAINING A LOW PROPORTION OF URONIC ACID

A typical feature of gums of this class is the presence of a central framework, or 'backbone', of galactose units, to which is attached a great variety of side chains. These may be quite complex, or may consist only of a single sugar residue.

In many cases, the periphery of the molecule consists largely of acid-labile residues, such as arabofuranose units. This being so, the technique of autohydrolysis is extremely useful in the examination of the low acid gums. On heating the aqueous solution of the gum, these peripheral groups are stripped off, leaving the degraded gum, a simpler structure which consists of the stable galactan framework with some residual side chains. The usual procedure is then to compare the results of methylation studies on the whole and degraded gums.

The Acacia genus includes gum arabic, which has probably been subjected to a closer investigation than any other gum (26, 40, 72, 77-81). In structure, it is fairly complex. The component sugar residues are D-galactose, D-arabinose, L-rhamnose and D-glucuronic acid. As far as is known at present, the galactose units of the main chain are glycosidically linked through the 1 and 3 positions, and the branches are attached through carbon atom 6. The rhamnose is present

entirely as end groups, and the arabinose partly as arabofuranose end groups, and partly linked through the 1 and 3 positions. Some arabopyranose units are also apparently present. The aldobiouronic acid isolated on hydrolysis is 6-O-β-D-glucuronosyl-D-galactose. No unique structure can be postulated for the gum, but the following is one of the possibilities.



where A = L-arabofuranose
Gal = D-galactopyranose
Rh = L-rhamnopyranose
GA = D-glucuronic acid.

The other gums of the genus are similar in many respects: they contain the same component sugar residues, although in varying proportions (see Table I, p.29). The aldobiouronic acid 6-O-β-D-glucuronosyl-D-galactose is common to all of them, but *Acacia karroo* (84) is apparently unique in the genus in

that it also gives 4-O-α-D-glucuronosyl-D-galactose on hydrolysis. Acacia pycnantha gum (86, 87), which is the only member of the genus besides gum arabic which has been the subject of detailed methylation studies, seems to be rather simpler in structure. It is less highly branched, some rhamnose units are attached directly to the backbone of the molecule, and the glucuronic acid occurs exclusively as end group.

The Prunus gums are a more diverse group. All contain residues of D-galactose, L-arabinose, D-xylose and D-glucuronic acid, but in cherry (90) and damson gums (14) D-mannose residues are also present. In these cases, the aldobiouronic acid isolated is 2-O-β-D-glucuronosyl-D-mannose. Where mannose is absent, however, the glucuronic acid is linked to the C6 group of a galactose residue, giving the same aldobiouronic acid as is isolated from the Acacia gums. The typical galactan chain is again present, with carbon atoms 1, 3 and 6 of the galactose residues taking part in linkages. The rather complex side chains vary fairly widely from one gum to another, but in general the pentose residues exist as acid-labile end groups, and are completely stripped off on autohydrolysis, leaving the degraded gum, containing galactose, glucuronic acid, and possibly mannose. In connection with the diversity observed within this genus, it is interesting to note that the English (90) and American varieties (94) of cherry exude gums which show considerable

variation in structure; for example, the former contains only traces of xylose, while the proportion of xylose in the latter is unusually high. Moreover, in the case of the gum from the Victoria plum tree, the proportions of the component sugars vary according to whether it is exuded from the bark, or from the fruit (36).

Gum ghatti, the exudate of Anogeissus latifolia (101-103), is in several respects different from either of the above groups. Its component sugar residues are D-galactose, D-mannose, L-arabinose, D-xylose and D-glucuronic acid, with traces of L-rhamnose. The glucuronic acid residues are linked in two ways; the aldobiouronic acids 2-O-β-D-glucuronosyl-D-mannose and 6-O-β-D-glucuronosyl-D-galactose, which are not usually found together in the Prunus genus, are both present in gum ghatti. The results of partial hydrolysis studies, mentioned earlier, suggest that the backbone of the molecule may be unusual in consisting largely of galactose units linked in the 1:6' positions, with possibly an occasional arabinose residue, linked through positions 1 and 3, incorporated into the main chain. In other respects, however, the structure follows the usual pattern of galactan backbone, with aldobio-uronic acid containing side chains, to which are attached acid-labile pentose end groups.

This general pattern is also followed by the gums containing

4-O-methyl glucuronic acid residues (Table II, p.32). The mode of linkage of the acid residues, however, is variable. In the Citrus gums (120-123), the aldobiouronic acids are 4-O-(4-O-methyl-α-D-glucuronosyl)-L-arabinose and 4-O-(4-O-methyl-α-D-glucuronosyl)-D-galactose. The latter is also isolated from gum myrrh (126), and both frankincense gum (124) and gum myrrh, which are closely related, also give rise to 6-O-(4-O-methyl-β-D-glucuronosyl)-D-galactose on hydrolysis. Further examples are shown in Table II.

The exudate of Combretum leonense (106) cannot readily be classified. It is slightly acetylated and contains L-arabinose, D-galactose and L-rhamnose in the proportions 9:10:1. Both glucuronic and galacturonic acid residues are present. A di- and a trisaccharide consisting of 1:6' linked galactose units have been isolated, as well as 3-O-D-galactopyranosyl-L-arabinose, and the aldobiouronic acid 6-O-β-D-glucuronosyl-D-galactose. Part of the arabinose is very easily removed under mild acid conditions. All this evidence suggests a general resemblance to the gums of the Acacia and Prunus genera, but, on the other hand, the aldobiouronic acid 2-O-α-D-galacturonosyl-L-rhamnose, and, possibly, the aldotriouronic acid O-D-galacturonosyl-(1→2)-O-L-rhamnosyl-(1→?)-D-galactose, have also been obtained, and these are typical of the high acid gums. Considering this, and the fact that the uronic acid

content has been found to vary between 14% and 20% (25), it seems fairly likely that the gum is not homogeneous, but consists of a mixture of two polysaccharides of different types.

TABLE I : GUMS CONTAINING D-GLUCURONIC ACID (Key - see p.31).

Source of Gum	GA	Gal	Ar	Rh	Ma	Xy	Other Sugars	Neutral Disaccharides	Aldobiouronic acids	Ref.
<u>Acacia gums</u>										
Acacia senegal (gum arabic)	1	3	3	1	-	-	-	Gal 1 - 3 Ar Gal 1 $\xrightarrow{\beta}$ 3 Gal	GA 1 $\xrightarrow{\beta}$ 6 Gal	26,40, 72,77-81
Acacia catechu	3	9	4	3	-	-	-	-	GA 1 $\xrightarrow{\beta}$ 6 Gal	82
Acacia cyanophylla	5	11	2	5	-	-	-	Gal 1 $\xrightarrow{\alpha}$ 3 Ar	GA 1 $\xrightarrow{\beta}$ 6 Gal	83
Acacia karroo	6	28	24	1	-	-	-	Ar 1 $\xrightarrow{\beta}$ 3 Ar	GA 1 $\xrightarrow{\beta}$ 6 Gal GA 1 $\xrightarrow{\alpha}$ 4 Gal	84
Acacia mollissima (black wattle gum)	1	5	6	1	-	-	-	Ar 1 $\xrightarrow{\beta}$ 3 Ar (tr)	GA 1 - 6 Gal	85
Acacia pycnantha	3	40	20	1	-	-	-	Gal 1 $\xrightarrow{\beta}$ 3 Gal	GA 1 $\xrightarrow{\beta}$ 6 Gal	86,87
Acacia sundra	2	5	4	2	-	-	-	-	GA 1 $\xrightarrow{\beta}$ 6 Gal	88
<u>Prunus gums.</u>										
Almond	1	3	4	-	-	2	-	-	GA 1 - 6 Gal	89
Cherry: English	1	2	6	-	1	tr	-	Ar 1 $\xrightarrow{\beta}$ 3 Ar	GA 1 $\xrightarrow{\beta}$ 2 Ma	90-93
Cherry: U.S.Wild Cherry	2	6	8	-	3	6	-	-	GA 1 - Ma	94
Damson	1	2	3	-	1	tr	-	-	GA 1 $\xrightarrow{\beta}$ 2 Ma	14,95,96
Egg plum	1	3	3	-	-	1	-	-	GA 1 - 6 Gal	97-99
Peach	1	5	6	tr	-	2	-	Ar 1 $\xrightarrow{\beta}$ 3 Ar Xy 1 $\xrightarrow{\beta}$ 5 Ar	GA 1 - 6 Gal	93,100
Plum	+	+	+	-	+	+	4MeGA (tr)	-	-	36

TABLE I (contd.)

Source of Gum	GA	Gal	Ar	Rh	Ma	Xy	Other Sugars	Neutral Oligosaccharides	Aldobiouronic Acids	Ref.
Other genera										
Anogeissus latifolia (gum ghatti)	1	3	5	tr	1	$\frac{1}{2}$	-	Gal 1 - 6 Gal Gal1-6Gal1-6Gal Gal 1 - 3 Ar Gal1-6Gal1-3Ar	GA 1 $\xrightarrow{\beta}$ 2 Ma GA 1 $\xrightarrow{\beta}$ 6 Gal	53, 101,102
Anogeissus schimperi	+	+	+	tr	+	+	Fu(tr) Ri(tr)	Gal 1 - 3 Gal Gal 1 - 3 Ar Gal 1 - 6 Gal Ar_f 1 - 3 Ar	GA 1 - 2 Ma GA 1 - Gal	103, 104
Combretum leonense	+	+	+	+	-	-	GalA	Gal 1 - 6 Gal Gal1-6Gal1-6Gal Gal 1 - 3 Ar	GA 1 - 6 Gal GalA 1 - 2 Rh (also GalA 1-2 Rh 1- Gal)	35, 105
Combretum verticillatum	+	+	+	-	-	-	-			106
Puya chilensis (Chagual)	15	22	5	-	-	23	-		GA 1 $\xrightarrow{\alpha}$ 2 Xy	10
Albizzia zygia	+	+	+	tr	+	-	4MeGA			107
Brachychiton diversifolium	5	3	-	2	-	-	-		GA 1 $\xrightarrow{\alpha}$ 2 Rh	34
Hakea acicularis	4	32	13	-	4	5	-		GA 1 $\xrightarrow{\beta}$ 2 Ma	108
Gleditschia triacanthes (Honey Locust)	5	9	16	-	-	-	-		GA 1 - Gal	11
Feronia elephantum (Ketha)	+	+	+	tr	-	+	-		GA 1 - 3 Gal	109,110

TABLE I (contd.)

Source of Gum	GA	Gal	Ar	Rh	Ma	Xy	Other Sugars	Aldobiouronic acids	Ref.
Melia azadirachta (Neem)	+	+	+	-	-	tr	Fu	GA 1 - 4 Gal	111
Phormium tenax	+	-	-	-	-	Xy	-		112
Moringa pterygosperma (Drum stick)	2	7	10	tr	-	-	-		113, 114
Grevillea robusta (Silk oak)	+	+	+	-	-	-	-	GA 1 - Gal	115
Sapota achras (Sapote)	+	-	+	-	-	+	-	GA 1 - Xy	116-118

Key:- GA = D-Glucuronic acid
4MeGA = 4-O-Methyl-D-glucuronic acid
GalA = D-Galacturonic acid
Gal = D-Galactose
Ar = L-Arabinose
Rh = L-Rhamnose
Ma = D-Mannose
Xy = D-Xylose
Fu = L-Fucose
Ri = Ribose
tr = trace.

Figures refer to molar proportions of sugar residues.

TABLE II : GUMS CONTAINING 4-O-METHYL-D-GLUCURONIC ACID

Source of Gum	4MeGA	Gal	Ar	Other Sugars	Neutral Disaccharides	Aldobiouronic Acids	Ref.
Lemon	12	34	17	-	Ar 1 $\xrightarrow{\beta}$ 3 Ar	4 MeGA 1 $\xrightarrow{\alpha}$ 4 Gal 4 MeGA 1 $\xrightarrow{\alpha}$ 4 Ar	119-122
Grapefruit	4	8	3	-	-	-	119
Fagara xanthoxyloides	+	+	+	-	-	-	33
Frankincense	4	7	1	Fu, Rh	-	4 MeGA 1 $\xrightarrow{\beta}$ 6 Gal	123
Myrrh	7	8	2	-	-	4 MeGA 1 $\xrightarrow{\beta}$ 6 Gal 4 MeGA 1 $\xrightarrow{\alpha}$ 4 Gal	124, 125
Prosopis juliflora (Mesquite Gum)	1	2	4	-	-	4 MeGA 1 $\xrightarrow{\beta}$ 6 Gal 4 MeGA 1 - 4 Gal	126-133
Lannea grandis (Modal gum)	1	5	1	-	-	4 MeGA 1 - 6 Gal	134
Spondias cythera (Golden apple gum)	+	+	+	Xy, Rh(tr) Fu(tr)	Ar 1 $\xrightarrow{\beta}$ 3 Ar Xy 1 $\xrightarrow{\alpha}$ 3 Ar Gal 1 $\xrightarrow{\beta}$ 6 Gal Gal 1 $\xrightarrow{\beta}$ 3 Gal	4 MeGA 1 $\xrightarrow{\beta}$ 6 Gal 4 MeGA 1 $\xrightarrow{\alpha}$ 3 Ar	135

Key:- 4 MeGA = 4-O-Methyl-D-glucuronic acid.
Gal = D-Galactose.
Ar = L-Arabinose.
Xy = D-xylose.
Fu = L-Fucose.
Rh = L-Rhamnose.
tr = trace.

TABLE III : GUMS CONTAINING D-GALACTURONIC ACID RESIDUES

Source of Gum	GalA	Gal	Rh	Ar	Other Sugars	Acidic Oligosaccharides	Ref.
Sterculia setigera	8	5	5	-	Ta (1)	Gal A 1 - 2 Rh Gal A 1 - 4 Gal	136, 137
Sterculia tormentosa	3	1	1	-	-	-	138
Sterculia urens (Karaya gum)	5	6	4	-	-	-	139
Khaya grandifolia	4	3	2	tr	4MeGA(1)	Gal A $1 \overset{\alpha}{-} 2$ Rh 4MeGA $1 \overset{\alpha}{-} 4$ Gal Gal A $1 \overset{\alpha}{-} 2$ Rh 1-4 Gal	140,59
Khaya senegalensis Major component	+	+	+	+	4MeGA	Gal A $1 \overset{\alpha}{-} 2$ Rh 4MeGA $1 \overset{\alpha}{-} 4$ Gal	17
Minor component	+	+	+	+	4MeGA	-	
Cochlospermum gossypium.	+	+	+	-	-	Gal A $1 \overset{\alpha}{-} 2$ Rh Gal A 1 – 4 Gal	141
Opuntia fulgida (Cholla gum)	1	3	tr	6	Xy(2)	-	93
Odina wodier (Gum jeol)	+	+	-	+	-	Gal A 1 - Gal	142, 143
Astralagus sp. (Gum tragacanth) I	-	1	-	1	-	-	49,144
II	+	-	-	-	Xy,Fu	-	145

Key:- GalA = D-Galacturonic acid Rh = L-Rhamnose Ta = D-Tagatose Fu = L-Fucose tr = trace
Gal = D-Galactose Ar = L-Arabinose Xy = D-Xylose 4MeGA = 4-O-methyl-D-glucuronic acid.

GUMS CONTAINING A HIGH PROPORTION OF URONIC ACID. (Table IV, p.43)

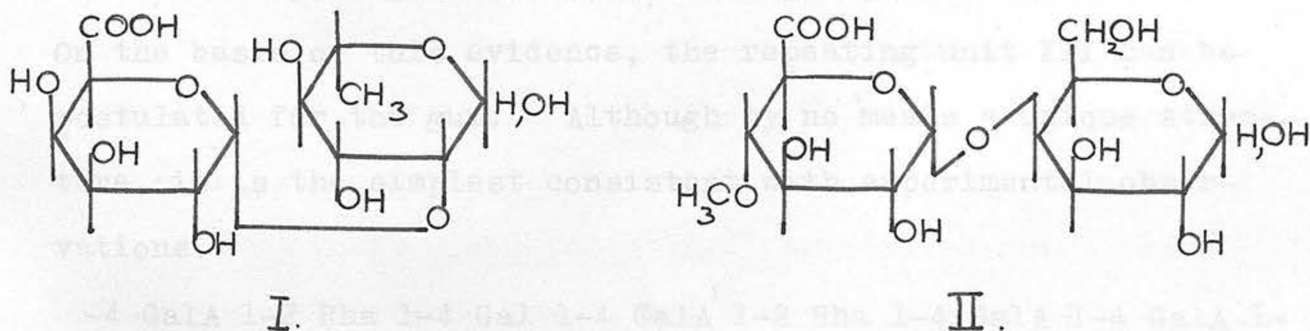
The high acid gums present, structurally, a very different picture. The main chain is not a galactan but is likely to contain a high proportion of uronic acid residues. The typical galactose linkage is 1,4, rather than 1,3 or 1,6. Acid-labile groups are present in very small proportion, if at all; the technique of autohydrolysis is therefore not applicable. Also, it has not so far been found possible to isolate neutral oligosaccharides by mild hydrolysis. Structural investigations have usually rested, therefore, on methylation studies and on identification of the aldobiouronic acids obtained on hydrolysis.

The gum exudate of Khaya grandifolia, the West African mahogany tree, is a typical high acid gum which has been fairly fully investigated by Aspinall, Hirst and Matheson (59). It is known to contain residues of D-galactose, L-rhamnose, D-galacturonic acid and 4-O-methyl-D-glucuronic acid, in the approximate proportions of 3:2:4:1, with traces of L-arabinose. The acidic material makes up 47% of the whole gum.

On methylation and hydrolysis, the polysaccharide gave rise to 2,3,4,6-tetra-O-methyl-D-galactose, 3-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-glucuronic acid, and 2,3-di-O-methyl-D-galacturonic acid.

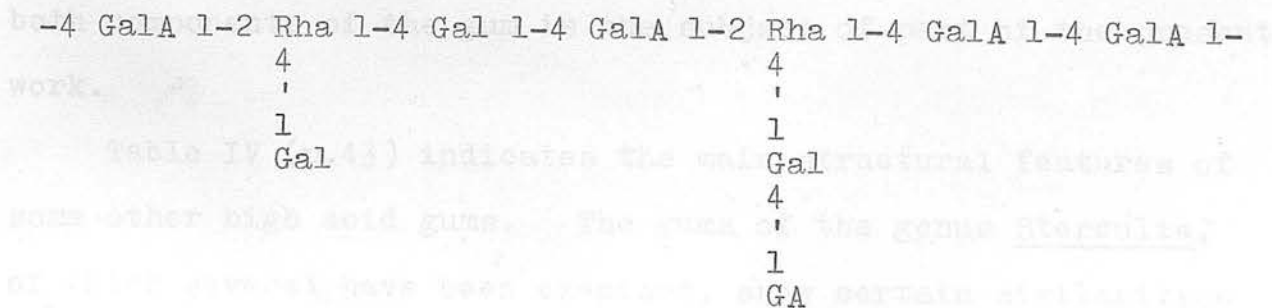
Partial hydrolysis of the gum gave a mixture of acidic oligosaccharides, which was found to contain galacturonic acid,

a mixture of aldobiouronic acids which could not be separated and an aldotriouronic acid. The aldobiouronic acid mixture, on methylation, reduction, remethylation and hydrolysis, gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose. On this evidence, two alternative pairs of aldobiouronic acids could be postulated; either 2-O-D-galacturonosyl-L-rhamnose (I), and 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose (II), or 4-O-D-galacturonosyl-D-galactose and 2-O-(4-O-methyl-D-glucuronosyl)-L-rhamnose. However, the facts that the aldobiouronic acids moved at the same rate on paper, and had similar ionophoretic mobilities, could only be explained by assigning to them structures I and II.



These acids are well-known hydrolysis products of plant gums and mucilages (119, 125, 132, 106, 136, 142, 146-148). The aldotriouronic acid was identified as O-D-galacturonosyl-(1→2)-O-L-rhamnosyl-(1→4)-D-galactose.

From the results of the methylation studies, it is clear that the molecule contains end groups of 4-O-methylglucuronic acid and of galactose residues, 1,4-linked galactose units, 1,4-linked galacturonic acid units and rhamnose branch points. The high uronic acid content necessarily implies that the main chain contains adjacent galacturonic acid residues. The isolation of 2-O-D-galacturonosyl-L-rhamnose (I) and of O-D-galacturonosyl-(1→2)-O-L-rhamnosyl-(1→4)-D-galactose on hydrolysis shows that the rhamnose residues are linked through positions 1 and 2 in the main chain, side chains being linked through position 4 and that some of the 1,4-linked galactose residues are also present in the main chain. The remainder of the 1,4-linked galactose, as is shown by the isolation of 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose, is interposed between the 4-O-methyl-D-glucuronic acid end groups and the main chain. On the basis of this evidence, the repeating unit III can be postulated for the gum. Although by no means a unique structure, it is the simplest consistent with experimental observations.



(GalA = D-galactopyranuronic acid, Gal = D-galactopyranose, Rha = L-rhamnopyranose, and GA = 4-O-methyl-D-glucopyranuronic acid.)

The exudate of another member of the genus, Khaya senegalensis, appears on preliminary examination to be very similar, since it contains the same monosaccharide residues, although in different proportions (59). It has, however, been found to be heterogeneous and can be separated by fractional precipitation into a major fraction containing over 40% uronic acid and a minor fraction containing only about 20%. The major fraction has been subjected to methylation studies by Stephen (17), who established the presence of the same methylated sugars as were isolated from K. grandifolia, together with smaller amounts of 2,3,5-tri-O-methyl-L-arabinose, 2,3,4-tri- and 2,4-di-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-D-glucuronic acid. Since glass-fibre paper ionophoresis showed that the material being examined was not quite homogeneous, it seems likely that the methylated sugars present in smaller amount arose from some of the minor fraction present as an impurity. If this is in fact the case, the major component of K. senegalensis gum must have very close structural similarities to K. grandifolia gum. Further examination of both components of the gum is the subject of part of the present work.

Table IV (p.43) indicates the main structural features of some other high acid gums. The gums of the genus Sterculia, of which several have been examined, show certain similarities

of structure to the Khaya gums. In the exudate of S. setigera, the cacao tree (136, 137), the D-galactose units are again present both as end groups and as members of straight chains, linked through positions 1 and 4, and the L-rhamnose residues exist as branch points. The D-galacturonic acid units, however, also take part in branching and some of the rhamnose exists as straight chain components. Partial hydrolysis studies give indications of the presence of 2-O-α-D-galacturonosyl-L-rhamnose, and of 4-O-D-galacturonosyl-D-galactose, but these fragments have not been unambiguously identified. An unusual feature is the presence of D-tagatose. This has been isolated from the hydrolysis product of the gum, but apparently does not survive the methylation procedures, since no methylated derivatives have been isolated; its structural significance is therefore not known. This may have some connection with the very high ratio (approximately 3:1) of branch point to end group residues, as obtained from the results of the methylation studies. It is evident that some residue which exists as end group in the gum has not been identified as such. A considerable amount of evidence therefore remains to be accumulated, before any attempt can be made to put forward a general structure for the gum.

The exudates of S. tormentosa (138) and of S. urens (Karaya gum)(139), both contain residues of D-galactose, L-rhamnose and D-galacturonic acid, in varying proportions but in neither case

has the presence of D-tagatose been observed.

It has already been mentioned that <u>Cochlospermum gossypium</u> gum closely resembles the Sterculia gums in physical properties. This resemblance is also evident on a molecular level. The work of Hirst and Dunstan (141) showed that the gum contained D-galactose residues linked through positions 1 and 4, D-galactose end groups, L-rhamnose linked through positions 1 and 2, and L-rhamnose branch points, involving carbon atoms 1,2 and 4. Some rhamnose was also present as end groups. As far as the acidic portion of the gum was concerned, the results of methylation studies were by no means clear, but it was suggested that D-galacturonic acid might be present both as branch points and linked through positions 1 and 4. Partial hydrolysis studies gave indications, but not definite proof, that galacturonic acid residues were linked to the C2 positions of rhamnose and to the C4 positions of galactose residues.

New light, however, has been shed on the structure of the gum by the immunological studies of Heidelberger and his co-workers (12), who have found that it undergoes mutual precipitation with the antipneumococcus sera Type II and Type XIV only. Co-precipitation with Type I antiserum would be expected, since the characteristic reactive group of the type is galacturonic acid. The <u>Khaya</u> gums undergo co-precipitation with Type I antiserum, presumably due to the presence of 1,4-

linked galacturonic acid residues; it would therefore seem that these particular residues are not present in <u>Cochlospermum gossypium</u> gum. The reactive groupings for Type II antiserum co-precipitation are multiple units of D-glucuronic acid end groups, 1,4,6-linked D-glucose, and 1,3-linked L-rhamnose. In view of the uncertainty connected with the acidic portion of the polysaccharide, it seems likely that the co-precipitation observed arises from the presence of the first of these groups and that both glucuronic and galacturonic acids are present in the gum. The reaction with Type XIV antiserum serves to confirm the presence of D-galactose end groups.

The gum from <u>Brachychiton diversifolium</u> (34) contains 50% of uronic acid and in some respects is a typical high-acid gum. It was formerly known as <u>Sterculia caudata</u>, and in fact does resemble the <u>Sterculia</u> gums in so far as methylation studies have shown that 1,2- and 1,2,4-linked rhamnose residues, 1,4-linked galactose residues and galactose end groups are present. As far as is known, however, the acidity of the gum is due entirely to D-glucuronic acid, almost all of which exists as end group. Partial hydrolysis studies suggest that the acid is linked to the C2 position of rhamnose residues.

Thus here, as in the case of <u>S. setigera</u>, there is a rather large disparity between the proportions of end group and of branch point, as estimated by methylation and hydrolysis. The

indications are that some other residues which have not yet been identified, also take part in branching. It is worthy of note that, although no D-galacturonic acid residues were found in the gum, the proportion of D-galactose in the hydrolysate increased markedly on reduction.

Gum tragacanth, the exudate of shrubs of the genus Astralagus, differs from the gums so far discussed in that it can very easily be separated into three components, a neutral polysaccharide, an acidic polysaccharide and a glycosidic component, which is apparently steroid in character. Fractionation has been carried out on the methylated gum, by means of the differences in solubility of the components (49).

The neutral polysaccharide is composed mainly of L-arabinose units, together with some D-galactose. The ease of hydrolysis indicates that the arabinose exists mainly in the furanose form. The isolation of 2,3,5-tri-O-methyl-L-arabinose, 2,3-di-O-methyl-L-arabinose and free L-arabinose after methylation and hydrolysis (145) suggests that the polysaccharide is an araban, containing double branch points. In this feature, it resembles cholla gum (93). The galactose residues also take part in branching; the polysaccharide is therefore not a simple araban.

The acidic polysaccharide, often referred to as tragacanthic acid, contains residues of D-galacturonic acid, D-xylose

and L-fucose (49). Methylation followed by hydrolysis gives 2,3,4-tri-O-methyl-L-fucose, 2,3,4-tri-O-methyl-D-xylose, 3,4-di-O-methyl-D-xylose, 2,3-di-O-methyl-D-galacturonic acid and a monomethyl-D-galacturonic acid. These must arise from a highly branched polysaccharide, with fucose and xylose end groups, 1,2-linked xylose and 1,4-linked galacturonic acid residues and galacturonic acid branch points.

TABLE IV : MAIN STRUCTURAL FEATURES OF SOME HIGH-ACID GUMS

Source of Gum	End Groups	Straight chain components	Branch Points	Acidic Oligosaccharides	Ref.
Sterculia setigera	Gal 1-	-4 Gal 1-	-4 GalA 1- 3 1	GalA 1 α 2 Rh	136,137
		-2 Rh 1 -			
			-4 Rh 1 - 2 or 3 1	GalA 1 - 4 Gal	
Brachychiton diversifolium	Gal 1-	-4 Gal 1-	-4 Rh 1- 2 1	GA 1 α 2 Rh	34
(S. caudata)	GA 1-	-2 Rh 1-			
Cochlospermum gossypium	Gal 1- Rh 1-	-4 Gal 1- -2 Rh 1-	-4 Rh 1- 2 1	GalA 1 - 2 Rh	141
		-GalA 1-?	-GalA 1-? 1	GalA 1 - 4 Gal?	
Khaya grandifolia	Gal 1- 4MeGA 1-	-4 Gal 1- -4 GalA 1-	-4 Rh 1- 2 1	GalA 1 α 2 Rh 4MeGA 1 α 4 Gal GalA 1-2 Rh 1-4 Gal	59
Khaya senegalensis	Gal 1-	-4 Gal 1-	-4 Rh 1- 2 1		17
(major component)	4MeGA 1-	-4 GalA 1-			
Gum Tragacanth	Fu 1-	-2 Xy 1-	-?GalA 1- ? 1		49
(Tragacanthic acid)	Xy 1-	-4 GalA 1-			

Key:- to sugar residues
Gal = D-Galactosyl
Rh = L-Rhamnosyl
Fu = L-Fucosyl
Xy = D-Xylosyl
GA = D-Glucuronosyl
4MeGA = 4-O-methyl-D-glucuronosyl
GalA = D-Galacturonosyl

OBJECT OF THE PRESENT INVESTIGATION

A very detailed examination had already been carried out on *Khaya grandfolia* gum, by Aspinall, Hirst and Matheson (59). The related gum from *Khaya senegalensis* had, on preliminary examination, been found to be heterogeneous, and methylation studies by Stephen on the major, high-acid component indicated that there were probably close similarities between the latter and *K. grandifolia* gum (17). It was considered to be of interest to complete this investigation by partial hydrolysis studies, and thus to ascertain whether the structural similarities within the *Khaya* genus are as close as, for example, in the *Acacia* genus. It was also hoped to make a fairly close examination of the minor, low-acid component of the gum.

It would also be of interest to make a comparison between genera, as well as within a single genus. The exudate of *Cochlospermum gossypium* had been studied by Hirst and Dunstan (141), and appeared to have several structural features in common with the *Khaya* gums, viz. 1,4-linked D-galactose units, D-galactose end groups and L-rhamnose branch points. One of the aldobiouronic acids of *Khaya grandifolia*, 2-O-α-D-galacturonosyl-L-rhamnose, was also thought to be present. The nature of the acidic portion of the polysaccharide, however, was not fully understood. It was therefore hoped to carry

out further investigations on this portion, and thus to be able to make a fuller comparison between the _Khaya_ and _Cochlospermum_ gums.

SECTION I

THE MAJOR COMPONENT OF KHAYA SENEGALENSIS GUM.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS

Paper chromatography was carried out on Whatman No 1 or No 4 filter paper. The following solvent systems were used:-

(A) Butan-1-ol - ethanol - water (4:1:5, v/v).

(B) Benzene - ethanol - water (169:47:15, v/v).

(C) Butan-1-ol - acetic acid - water, (8:2:5, v/v).

(D) Butan-1-ol - acetic acid - water (4:1:5, v/v).

(E) Ethyl acetate - pyridine - water (10:4:3, v/v).

(F) Ethyl acetate - acetic acid - formic acid - water (18:3:1:4, v/v).

(G) Butan-2-one half-saturated with water.

(H) Butanol - pyridine - water - benzene (5:3:3:1, v/v).

In the case of two-phase solvents, the upper, organic layer was used. Chromatograms of reducing sugars were sprayed with a saturated aqueous solution of aniline oxalate, and developed by heating for 10 min. at 100-120°, when spots of various characteristic colours appeared.

Where sugar alcohols were present, chromatograms were developed by successive immersion in saturated solutions of silver nitrate in acetone and sodium hydroxide in ethanol. The black spots indicating the positions of the polyhydroxy compounds appeared in about two minutes. The papers were

then treated with aqueous sodium thiosulphate solution, and washed for 30 min. in running water.

The rate of movement of methylated sugars was given relative to that of 2:3:4:6-tetra-O-methyl-D-glucose in solvent A, and was referred to as the R_G value. That of non-methylated sugars was given relative either to the solvent front (R_f), or to some convenient standard sugar (e.g. $R_{galactose}$).

Quantitative separation of sugars on thick paper (Whatman No3 MM)

The position of the sugars after separation was ascertained by cutting out and spraying a narrow centre strip. The sugars were eluted from the paper with cold water.

In the case of uronic acids, the papers were dried in air until completely free from solvent, and sprayed with bromothymol blue, which caused the areas containing uronic acid to show up as yellow patches on a blue background. After elution of the paper, the sugar solutions were treated with charcoal in order to remove the dye.

Cellulose columns were in general packed dry, and washed with water, followed by the solvent to be used. Butan-1-ol for use on columns was purified by heating under reflux for one hour with sodium hydroxide (10 g./litre of solvent), and distilling; light petroleum (b.p. 100-120°) was shaken with three portions (each 10% by volume) of concentrated sulphuric acid, washed free from acid, and distilled.

Fractions of 15-20 ml. were collected from columns, and an 8 ml. sample from every fifth tube was evaporated to dryness and examined chromatographically. Fractions were bulked accordingly, and evaporated to dryness, and the syrups obtained were cleaned by dissolving in water and treating with charcoal.

Paper ionophoresis (66, 67) was carried out in borate buffer solution of pH 10, at a potential of 500 volts. Papers were run for 5 hours, and sprayed with a saturated aqueous solution of aniline oxalate, acidified with glacial acetic acid.

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

Hydrolyses were carried out at 100°. Where sulphuric acid was used, barium hydroxide solution was added until the solution was almost neutralised, and neutralisation was completed by addition of solid barium carbonate. The barium sulphate and excess barium carbonate were filtered off, and the filtrate was evaporated to dryness.

Small scale hydrolyses on 5 mg. of sugar were carried out in sealed tubes, using hydrochloric acid (1 ml.). Neutralisation was effected by adding excess silver carbonate, filtering, passing hydrogen sulphide, filtering off the silver sulphide formed, evaporating to dryness, and taking up in a minimum of

solvent and refiltering if necessary. Products of methanolysis were neutralised in the same manner.

Optical rotations were observed at 18±2°, in aqueous solution unless otherwise stated.

Methoxyl determinations were carried out by the Zeisel semi-micro procedure (149).

Aniline derivatives were prepared by dissolving equimolecular quantities of the sugar and redistilled aniline in dry ethanol, and refluxing the solution for 20 min. under nitrogen, in the absence of light. Evaporation of the solvent gave the aniline derivative, which was recrystallised from ethanol. P-Toluidine derivatives were prepared in the same manner.

Lactones of aldonic acids of methylated sugars were prepared by oxidation with bromine water. The sugar (10 - 100 mg.) was dissolved in water (2 - 3 ml.), bromine (5 - 20 drops) was added, and the mixture was kept in the dark at room temperature for three days. Excess bromine was removed by aeration, and the solution was neutralised with silver carbonate in the usual manner, and evaporated to dryness. The residue was extracted with ethanol or ethyl acetate, and the lactone was recrystallised from one of a variety of solvents.

Potassium borohydride reductions (150) were effected by

dissolving equal weights of the sugar and the reducing agent in water, and allowing the solution to stand overnight at room temperature. Excess hydride was then destroyed, and potassium ions removed, by shaking the solution with Amberlite resin IR-120(H) for 30 min. This was followed by filtration and evaporation. Borate ions were removed by the repeated addition and evaporation of methanol.

Tetrahydrofuran for lithium aluminium hydride reductions was purified by allowing to stand over sodium wire for 48 hours, distilling off fresh sodium, and finally distilling off lithium aluminium hydride.

Small scale periodate oxidation of methylated sugars was carried out by the method of Lemieux and Bauer (151). The syrup (1 mg.) was dissolved in 0.5N sodium metaperiodate solution (0.12 ml.), and kept at 0° for 1 hour. Ethylene glycol (1 drop) was added, and the solution, after 5 min., was made alkaline to phenolphthalein with 0.5N sodium hydroxide solution. The whole of the solution was spotted on paper, and examined chromatographically in solvent A. Results were obtained as follows for some standard sugars:-

Sugar	Oxidation Product R_G	Colour
2-O-methylgalactose	0.15	lemon yellow
3-O-methylglucose	0.35	wine red

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Sugar	Oxidation	Product
4-O-methylglucose	0.53	yellow-brown
2,3-di-O-methylgalactose	0.66,0.78, 0.87	grey, brown grey resp.
2,6-di-O-methylgalactose	0.15	lemon yellow

Degradation of methylated hexoses to the corresponding methylated pentoses was carried out by the method of Jones and Perry (152). The sugar (2 mg.) was reduced with potassium borohydride in the usual manner to the corresponding hexitol. Sodium periodate (0.03M, 3 ml.) was added, and oxidation was allowed to proceed for 30 min. at room temperature, in the absence of light. Excess periodate was destroyed with ethylene glycol, and the solution was de-ionised, evaporated, and examined chromatographically in solvent A, together with a standard treated in the same manner.

Demethylations (153) were carried out by heating the methylated sugars (ca. 5 mg.) with hydriodic acid (1 ml.) at 100°, for 8 min. The solutions were then cooled, diluted with water, neutralised with silver carbonate, treated with hydrogen sulphide, filtered and evaporated to dryness, and the products were identified chromatographically in solvent E.

PURIFICATION AND FRACTIONATION OF *K. SENEGALENSIS* GUM

The gum was obtained in the form of yellowish nodules of varying size. A certain quantity of bark still adhered to some of the nodules. Since previous work (59) had shown the gum to exist in a partially acetylated form, it was first de-acetylated as follows.

The gum (75 g.) was finely ground, and allowed to swell for three days in water (1 l.), the product being a thick gel. Sodium hydroxide (40 g.) was added slowly, and stirring was continued for 7 hours, after which time most of the gum had gone into solution. The insoluble part, together with mechanical impurities, was filtered off through 4 thicknesses of muslin. The filtrate was added with stirring to methylated spirits (3180 ml.) containing concentrated hydrochloric acid (150 ml.), to give a white flocculent gel. This was allowed to settle and coagulate overnight at 0°, and separated from the supernatant liquid by decantation and centrifuging. The supernatant liquid (as also from subsequent reprecipitations) was immediately neutralised by the addition of excess calcium carbonate.

The gel was dispersed in water, and reprecipitated by pouring slowly into a volume of ethanol sufficient to give a net ethanol concentration of 70%. Four more similar precipitations, in which the ethanol concentration was about 60%, were

carried out. A sample of the final product was dissolved in water, the solution was de-ionised with Amberlite resins IR-120(H) and IR-4B(OH) in succession, and the polysaccharide was precipitated from ethanol, redispersed in water and freeze-dried, to give a white product, of $[\alpha]_D +133^\circ$ (c.,0664), equiv. weight 344.

The bulk of polysaccharide I, or the major component of the gum, was washed several times with ethanol and with ether, and dried in vacuo, to give a slightly coloured powder.

The combined supernatant liquors now contained a precipitate consisting of the calcium salt of polysaccharide II, together with excess calcium carbonate. The precipitate was removed at the centrifuge, and stirred with two portions of water, in order to bring all the calcium salt of the polysaccharide into solution. The calcium carbonate was filtered off, and the filtrate de-ionised with Amberlite resins IR-120(H) and IR-4B(OH). Polysaccharide II, or the minor component of the gum, was isolated by precipitation from ethanol, washed with ethanol and ether, and dried in vacuo. A sample of the de-ionised solution was freeze-dried, and the product had $[\alpha]_D -0.5^\circ$ (c. 1.001), equiv. weight 1080.

The supernatant liquors from the fractionation of the gum were evaporated to small volume, and polysaccharide III was precipitated from ethanol. A sample was dissolved in water,

de-ionised and freeze-dried, to give a product having $[\alpha]_D$ 0°, (c.,0.81) and very high equiv. weight. The bulk of the polysaccharide was isolated by washing with ethanol and ether, and drying *in vacuo*.

THE MAJOR COMPONENT OF KHAYA SENEGALENSIS GUM: PARTIAL HYDROLYSIS STUDIES

HYDROLYSIS OF THE POLYSACCHARIDE, AND ISOLATION OF THE ACIDIC FRACTION.

The polysaccharide (15 g.) was hydrolysed at 100° with N sulphuric acid (400 ml.) for 6 hr., after which time a certain quantity remained undissolved. The insoluble part was hydrolysed for a further 6 hr. with fresh acid, and the remaining gel was centrifuged off. The combined solutions were neutralised with barium hydroxide solution and barium carbonate, filtered, evaporated to small volume, passed through a column of Amberlite resin IR-120(H) in order to remove barium ions, and evaporated to a syrup which on examination by paper chromatography in solvent E was shown to contain galactose, rhamnose, arabinose (trace), and a mixture of acidic oligosaccharides.

The separation of neutral sugars from acidic components was first attempted using a column of Amberlite resin CG-400 (100-200 mesh) in the acetate form. It was found, however, that the acidic fragments were incompletely adsorbed, and were in part eluted with water, along with the neutral sugars. Conversion of the uronic acids to their sodium salts gave no better results.

An attempt was made to fractionate that part of the acidic portion which had been adsorbed, by gradient elution of the column, from water to 10% acetic acid. This also was unsuccessful; the eluate fractions were therefore recombined and evaporated to dryness.

A successful separation of neutral and acidic components of the hydrolysate was carried out on a column of the weakly basic Amberlite resin CG-45. Before use, a 1% glucose solution was passed through the column in order to block any reactive centres in the resin, and prevent loss of product due to irreversible sorption. The syrup was then placed on the column, the neutral sugars eluted with water, and the acid fraction washed off with 15% formic acid and evaporated to a syrup (2.12 g.).

FRACTIONATION OF THE ACIDIC MIXTURE ON A CELLULOSE COLUMN

The cellulose column (60 x 3.5 cm.) was packed wet. Cellulose powder was stirred up in acetone until free from air bubbles, and the slurry was packed into the column about an inch at a time, using air pressure on top of the column, and taking care not to allow the solvent level to drop below the top of the cellulose. The solvent was changed from acetone to water, and from water to the eluting solvent.

The acidic fraction (2.12 g.) of the gum hydrolysate was

adsorbed on to circles of thick filter paper, placed on the column, and covered by a further inch of cellulose powder. The column was eluted with ethyl acetate - acetic acid - water (9:2:2 v/v), and the contents of every fifth tube were examined in solvent C. Six fractions were obtained. Each was shaken with an equal volume of water, and the organic layer was discarded. The aqueous layer was evaporated to small volume, shaken with ether in order to remove most of the ethyl acetate and acetic acid, and evaporated to dryness.

Fraction 1. (0.041 g.) 4-O-methyl glucuronic acid.

This gave a single yellow-brown spot, moving just behind rhamnose ($R_{rhamnose}$ 0.92). The syrup was left overnight in contact with a dry methanolic solution of hydrogen chloride (2.7%, 10 c.c.), refluxed for 7 hr. and neutralised, and the methyl ester methyl glycoside was isolated on evaporation.

This was reduced with potassium borohydride, and the methyl glycoside obtained was hydrolysed for 2 hours in N hydrochloric acid, to give a product (0.022 g.) running on paper at the same rate as 4-O-methyl glucose. The p-toluidine derivative was prepared, but no crystalline product was obtained.

A small-scale periodate oxidation was carried out on the sugar, and on 3-O-methyl and 4-O-methyl glucose as standards. Chromatographic examination of the products in solvent A showed identical patterns of spots for the sample and 4-O-methyl glucose.

Fraction 2. (0.040 g.).

Mainly 4-O-methyl glucuronic acid, with traces of slower moving components.

Fraction 3. (0.187 g.).

This was a complex mixture of acidic fragments, none present in very high proportion. It streaked badly on paper, and it was impossible to identify any component.

Fraction 4. (0.031 g.).

The same sugars were present as in fraction 5, with traces of faster components.

Fraction 5. (0.300 g.).

This gave a pink and a brown spot, running almost together, at about the rate of galactose, and was assumed to be a mixture of two aldobiouronic acids.

Fraction 6. (0.960 g.).

When run on paper in solvent C, this fraction gave a streaking spot near the starting line. Other solvent proportions, butan-1-ol - acetic acid - water (1:1:1) and butan-1-Ol (2 parts):acetic acid (1 part), saturated with water, were used; these gave more movement, but no better resolution. The fraction was suspected of consisting of a mixture of acidic oligosaccharides containing three or more sugar units.

A portion was subjected to fairly strong acid hydrolysis (2N sulphuric acid, 6 hours). Chromatographic examination of the hydrolysate in solvent C showed the presence of D-galactose, L-rhamnose, a trace of L-arabinose, and three acid fractions, one very heavy, with $R_{galactose}$ 0.81, probably galacturonic acid, and two less heavy, with $R_{galactose}$ 1.16 and 1.25.

A second portion was treated with an excess of potassium borohydride, in order to convert all reducing end-group sugars to the corresponding alcohols, which would not give coloured spots with aniline oxalate spray. The reduced product was hydrolysed with N sulphuric acid for 5 hours, neutralised, de-ionised, and run in solvents C and E. Most of the galactose seemed to have been removed, but no clear evidence was obtained.

THE ALDOBIOURONIC ACIDS

Small Scale Hydrolysis of the Aldobiouronic Acid Mixture

Fraction 4 (5 mg.) was hydrolysed for 6 hours with 2N sulphuric acid, neutralised with barium carbonate, de-ionised with Amberlite resin IR-120(H), and examined chromatographically in solvent E. The spots obtained corresponded to galactose, rhamnose, a trace of arabinose, and a mixture of uronic acids. An unidentified yellowish spot was observed, with R_f value similar to that of glucose. It was possible

that this might be a degradation product of galactose when left in contact with barium carbonate, but a control experiment, gave no such spot. Other workers, however, have observed such a spot as a degradation product of galacturonic acid when treated with 2N acid.

Small Scale Reduction and Hydrolysis

Fraction 4 (8 mg.) was left overnight in contact with a dry 2.7% methanolic solution of hydrogen chloride (2 c.c.), heated under reflux for 7 hours, neutralised, and evaporated to give the methyl ester methyl glycosides of the acids. The syrup was dissolved in water (5 ml.), and reduced with potassium borohydride to the methyl glycosides of the corresponding disaccharides. These were hydrolysed with N sulphuric acid for 5 hours, and the hydrolysate was neutralised with barium carbonate. The product was examined chromatographically in solvents C and E, and was observed to contain galactose in high proportion, rhamnose, a trace of arabinose, and 4-O-methyl glucose. Again an unidentified spot appeared just in front of galactose.

Methylation of the Mixture of Aldobiouronic Acids

The acids were methylated by Haworth's procedure. The syrup (300 mg.) was dissolved in water (6 ml.), and dimethyl sulphate (1 ml.) and sodium hydroxide solution (1.5 ml. 30%)

were added dropwise over 2 hours, care being taken to avoid excess of sodium hydroxide over the initial period, until the reducing groups were protected by glycoside formation. Three further additions of dimethyl sulphate (7 ml.) and sodium hydroxide solution (10 ml., 30%) were made, each over 6 hours. Stirring was continuous between and during these additions. Excess dimethyl sulphate was destroyed by heating on a boiling water-bath for 30 min., and the solution was cooled, acidified, and extracted five times with chloroform and three times with ethyl acetate. The combined extracts were dried and concentrated, giving 250 mg. of methylated aldobiouronic acids.

Reduction of the Methylated Aldobiouronic Acids

Powdered lithium aluminium hydride (100 mg.) was added to the solution of the syrup (250 mg.) in dry tetrahydrofuran (40 ml.), and the mixture was refluxed for two hours, a further 50 mg. of lithium aluminium hydride being added after the first hour. The excess lithium aluminium hydride was then destroyed by the cautious addition of water, the hydroxide precipitates were filtered off and extracted repeatedly with dry acetone and dry chloroform, and the extracts and mother liquors were bulked and evaporated to dryness. Re-extraction with dry chloroform, filtration and evaporation gave the methylated disaccharides (183 mg.).

Remethylation of the Reduced Methylated Product

The dry syrup (183 mg.) was dissolved in a minimum quantity of purified methyl iodide. Gentle reflux was maintained, and dry, freshly-prepared silver oxide (500 mg.) was added over 6 hours in small quantities, with frequent shaking. The silver oxide was then filtered off, boiled up with two portions of dry chloroform, and finally extracted with chloroform for 8 hours in a Soxhlet extractor. The combined extracts were bulked with the mother liquors, evaporated to small volume, filtered through Whatman No.42 filter paper, and taken to dryness, to give the fully methylated disaccharides.

Hydrolysis of the Fully Methylated Disaccharides

A trial hydrolysis was carried out on 1 mg. of the syrup, with N hydrochloric acid, for 4 hours. After neutralisation and evaporation to dryness, the product was run in solvent A, and on developing, the chromatogram showed five spots, of R_G value 1.0 (pink), 0.89 (pink), 0.86 (yellow), 0.70 (pink), and 0.58 (yellow).

The bulk of the material was hydrolysed in the same manner. Since two of the methylated sugars ran so close together on paper, an attempt was made to fractionate the mixture on a celite column, using solvent B as eluant. This, however, proved unsuccessful; the mixture of methylated sugars was therefore fractionated on a cellulose column (45 x 2.5 cm.),

using as eluant butan-1-ol (70%) - light petroleum (b.p. 100-120°) (30%), saturated with water. Fractions were obtained as follows:-

(a) 2,3,4,6-tetra-O-methyl-D-glucose.

(19 mg.) R_G 1.0.

Crystallised out on seeding. M.p. 81-2°, not lowered on admixture with authentic sample.

(b) This fraction gave three spots, and was therefore refractionated on thick paper in solvent B, to give three fractions.

(i) 2,3,4,6-tetra-O-methyl-D-galactose.

(25 mg.) R_G 0.89.

Identified by conversion to the aniline derivative, which had m.p. and mixed m.p. 187-8°.

(ii) 3,4-di-O-methyl-L-rhamnose.

(20 mg.) R_G 0.86. M_G 0.36 (authentic specimen had M_G 0.38).

Partially crystalline. Oxidised to 3,4-di-O-methyl-L-rhamnono-1,5-lactone, which crystallised on seeding. M.p. and mixed m.p. 70-72°.

(iii) 7 mg. Combined with fraction (c).

(c) 2,3,6-tri-O-methyl-D-galactose.

(10 mg.) R_G 0.70.

Oxidised to 2,3,6-tri-O-methyl-D-galactono-1,4-lactone, which crystallised out on seeding. M.p. and mixed m.p. 95-6°.

(d) 2,3,4-tri-O-methyl-D-galactose.

(2.7 mg.) R_G 0.65.

Partially crystalline. Identified by potassium borohydride reduction, followed by periodate oxidation of the sugar alcohol, with an authentic specimen as control. Both samples gave one mole of formaldehyde, and chromatographic examination of the oxidation products in solvent A showed identical spots, of R_G value 0.82, corresponding to 2,3,4-tri-O-methyl lyxose.

(e) Traces of a sugar of R_G 0.58, corresponding to monomethyl rhamnose, were observed, but it was not isolated in quantity sufficient for identification.

PERIODATE OXIDATION OF K. SENEGALENSIS GUM, MAJOR COMPONENT.

The polysaccharide (2 g.) was dissolved in water (100 ml.) Sodium metaperiodate (7 g.) was dissolved in water (117 ml.), and 100 ml. of the solution was added to the polysaccharide solution, that remaining being diluted to twice its volume for use as a standard in periodate uptake measurements. The solutions were kept in the dark at room temperature.

The rate of uptake of periodate by the polysaccharide was measured by a spectrophotometric method, depending on the absorption by the periodate ion at 223 mμ (154). The sodium metaperiodate solution was divided into two portions, and half was treated with excess fructose, to give a sodium iodate solution of the same molarity as the periodate solution and the experimental solution.

For each determination, a 1 ml. aliquot was removed from each solution, and diluted by 1500, and the optical density of the resulting solution was measured in the Unicam spectrophotometer at λ =223mμ. The values obtained for the standard solutions gave a straight line, from which the amount of periodate so far taken up was found. The results were in turn plotted against time. Details of results are shown below, and in the accompanying diagrams.

	Optical density after dilution.			
Time	Periodate solution	Iodate solution	Experimental solution	Moles IO_4^- reduced per 10 sugar units
18 hr.	0.940	0.140	0.753	5.9
25 hr.	0.940	0.138	0.700	7.5
42.5 hr.	0.952	0.147	0.684	8.4

Oxidation was therefore considered to be complete after 42 hours, when 8.4 moles of periodate per 10 monosaccharide units had been consumed. (A rough calculation from the results of methylation gave a value of about 9 moles.)

Excess periodate was destroyed by the addition of ethylene glycol (1.5 g.) and the solution was dialysed until free from iodate ions, and evaporated to small volume. The oxidised polysaccharide was then precipitated from acetone and dried with ether.

Reduction was carried out by allowing the polysaccharide to stand overnight in aqueous solution containing potassium borohydride (1.4 g., 50% excess). Excess borohydride was destroyed by stirring with Amberlite resin IR-120(H) and all potassium ions were removed from the solution by passing it through a column of the same resin.

After evaporation to small volume, the solution was made up to N with sulphuric acid, and allowed to stand at room temperature for 1 hour. It was then neutralised with barium carbonate, centrifuged, re-evaporated to small volume and poured into excess acetone. The high molecular weight material, which was precipitated in appreciable amount, was centrifuged off and the centrifugate was evaporated to dryness, giving only 200 mg. of low molecular weight material.

A small scale hydrolysis was carried out and both the

hydrolysate and the unhydrolysed material were examined chromatographically in solvents E, F, and G, using both aniline oxalate and alkaline silver nitrate as developers. Only galactose, rhamnose, threitol and glycerol were identified.

Two 100 mg. portions of the high molecular weight material were subjected to further hydrolysis for 5 hours, one with N acid, at room temperature and the other with N/20 acid, at 100°. In neither case was there any change in the optical rotation of the solution.

DISCUSSION

The exudate of the West African tree, Khaya senegelinsis, has already been the subject of some chemical investigation. A preliminary examination by Matheson (59) showed that the gum had an equivalent weight of 412. It was partially acetylated, and contained 1.2% of methoxyl groups. Like the related Khaya grandifolia gum, it was composed of residues of D-galactose, L-rhamnose, L-arabinose and acidic sugars. Chromatographic evidence suggested that the acidity was due at least in part to the presence of residues of 4-O-methylglucuronic acid.

Further work by Stephen (17) showed that the gum was not homogeneous, but could be separated by solubility differences in aqueous ethanol into two fractions, the major, less soluble fraction containing over 50% uronic acid residues and the minor, more soluble fraction, about 20%. These fractions, on examination in the ultracentrifuge and in the Antweiler microelectrophoresis apparatus (155), were shown to have slightly different properties and each appeared to be homogeneous. The whole gum, however, could not be completely resolved into two components by either of these methods, so this could not be taken as proof that the fractionation by precipitation had been completely efficient. By the criterion of glass fibre paper

ionophoresis, the whole gum contained two components; the minor fraction, as isolated by fractional precipitation, was homogeneous, and the major fraction contained about 5% of the minor component.

Stephen also carried out methylation studies on the major fraction of the gum and obtained evidence for the presence of D-galactose end groups and 1,4-linked D-galactose units, L-rhamnose branch points linked in the C 1, 2 and 4 positions, 1,4-linked D-galacturonic acid residues and D-glucuronic acid end groups. From the study of partially methylated acidic oligosaccharides isolated from the methylated gum, it appeared that the glucuronic acid end groups were glycosidically linked to the C 4 position of galactose residues, and the galacturonic acid residues to the C 2 or the C 4 position of the rhamnose branch points.

Evidence was also provided for the existence in small amount, in the major fraction of the gum, of 1,6- and 1,3,6-linked D-galactose residues, L-rhamnose end groups and 1,2-linked L-rhamnose residues, L-arabinose end groups and 1,2-linked glucuronic acid units.

In the present work, the major fraction of the gum was subjected to partial hydrolysis and a study was made of the acidic substances obtained. Unsuccessful attempts were also made to isolate degradation products of the periodate-oxidised

polysaccharide.

The gum, which was obtained in the form of hard yellowish nodules, was crushed to a powder and deacetylated by treatment with N-sodium hydroxide solution. The major, high-acid fraction of the gum was precipitated by the addition of acidified ethanol to give an ethanol concentration of 70% and the minor fraction was precipitated from the centrifugate as the insoluble calcium salt. The major fraction was purified by repeatedly dissolving in water and precipitating from 60% ethanol and after purification had $[\alpha]_D + 133^\circ$ and equivalent weight of 344, corresponding to a uronic anhydride content of 51%.

This polysaccharide was hydrolysed with N-sulphuric acid for 12 hours and the low molecular weight material obtained was fractionated into its neutral and acidic components by absorption of the acids on an anion exchange resin column. Chromatographic evidence was obtained for the presence of galactose, rhamnose and arabinose in the neutral fraction.

The acids were separated on cellulose. Two fractions were isolated in quantity sufficient for further investigation: an O-methylhexuronic acid and a mixture of aldobiouronic acids. The O-methylhexuronic acid, on conversion to the methyl ester methyl glycoside and reduction with potassium borohydride, gave rise to a sugar chromatographically identical to 4-O-

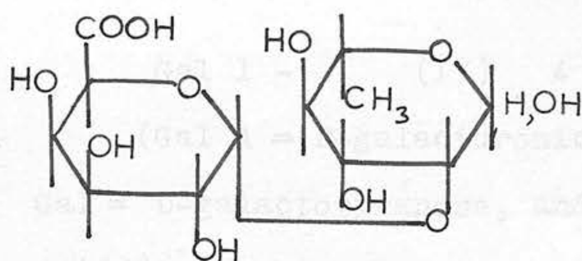
methylglucose. Chromatographic examination of the periodate oxidation products confirmed its identity as such. From this, it is evident that the glucuronic acid end groups shown to be present by the methylation studies are themselves methylated at the C4 position in the naturally occurring polysaccharide, as in K. grandifolia gum. The existence of methoxyl groups in small amount in the polysaccharide is thus accounted for.

The mixture of aldobiouronic acids on hydrolysis gave rise to galactose, rhamnose and an acidic fraction. On reduction and hydrolysis of the derived methyl ester methyl glycoside, rhamnose, 4-O-methylglucose and an increased proportion of galactose were obtained.

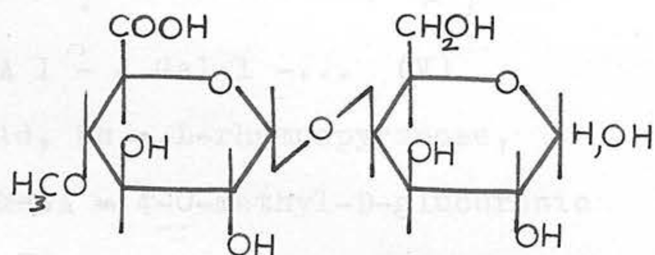
The aldobiouronic acids were methylated with dimethyl sulphate and sodium hydroxide, reduced with lithium aluminium hydride and remethylated with methyl iodide and silver oxide, to give a mixture of fully methylated disaccharides. This mixture, on hydrolysis, gave 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose, all identified by the formation of crystalline derivatives. The small quantity of 2,3,4-tri-O-methyl-D-galactose which was also isolated probably arose from incomplete methylation at the C 6 group after reduction of galacturonic acid residues.

Since methylation studies have shown that galacturonic acid

residues are linked to rhamnose in the polysaccharide and 4-O-methyl-glucuronic acid to galactose, it is evident that the aldobiouronic acids isolated on partial hydrolysis are 2-O-D-galacturonosyl-L-rhamnose (I) and 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose (II).



I.

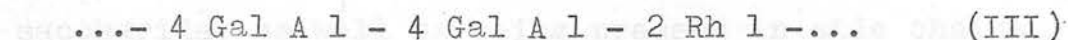


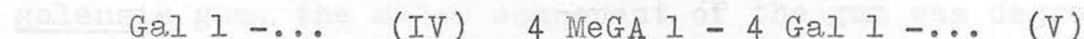
II.

These two acids were also isolated from the product of partial hydrolysis of K. grandifolia gum. The acid II has not so far been isolated from any other high acid gum, but I has been found in the hydrolysis products of Sterculia setigera and Cochlospermum gossypium gums.

From the combined results of methylation and partial hydrolysis studies, it is clear that the major component of K. senegalensis gum consists for the most part of a chain (III) of 1,4-linked D-galacturonic acid residues, with occasional 1,2-linked L-rhamnose residues, carrying side chains at the C 4 position. The side chains must involve the D-galactose and 4-O-methyl-D-

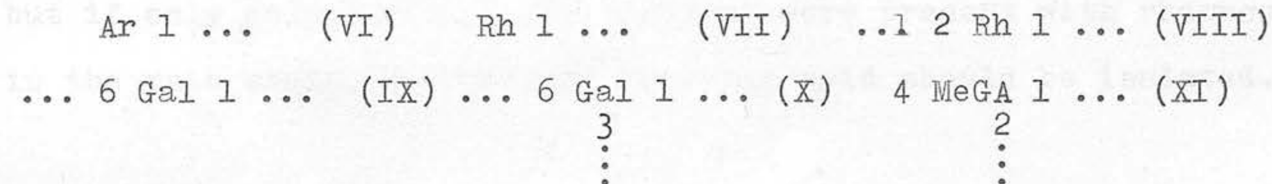
glucuronic acid end groups. There is no reason to believe that the D-galactose end groups (IV) are not linked directly to the L-rhamnose branch points, but at least one 1,4-linked D-galactose residue must lie between the 4-O-methylglucuronic acid and the main chain, as in V.





(Gal A = D-galacturonic acid, Rh = L-rhamnopyranose, Gal = D-galactopyranose, and 4 MeGA = 4-O-methyl-D-glucuronic acid.)

If these three structural fragments are taken as representing between them a general picture of the molecular structure of the polysaccharide, it is evident that the resemblance to <u>K. grandifolia</u> gum is extremely close. The fragments VI - XI, however, which were isolated in minor amount from the methylated polysaccharide, must also be taken into account.



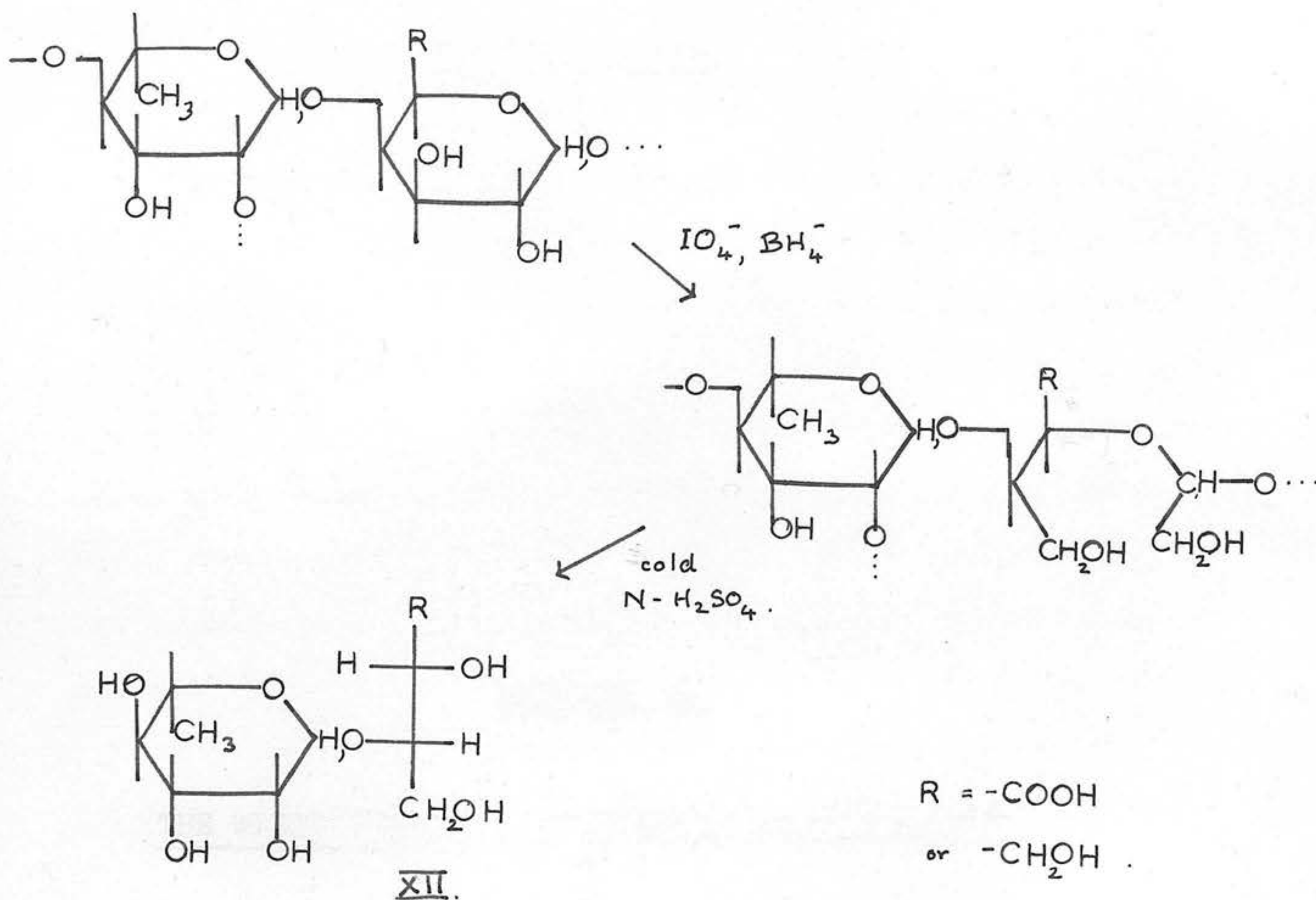
(Ar = L-arabinofuranose).

They may form an integral part of the structure of the major component of the gum; it seems more likely, however, considering the known heterogeneity of the polysaccharide after

fractionation, that at least some of them may arise from the small proportion of the minor component present.

In the investigations on K. grandifolia gum, partial hydrolysis studies showed that some of the 1,4-linked galactose residues were joined to rhamnose in the main chain of the polysaccharide, as well as being present in side chains. In an attempt to discover whether this was also the case in K. senegalensis gum, the major component of the gum was degraded using the method of Smith and his co-workers (72).

The polysaccharide was oxidised with periodate and the product was reduced with potassium borohydride. This should give a degraded polysaccharide with only the rhamnose residues intact. Very mild acid treatment of this polysaccharide with mineral acid should cleave only the acetal linkages; it should therefore be possible to isolate the fragment XII. Identification of this as a rhamnosyl threitol would imply the existence of galactose residues adjacent to rhamnose branch points, but if only galacturonic acid residues were present with rhamnose in the main chain, a rhamnosyl threonic acid should be isolated.



In practice, however, it was found that in polysaccharides of such high uronic acid content as the Khaya gums, such cleavage did not take place. Very little low molecular weight material was isolated after the acid treatment; it did in fact contain threitol and glycerol but there was no evidence for the presence of fragments of higher chain length. In view of these results, the question of whether galactose residues are present in the backbone of the molecule is one which must be left open.

SECTION II

THE MINOR COMPONENT OF *KHAYA SENEGALENSIS* GUM

EXPERIMENTAL

The fraction of the gum soluble in 60% aqueous ethanol had been isolated, as previously described, in the form of the calcium salt. The free acid form of the polysaccharide was obtained by passing the salt in aqueous solution through a column of Amberlite resin IR-120(H). The solution was then evaporated to small volume, and the polysaccharide was precipitated from ethanol, washed with ether, and dried in vacuo. The uronic anhydride content, as measured by the carbazole method (156), was 24.7%.

ATTEMPTED FRACTIONATION OF THE MINOR COMPONENT OF THE GUM

I. Fractional Precipitation from Aqueous Solution with Ethanol

The polysaccharide (1 g.) was dissolved in water (100 ml.), and ethanol was added in 20 ml. portions. As soon as precipitation occurred, the polysaccharide was centrifuged off, dissolved in water and freeze-dried, and addition of ethanol to the centrifugate was continued, after evaporation if necessary.

No.	Wt. of fraction	Ethanol:water ratio	Equiv. Wt.
1.	120 mg.	1.4 : 1	793
2.	6 mg.	1.8 : 1	925

No.	Wt. of fraction	Ethanol:water ratio	Equiv. Wt.
3.	460 mg.	2 : 1	950
4.	55 mg.	2.8 : 1	935
5.	140 mg.	3.6 : 1	944
6.	48 mg.	4.1 : 1	922
7.	7 mg.	8 : 1	-

II. Precipitation with 'Cetavlon' (Cetyltrimethylammonium Bromide).

The polysaccharide (1 g.) was dissolved in water (30 ml.) and the solution was neutralised to exactly pH 7 with dilute ammonium hydroxide solution. On addition of a 10% solution of 'Cetavlon' (30 ml.), the cetyltrimethylammonium salt of the polyuronic acid came down as a heavy white precipitate. This was centrifuged off, washed, and dissolved in 2N-acetic acid (10 ml.) in order to destroy the complex, and the polysaccharide was precipitated from excess ethanol. The precipitate was centrifuged off, washed successively with acetic acid/ethanol, ethanol and ether, and dried in vacuo.

Yield : 0.711 g. Equivalent weight : 1028.

For precipitation of any neutral polysaccharide with 'Cetavlon', it was necessary to form the borate complex of the polysaccharide. The mother liquors from the previous precipitation still contained a large excess of 'Cetavlon'. Addition of an equal volume of 1% borate buffer solution (pH 8) gave no

precipitation. Addition of pH 10 buffer solution gave a small precipitate. The complex was destroyed as before, and the polysaccharide was precipitated and dried.

Yield : 0.029 g. Equivalent weight : 1104.

It therefore appeared that no neutral polysaccharide was present.

III. Precipitation with Copper Acetate

The polysaccharide (1 g.) was dissolved in water (20 ml.) and cupric acetate solution (7% w./v. in water) was added, up to a maximum concentration of 0.40% of cupric acetate. No precipitation occurred; ethanol was therefore added in portions, each precipitate so formed being removed before the addition of further precipitant. The precipitates were dissolved in water, a few drops of hydrochloric acid were added to decompose the copper complex, and reprecipitation was brought about by the addition of excess ethanol. The polysaccharide fractions so obtained were washed with ethanol until free from chloride ions, then twice with ether, and dried in vacuo.

Fraction	Weight	Equivalent weight
1.	116 mg.	521
2.	20 mg.	735
3.	312 mg.	962
4.	338 mg.	954
5.	84 mg.	944

IV. Glass Fibre Paper Ionophoresis

Ionophoresis was carried out on glass fibre paper, in 2N-sodium hydroxide solution. The ionophoretogram was developed with potassium permanganate spray. The minor fraction of the gum showed one discrete spot, and was therefore assumed to consist of a homogeneous polysaccharide.

PARTIAL HYDROLYSIS STUDIES

The polysaccharide (200 mg.) was hydrolysed with N-sulphuric acid for 6 hours at 100°, neutralised, de-ionised and evaporated to dryness, to give 183 mg. of syrup, which was shown by chromatography in solvent E to contain galactose, rhamnose, arabinose and acidic sugars. The acidic fraction was isolated by chromatographic separation of the mixture on Whatman No.17 paper, in solvent E.

Chromatographic examination of the acidic fraction in solvents C and F showed it to be a complex mixture, containing at least 7 components. These had R_{GalA} values as follows in solvent F: 0.96 (faint), 0.75, 0.65, 0.56 (present in high proportion), 0.48, 0.21 (faint), and 0.17 (streaking from origin). No component was chromatographically identical to galacturonic acid, glucuronic acid, or 2-O-α-D-galacturonosyl-L-rhamnose.

The mixture was separated on Whatman No.31 paper (thick, acid-washed) in solvent F, into three fractions. A portion of each was hydrolysed with 2N-sulphuric acid for 5 hours, and the products of hydrolysis were identified chromatographically in solvent E. The remainder was subjected to methanolysis, borohydride reduction and hydrolysis with N-hydrochloric acid for 2 hours, and again the products were identified chromatographically in solvent E.

(i) 14 mg. R_{GalA} 0.75, 0.65 and probably 0.56.

On hydrolysis, galactose, rhamnose and an acidic fraction were shown to be present. On reduction of the acidic sugars, galactose, 4-O-methylglucose, and rhamnose (in small proportion) were obtained.

(ii) 12 mg. R_{GalA} mainly 0.56, minor component at 0.48.

Galactose was the only neutral sugar observed on hydrolysis. Methanolysis, reduction and hydrolysis gave galactose and 4-O-methylglucose in approximately equal proportions.

(iii) 8.5 mg. R_{GalA} 0.21, and slower-moving sugars.

Hydrolysis gave only galactose. Methanolysis, reduction and hydrolysis gave galactose in high proportion, with glucose, 4-O-methylglucose and rhamnose as minor components, and a trace of arabinose.

METHYLATION STUDIES

METHYLATION OF THE MINOR FRACTION OF K. SENEGALENSIS GUM

The polysaccharide (20 g.) was dissolved in a minimum quantity of water, and 30% aqueous sodium hydroxide solution (400 ml.) and dimethyl sulphate (280 ml.) were added dropwise over a period of 8 hours, in an atmosphere of nitrogen, with efficient stirring, and cooling in ice-water. Stirring was continued overnight, and two further methylations were carried out on two succeeding days. The reaction mixture was then dialysed until the concentration of ions was small, and evaporated to small volume, and three further methylations were carried out.

Excess dimethyl sulphate was destroyed by heating to 70°, and the solution was brought to pH 6 with sulphuric acid, dialysed until free from sulphate ions, evaporated to small volume, and freeze-dried, to give the partially methylated polysaccharide (9 g.), OMe 34.9%, sulphated ash 3.5%.

This was dissolved in water, and neutralised with silver carbonate. Excess silver carbonate was filtered off, and the solution was freeze-dried to give the silver salt of the polysaccharide (about 10 g.) as a brown glassy solid.

The silver salt was suspended in a mixture of methyl iodide and methanol, and silver oxide (9 g.) was added

portionwise to the refluxing solution over a period of 6 hours. The residue was filtered off and extracted 3 times with hot chloroform, and the combined extracts and filtrate were evaporated to dryness.

The product was only partially soluble in methyl iodide. This was thought to be due to the presence of sodium salt of the polysaccharide. The insoluble part, therefore, together with the silver oxide residues, was extracted several times with methanol, and the product was de-ionised with Amberlite resin IR-120(H), and converted as before to the silver salt, which was now freely soluble in methyl iodide. This solution was combined with that previously obtained, and a further Purdie methylation was carried out, to give the fully methylated polysaccharide (5.21 g.), $[\alpha]_D -21^o$; OMe 40.5%, not raised on further methylation.

HYDROLYSIS OF THE METHYLATED POLYSACCHARIDE, AND SEPARATION INTO NEUTRAL AND ACIDIC FRACTIONS

The methylated polysaccharide (5 g.) was heated under reflux for 12 hours with a 2% solution of hydrochloric acid in methanol (250 ml.). The solution was concentrated to 50 ml., and diluted with water and reconcentrated several times to remove as much of the methanol as possible. Finally, hydrochloric acid was added to give a N solution.

This solution was heated for 12 hours at 100°, cooled, neutralised with silver carbonate, filtered, evaporated to dryness, taken up in methanol and re-evaporated. The product was dissolved in water, de-ionised with Amberlite resin IR-120 (H), and neutralised with barium carbonate. Excess barium carbonate was filtered off and the solution was evaporated to dryness.

The product was repeatedly extracted with 150 ml. portions of ether, over a period of about 5 days, until the weight of neutral methylated material obtained from successive extracts began to decrease. A total weight of 3 g., comprising the greater part of the neutral methylated sugars, was obtained.

The residue was placed on a small cellulose column (45 x 2.5 cm.), which was then eluted with butanol half-saturated with water. Fractions of about 200 ml. were collected, each being evaporated to dryness and examined chromatographically in solvent A. The following fractions were obtained.

(i) 29 mg. R_G 1.01, 0.93.

(ii) 286 mg. R_G 0.77, 0.55, 0.36.

(iii) 42 mg. R_G 0.55, 0.38, 0.29.

(iv) 35 mg.)
) mainly methylated acidic sugars.
(v) 81 mg.)

The column was then washed through with water, in order to remove the methylated uronic acids. On evaporation, these

were obtained in the form of the barium salt, as a brownish, glassy solid (0.740 g.).

EXAMINATION OF THE NEUTRAL METHYLATED SUGARS

The syrup (3 g.) was dissolved in a little water, freeze-dried on to cellulose, and placed on a cellulose column (65 x 3.5 cm.). The column was eluted with light petroleum (b.p. 100-120°) / butan-1-ol, saturated with water, in the proportions of 9:1, 4:1, 7:3 and 1:1 in succession, and finally with butan-1-ol half-saturated with water. The following fractions were obtained.

Fraction 1. 0.234 g. R_G 1.03.
$[\alpha]_D = +61^{\circ}$ (c., 2.34).

A portion was subjected to further hydrolysis with N-hydrochloric acid, and the product was examined chromatographically in solvent D. Two acidic components, of R_G 0.82 and 0.91, were observed, together with some unchanged material. The product was treated with 0.5N-sodium hydroxide solution at 50°, for 30 min. Chromatographic examination of the product in solvent D revealed four components, of R_G 1.0, 0.93, 0.78 and 0.68. The first was chromatographically identical with 2,3,5-tri-O-methylarabinose.

Fraction 2. 0.103 g. R_G 1.03 and 0.98.

The syrup was treated with 0.5N-sodium hydroxide solution as before. The product was fractionated on thick paper, in solvent B, and the major fraction, which was chromatographically indistinguishable from 2,3,5-tri-O-methylarabinose, was isolated. It had $[\alpha]_D = -34^\circ$ (c., 0.25). Oxidation with bromine water gave rise to the lactone, which was partially crystalline. This was converted to the amide, which had m.p. 127-129°, not depressed on admixture with 2,3,5-tri-O-methyl-L-arabonamide.

<u>Fraction 3.</u> 0.022 g. R_G 0.97.
$[\alpha]_D = 0^\circ$ (c., 0.22).

This fraction was chromatographically non-identical with 2,3,5-tri-O-methylarabinose, since it gave a pink colour with aniline oxalate spray. On chromatographic examination in solvent D of the product of alkaline hydrolysis, it was found that the fraction had been wholly converted to acidic sugars.

<u>Fraction 4.</u> 0.032 g. R_G 0.98 and 0.89.
$[\alpha]_D = +29.5^\circ$ (c., 0.44).

Demethylation gave galactose. The rotation corresponded to a mixture of 7 parts of the components of fraction 3, to 3 parts of 2,3,4,6-tetra-O-methylgalactose, from which the slower-moving component was chromatographically indistinguishable.

<u>Fraction 5</u>. 0.333 g. R_G 0.89, with a trace at 0.98.

$[\alpha]_D = +99.5^{\circ}$ (c., 3.08).

The syrup (100 mg.) was fractionated on thick paper, in solvent B, which showed two minor components, one faster than the main component, and one slower. The main component was isolated. This had $[\alpha]_D = +110^{\circ}$ (c., 0.49), and was chromatographically identical to 2,3,4,6-tetra-O-methylgalactose.

The aniline derivative was prepared, and, after recrystallisation from ethyl acetate, had m.p. 192-193°, not lowered on admixture with an authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine.

The slower-moving component was chromatographically identical in solvent B to 3,4-di-O-methylrhamnose and had the same ionophoretic mobility.

<u>Fraction 6</u>. 0.061 g. R_G 0.91 and 0.83.

$[\alpha]_D = +50^{\circ}$ (c., 0.92).

Chromatographic examination in solvent B showed that the fraction consisted largely of 2,3,4,6-tetra-O-methylgalactose, with two other components, one moving slightly faster on paper, the other, giving a yellow colour with aniline oxalate spray, moving at a slightly slower rate, and chromatographically and ionophoretically identical to 3,4-di-O-methylrhamnose.

<u>Fraction 7</u>. 0.041 g. R_G 0.92 and 0.84, streaking.

$[\alpha]_D = +33^\circ$ (c., 0.63).

Chromatographic examination in solvent B showed the main component as a streaking spot, moving at approximately thesame rate as tetra-O-methylgalactose. The minor component observed was slower-moving on paper than 3,4-di-O-methylrhamnose. Demethylation gave mainly galactose, with a trace of arabinose, and an acidic component.

Fraction 8. 0.045 g. R_G 0.84 - 0.92, streaking.
$[\alpha]_D = +31^\circ$ (c., 0.48).

Demethylation gave galactose, with a trace of arabinose. Chromatography in solvents A and B showed that neither 2,3,4-tri-O-methylarabopyranose, nor 2,5- nor 3,5-di-O-methylarabinose was present.

Fraction 9. 0.175 g. R_G 0.84 - 0.92, and 0.77.
$[\alpha]_D = +49^\circ$ (c., 1.18).

The syrup was fractionated on thick paper, in solvent B, to give the faster- and slower-moving components in the ratio of 2 : 3.

The faster-moving component was chromatographically very similar to those present in fractions 7 and 8, and had $[\alpha]_D = +23^\circ$. Galactose and arabinose were obtained on demethylation. A small portion was hydrolysed with N acid for 4 hours. Chromatography of the product in solvent A showed the presence of

2,3,5-tri-O-methylarabinose, 2,3,6- and 2,3,4-tri-O-methylgalactose and a di-O-methylgalactose, in approximately equal proportions.

The slower-moving component was chromatographically indistinguishable in solvents A and E from 2,3,6-tri-O-methylgalactose, and chromatographic examination of the product of borohydride reduction followed by periodate oxidation showed the same pattern as was obtained from an authentic sample, similarly treated. The lactone was prepared, and had m.p. and mixed m.p. 97-99° with authentic 2,3,6-tri-O-methyl-D-galactonolactone.

<u>Fraction 10.</u> 0.160 g. R_G 0.81 and 0.72.
$[\alpha]_D = + 98^{\circ}$ (c., 1.37).

Demethylation gave galactose, and a trace of arabinose. The faster-moving component, present in small proportion, was chromatographically similar to the products of incomplete hydrolysis found in previous fractions.

The slower-moving, major fraction was chromatographically indistinguishable in solvents A and E from 2,3,4-tri-O-methylgalactose. Its identity as such was confirmed by chromatography of the product of borohydride reduction, and periodate oxidation. No 2,3,6-tri-O-methylgalactose appeared to be present, by these criteria.

<u>Fraction 11</u>. 0.245 g. R_G 0.82 and 0.73.

$[\alpha]_D = + 126^o$ (c., 1.07).

Only traces of the faster moving component were present. The major component, after purification on thick paper in solvent A, was chromatographically identical, in solvents A and E, with 2,3,4-tri-O-methylgalactose, and non-identical in solvent E with 2,3,6-tri-O-methylgalactose, and had $[\alpha]_D = + 113^o$ (c., 0.75). Periodate oxidation of the borohydride-reduced sugar gave a chromatographic pattern identical to that obtained from authentic 2,3,4-tri-O-methyl-D-galactose, and different from that obtained from the 2,3,6-tri-O-methyl- derivative.

The aniline derivative was prepared, and after recrystallisation from ethyl acetate gave colourless plates, having m.p. and mixed m.p. with authentic 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine of 165-167°.

Examination of the material by vapour phase chromatography (see Fig.3, opposite) showed the presence of 2,3,4-tri-O-methyl-galactose, together with traces of a dimethylgalactose, but no 2,3,6-tri-O-methylgalactose was present.

<u>Fraction 12</u>. 0.079 g. R_G 0.74 and 0.62.

The syrup was fractionated on thick paper in solvent B, to give approximately equal quantities of the two components. The faster-moving was chromatographically indistinguishable from 2,3,4-tri-O-methylgalactose.

The slower moving component was shown by chromatography, ionophoresis, and chromatography of the periodate oxidation product, to be identical to 3-O-methylrhamnose. It had $[\alpha]_D = +34^\circ$ (c., 0.75), and crystallised out on seeding to give needles, melting at 110-112°, m.p. not lowered on admixture with an authentic sample of 3-O-methyl-L-rhamnose.

<u>Fraction 13.</u> 0.093 g. R_G 0.73, 0.61 and 0.53.

$[\alpha]_D = +70^\circ \rightarrow +72^\circ$ (c., 0.93).

From chromatographic evidence, and from the rotation, the fraction appeared to be a mixture of 2,3,4-tri-O-methylgalactose, 3-O-methylrhamnose and 2,4-di-O-methylgalactose.

<u>Fraction 14.</u> 0.531 g. R_G 0.51.

$[\alpha]_D = +106^\circ \rightarrow +86^\circ$ (c., 1.21).

The syrup crystallised out very easily on standing, to give white plates, melting at 90-97°. Periodate oxidation of the product of borohydride reduction gave a chromatographic pattern corresponding to a dimethylpentose, together with a trace of a dimethyltetrose, indicating that the fraction consisted mainly of 2,4-di-O-methylgalactose, with traces of 2,3-di-O-methylgalactose.

After two recrystallisations from ethyl acetate, the crystals had m.p. and mixed m.p. 102-105° with authentic 2,4-di-O-methyl-D-galactose hydrate. The purified product was

unaffected by periodate. The aniline derivative had m.p. and mixed m.p. 212-214°.

<u>Fraction 15.</u> 0.080 g. R_G 0.50 and 0.43.

The syrup crystallised on standing, and after several re-crystallisations, had m.p. 102-104°, not lowered on admixture with an authentic sample of 2,4-di-O-methyl-D-galactose hydrate. Chromatography of the periodate oxidation product gave no information concerning the identity of the minor components, which were present in very small proportion.

<u>Fraction 16.</u> 0.076 g. R_G 0.35, with traces at 0.58 and 0.50.

The material crystallised out on standing and was purified by recrystallisation from methanol. The pure product was chromatographically identical with 2-O-methylgalactose, and had $[\alpha]_D = +56^\circ \rightarrow +74^\circ$ (c., 0.44), and m.p. and mixed m.p. 147-149°. Chromatographic examination of the product of periodate oxidation gave the characteristic lemon-yellow colour of methoxymalondialdehyde, which was also obtained on oxidation of authentic 2-O-methyl-D-galactose.

Fraction (ii) from the small column, which contained some neutral sugars which were not extracted with ether, was also examined. It was separated on Whatman No.17 paper, in solvent A, into three components.

(a) 0.047 g. R_G 0.73.

This was chromatographically identical to the main component of fraction 11, and to authentic 2,3,4-tri-O-methylgalactose, in solvents A and E.

(b) 0.081 g. R_G 0.51.

The syrup, which was chromatographically identical to 2,4-di-O-methylgalactose, crystallised rapidly on standing, and after recrystallisation its m.p. (101-103°) was not lowered on admixture with a sample from fraction 14.

(c) 0.044 g. R_G 0.34.

This fraction was chromatographically identical to 2-O-methylgalactose, and crystallised out on standing to give needles melting at 143-147°, m.p. not lowered on admixture with a recrystallised sample from fraction 16.

<u>EXAMINATION OF THE METHYLATED ACIDIC FRACTION</u>

The acidic portion of the hydrolysed methylated gum had been isolated in the form of the barium salt. Barium ions were removed with Amberlite resin IR-120(H), and the product was subjected to methanolysis, followed by reduction with lithium aluminium hydride, in tetrahydrofuran solution. The lithium aluminium hydride (about 2.5 moles) was added in two portions, and the mixture was maintained under reflux for two

hours in all. Excess lithium aluminium hydride was destroyed by the addition of water, and the reduced product (0.489 g.) was isolated by extraction of the insoluble residues with dry acetone and chloroform, and by evaporation of the filtrate.

All except 70 mg. of the reduced material was hydrolysed with N-hydrochloric acid, for 4 hours, neutralised in the usual manner, and evaporated to dryness, to give a mixture of partially methylated neutral monosaccharides. This mixture (0.320 g.) was fractionated on a cellulose column, using as eluant light petroleum (b.p. 100-120°) butan-1-ol (1:1), saturated with water, followed by butan-1-ol half-saturated with water. The following fractions were obtained.

<u>Fraction 1</u>. 0.096 g. R_G 0.85.
$[\alpha]_D = + 68^{o}$ (c., 0.46).

The syrup was chromatographically indistinguishable from an authentic sample of 2,3,4-tri-O-methylglucose, and was characterised by conversion to the aniline derivative, m.p. 129-131°, mixed m.p. 130-132° with an authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine.

<u>Fraction 2</u>. 0.081 g. R_G 0.85 and 0.74.

The faster-moving sugar was identified chromatographically as 2,3,4-tri-O-methylglucose. The slower-moving component was isolated by separation of the mixture on filter sheets in

solvent A. It had $[\alpha]_D = +92^o$ (c., 0.56). Examination in solvent E showed it to consist mainly of 2,3,6-tri-O-methyl-galactose, with some of the 2,3,4- derivative. Periodate oxidation of the product of borohydride reduction gave, according to chromatographic evidence, mainly 2,3-di-O-methylthreose (R_G 0.91), with a little 2,3,4-tri-O-methyllyxose (R_G 0.80).

The major component of the mixture of trimethyl galactoses was isolated by fractionation of the mixture on thick paper, in solvent E, and was characterised by oxidation with bromine water, to give 2,3,6-tri-O-methyl-D-galactonolactone, m.p. and mixed m.p. 98-99°.

<u>Fraction 3</u>. 0.034 g. R_G 0.62 and 0.59.

The syrup crystallised partially on standing and was chromatographically identical in solvents A and B to a mixture of 3,4-di-O-methylglucose and 3-O-methylrhamnose. The faster-moving major component (0.022 g.) was isolated by fractionation of the mixture on filter sheets in solvent B.

This sugar had $[\alpha]_D = +61^o$ (c., 0.65), and gave only glucose on demethylation. Chromatography of the product of periodate oxidation showed a pattern identical to that obtained on oxidation of 3,4-di-O-methylmannose. The sugar was recrystallised from ethyl acetate, to give a product melting at 112-115°. The value given in the literature for the m.p. of 3,4-

di-O-methyl-D-glucose (157) is 113°.

<u>Fraction 4.</u> 0.011 g. R_G 0.59.

$[\alpha]_D$ = + 37° (c., 0.80).

The syrup was chromatographically indistinguishable from 3-O-methylrhamnose in solvents A and B, and gave the same chromatographic pattern on periodate oxidation. Its ionophoretic mobility was also identical to that of 3-O-methylrhamnose. On seeding, the syrup crystallised, to give needles melting at 110-113° (m.p. not lowered on admixture with authentic 3-O-methyl-L-rhamnose).

<u>Fraction 5.</u> 0.030 g. R_G 0.48.

Demethylation gave only galactose. Chromatographic examination of the product of periodate oxidation showed that the fraction consisted of a mixture of 2,3- and 2,4-di-O-methyl-galactose, with a possible trace of the 2,6- derivative (shown by the presence of methoxymalondialdehyde). The syrup was heated under reflux with an equimolar quantity of aniline in dry ethanol. On evaporation of the solvent, a partially crystalline product was obtained. The crystals were isolated by washing with acetone, and, after recrystallisation, melted at 213-215°, m.p. not lowered on admixture with authentic 2,4-di-O-methyl-N-phenyl-D-galactosylamine. The remaining syrup, which was present in small proportion, did not crystallise out on

standing.

Fraction 6. 0.008 g. R_G 0.46.

The syrup crystallised on standing, and after recrystallisation from ethyl acetate, melted at 101-103°, m.p. not lowered on admixture with authentic 2,4-di-O-methyl-D-galactose. The sugar did not reduce periodate.

Fraction 7. 0.008 g. R_G 0.46 and 0.30.

The fraction was shown by chromatography to consist of a mixture of 2,4-di-O-methylgalactose and 2-O-methylgalactose.

Fraction 8. 0.007 g. R_G 0.30.

The fraction was chromatographically pure, and indistinguishable from 2-O-methylgalactose. Chromatography of the periodate oxidation product showed the presence of methoxymalondialdehyde, arising from a 2-O-methylaldose.

DISCUSSION

The minor component of Khaya senegalensis gum, which was left in solution after precipitation of the major component from aqueous ethanol, was isolated as the insoluble calcium salt. The polysaccharide itself was obtained by removal of calcium ions with cation exchange resin.

This fraction of the gum appeared to contain a much lower proportion of uronic acid residues than the major fraction; the carbazole method of uronic anhydride determination gave a value of 24.7%, while determinations of neutralisation equivalent gave results of about 1000, corresponding to less than 20% of uronic acid residues in the polysaccharide. The optical rotation of the polysaccharide in aqueous solution was also very different from that of the major fraction, being close to 0°.

The variable results obtained for the neutralisation equivalent suggested that the minor fraction of the gum might be heterogeneous, a possible explanation being that it consisted of a neutral polysaccharide, together with varying amounts of the major component. Attempts were therefore made to fractionate it, using a variety of methods.

Simple fractional precipitation with ethanol from the aqueous solution gave no evidence of heterogeneity. The first

fraction had a slightly low equivalent (793), but those of subsequent fractions, representing the bulk of the material, were fairly closely scattered about an average of 935.

The polysaccharide was next treated with the long chain quaternary ammonium salt, 'Cetavlon', or cetyltrimethylammonium bromide. Almost the whole of the sample was precipitated as the acidic polysaccharide-'Cetavlon' complex, and no neutral polysaccharide was precipitated on the addition of borate ions to the system.

Fractional precipitation of the copper complex of the polysaccharide from aqueous ethanol also failed to give any true fractionation. The first two fractions had low equivalents, suggesting the presence of some of the major fraction as an impurity but, as before, later fractions had equivalents of about 950 and no neutral polysaccharide was isolated.

At this stage, glass fibre paper ionophoresis was carried out on the gum and its fractions. Since the method was capable of showing heterogeneity in the whole gum, and in the major fraction, the appearance of only one component on the ionophoretogram for the minor fraction was taken as a fairly certain indication that it consisted of a single homogeneous polysaccharide.

Preliminary partial hydrolysis studies were carried out on the polysaccharide. Galactose was present in high proportion

in the hydrolysis product, along with arabinose and rhamnose, and an acidic fraction. This acidic fraction, on chromatographic examination in acid solvent, was shown to be a complex mixture of acidic fragments. That present in highest proportion was chromatographically indistinguishable from 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose. On hydrolysis, galactose was the only neutral sugar obtained and reduction and hydrolysis gave galactose and 4-O-methylglucose in equal amounts; the major acidic component obtained on hydrolysis of the polysaccharide is therefore an aldobiouronic acid consisting of 4-O-methylglucuronic acid linked to a galactose residue.

Two other, faster moving components were also present, in approximately equal quantities. Hydrolysis of these together gave galactose, a smaller amount of rhamnose, and an acidic fraction; on reduction followed by hydrolysis, galactose, 4-O-methylglucose and rhamnose were obtained, indicating that the 4-O-methylglucuronic acid residues present in the polysaccharide probably take part in more than one glycuronosyl linkage. Neither component was chromatographically identical to galacturonic, glucuronic or 4-O-methylglucuronic acid, or to 2-O-D-galacturonosyl-L-rhamnose.

A mixture of very slow moving acidic fragments, probably of higher chain length than the major component of the

hydrolysis product, was also examined. After reduction and hydrolysis, the sugars obtained were galactose in high proportion, 4-O-methylglucose and glucose in approximately equal amounts, some rhamnose and a trace of arabinose.

From these results, it is apparent that the acidity of the polysaccharide is largely due to the presence of glucuronic acid residues, most, although not all, of which are methylated at the C4 position. It cannot be stated whether or not galacturonic acid residues are also present.

The polysaccharide was methylated as fully as possible, using Haworth's and Purdie's reagents in succession. The product, which contained 40.5% of methoxyl groups, was then hydrolysed to give a complex mixture of neutral methylated sugars and methylated acidic oligosaccharides.

By exhaustive extraction of this mixture with ether, most of the neutral methylated sugars were removed, together with some of the acidic material in the form of unhydrolysed ester. The neutral sugars remaining in the residue after extraction were separated from the bulk of the acidic material by chromatography on cellulose, and were identified as 2,3,4-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose and 2-O-methyl-D-galactose.

The product of ether extraction was fractionated by partition chromatography on cellulose, giving 2,3,5-tri-O-

methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-galactose, 3,4-di-O-methyl-rhamnose, 2,3,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose, 3-O-methyl-L-rhamnose, 2,4- and 2,3-di-O-methyl-D-galactose and 2-O-methyl-D-galactose, together with a small proportion of acid derivatives, which were not identified. All the neutral sugars except the 3,4-di-O-methylrhamnose and the 2,3-di-O-methylgalactose were identified by the formation of crystalline derivatives, or by the crystallinity of the sugars themselves.

Since many of the fractions obtained were mixtures, which had to be reseparated, it was possible only to estimate a very approximate value for the weight of each sugar present.

	Approx. wt.	Approx. Molar Propns.
2,3,4,6-tetra-O-methyl-D-galactose	0.34 g.	8
2,3,4-tri-O-methyl-D-galactose	0.55 g.	14
2,3,6-tri-O-methyl-D-galactose	0.14 g.	3.5
2,4-di-O-methyl-D-galactose	0.77 g.	20
2,3,5-tri-O-methyl-L-arabinose	0.11 g.	3
3-O-methyl-L-rhamnose	0.07 g.	2

The 3,4-di-O-methylrhamnose and the 2,3-di-O-methylgalactose were present only in very small amounts. The proportion of 2-O-methylgalactose obtained was also small relative to the amounts of the other galactose derivatives and it was

impossible to say whether it was a product of incomplete methylation, or whether it was true structural significance.

The results given in the table indicate the presence in the polysaccharide of large amounts of galactose, existing as end groups, 1,6-linked units, and 1,3,6-linked branch points, with a smaller proportion linked through positions 1 and 4. The small quantities of 2,3,5-tri-O-methylarabinose and 3-O-methylrhamnose isolated represent arabofuranose end groups and rhamnose branch points respectively, present in small proportion in the polysaccharide. The amount of 1,2-linked rhamnose present is probably very small.

The methylated acidic sugars were converted to the methyl ester methyl glycosides, which were then reduced with lithium aluminium hydride, to give the corresponding methylated neutral oligosaccharides. These, on hydrolysis, gave rise to a mixture of neutral methylated sugars which was resolved on cellulose into 2,3,4-tri- and 3,4-di-O-methyl-D-glucose, 2,3,6- and 2,3,4-tri-O-methyl-D-galactose, 2,3- and 2,4-di-O-methyl-D-galactose, with a possible trace of the 2,6- derivative, 2-O-methyl-D-galactose, and 3-O-methyl-L-rhamnose. Of these, the tri-O-methylglucose, the 2,3,6-tri- and 2,4-di-O-methylgalactose and the 3-O-methylrhamnose were identified by means of crystalline derivatives; for the others, identification was by chromatographic means.

The approximate weights of the various methylated sugars were estimated to be as follows, although again no accurate estimation was possible.

	Approx. wt.	Approx. Molar Proportions
2,3,4-tri-O-methyl-D-glucose	0.110 g.	8
3,4-di-O-methyl-D-glucose	0.027 g.	2
2,3,6-tri-O-methyl-D-galactose	0.045 g.	3
2,3,4-tri-O-methyl-D-galactose	0.015 g.	1
2,4-di-O-methyl-D-galactose	0.030 g.	2
2,3-di-O-methyl-D-galactose	0.010 g.	1
2-O-methyl-D-galactose	0.012 g.	1
3-O-methyl-L-rhamnose	0.018 g.	1.5

These products must have arisen from a complex mixture of partially methylated acidic fragments, some of which still contained neutral sugar residues, the glycuronosyl links having resisted hydrolysis. The 2,3,6-tri-O-methylgalactose and 3-O-methylrhamnose, for example, must represent 1,4-linked galactose and 1,2,4-linked rhamnose units to which acid residues are glycosidically attached in the polysaccharide. From the proportions of methylated sugars present in the mixture, the 2,3,6-tri-O-methylgalactose can only have been joined to the 2,3,4-tri-O-methylgalactose. When taken into conjunction with the results of the partial hydrolysis studies, this

evidence points to the existence in the polysaccharide of an aldobiouronic acid fragment, consisting of a 4-O-methylglucuronic acid end group joined to a 1,4-linked galactose unit and accounting for a high proportion of the uronic acid content.

The 3,4-di-O-methylglucose must have arisen from 1,2-linked 4-O-methylglucuronic or glucuronic acid residues. The 2,3-di-O-methylgalactose is present in significant amount only in the acidic fraction and cannot have arisen from a neutral sugar. It must therefore indicate the presence of 1,4-linked galacturonic acid residues in small proportion in the polysaccharide.

The other galactose derivatives may be products of reduction of other methylated galacturonic acid fragments, but some may equally well arise from neutral sugars, since all have been isolated in the neutral fraction of the hydrolysed methylated polysaccharide.

The minor fraction of K. senegalensis gum, therefore, may be said definitely to contain the following residues.

```
    Gal 1 -          - 6 Gal 1 -          - 6 Gal 1 -
                                                3
                                                '
    GA 1 - 4 Gal 1 -     Ar 1 -           - 4 Rh 1 -
                                                2
                                                '
    - 2 GA 1 -       - 4 Gal A 1 -
```

(Gal = D-galactopyranose, Ar = L-arabofuranose, Rh = L-

rhamnopyranose, GA = 4-O-methyl-D-glucuronic acid or D-glucuronic acid, Gal A = D-galacturonic acid).

There is a possibility that galacturonic acid may also be present as end groups and as 1,3-linked units.

It is impossible on the basis of investigations so far carried out to propose a general structure for the minor component of _K. senegalensis_ gum, but it is clear that the two polysaccharides present in the gum are markedly different in character. The major component is typical of gums containing a high proportion of galacturonic acid residues, or of high acid content generally, whereas the picture which emerges for the structure of the minor component is that of a basic framework of galactose units, the characteristic linking points being carbon atoms 1,3 and 6, with arabinose and uronic acid residues occupying peripheral positions; in other words, a typical low-acid gum polysaccharide.

Although the order of linkage of the various units is not known, the indications are that the molecular structure is similar in many respects to those of the _Acacia_ and _Prunus_ gums (Table I, p.29) and mesquite gum (p.32), all of which also consist of a galactan backbone, with 1,3- and 1,6-links predominating, and with side chains of varying composition, usually containing glucuronic or 4-O-methylglucuronic acid and arabinose. In all these gums, however, the arabinose is

present in fairly high proportion, and exists as non-terminal units as well as end groups, and the galactose seldom occupies a terminal position, as it does in the K. senegalensis polysaccharide.

But perhaps the most unusual features of the polysaccharide under study are the 1,4-linked galacturonic acid, and the 1,2,4-linked rhamnose residues, which are more typical of the high acid gums, and are found in the major component of the gum. Galacturonic as well as glucuronic acid has been isolated from Combretum leonense gum (p.27), although it, too, is a low acid gum containing a fairly high proportion of 1,6-linked galactose units, but it appears to be linked in the typical manner to the C2 position of rhamnose, which seems not to be the case here. Rhamnose exists only in trace amount, if at all, in the 4-O-methylglucuronic acid containing gums and in those of the genus Prunus; in the Acacia gums, appreciable amounts have been found but always in peripheral positions and easily removable by autohydrolysis.

At this stage, it is necessary to re-examine the question of the homogeneity of the polysaccharide. It is possible that these non-typical residues, present in small proportion, may be due to contamination by the major component of the gum. The aldobiouronic acid fragment 4-O-(4-O-methyl-D-glucuronosyl) -D-galactose is also isolated from the major component, but is

present in the minor fraction in such high proportion that it cannot have arisen solely from the small quantities of contaminant which may be present and must therefore be an integral part of the structure of the polysaccharide.

A considerable amount of work remains to be carried out on the minor component of the gum, in order to obtain a clearer picture of its molecular structure. Periodate oxidation studies should be rewarding, since it may be possible to isolate a periodate-resistant nucleus of 1,3-linked galactose units. It should also be possible, by graded hydrolysis, to obtain neutral oligosaccharides, the identification of which would help considerably in the elucidation of the polysaccharide structure. Finally, it will be necessary to carry out a more detailed examination of the acidic fragments obtained on partial hydrolysis of the polysaccharide.

SECTION III

COCHLOSPERMUM GOSSYPIUM GUM

EXPERIMENTAL

ATTEMPTED REDUCTION OF COCHLOSPERMUM GOSSYPIUM GUM

The powdered gum was first deacetylated as follows. It was added slowly with stirring to warm water, to give an extremely viscous gel. This was made up to 5% with concentrated sodium hydroxide solution, kept at about 40° for 7 hours and allowed to stand at room temperature for a further 20 hours. At the end of this time, the viscosity had decreased considerably and on acidification with hydrochloric acid, it decreased further. The solution was filtered in order to remove mechanical impurities and the gum was precipitated from methylated spirits (4 vol.). The precipitate was centrifuged off, washed with methylated spirits until free from chloride ions, washed with methanol and ether in succession, and dried in vacuo, to give the deacetylated polysaccharide as a whitish powder.

The deacetylated polysaccharide (15 g.) was added with stirring to water (500 ml.), to give a viscous product, ethylene oxide (75 ml.) was added and the mixture was allowed to stand at room temperature for 9 days. At the end of this time, the pH of the solution had risen from 2 to 7 and the solution was no longer viscous. The glycol ester was precipitated by adding the solution slowly with stirring to 4 volumes

of methylated spirits. It came down as a fibrous mass, which was washed several times with ethanol and with ether and dried in vacuo. The mother liquors were evaporated to small volume and a further quantity of esterified polysaccharide was precipitated from methylated spirits and isolated in the same manner as before.

Total yield of glycol ester - 15.6 g.

The glycol ester of the polysaccharide was dissolved in water (350 ml.), glycerol (5.5 g.) was added, the solution was cooled to 0°, and a solution of potassium borohydride (5 g.) in water (30 ml.) was added. After standing for 16 hours at room temperature the solution was de-ionised by passing it through alternate columns of the Amberlite resins IR-120(H) and IR-400, until a neutral eluate was obtained. The solution was evaporated to small volume and the reduced polysaccharide was precipitated from ethanol, washed with ethanol and ether and dried in vacuo, to give a yield of 11.2 g.

The uronic anhydride content was measured by Kaye and Kent's method (39) and was found to have been lowered to 19.3%, from an original value of 37%.

Two further reductions were carried out in the same manner. The final product (7 g.) had a uronic anhydride content of about 14%.

A portion was hydrolysed with N hydrochloric acid for

6 hours and the hydrolysate was examined chromatographically in solvents F and H. The presence of galactose, rhamnose and glucose, together with two acidic fractions, was detected.

Since the reductions were very inefficient beyond a uronic anhydride content of 15% and losses of product were high, no more were carried out.

PARTIAL HYDROLYSIS OF COCHLOSPERMUM GOSSYPIUM GUM, AND IDENTIFICATION OF THE ACIDIC FRAGMENTS.

TRIAL HYDROLYSIS

The polysaccharide (100 mg.), in its original acetylated form, was hydrolysed at 100° with N-sulphuric acid. After 6 hours, the solution was neutralised with barium hydroxide and barium carbonate and centrifuged. Barium ions were removed with Amberlite resin IR-120(H) and the solution was evaporated to dryness. The product was fractionated into its neutral and acidic components on thick paper, in solvent E.

The acidic fraction, which remained on the starting line of the chromatogram, was eluted from the paper and a portion was examined chromatographically in solvent F. Three spots were observed, one (A) chromatographically identical with D-galacturonic acid, the second (B) having R_{GalA} 0.76 and the third (C) very slow, having R_{GalA} 0.14.

The bulk of the acidic material was separated on thick paper, in solvent F, into these three fractions. Each was subjected to methanolysis, potassium borohydride reduction and hydrolysis with N-hydrochloric acid and in each case the product was examined chromatographically in solvent E.

A. The fastest fraction gave mainly galactose, with slight traces of rhamnose, probably due to incomplete separation.

B. This fraction gave galactose and rhamnose in equal proportion, and was probably a galacturonosyl rhamnose.

C. The slow fraction gave galactose, a smaller amount of glucose, and a trace of rhamnose. An unidentified pink spot was observed, having $R_{rhamnose}$ 1.03. This was chromatographically non-identical with glucurone and 4-O-methylglucose. Determination of methoxyl content of the original gum by Zeisel's method, using infra-red spectroscopic techniques, showed that no methoxyl groups were present; the spot therefore did not arise from a methylated sugar. The proportion of glucose in the hydrolysate did not increase on further hydrolysis.

LARGE SCALE HYDROLYSIS

The powdered gum (2.7 g.) was allowed to swell in water (50 ml.) for 2 days. 2N-Sulphuric acid (50 ml.) was added and the suspension was warmed. After a short time, a clear, mobile solution was obtained and mechanical impurities were filtered off. The solution was then heated at 100° for 12 hours, neutralised with barium hydroxide and barium carbonate, de-ionised with Amberlite resin IR-120(H) and evaporated to dryness, to give 1.47 g. of syrup.

This was placed on a column of Amberlite resin CG-45 (formate form). The column was washed through with carbonate-free water in order to remove the neutral sugars completely.

The acidic fraction of the hydrolysate was then eluted with 15% formic acid. Evaporation to dryness yielded 0.646 g. of product.

The acidic mixture was fractionated chromatographically, on Whatman No. 17 paper. The following fractions were obtained.
I. 0.022 g. This fraction gave only galactose on reduction and was chromatographically identical to galacturonic acid.

II. 0.165 g. The acid was methylated three times by Haworth's method. Each methylation involved the addition of dimethyl sulphate (9 ml.) and 30% sodium hydroxide solution (20 ml.). The temperature of the reaction mixture was kept below 10°, and vigorous stirring was maintained during and between methylations.

After the final methylation, the solution was heated on a boiling water bath for 30 min., cooled and just acidified with dilute sulphuric acid. Sodium sulphate was precipitated by pouring the solution into an excess of methylated spirits. The precipitate was filtered off and washed with methylated spirits, and the combined filtrate and washings were made slightly alkaline and evaporated to a volume of 50-100 ml. The solution was re-acidified and extracted 4 times with chloroform. The combined extracts were evaporated to small volume and filtered and the methylated acid (0.124 g.) was precipitated by the addition to the filtrate of light petroleum (b.p. 60-80°).

After recrystallisation from light petroleum, almost colourless plates were obtained, melting at 67-69°, m.p. not depressed on admixture with an authentic sample of methyl 2-O-α-D-galactopyranosyluronic acid-L-rhamnoside pentamethyl ether. The pure product had $\alpha_D = +92°$ (c.,0.49 in chloroform), and gave an X-ray powder photograph identical to that obtained from the authentic sample.

III. 0.191 g. The same procedure was followed as for the methylation of fraction II, except that in each methylation, dimethyl sulphate (11 ml.) and sodium hydroxide solution (25 ml.) were added.

The product (86 mg.) was dissolved in dry tetrahydrofuran and reduced by the addition of lithium aluminium hydride (40 mg.) before heating was begun, and of a further portion (40 mg.) after 1 hour. Heating was continued for another hour. Excess lithium aluminium hydride was destroyed by the addition of water. The precipitated hydroxides were filtered off and extracted with hot acetone and chloroform. The filtrate was evaporated to dryness and also extracted. The combined extracts on evaporation gave the fully reduced product (76 mg.).

A small scale hydrolysis was carried out, using N-hydrochloric acid, for 4 hours. Chromatographic examination of the product showed methylated sugars having R_G values of 0.89, 0.73

and 0.47, with traces of others.

The bulk of the reduced product was similarly hydrolysed and a portion was examined by vapour phase chromatography (see Fig.4, opposite). The peaks represented the methyl glycosides of the following methylated sugars. (R_Q represents the rate of flow compared to that of the standard, quinoline).

A,B,C.(R_Q 0.73,1.02,1.24) - unidentified pentose or deoxyhexose derivatives.

D,F.(R_Q 1.57,2.12) - 2,3,4-tri-O-methylglucopyranose.

E,G.(R_Q 1.91.2.44) - 2,3,6-tri-O-methylgalactofuranose.

H,I.(R_Q 2.60,2.97) - 2,3,6-tri-O-methylgalactopyranose.

I,J.(R_Q 2.97,3.33) - 2,3,4-tri-O-methylgalactopyranose.

K,L. - a di-O-methylgalactopyranose, probably the 2,4- derivative.

The hydrolysate (45 mg.) was separated on thick paper, in solvent A, into three fractions, corresponding approximately to the R_G values given previously.

(a) The fastest-moving fraction was shown by chromatography in solvent B and by ionophoresis to consist of a mixture of 2,3,4-tri-O-methylglucose and 3,4-di-O-methylrhamnose.

(b) The fraction having an R_G value of about 0.74 gave only galactose on demethylation. Chromatography in solvent E indicated the presence of a mixture of 2,3,6- and 2,3,4-tri-O-methylgalactose.

(c) The slow-moving fraction gave galactose on demethylation and was unattacked by periodate. Periodate oxidation of the product of borohydride reduction gave a chromatographic spot (presumably a dimethylpentose) identical to that obtained from authentic 2,4-di-O-methylgalactose and different from the dimethyltetrose spot obtained from authentic 2,3-di-O-methyl-D-galactose.

IV. 0.031 g. This fraction was extremely slow-moving, its $R_{galacturonic\ acid}$ value being less than 0.05. A small portion was hydrolysed with N-hydrochloric acid for 4 hours. Chromatographic examination of the product in solvent E showed the presence of galactose, rhamnose and an acidic fraction. Another portion (5 mg.) was subjected to methanolysis, reduction with potassium borohydride and hydrolysis with 2N-hydrochloric acid for 6 hours. The product, on chromatographic examination, was found to contain galactose, rhamnose and glucose.

The fraction was examined chromatographically in ethyl acetate/acetic acid/formic acid/water (18:8:3:9), along with an authentic sample of O-D-galacturonosyl-(1→2)-O-L-rhamnosyl-(1→4)-D-galactose. The latter was shown to be absent and fraction IV to consist of three components, all slower-moving than the aldotriouronic acid.

THE METHYLATION OF *COCHLOSPERMUM GOSSYPIUM* GUM

The powdered gum (20 g.) was added slowly with constant stirring to water (150 ml.), which caused it to swell considerably, to give a very viscous product. Deacetylation was carried out by the addition of sodium hydroxide (67.5 g.) in concentrated solution. Since the gel was still too thick to be workable, it was placed in the methylation vessel, diluted gradually with stirring to a volume of about 700 ml. and stirred overnight to give a homogeneous solution of fairly high viscosity.

This was methylated by the dropwise addition of a further 175 ml. of 30% sodium hydroxide solution together with dimethyl sulphate (250 ml.) over a period of 7 hours, with efficient stirring, the temperature being maintained below 15°. Stirring was continued overnight and the solution was dialysed for three days against running water, evaporated to small volume and methylated three times more in the same manner. Each methylation involved the addition of 30% sodium hydroxide solution (400 ml.) and dimethyl sulphate (280 ml.), with stirring during and between methylations.

The product was heated to boiling point to destroy excess dimethyl sulphate, allowed to cool, and acidified with 25% sulphuric acid. Under these conditions, the free acid form

of the partially methylated polysaccharide came out of solution. The precipitate was extracted first with chloroform and then with acetone, to give a chloroform-soluble fraction (11.9 g.,OMe 31%), and an acetone-soluble fraction (2.4 g., OMe 27.5%). The latter was remethylated once, giving a chloroform-soluble product, which, together with that obtained by chloroform extraction of the mother liquors, was combined with the major fraction. Dialysis and evaporation of the mother liquors gave a negligible yield of polysaccharide.

The partially methylated polysaccharide was dissolved in water and neutralised with silver carbonate and the filtrate was freeze-dried to give a brown glassy product (12.6 g.), which was methylated in two portions of 7 g. and 5.6 g., by Purdie's method. The silver salt (7 g.) was finely ground and heated under reflux with dry methyl iodide (75 ml.), with the addition in small portions of freshly prepared ether-dried silver oxide (7 g.), over a period of 7 hours. The supernatant liquid was then decanted, the residue was extracted three times with hot chloroform and the combined solutions were evaporated to dryness. Three more methylations were carried out, and four on the other portion of silver salt in the same manner, to give the methylated polysaccharide as a pale yellow glassy solid (8.3 g.), OMe 37.4%, not raised by further methy-

lation.

The product was fractionated by heating under reflux with a series of solvent mixtures containing different proportions of light petroleum (b.p. 100-120°) and chloroform. Fractions were obtained as shown below.

	% Petroleum in solvent.	% $CHCl_3$ in solvent.	Wt. of Fraction	OMe(%)	$[\alpha]_D$ in MeOH
1.	100	-	-	-	-
2.	95	5	0.83 g.	37.7	52.5°(c.,0.446)
3.	90	10	1.15 g.	37.7	57.9°(c.,0.542)
4.	85	15	5.14 g.	37.1	57.6°(c.,0.232)
5.	80	20	1.16 g.	35.9	52.1°(c.,0.402)
6.	75	25	0.04 g.	-	-

Fractions 3 and 4 were combined and remethylated to give a product of methoxyl content 37.6%, not raised on further methylation.

THE HYDROLYSIS OF THE METHYLATED GUM

The fully methylated polysaccharide (5.05 g.) was hydrolysed by heating at 100° with 90% formic acid (200 ml.), for 25 hours. The solution had begun to darken after 6 hours; however, chromatographic examination at this stage showed that hydrolysis was not nearly complete. After completion of

hydrolysis, most of the formic acid was removed by repeated dilution and evaporation of the solution under reduced pressure, the final traces being removed by evaporation with methanol.

Formyl esters of the methylated sugars were decomposed by heating on a boiling water bath for 2 hours with 0.5N sulphuric acid. The solution was neutralised with barium carbonate, filtered and the filtrate evaporated to dryness, to give a brown syrup. This was freeze-dried in admixture with cellulose powder, and placed on a cellulose column (35 x 600 mm.). Complete removal of methylated neutral sugars was effected by washing with 4 litres of butanol half-saturated with water. Chromatographic examination in solvent A showed the presence of the methylated sugars previously identified, viz. :-

2,3,4-tri-O-methyl-L-rhamnose (R_G 1.01)
2,3,4,6-tetra-O-methyl-D-galactose (R_G 0.88)
3,4-di-O-methyl-L-rhamnose (R_G 0.84)
2,3,6-tri-O-methyl-D-galactose (R_G 0.75)
3-O-methyl-L-rhamnose (R_G 0.60)

A small amount of free L-rhamnose (R_G 0.32) was also observed.

The mixture of methylated uronic acids was eluted with water, evaporation giving 1.8 g. of syrup. Chromatographic examination of a portion in solvent C showed the presence of

three main fractions, having R_G values of 0.83, 0.45 and 0.40. Three other fractions, of R_G values 0.90, 0.65 and 0.20, were present in small amount.

REDUCTION OF THE MIXTURE OF METHYLATED URONIC ACIDS

The dry syrup was allowed to stand overnight in solution in a dry 2.6% methanolic solution of hydrogen chloride, heated under reflux for 6 hours, neutralised and evaporated to dryness. The product (1.7 g.) was dissolved in pure dry tetrahydrofuran (30 ml.) and a slurry of lithium aluminium hydride (2 g.) in tetrahydrofuran (30 ml.) was added to the refluxing solution over a period of 1 hour. Heating was continued for a further hour, the mixture was allowed to cool and excess lithium aluminium hydride was destroyed by the addition of ethyl acetate and water in succession. The precipitated hydroxides were filtered off, and extracted several times with dry acetone and dry chloroform. The filtrate was evaporated to dryness, and also extracted. All the extracts were combined and evaporated to give a yellow syrup (1.0 g.), which on chromatographic examination was shown to contain methylated sugars of R_G values 0.86, 0.60 (trace), 0.31 and 0.24. Examination in solvents B and G showed that 3,4-di-O-methyl rhamnose, which would be obscured in solvent A, was not present in the mixture.

SEPARATION OF THE METHYLATED SUGARS OBTAINED ON REDUCTION

The syrup was placed on a cellulose column (2.5 x 45 cm.), which was eluted with light petroleum (b.p. 100-120°)(50%)/ butan-1-ol (50%), saturated with water. For the slower components, the eluting solvent was changed to butan-1-ol half-saturated with water. The eluate was examined chromatographically in solvent A, and bulked accordingly to give the following fractions.

Fraction 1. 0.011g. R_G 0.99

$[\alpha]_D = +98^o$.

The yellow-brown colour obtained with aniline oxalate spray did not correspond with any of the known methylated monosaccharides. A portion of the syrup was warmed for 10 min. with normal sodium hydroxide solution. The solution was de-ionised with Amberlite resin IR-120(H) and evaporated to dryness and the product was examined chromatographically in solvent A. Two spots were observed, having R_G 0.87 and 0.76. These suggested the possible presence of 2,3,4-tri-O-methyl-glucose and 2,3,6-tri-O-methylgalactose linked in a methylated disaccharide. Examination in solvent F showed that no acids were present.

Fraction 2. 0.196g. R_G 0.85

$[\alpha]_D = +66^o$ (c.,1.84).

OMe = 35.3% (Calc. 41.8% for a tri-O-methylhexose).

Chromatography in solvent A showed a pink spot identical to 2,3,4-tri-O-methylglucose. Demethylation gave glucose only. Periodate oxidation of the product of potassium borohydride reduction gave a product chromatographically identical to 2,3,4-tri-O-methylxylose. This was also obtained from an authentic sample of 2,3,4-tri-O-methylglucose, similarly treated.

The aniline derivative was prepared, but was not successfully crystallised. The free methylated sugar was regenerated by heating under reflux in ethanolic solution with Amberlite resin IR-120(H), and the potassium borohydride reduction and periodate oxidation were repeated on the bulk of the material. The product was again chromatographically identical with 2,3,4-tri-O-methylxylose, but all attempts to crystallise it were unsuccessful.

Fraction 3. 0.073g. R_G 0.78, 0.71 and 0.63.

Chromatography in solvent A gave streaking, but better separation was obtained in solvent B. The mixture was fractionated on filter sheets in solvent B, to give the following sub-fractions.

Fraction 3 (a). 0.024g. R_G 0.78.

Only galactose was obtained on demethylation. The fraction was chromatographically identical to 2,3,6-tri-O-methyl-D-galactose, in solvents A and E.

Fraction 3 (b). 0.012g. R_G 0.71.

Only galactose was obtained on demethylation.

Fraction 3 (c). 0.011g. R_G 0.63.

The sugar gave a brown spot with aniline oxalate spray. Demethylation gave glucose only. A portion was treated with periodate and was found to be attacked by it. Examination of the product in solvent A showed a pattern identical to that obtained from 3,4-di-O-methylmannose.

Fraction 4. 0.012g. R_G 0.59.

$[\alpha]_D = +57^o$ (c.,1.2).

Paper ionophoresis showed the presence of 3-O-methyl-rhamnose (M_G 0.41) as the main component, together with impurities of M_G 0.59 and 0.12. The identity of the 3-O-methyl-rhamnose was confirmed by chromatographic examination of the product of periodate oxidation.

Fraction 5. 0.014g. R_G 0.50.

Only galactose was obtained on demethylation. Paper chromatography and ionophoresis indicated 2,3-di-O-methylgalactose. Its presence was confirmed by periodate oxidation followed by chromatographic examination, and by potassium borohydride reduction followed by periodate oxidation and chromatographic examination of the product.

Fraction 6. 0.023g. R_G 0.42 approx.

The fraction was apparently a mixture, since it showed both a brown and a pink colour with aniline oxalate spray, but it

could not be fractionated chromatographically. Demethylation gave galactose only.

Chromatographic examination of the product of periodate oxidation showed a pattern corresponding mainly to a di-O-methylpentose, which would arise from a di-O-methylhexose not methylated in positions 1 and 2. Periodate oxidation of the product of borohydride reduction followed by chromatographic examination showed mainly the same pattern as obtained for authentic 3,4-di-O-methylgalactose. Ionophoresis showed that the major component of the fraction had the same mobility as authentic 3,4-di-O-methylgalactose.

Fraction 7. 0.060g. R_G 0.31.

$[\alpha]_D = +68^{\circ}$ (c.,0.34).

OMe 15.6% (Calc. 16% for a monomethylhexose).

Demethylation gave galactose. The sugar was crystalline and was chromatographically identical to 2-O-methylgalactose. Chromatographic examination of the product of periodate oxidation showed a pattern identical to that obtained from an authentic sample of 2-O-methylgalactose. The methyl glycoside took up approximately 1 mole of periodate. The sugar was recrystallised from glacial acetic acid, to give a product with m.p. 143 - 7°, alone and in admixture with 2-O-methylgalactose.

Fraction 8. 0.138g. R_G 0.24.

$[\alpha]_D = +86^{\circ}$ (c.,0.44).

OMe = 12.3% (Calc. 16% for a monomethylhexose).

Galactose was obtained on demethylation. Chromatographic examination of the sugar and of the product of periodate oxidation indicated the presence of 3-O-methylgalactose. The methyl glycoside was unattacked by periodate; this excludes all other monomethyl ethers of galactose. The sugar crystallised out on standing and was recrystallised from ethanol/acetone to give a product melting at 140-142°, m.p. not lowered on admixture with an authentic sample of 3-O-methyl-D-galactose.

DISCUSSION

Cochlospermum gossypium gum, sometimes known as kutira gum, is of Indian origin and because of similarities in properties has been used as a substitute for karaya gum, the exudate of Sterculia urens. It has already been examined by Hirst and Dunstan (141). The gum was found by these authors to be a partially acetylated acidic polysaccharide, of equivalent weight 470, corresponding to a uronic anhydride content of 37%. The fact that purified samples from different nodules showed no significant variation in properties suggested that the gum was homogeneous. Evidence was obtained for the presence of residues of D-galactose, L-rhamnose and D-galacturonic acid, in approximately equal proportions.

The gum was methylated by these workers and the modes of linkage of the neutral sugars were definitely established. Galactose was found to exist in the form of end groups (2 parts), and as 1,4-linked units (1 part), and rhamnose as end groups (1 part), 1,2-linked straight chain components (1 part), and 1,2,4-linked branch points (1 part). The position as regards the methylated uronic acid residues, however, was by no means clear; it could only be tentatively suggested that galacturonic acid residues might exist as branch points, and 1,2- or 1,3-linked straight chain components.

The examination of hydrolysis products did not clarify the situation appreciably. No pure aldobiouronic acids were isolated, but on methylation and hydrolysis of the acidic portion of the hydrolysate, 2,3,6-tri-O-methyl-D-galactose, 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-galacturonic acid were identified, suggesting the possible presence of 2-O-D-galacturonosyl-L-rhamnose, 4-O-D-galacturonosyl-D-galactose, and 4-O-D-galacturonosyl-D-galacturonic acid.

In the present work, it was hoped to elucidate the structure of the acidic portion of the gum. If this was indeed largely composed of branch points and 1,3-linked units, it should be a suitable subject for periodate oxidation studies. First, however, it would be necessary to reduce the gum completely, to the corresponding neutral polysaccharide. Since, of the neutral sugar residues, only a proportion of the rhamnose would be resistant to periodate oxidation, the isolation of a periodate-resistant nucleus of galactose units in the reduced polysaccharide would indicate the presence in the original gum of a 1,3-linked chain of galacturonic acid residues. The method of reduction used (74) involved the esterification of the carboxyl groups in the deacetylated gum with ethylene oxide and reduction of the glycol ester with potassium borohydride. Unfortunately, after slightly more than half the carboxyl groups had been reduced, it was found that very little further reduction was

possible, and in the absence of a more efficient method, the project had to be abandoned.

One interesting piece of information, however, arose during the course of the experiment. Chromatography of the hydrolysate of the partially reduced polysaccharide showed the presence of glucose, which was not obtained on hydrolysis of the original gum, and must therefore have arisen from reduced glucuronic acid units. Evidence was thus provided for the existence in the gum of residues of glucuronic acid, as well as of galacturonic acid. This evidence is in agreement with the findings of Heidelberger et al. in their immunological studies (12); the co-precipitation observed to take place between C. gossypium gum and anti-pneumococcus serum Type II strongly suggests the presence of D-glucuronic acid end groups in the gum. In this respect, therefore, C. gossypium gum resembles the Khaya gums, which also contain two different uronic acid residues. In the latter, however, the glucuronic acid is methylated in the C4 position, whereas methoxyl groups have been shown to be absent in C. gossypium gum.

Partial hydrolysis of the gum gave galactose, rhamnose and an acidic fraction, which was separated chromatographically on filter sheets, into four components. Two of these were present in relatively large amount, while the others were only minor components. One of the small fractions was fast moving

and was shown chromatographically to consist entirely of galacturonic acid. The other was very slow moving, and gave galactose, rhamnose and an acidic fraction after hydrolysis, and galactose, rhamnose and glucose after reduction and hydrolysis. On chromatographic examination, it proved to be a mixture of three components, all slower moving than the aldotriouronic acid O-D-galacturonosyl-(1→2)-O-L-rhamnosyl(1→4)-D-galactose.

The two fractions present in major amount were thought, on chromatographic evidence, to be two aldobiouronic acids, a galacturonosyl rhamnose and a glucuronosyl galactose. The faster moving was conclusively identified as 2-O-α-D-galacturonosyl-L-rhamnose, by formation of the crystalline methylated derivative, methyl 2-O-α-D-galactopyranosyluronic acid-L-rhamnoside pentamethyl ether.

The second large fraction, however, on methylation, followed by reduction and hydrolysis, gave a product which was shown chromatographically to contain at least three partially methylated neutral sugars, indicating that it was a mixture of at least two chromatographically indistinguishable acidic oligosaccharides. A portion of the product was converted to the more volatile methyl glycosides of the sugars present, and was examined chromatographically in the vapour phase, by Dr. C.T. Bishop. The results obtained (see Fig 4, facing p.119) indicated the presence of 2,3,4-tri-O-methylglucose, 2,3,6-tri- and

2,3,4-tri-O-methylgalactose, a di-O-methylgalactose and an unidentified sugar. Paper chromatographic methods confirmed the identity of the tri-O-methylhexoses, identified the di-O-methylgalactose as the 2,4- derivative and showed that the previously unidentified sugar was 3,4-di-O-methylrhamnose. From the amount of 2,4-di-O-methylgalactose present, there could be no doubt that it was of structural significance, and not a product of incomplete methylation. The methylated sugars, therefore, arose from the following groups in the mixture of acidic oligosaccharides.

GA 1 -	- 2 Rh
GalA 1 -	- 4 Gal
	- 3 GalA

Since the stability of galacturonosyl linkages is much higher than that of glycosidic links, it seems fairly certain that the 1,3-linked galacturonic acid unit arises from a fragment containing adjacent galacturonic acid residues and not from the reducing end of a trisaccharide containing a neutral sugar residue. This fragment may be a 1,3-linked digalacturonic acid, or may be a trisaccharide, with a further neutral sugar at the reducing end. Beyond this, no conclusions can be drawn regarding the mode of linkage of the residues found to be present in the mixture.

The gum was methylated as fully as possible and afterwards

hydrolysed. The neutral and acidic portions of the product were separated on cellulose and chromatographic evidence was obtained for the presence in the former of 2,3,4-tri-O-methyl-rhamnose, 3,4-di-O-methylrhamnose, 3-O-methylrhamnose, 2,3,4,6-tetra-O-methylgalactose and 2,3,6-tri-O-methylgalactose, with 2,6-di-O-methylgalactose in small amount. These results were in agreement with the findings of Hirst and Dunstan. A small proportion of free rhamnose was also observed, probably due to undermethylation; this was also the probable source of the 2,6-di-O-methylgalactose.

The acidic fraction was a fairly complex mixture, containing three major, and at least three minor components. Conversion to the methyl ester methyl glycosides, followed by reduction and hydrolysis, gave a mixture of methylated neutral sugars, which was fractionated on cellulose. The components were estimated to be present in the following approximate proportions.

2,3,4-tri-O-methylglucose	0.196 g.	12
3,4-di-O-methylglucose	0.015 g.	1
2,3,6-tri-O-methylgalactose	0.025 g.	1.5
2,3-di-O-methylgalactose	0.014 g.	1
3,4-di-O-methylgalactose	0.015 g.	1
2-O-methylgalactose	0.060 g.	4
3-O-methylgalactose	0.138 g.	10
3-O-methylrhamnose	0.012 g.	1

Thus, the main components of the reduced acidic portion of the methylated gum are 2,3,4-tri-O-methylglucose and 3-O-methylgalactose, arising from glucuronic acid end groups and 1,2,4-linked galacturonic acid branch points respectively. Galacturonic acid is to a lesser degree linked through positions 1,3 and 4.

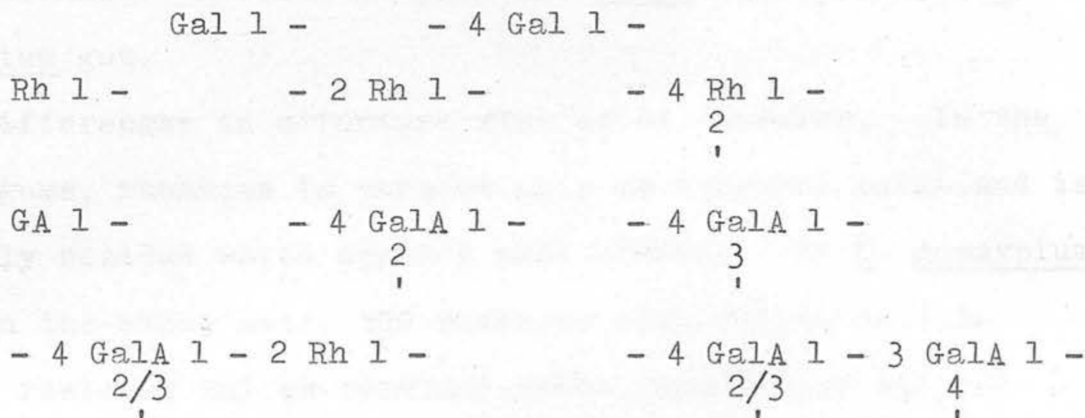
Of the minor components, the 2,3- and 3,4-di-O-methylgalactose derivatives must represent small quantities of 1,4- and 1,2-linked galacturonic acid in the gum. The 3,4-di-O-methylglucose may have arisen from reduction of a 1,2-linked glucuronic acid residue, but the possibility of its being a product of incomplete methylation cannot be discounted.

The 2,3,6-tri-O-methylgalactose and 3-O-methylrhamnose are also present in the neutral fraction of the gum, and obviously arise from 1,4-linked galactose units and 1,2,4-linked rhamnose branch points taking part in aldobiouronic acid fragments. It is probably this 1,2,4-linked rhamnose, rather than the 1,2-linked residue, which takes part in the 2-O-α-D-galacturonosyl-L-rhamnose isolated after partial hydrolysis of the gum.

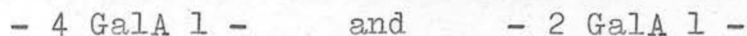
According to the results of methylation, the only group which could be responsible for the 2,4-di-O-methylgalactose isolated from the other product of partial hydrolysis, after methylation, reduction and hydrolysis, is the 1,3,4-linked

galacturonic acid branch point. The work of Hirst and Dunstan gave fairly strong indications of the presence of 2,4-di-O-methylgalacturonic acid in the hydrolysed methylated gum, but no evidence has been found in the present work for the presence of 1,3-linked galacturonic acid residues in the gum.

From the combined results of methylation and partial hydrolysis studies, therefore, the polysaccharide molecule is known to include the following fragments:-



and, in small quantity, the residues



(Gal = D-galactopyranose, Rh = L -rhamnopyranose, GA = D-glucuronic acid, GalA = D-galacturonic acid).

1,2-linked glucuronic acid residues may also be present in small amount.

In many respects, C. gossypium gum apparently resembles the high acid gum polysaccharides of the genus Khaya. Like

them, it seems to consist of a backbone of galacturonic acid residues, containing rhamnose branch points, to which are appended side chains, with galactose and glucuronic acid (in this case unmethylated) in terminal positions. 1,4-Linked galactose units are present in significant proportion in both cases. In the Khaya gums, they carry the glucuronic acid end groups and it is not impossible that this may also be the case here. The aldobiouronic acid 2-O-α-D-galacturonosyl-L-rhamnose has been isolated from the Khaya gums and from C. gossypium gum.

Differences in structure also exist, however. In the Khaya gums, rhamnose is present only as a branch point and is the only residue which carries side chains. In C. gossypium gum, on the other hand, the rhamnose also exists as 1,2-linked residues and as terminal units, and almost all the galacturonic acid residues also take part in branching. On the whole, however, the polysaccharides appear to have many features in common, although on the basis of the investigations so far carried out it is impossible to say whether the residues are joined in the same order. It is obvious from present evidence that the backbone, or central part of the C. gossypium gum molecule is much more complex than those of the Khaya gums.

Certain resemblances towards other high acid gums, e.g. Sterculia setigera and Brachychiton diversifolium, may also be

noted. The former apparently contains no glucuronic acid end groups, but contains galacturonic acid as branch points, 1,2- and 1,2,4-linked rhamnose, 1,4-linked galactose and galactose end groups and gives 2-O-α-D-galacturonosyl-L-rhamnose on hydrolysis. In the latter, on the other hand, all the acidity appears to be due to glucuronic acid end groups but the galactose and rhamnose exist in the same types of residue as in _S. setigera_ and in _C. gossypium_ gums. There is no doubt that in spite of individual differences, all these high acid gums are of the same general structural pattern as the _Khaya_ gums, although the latter would appear on the basis of present knowledge to be the simplest in structure.

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