THE MOLECULAR STRUCTURE OF PLANT GUMS

WITH SPECIAL REFERENCE TO

COMBRETUM LEONENSE GUM.

by

V. P. BHAVANANDAN, B.Sc. (CEYLON).

Thesis submitted for the degree of
Doctor of Philosophy.

University of Edinburgh. May, 1962.

ABSTRACT OF THESIS

Name of Candidate VEERASINGHAM P. BHAVANANDAN

Address Dept.of Chemistry, West Mains Road, Edinburgh, 9.

Degree Doctor of Philosophy *Date* May, 1962

Title of Thesis The molecular structure of Plant Gums, with special reference to Combretum leonense Gum.

Combretum leonense gum contains residues of D-galactose, L-arabinose, L-rhamnose, D-galacturonic acid and D-glucuronic acid. Autohydrolysis of the gum acid affords a substantially arabinose free degraded gum and a mixture of sugars including the disaccharide 3-O-β-L-arabinopyranosyl-L-arabinose. Graded acid hydrolysis gave a mixture of neutral and acidic oligosaccharides amongst which the following have been characterised:

the first two members of the series
O-β-D-galactopyranosyl-[(1→6)-O-β-D-galactopyranosyl]-(1→6)-D-galactose (n=0-3),
3-O-β-D-galactopyranosyl-L-arabinose,
2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, and
6-O-(β-D-glucopyranosyluronic acid)-D-galactose.

In addition the following oligosaccharides, present in minor amounts have been tentatively identified:
4-O-β-D-galactopyranosyl-D-galactose,
O-α-D-galactopyranosyluronic acid-(1→2)-O-L-rhamnopyranosyl-(1→4)-D-galactose, and
O-D-galactopyranosyl-(1→3 or 4)-O-α-D-galactopyranosyluronic acid-(1→2)-L-rhamnose.

Hydrolysis of methylated degraded gum yields

2,3,4,6-tetra-O-methyl-D-galactose,
2,3,4-tri-O-methyl-D-galactose,
2,3-di-O-methyl-D-galactose,
3,4-di-O-methyl-L-rhamnose,
3-mono-O-methyl-L-rhamnose, and
2,3,4-tri-O-methyl-D-glucuronic acid, together with traces of other sugars.

Hydrolysis of the methylated gum affords

2,3,5-tri-O-methyl-L-arabinose,
2,3-di-O-methyl-L-arabinose,
2,5-di-O-methyl-L-arabinose,
2,3,4-tri-O-methyl-D-galactose,
2,3,6-tri-O-methyl-D-galactose,
2,3-di-O-methyl-D-galactose,
2,4-di-O-methyl-D-galactose,
2,6-di-O-methyl-D-galactose,
2-mono-O-methyl-D-galactose,
3-mono-O-methyl-L-rhamnose
L-rhamnose, and
2,3-di-O-methyl-D-glucuronic acid,
together with smaller amounts of other sugars.

Partial structures for the gum, in terms of the galactan framework, acidic fragments and the acid-labile periphery, are discussed in the light of these results.

The structural features of the gum have been compared with those of other gums. Special reference being made to its relationship wit'

Use other side if necessary

cont.

the gums of the Combretaceae family and the similarly constituted Virgilia oroboides gum.

The nature of heterogeneity that exists in the gum was investigated by fractionating the gum on anion-exchange cellulose columns.

The periodate resistant portion of the gum was also investigated.

TO MY PARENTS

CONTENTS.

Acknowledgements.

I wish to express my sincere debt of gratitude to Professor E. L. Hirst, C. B. E., F. R. S. for providing the opportunity for the pursuit of these studies and for his continued interest in this research, and to Dr. G. O. Aspinall under whose expert guidance this work was carried out, for the invaluable advice and encouragement which he gave so willingly at all times.

My grateful thanks are also due to Dr. C. A. Beevers for the help in taking X-ray powder photographs, to Dr. C. T. Greenwood for carrying out the ultracentrifugal measurements, and to other members of the staff (teaching and technical) and research workers of the chemistry department for their kind co-operation and help.

Finally, I wish to thank the University of Edinburgh for the award of a Post-Graduate Studentship.

V.P.B.

I N T R O D U C T I O N

I N T R O D U C T I O N.

A wide variety of substances having gummy characteristics are termed as gums. Thus the terpenoid resins, rubber, hemicelluloses, seed extracts, seaweed polysaccharides and the polysaccharide exudates from plants have all been called gums. In this thesis the word gum refers to the last of the types mentioned above. Thus gums may be defined as "those carbohydrate materials which are exuded from the outer portions of trees and fruits spontaneously, after mechanical injury or after attack by micro-organisms."

The plants after injury exude a rather mobile solution which soon becomes a sticky syrup and dries slowly to give hard and brittle nodules. These nodules are composed almost entirely of carbohydrate material, together with small amounts of impurities such as terpenoid resins (which colours the nodule yellow, brown or red), proteins, dust, pieces of wood etc. If the nodules contain acetylated polysaccharides then they smell faintly of acetic acid due to decomposition. Most gums dissolve in water to give highly viscous solutions and others swell up to give gels. Almost all gums are soluble in dilute alkali and insoluble in organic solvents. Molecular weights of the gums are usually very high, as high as 9,500,000 has been quoted for Karaya gum (1).

The gums may consist of more than one polysaccharide and these are heteropolysaccharides as their molecules comprise anything from two to four main neutral sugars and one or two uronic acid residues. The most common neutral sugars are D-

galactose, L-arabinose, L-rhamnose, D-mannose, and D-xylose, whereas L-fucose occurs in rare cases. The uronic acids are D-glucuronic acid (sometimes as its 4-O-methyl ether) and D-galacturonic acid. Each of these sugars may be involved in more than one type of linkage in the same polysaccharide.

The gum polysaccharides have a highly branched molecular structure and they are amongst the most complicated known to organic chemists. Their structure resembles closely those of the bacterial polysaccharides and the mucilages. In fact, there are no general structural differences between plant gums and mucilages and they are usually reviewed together (2-6), in spite of the difference in their mode of origin and their function in the plant. Mucilages are products of normal plant metabolism, they are isolated by extraction of different parts of the plant in which they are known to serve as food reserves or as reservoirs of water.

The origin of gum exudates, however, is still in doubt. The gums could, like the mucilages be the product of normal plant metabolism, or they could arise from pathological conditions. There is evidence to favour both these views. For example, gum tragacanth is produced immediately after mechanical injury and is thus probably a product of the tree itself. Also the exudates from one tree or different trees of the same species, are remarkably similar in their composition, whereas one might expect different bacteria to produce different gums on trees of the same species. The latter view, which is the less favoured of the two (5) has its main support from experiments carried out by innoculating

bacteria into trees, thus producing gummosis. The observation that healthy Acacia trees do not produce any gums, whereas the ones growing under adverse conditions such as high elevation and lack of moisture produce gums in great quantities, is claimed as a support for this view. This, however, could be due to the fact that under such conditions the bark of the tree cracks, causing gum exudation. In this context it is interesting to note that the supposedly infected and diseased Acacia trees continue to survive and propogate.

It is not possible at the moment to draw conclusions on this problem, and it is not unlikely that there is more than one reason for the production of gum by plants.

The most likely function of gum formation is to prevent loss of moisture by sealing off the injured portion and to prevent invasion of the tissue by micro-organisms (3). The capsular polysaccharides of pneumococcus are known to offer protection to the pneumococcus organism; gums which are very similar to the above polysaccharides have been suggested to have a similar function of protecting the plant tissues (2).

Some of the other problems facing the carbohydrate chemists are the source of structural material for gum within the plant, and whether gums are formed at the site of injury or whether they are generated elsewhere in the plant and then transported to the injured site. Some understanding of these problems could be expected if the structural relationship of the various carbohydrate materials present in the plant, such as starch, cellulose, hemicellulose, gum and pectin were investigated.

Whatever the exact origin of the gum exudates may be, it is believed that they are more probably formed by some type of enzymic polymerisation, rather than by direct chemical polymerisation (2).

There is no generally accepted system of classification of gums, even though classifications based on physical properties such as solubility and those based on chemical composition, such as nature of uronic acid, are available.

A large number of plants, particularly the fruit trees and the thorny shrubs which are found in hot dry climates are capable of producing gums. The bulk of the gum for industry is collected from wild trees, by hand picking. Some of it comes from trees specially grown for this purpose in gardens. Over one hundred species of genus Acacia produce gums known commercially as Gum Arabic. Other gums of commercial importance are from the species of Astragalus, Prunus, Streculia and Combretaceae. The marketing of gums under collective names leads to confusion in structural work and definite conclusions cannot be drawn unless botanical origin of a particular sample is known.

Gums have been of use to man from ancient times. Egyptians used them for paint thickening and embalming purposes, as far back as 2000 B.C. At present, they are used in industry for a variety of purposes, such as textile printing, paper-making, adhesives and emulsifiers. Being tasteless and harmless they are also used in pharmaceuticals, confectionery and cosmetics.

The scientific investigation of gums is desirable from the point of view of their importance in industry and their close

similarity to bacterial polysaccharides. But perhaps the most important reason lies in the fact that "it leads to some of the fundamental problems in carbohydrate chemistry, namely, the mechanisms by which the primary products of photosynthesis, which appears to be based on D-glucose, are transformed into other hexoses, uronic acids, methyl pentoses and pentoses." (5)

PURIFICATION AND FRACTIONATION.

The polysaccharide material in the gum usually exists as the neutral or slightly acid salts of cations such as calcium, magnesium, sodium and potassium.

The powdered gum is dissolved in water or in dilute sodium hydroxide and the insoluble extraneous matter such as pieces of wood, insects and sand are filtered off. The free gum acid is then precipitated by addition of acidified organic solvent, usually ethanol, leaving the low molecular weight material in solution. Other organic solvents such as methanol, acetone and acetic acid have also been used. Further purification of the polysaccharide may be effected by reprecipitations, dialysis or by treatment with ion exchange resins. The purified polysaccharide is either freeze dried or dried by trituration with absolute alcohol or acetone followed by drying in a vacuum pistol. However, it is reported (8) that carbohydrate materials which have been either freeze dried or vacuum dried up to temperatures at which the decomposition of the material begins, still retain water and organic solvents. Also, the solvent used for purification might have some effect on the polysaccharide. Drummond and Percival (9) found a fast moving sugar corresponding to a mono-O-ethyl sugar in the partial acid hydrolysate of the gum exudate of Albizzia zygia, which they had purified from ethanol. They consider this sugar to be an artefact produced by the ethanol, since this sugar was not detected when propan-2-ol was used instead of ethanol for purification.

In all polymer chemistry the most important and at the same

time the most difficult problem is to establish the homogeneity of the starting material. Unfortunately complete homogeneity of a sample cannot be established directly. However deviations from it may be detected by examining the sample by as many as possible of the methods available to assess homogeneity. In polysaccharide work the necessity to assess homogeneity is even greater now that new chemical and biochemical techniques of high degree of precision have been developed to investigate the fine structures. Several polysaccharides, including some gums, are known definitely to be heterogeneous. For example, gum tragacanth (13) and Khaya senegalensis gum (12) have been fractionated into polysaccharides with entirely different chemical structures. In other cases, the polysaccharides are assumed to be homogeneous mainly because of the failure to effect fractionation by any of the several methods available. The heterogeneity of a polysaccharide may be of three different types. The polysaccharide may be made up of a mixture of

(i) polysaccharides containing different sugar components or the same sugar components linked in different ways,

(ii) polysaccharides containing the same component sugars linked in the same way but in different proportions,

(iii) polysaccharides differing only in their molecular sizes.

The last type of heterogeneity is not very serious in that it does not prevent correct interpretation of the results.

In many instances purification and fractionation of polysaccharides are done in one operation. Thus for example if precipitation or extraction of polysaccharides, which are essentially

purification techniques, are carried out in stages it might be possible to isolate two different polysaccharides. There are several such techniques by which purification (and fractionation) of polysaccharides may be effected on a preparative scale.

Graded extraction has often been used to isolate pure polysaccharides from the raw materials. Cold or hot water, alkali, alkali-borate mixtures are the common solvents used for extraction. However, if the conditions of extraction are too drastic, modification of structure and or molecular weight might occur. Dimethyl sulphoxide is considered to be a good solvent for preferential extraction of polysaccharides (10), especially for those containing O-acetyl groups.

Another method commonly used for isolating pure polysaccharides is fractional precipitation. By gradual addition of organic solvents such as ethanol to aqueous solution of the gum and immediate removal of any precipitate formed, components of different solubilities can be separated. The separation is rough and several reprecipitations may be necessary to obtain the fractions pure. The two components of Olibanum gum (11) and the Khaya senegalensis gum (12) have been fractionated by this method. This type of fractionation can also be used to fractionate mixtures of acetylated, methylated, or nitrated polysaccharides. For example, gum tragacanth (13) has been fractionated into an acidic polysaccharide, an arabogalactan and a glycoside, after complete methylation. Purification of polysaccharides can also be achieved by adding salts to aqueous solution of the mixture. For example, corn hull hemicellulose (14) has been precipitated by

addition of ammonium sulphate to an aqueous extract. Most polysaccharides form insoluble complexes with certain inorganic compounds and are thus precipitated out of solution. Fehling solution (15), cupric acetate (16), cupric sulphate (17), cetyl trimethyl ammonium bromide (Cetavlon) (18), and Cetavlon-boric acid mixtures (19) have all been used to effect fractionation. Some polysaccharides are also capable of complexing with other polysaccharides and with certain proteins. This fact has been used to purify polysaccharides in a few instances. Wider application of this method is expected in the future (2).

Enzymes can also be used to purify polysaccharides, by enzymolysis of the unwanted component by specific enzymes. For example, the glucomannan in Iles mannan can be purified by decomposition of amylose-like impurities with amylase (2).

Adsorption or displacement chromatography has had only little success in separating mixtures of polysaccharides. Some instances in which it has been used are, the separation of mucopolysaccharides on cellulose columns by gradient elution with increasing concentrations of ethanol containing 0·3% barium acetate solution (23), the fractionation of sugar beet araban on a charcoal column (24), and of polysaccharides from Mycobacterium tuberculosis on silica gel column (25).

A more efficient chromatographic procedure for polysaccharide has been recently developed by Neukom et al. (26). Their method, which makes use of anion-exchange cellulose, was developed for analytical purposes, but can be extended to small scale preparative separations. They found, in analogy to the precipitation of

acidic and neutral polysaccharides with quaternary ammonium salts, that acidic polysaccharides were absorbed readily on anion-exchange cellulose at neutral pH values, whereas neutral polysaccharides were not or only weakly absorbed. Thus by using DiEthylAminoEthyl (DEAE-) cellulose in different forms (borate, phosphate, hydroxyl etc.) together with a suitable elution medium at differing pH values and electrolytic concentration they were able to fractionate wheat starch dextrin, a mixture of sugar beet araban and pectic acid, the cold water soluble wheat flour polysaccharides, and other similar mixtures (26). They have also shown that in the case of pectic substances the extent of absorption on to the cellulose derivative depends on the degree of esterification, the degree of polymerisation and the content of side groups (27). This method has also been used to assess the homogeneity of gum polysaccharides. In the case of Khaya senegalensis gum a complete separation of the two component polysaccharides has been achieved by this method (28); fractional precipitation gives only a rough separation of these components (12).

The ultrafiltration technique using membranes of graded pores size would probably be useful in separating mixtures of polysaccharides of different molecular size. Using this method Jones and co-workers (20) have separated dextran into fractions of different molecular weights.

There is no unambiguous method to assess the homogeneity of polysaccharide obtained by the above methods of fractionation. However, there are several tests of differing criteria available, to which the fractions could be subjected. Chemical analysis

(qualitative and quantitative) of component sugars and determination of physical constants of the polysaccharide (for example optical rotation, uronic anhydride content) are often useful to detect differences between fractions. The method of anion-exchange cellulose chromatography as described earlier can also be used as a diagonistic test for homogeneity.

Electrophoresis (ionophoresis) using filter paper and borate buffer, has been used in a few instances to test the homogeneity of polysaccharides (30, 31, 32). This method, however, is restricted because of the absorption of polysaccharides on to the paper and because it requires specific spray reagents for detecting the polysaccharides that are not interferred with by the cellulose paper. To overcome these difficulties an inert support such as glass fibre paper has been used (32, 33). Using this technique with 2N-sodium hydroxide as electrolyte and alkaline permanganate as spray gum ghatti, gum tragacanth, the gums of Acacia pycnantha, Acacia senegal, and Acacia arabicum has been claimed to be heterogeneous, since they all showed more than one spot (34). Electrophoresis in Tiselius apparatus (29) and on thick glass fibre paper or column of powdered glass (35) could be used to effect separations of polysaccharides on a preparative scale.

Another important analytical test for homogeneity depends on the fact that antipneumococcus horse sera will give precipitate with polysaccharides which are structurally related to the antipneumococcus polysaccharides. Thus gum arabic gives a precipitate with Type II antipneumococcus serum from which a

polysaccharide having less rhamnose than the original gum can be recovered (21).

The molecular weight distribution of a mixture of polysaccharides can be investigated by sedimentation analysis in an ultracentrifuge (7). This method also gives an estimate of the molecular weights of the components. Molecular weight distribution could also be studied by turbidimetric measurements using light-scattering photometers.

A few investigations of the inter-nodule variations in gums have been reported in the literature. Torto (36) examined nodules from different trees of Fagara xanthoxylides and found no significant variations in optical rotations and equivalent weights. Three different nodules from Brachychiton diversifolium examined for ash, nitrogen, methoxyl, and acetyl contents did not show any significant difference (37). Some samples of Acacia pycnantha gum have been shown to be heterogeneous by glass fibre ionophoresis (34, 38). An examination of several gum nodules from Combretum leonense has shown significant variations, among other things in uronic anhydride content, much greater than can be explained by analytical error (39).

In view of the possibility of heterogeneity in gums, caution must be exercised when interpreting the experimental results. Thus most if not all interpretations of results, specially those regarding the fine structure of polysaccharides, represent only the most probable one, and not the only one. It has not been possible to assign a unique structural formula for any gum. However, the main structural features of many gums have been

elucidated and compared with other gums of the same or related botanical origin. It has been suggested that gums from related botanical species, as in the case of the hemicellulose group of polysaccharides, may have similar basal units of molecular structure and differ considerably in fine structure (38). Such similarities in backbone have been shown to exist in gums from botanically related species, such as between gum arabic and Acacia pycnantha gum (38) and between gum ghatti and Anogeissus schimperi gum (40). These pairs of gums also show remarkable similarities in other important structural features, such as the aldobiouronic acid units and the acid labile units.

STRUCTURAL INVESTIGATION OF POLYSACCHARIDES.

An idea of the general nature of the polysaccharide under investigation can be obtained by determination of physical constants. This involves the measurement of optical rotation, neutralisation equivalent, viscosity coefficient, uronic acid anhydride, acetyl, methoxyl, nitrogen, and ash contents.

For the structural investigation of a purified polysaccharide, a number of different techniques are required. Firstly, the nature and proportions of the sugar components are determined by examination of the total acid hydrolysate of the polysaccharide. Secondly, information on the mode of linkage of each sugar unit and in many instances, on the ring structure of sugars and the proportion of non-reducing terminal groups, are obtained by methylation procedure. Finally, the order of the sugar units and the linkages are determined by partial hydrolysis studies. Supplementary and additional information can also be obtained from periodate oxidation, alkaline degradation, enzymic degradation, and immunological studies. In many instances valuable information can be obtained from investigation of the modified (reduced, oxidised or acid degraded) polysaccharide.

Composition of the polysaccharides.

Characterisation of the sugar components is done by total hydrolysis of the polysaccharide with 1 or 2N sulphuric acid at 100°, separation of the products by column chromatography (22) and preparation of crystalline derivatives.

The proportions of the neutral sugars are best estimated by the method developed by Flood, Hirst and Jones (41). The

product of total hydrolysis of the polysaccharide is chromatographed on paper and the sugar components are located by spraying side strips. The sugars are then eluted from the paper and estimated independently by some micro volumetric methods (42,43) or colorimetric methods (44-46). Any error in this method of determining proportion is due to incomplete breakdown of the neutral sugars linked to uronic acid and to decomposition of sugars during hydrolysis. From the results of this determination and the uronic anhydride determination it is possible to calculate the total composition of the polysaccharide.

METHYLATION PROCEDURE.

The problem of determining the mode of linkages present in the polysaccharide is best solved by the classical method of methylation. Complete methylation blocks all the hydroxyl groups which are not involved in any glycosidic linkages by converting them into non-reactive methyl ethers. Hence on hydrolysis of the resulting methylated polysaccharide the methyl sugars obtained will carry free hydroxyl groups only on those carbon atoms which were involved in glycosidic linkages or ring formation. Identification of these partly methylated sugars will then provide evidence as to the nature of the sugar residues, and to their modes of linkage in the polysaccharide if the ring size of the sugar residues is known. The methylation results will also indicate the non-reducing terminal residues, the points at which branching occurs and the number of residues per average building unit. Thus in the case of a homopolysaccharide it is possible

to construct the building unit with a fair significance, from methylation results alone. In the case of heteropolysaccharides, however, it is necessary to know the order in which the different sugar residues occur before such a unit can be constructed.

The standard method of methylation is still the original procedure initially developed by Haworth (47), using methyl sulphate and aqueous sodium hydroxide. The above method even though efficient does not usually give complete methylation, especially with acidic polysaccharides. The methylation can then be completed by Purdie's method (52), using methyl iodide and silver oxide. Modifications of the Purdie methylation by Kuhn makes use of N,N-dimethyl formamide (53) as a solvent and barium oxide instead of silver oxide (54).

Other less commonly used methods available for methylation make use of thallium hydroxide and methyl iodide (56), diazomethane (57) and methyl iodide with sodium in liquid ammonia (58). The last method has been adopted to the micro scale (2-10 mg.) by Isbell et al. (60).

The fully methylated polysaccharides are insoluble in hot inorganic acids and therefore cannot be directly hydrolysed. This difficulty may be overcome by preliminary methanolysis, or formolysis or treatment with cold acid (61, 62). The products of hydrolysis can be separated by the various chromatographic techniques available and the methylated sugars characterised by means of crystalline derivatives.

PARTIAL HYDROLYSIS. (LINKAGE ANALYSIS).

By methylation studies it is not possible to determine the order in which the sugar units are linked together in the polysaccharide. Especially in a heteropolysaccharide containing five or six different sugar residues the task of determining the order is a difficult one. The method of partial hydrolysis is by far the most useful for this purpose and probably the only unambiguous method available. In addition to the order of sugar residues, the order of glycosidic chain linkage and the anomeric configuration of the linkages in the polysaccharide can be determined by this method. Since the development of the various chromatographic techniques (22) required for the separation of fragments liberated on partial hydrolysis, the method has become even more important.

The glycosidic linkages in a polysaccharide display extreme differences in stability, enabling one to carry out a stepwise degradation of the polysaccharide. Thus, by this method it is possible to get information on different portions of the molecule such as the periphery, the backbone and the acid resistant core.

Sugars existing in the furanoside ring form are much more acid-labile than those in the pyranose form. Thus these furanose sugars are easily removed by heating a solution of the polysaccharide with very dilute (ca.0·01N) acid or in the case of acidic polysaccharides by just heating an aqueous solution. This latter process is known as autohydrolysis. The degraded polysaccharide obtained after the removal of the acid-labile groups

as mono- and di-saccharides, is less complicated than the original polysaccharide. A comparison of the methylation results of the original and degraded polysaccharides will provide information regarding points of attachment of the acid labile groups (71 and 72).

The degraded gum after autohydrolysis is composed of monosaccharides linked by pyranoside and uronic acid glycosidic linkages. The pyranoside linkages can be selectively hydrolysed with stronger acids (ca. 0·1N) giving rise to monosaccharides and oligosaccharides containing only neutral sugars. By vigorous hydrolysis (ca. 1·0N) of the degraded gum obtained after the above hydrolysis, fragments containing uronic acids can be isolated. This is made possible by the stability of the glycosiduronic acid linkages. In addition to the aldobiouronic acids in good yields, smaller amounts of aldotriouronic acids have also been isolated (40, 61).

The structural significance of oligosaccharides present in minute quantities in the partial acid hydrolysates are doubtful, because acid hydrolysis is a reversible reaction. Thus when a solution of one or more monosaccharides is heated with acids oligosaccharides are formed (73). However, a possible way of distinguishing acid reversion products is that they reach an equilibrium concentration and thus do not disappear on prolonged heating (74). During acid hydrolysis artefacts could also arise from acid catalysed trans glucosylation of oligosaccharides (75).

The differing stability of glycosidic linkages in polysaccharides, which allows great degrees of selectivity in

hydrolysis, has its disadvantage too. Thus it is very difficult to determine the nature of the very acid-labile linkages. This is because this linkage will be among the first to be cleaved on acid hydrolysis and will not be present in any of the oligosaccharides isolated. To obtain information about such linkages it is therefore necessary to resort to other methods than direct partial acid hydrolysis.

Partial acetolysis of polysaccharide with a mixture of acetic anhydride, acetic acid and sulphuric acid, followed by deacetylation of products give oligosaccharides which cannot be obtained by partial acid hydrolysis. Thus the rhamnose residue in gum arabic, which is acid labile, has been shown to be attached to position 4 of the glucuronic acid by isolation of the oligosaccharide 4-O-L-rhamnopyranosyl-D-glucose from partial actolysis products of the reduced gum arabic (76). Mercaptolysis and methanolysis have been similarly used to study complex seaweed polysaccharides that contain acid-labile anhydro sugars.

Other disadvantages of direct partial acid hydrolysis, are the destruction of sugars which results in low yields of oligosaccharides and the removal of functional groups such as sulphate and N-acetyl. An elegant method has been developed by Painter to overcome this difficulty, by carrying out hydrolysis in a dialysis bag and continually removing the products. Thus by carrying out auto-hydrolysis of sulphated polysaccharide (77) in a dialysis bag he was able to isolate sulphated mono and oligosaccharides. This method has been extended to other polysaccharides by carrying out the hydrolysis in the dialysis bag with

water soluble non-dialysable polystyrene-sulphonic acid (78). The partial hydrolysis of inulin with high yields (40-60%) of di- to octa-saccharides (78) and of a mucopolysaccharide without appreciable N-deacetylation of hexosamine (79) has been achieved by this method. Another advantage of this method is that it eliminates the possibilities of acid reversion of the monosaccharides produced.

A novel method of determining the mode of attachment of very acid labile groups to the adjacent sugar residues has been developed by Aspinall et al.(80). This is done by conversion of acid-labile furanoside linkage in polysaccharide to the acid resisting furanosiduronic acid linkage by oxidation with oxygen in the presence of a platinum catalyst. It is now possible to isolate and identify aldobiouronic acids from the acid hydrolysate of the oxidised polysaccharide, indicating the nature of the original glycosidic linkage.

Hydrolysis of polyuronides such as pectic acid, alginic acid or gums of high uronic acid content without considerable degradation is impossible. These polysaccharides are more easily hydrolysed if they are converted to the corresponding neutral polysaccharide by reduction. Sisal pectic acid has been converted to a galactan (containing 5-6% uronic anhydride) by first treating with ethylene oxide followed by reduction of the ethylene glycol ester with potassium borohydride (81). Reduction of acidic polysaccharide can also be achieved with diborane if a derivative of the polysaccharide (acetate, propionate) soluble

in ether type of solvent can be prepared. For example, mesquitic acid acetate has been almost completely reduced in this manner (82).

In comparison to acid hydrolysis, enzymic hydrolysis (enzymolysis) has the advantage of specificity. For example, the action of β-amylase on 1:4'-linked unbranched glucan amylose gives maltose as the only final product. However, acid hydrolysis of amylose gives glucose and maltosaccharides arising through random scission of bonds. In the case of homopolysaccharides such as starch and glycogen enzymolysis has been used with success. But in the case of heteropolysaccharides which contain a diversity of building units and linkages the use of enzymes have been restricted. However, if pure enzyme preparations are available it will be possible to obtain valuable information, such as to the presence or absence of a linkage for which the enzyme is specific. Also, since a pure enzyme will split only one particular bond out of a variety, it will be possible to obtain fragments of great structural significance.

The mixture of neutral oligosaccharides from partial hydrolysis can be separated from monosaccharides and from one another by chromatography on charcoal-celite (88), cellulose (64) or resin (89) columns. The acidic oligosaccharides are separated from the neutral sugars by absorbing them on weakly basic anion exchange resin columns and washing the column with water till free of neutral sugar. The absorbed acidic oligosaccharides may then be fractionated by eluting the column with increasing concentrations of formic acid (90). Further separation of the

oligosaccharides may be effected by thick paper chromatography.

The component sugars of the oligosaccharides are identified by hydrolysis, after reduction for acidic oligosaccharide, followed by paper chromatography of the products. It is also possible to detect the reducing end group of the oligosaccharide by first reducing it to the glycitol followed by hydrolysis and identification of the products.

An idea as to the identity of the oligosaccharide can be obtained from its chromatographic speed in different solvent systems, ionophoretic behaviour and optical rotation. The nature of the linkages can be established by complete methylation of the oligosaccharide, followed by hydrolysis (after reduction with lithium aluminium hydride for acidic oligosaccharides) and identification of the resulting methylated sugars. When only very small quantities of oligosaccharide are available, it is possible to get an idea of the linkage by carrying out the methylation on a micro scale (2-3 mg.), methanolysing and examining the resulting methyl glycosides by gas-liquid partition chromatography.

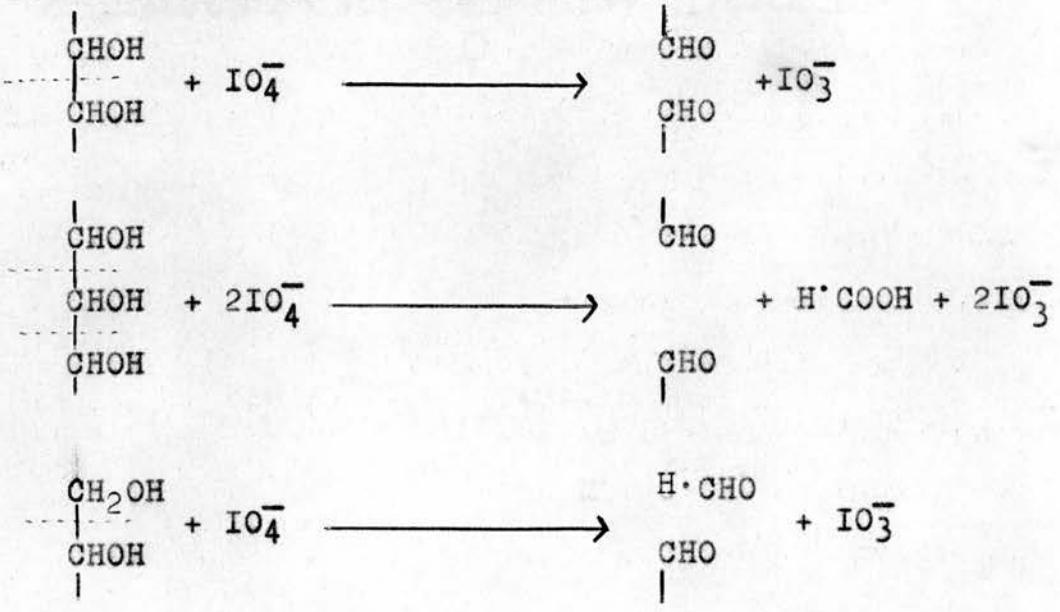
Periodate (83) and lead tetra acetate (84) oxidations can also be carried out on small amounts of oligosaccharides. The consumption of oxidant and the formation of products (formaldehyde, formic acid) depend on the type of substitution of the reducing residue and thus the measurements of these values will give information about the linkage. Periodate oxidation is better done on a derivative of the oligosaccharide such as osazone (86), acid or alcohol (87). By degrading the

disaccharides to the glycer itol glycosides with lead tetra acetate and comparing with authentic specimens, Perlin et al.(85) have been able to assign the anomeric configuration of disacharides. Many oligosaccharides and their derivatives (osazone, acetate, methyl ether) are crystalline and thus can be identified by comparing with authentic synthetic specimens.

Periodate Oxidation.

Valuable structural information can be obtained from a study of the action of periodate ion on the polysaccharide. Because of the aqueous solvent required and the mild nature of the reaction which does not affect the sensitive carbohydrate structure, periodate oxidation is widely used in carbohydrate work (83).

Periodic acid and its salts are capable of cleaving the carbon-carbon bonds in 1,2-diol and 1,2,3-triol groups. The general reaction schemes are shown below.



Thus in general, the arrangement of diol or triol groups may be deduced from measurements of the consumption of oxidant and

determination of the nature and amount of oxidation products (formaldehyde, formic acid, carbon dioxide). In many instances these deductions will give information on the nature and proportion of the repeating and interchain linkages. From periodate oxidation studies it is also possible to get information on molecular weights, degree of polymerisation and chain length of the molecule. The periodate oxidation is influenced by temperature, pH, concentration of reactants and light (83). If the oxidation is carried out in excess oxidant or at higher (5°) temperature in alkaline solution (pH 8) and presence of light, then 'over-oxidation' will occur, that is oxidation proceeds beyond the ideal reaction, with over-consumption of periodate and further oxidation of the normal products such as formic acid and formaldehyde. In certain cases useful information can be obtained by causing deliberate over-oxidation (91). But in normal cases it is essential to avoid under- or over-oxidation and hence the reaction conditions must be controlled carefully.

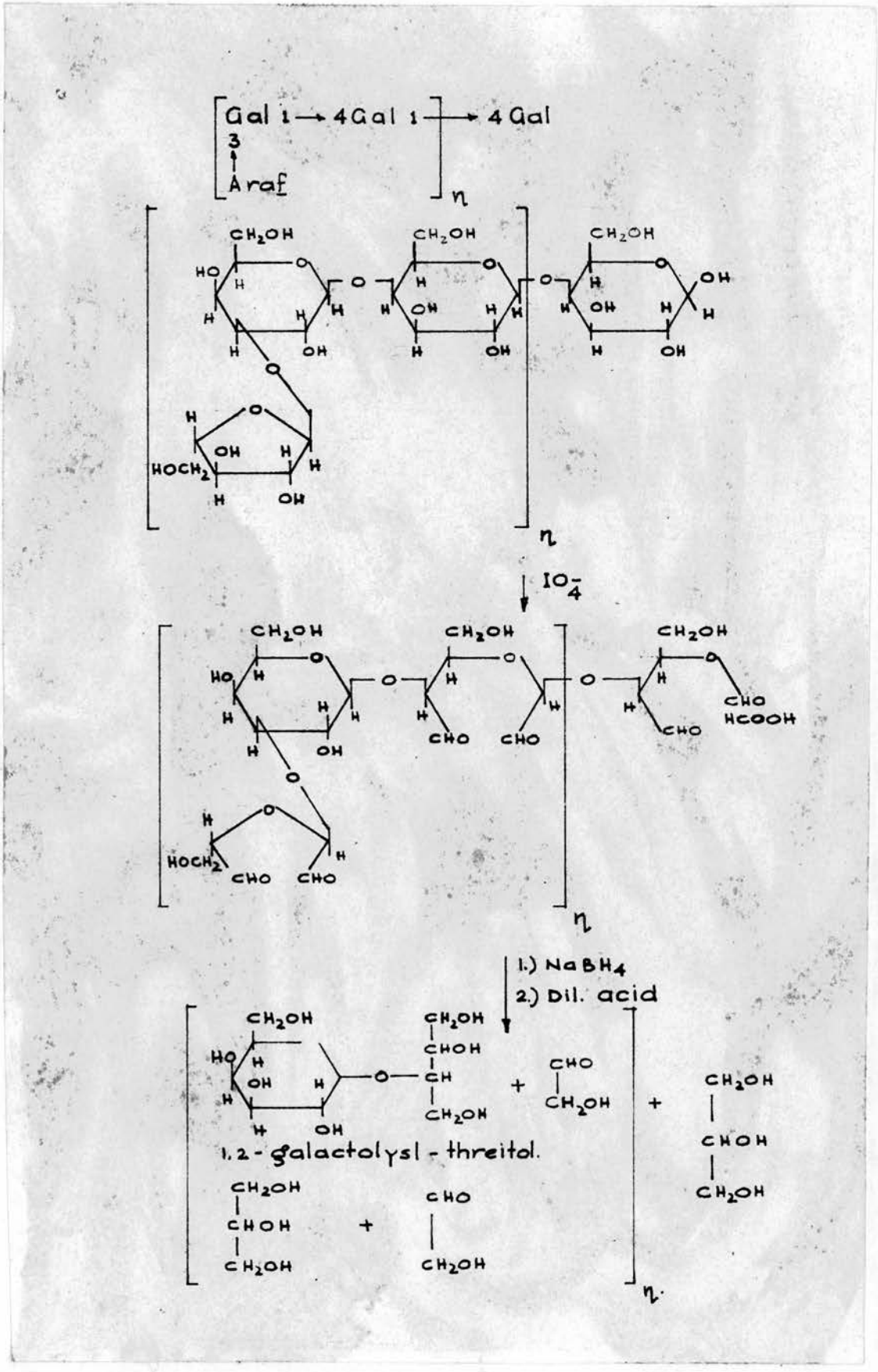
In the case of gums and other similar highly branched heteropolysaccharides, many different interpretations of the analytical data are possible. Also, polysaccharides which have uronic acid residues are liable to behave abnormally, since over-oxidation is a common feature with uronic acid residues (92). Thus in these cases the results should preferably be interpreted in the light of information obtained by other methods.

Alternative periodate methods involve examination of the residual oxidised polysaccharide. Thus for heteropolysaccharides

such as gum, the proportion of unoxidisable residues of each type of monosaccharide may be determined by hydrolysis of the oxidised polysaccharide (polyaldehyde). It should be also possible to obtain a simpler polysaccharide, for further structural investigation, by removing the oxidised sugar residues from the polyaldehyde by mild hydrolysis. However, on such direct hydrolysis the polyaldehyde decomposes to a great extent and also gives rise to interfering products.

Barry (93) has shown that treatment of the periodate oxidised polysaccharide with phenylhydrazine removes the fragments of the cleaved residues as phenylosazones. Thus, in the case of complex polysaccharides it is possible to cause stepwise degradation by a series of oxidation with periodate and treatment with phenylhydrazine. For example, after three such treatments gum arabic gave a material which was resistant to further oxidation indicating the 1,3'-linked galactan backbone of the gum.

A similar method developed recently by Smith (94) is generally preferred in being experimentally cleaner. It involves the reduction of the polyaldehyde to a polyalcohol by borohydride followed by treatment with dilute mineral acid at room temperature. Under such conditions only the acetal linkages of the oxidised residues are cleaved leaving the normal glycosidic linkages intact. An illustration of such a degradation of a hypothetical polysaccharide molecule is shown in the following scheme.


Gal 1→4Gal 1→4Gal
3
↑
Araf
η
IO4−
1.) NaBH4
2.) Dil. acid
1.2-galactolysl-threitol.

Other Methods.

In addition to the general methods of structural investigation discussed above, a number of other procedures are available. At least one or two of these special methods need mentioning.

Polysaccharides on treatment with alkali undergo degradation. In general the degradation begins at the reducing end and proceeds in the stepwise manner. The saccharinic acids produced on such degradation are characteristic of the linkages in the polysaccharide and thus afford valuable structural information (95).

The ability of polysaccharides to give co-precipitation reactions with antipneumococcus sera has been correlated to the presence of particular groupings in the polysaccharide (96). Thus, it is possible by use of antisera precipitation reactions to predict or confirm the presence of structural features in polysaccharides.

Object of the Present Investigation.

Preliminary investigations (98) of the Combretum leonense gum showed it was composed of D-galactose, L-rhamnose, L-arabinose, D-galacturonic acid and D-glucuronic acid. In this work, it was intended to carry out a detailed structural investigation of this gum and compare its structural features with those of gum ghatti and gum of Anogeissus schimperi, both of which belong to the same family (Combretaceae) as the above gum, but to a different genus, (Anogeissus).

Such comparisons of structures will enable one to understand the structural relationships of the vastly complicated gums belonging to related and unrelated botanical species.

This work was also intended to study the nature of heterogenity of the gum, in view of the inter-nodular differences noted by previous workers (39).

D I S C U S S I O N

DISCUSSION.

The sample of Combretum leonense gum used in the present investigation was collected in Nigeria (1952) and kindly provided by Dr. R. J. McIlroy. The consignment consisted of large clear glassy nodules, varying in colour from reddish brown to light yellow and developed a distinct odour of acetic acid when stored in a closed bottle.

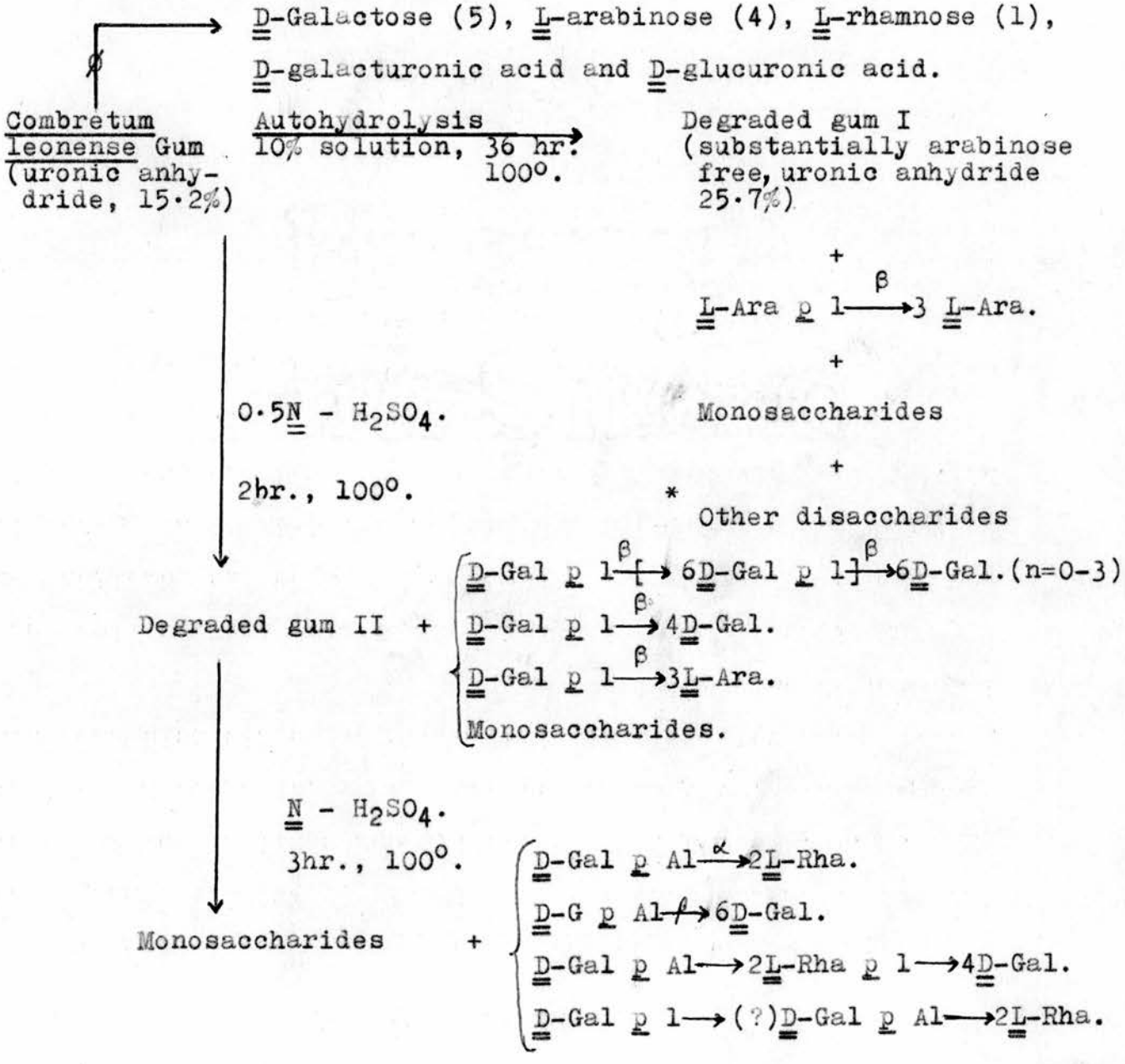
Preliminary investigations showed that the purified gum had uronic acid anhydride, 14·9-20·1%; specific rotation, -6 - 15°; nitrogen, 0·03-0·05%; acetyl, 1·7-4·4%; and methoxyl, nil. The equivalent weight calculated from the free titratable acidity was found to vary from 870 to 1168 (39). The neutral sugars present in the gum were identified by preparing crystalline derivatives as D-galactose, L-arabinose, and L-rhamnose, which were present in an approximate ratio of 5:4:1 (98 and 109). The main uronic acid present in the gum was identified as D-galacturonic acid and the presence of D-glucuronic acid was indicated by chromatographic evidence (109).

The crushed gum nodules dissolved in water with comparative ease and solutions up to 10% (w/v) could be made at room temperatures. The purified gum polysaccharide was obtained as a creamy white amorphous powder by precipitation with acidified ethanol.

Preliminary studies (98) showed that the gum contains three types of glycosidic linkages with distinctly different rates of hydrolysis. This is a common feature in many other gums, and

advantage had been taken of this fact to obtain selective hydrolysis of the linkages. In the present investigation the gum was subjected to a stepwise degradation, as shown in the flow sheet below, to yield fragments from various parts of the gum molecule.

Scheme showing graded hydrolysis.



* These disaccharides were not fully identified, but arabinobioses and an arabinosyl-galactose were probably present.

Ø Work carried out by King (109), numbers within brackets denotes approximate molecular proportions of the sugars.

Hydrolysis results.

An aqueous solution (4%) of the purified gum acid was heated on a boiling water bath and the autohydrolysis was followed by measuring the change in optical rotation. The changes in optical rotation were large at the beginning and after 48 hr. the changes were very small, indicating that most of the acid-labile groups had been released by the end of this time. It was found that in addition to large amounts of arabinose residues, some galactose and rhamnose residues were also released after only 4 hr. heating. The amount of rhamnose residues released did not seem to change, whereas the amounts of arabinose and galactose residues released increased with increasing heating time. A pentose disaccharide (subsequently identified as 3-O-β-L-arabinopyranosyl-L-arabinose) was also detected after 12 hr. autohydrolysis and the amount of this disaccharide released reached a maximum at 48 hr. and then decreased.

The proportion of arabinose residues relative to galactose residues in the degraded polysaccharide decreased with increasing time of hydrolysis and the polysaccharide remaining after 96 hr. autohydrolysis had a very small proportion of arabinose residues in it. The proportion of rhamnose residues in the

degraded polysaccharide, again appeared to remain constant. It is probable, therefore, that a part of the rhamnose residues present in the gum are acid labile and are thus easily removed on autohydrolysis and the rest of the rhamnose residues are in the acid resistant core and are not removed even on prolonged autohydrolysis. Even though some of the galactose residues were easily removed on autohydrolysis, subsequent methylation studies showed that all the galactose residues in the gum were present in the pyranose ring form. In this connection it may be noted that Bouveng (110) failed to find any significant difference in the rates at which the galactopyranosidic residues and arabinofuranosidic residues were released from the arabinogalactan of Western larch on mild hydrolysis.

A large scale autohydrolysis was carried out by heating a 10% solution of the gum acid for 36 hr. (constant rotation) and the degraded gum I was precipitated with ethanol. The mixture of mono- and oligosaccharides was fractionated by chromatography on charcoal-Celite. Most of the fractions obtained were mixtures and even after several fractionations by partition chromatography on cellulose, these fractions were not obtained in a chromatographically pure form.

Only one disaccharide was obtained chromatographically pure and in sufficient amount for detailed structural investigation. Its structure was proved to be 3-O-β-L-arabinopyranosyl-L-arabinose, even though it had a specific rotation (+164°) lower than the values quoted in the literature (ca.

+200°) for the disaccharide. This low specific rotation was probably due to contamination of the disaccharide with non-carbohydrate material.

The disaccharide gave a phenylosazone which was identical (mixed m.p. and X-ray powder photograph) with that prepared from an authentic sample of 3-O-β-L-arabinopyranosyl-L-arabinose. The phenylosazone on periodate oxidation yielded formaldehyde but no mesoxaldialdehyde 1,2-bisphenylhydrazone. This result is in agreement with those for a 1,3'-linked pentose disaccharide, since both the 1,4'- and 1,5'-linked pentose disaccharides will not yield formaldehyde on periodate oxidation (86). The structure was confirmed by methylation of the disaccharide, followed by methanolysis of the fully methylated disaccharide when the methyl glycosides of the following methylated sugars:- 2,3,4-tri-O-methylarabinose; 2,4-di-O-methylarabinose and 2,5-di-O-methylarabinose (trace); were detected by vapour phase chromatography. The mixture of methyl glycosides was hydrolysed and the 2,4-di-O-methyl-L-arabinose separated by chromatography on filter sheets was characterised as its aniline derivative.

Small amounts of various other disaccharides liberated on autohydrolysis were not fully characterised as they were not obtained in a sufficiently pure form. One of the fractions contained a disaccharide which was chromatographically identical to 3-O-arabinofuranosyl arabinose isolated from *Anogeissus schimperi* gum (40).

Another fraction contained a sugar, which was chromatogra-

phically pure and indistinguishable from 3-O-β-D-galactopyranosyl-L-arabinose and gave only galactose and arabinose on hydrolysis. But on reduction with potassium borohydride followed by hydrolysis, the fraction gave mainly galactitol and arabinose in addition to traces of arabitol and galactose. This indicates the presence of a (?)-O-arabinosyl galactose in addition to some 3-O-galactopyranosyl arabinose in this fraction.

Partial hydrolysis of the gum with 0·5N sulphuric acid gave the degraded gum II and a mixture of mono- and oligosaccharides. The oligosaccharides were obtained pure by fractionation on a charcoal column, followed by partition chromatography on cellulose.

The main products were members of an homologus series (I) of galactose-containing oligosaccharides.
O-β-D-galactopyranosyl-[(1→6)-O-β-D-galactopyranosyl]$_n$-(1→6)-D-galactose (I).

The first member was a disaccharide, which on complete methylation followed by hydrolysis gave equimolecular proportions of 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-D-galactose proving the structure of the disaccharide to be 6-O-β-D-galactopyranosyl-D-galactose (I, n = 0). The presence of a β-linkage was indicated by the low positive rotation ($[\alpha]_D$+30°) of the disaccharide, which was similar to that recorded for 1,6-linked galactobiose isolated from partial acid hydrolysate of gum ghatti (102). The disaccharide gave a phenylosazone which was identical (mixed m.p. and X-ray powder photograph) with that prepared from the

corresponding disaccharide from gum ghatti.

The second member of the series was a trisaccharide which on partial hydrolysis gave galactose and 6-O-β-galactosylgalactose. Hydrolysis of the fully methylated trisaccharide gave 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-D-galactose in the proportion 1:2 (approx.), thus proving the structure of the trisaccharide to be O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose. (I, n = 1).

The third member of the series (I, n = 2) was chromatographically indistinguishable from the 1,6'-linked galactotetraose isolated from gum ghatti (102). Partial hydrolysis gave only one disaccharide, the 6-O-β-galactosylgalactose.

The fourth member of the series (I, n = 3) was chromatographically indistinguishable from the 1,6'-linked galactopentaose isolated from Anogeissus schimperi gum (40). Again 6-O-β-galactosyl galactose was the only disaccharide obtained on partial hydrolysis.

In addition to the above series of galactose-containing oligosaccharides, small amounts of two other disaccharides were isolated from the partial acid hydrolysate of the gum. One of these disaccharides was chromatographically identical to 3-O-β-D-galactopyranosyl-L-arabinose isolated from gum ghatti (102). The disaccharide was obtained crystalline which was identical (mixed m.p. and X-ray powder diagram) with an authentic crystalline sample of 3-O-β-D-galactopyranosyl-L-arabinose. However the disaccharide had a m.p. (175°) and specific rotation

(+22·4°), which are lower than those quoted in the literature (102). These low values are probably due to contamination of the disaccharide with non-carbohydrate material.

The second minor disaccharide is tentatively identified as 4-O-β-D-galactopyranosyl-D-galactose from the following evidence. The disaccharide was chromatographically and ionophoretically identical to 4-O-β-D-galactopyranosyl-D-galactose and distinguishable from 3-O-β-D-galactopyranosyl-D-galactose and 4-O-α-D-galactopyranosyl-D-galactose. Lead tetra-acetate oxidation of the disaccharide followed by hydrolysis gave a tetrose in addition to other products, which can arise only from a 1,4'-linked galactobiose. A 1,3'-linked galactobiose would have yielded lyxose on the same treatment. Methylation of a small sample of the disaccharide followed by methanolysis and examination of the products by vapour phase chromatography showed that methyl-2,3,6-tri- and methyl-2,3,4,6-tetra-O-methyl galactosides were the major components.

Hydrolysis of the degraded gum II with N-sulphuric acid gave a mixture of acidic oligosaccharides. The acids were separated from neutral sugars by adsorbing the acids on basic resins and eluting with dilute formic acid. Further fractionations of the acids were effected by chromatography on filter sheets.

The main component was the aldobiouronic acid, 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose whose structure was proved as follows. Reduction of the derived methyl ester

methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. Methylation afforded the crystalline methyl glycoside penta methyl ether of the acid, which was identical (mixed m.p., specific rotation and X-ray powder diagram) with a sample prepared from the same aldobiouronic acid isolated from the pectic acid of Medicago sativa (105). Reduction of this methylated acid with diborane, followed by hydrolysis gave equimolar proportions of 2,3,4-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose.

The second acid fraction gave galactose, glucose and a trace of rhamnose on potassium borohydride reduction of the derived methyl ester methyl glycosides followed by hydrolysis. The trace of rhamnose was probably due to contamination by the aldotriouronic acids, since on complete methylation the fraction afforded a crystalline derivative identical (mixed m.p., specific rotation and X-ray powder diagram) to an authentic sample of methyl ester methyl glycoside of 6-O-(β-D-glucopyranosyl uronic acid)-D-galactose hexa methyl ether (kindly provided by Prof. F. Smith).

The third acidic component isolated was chromatographically and ionophoretically homogenous and appeared to be an aldotriouronic acid. Hydrolysis with N-acid gave galactose, rhamnose, and 2-O-galacturonosyl rhamnose. Reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave galactose and rhamnose. But reduction of the potassium salt of the acid to the glycitol

followed by hydrolysis gave galactitol, rhamnitol, galactose, rhamnose and 2-O-galactouronosyl rhamnose suggesting that there was more than one acid component in this fraction. On the basis of the above evidence, some probable structures for the aldotriouronic acids in the mixture are IV, V and VI.

Gal Al⟶2 Rha 1⟶Gal (IV)

Gal 1⟶Gal Al⟶2 Rha (V)

Gal Al⟶2 Rha (VI)
↑
Gal

In a trial experiment it was found that 2-O-galacturonosyl-rhamnose was unattacked by alkali. This is in agreement with the known fact that reducing sugars with position 2 blocked are not degraded by cold alkali. Hence, the aldotriouronic acids V or VI will not be attacked by alkali, whereas the aldotriouronic acid IV would be degraded to 2-O-galacturonosyl-rhamnose and a saccharinic acid, provided the rhamnose is attached to positions 3 or 4 of the galactose residue.

The mixture of aldotriouronic acids on treatment with alkali was partly converted to 2-O-galacturonosylrhamnose while the other part was unattacked. This indicates that the mixture contains an aldotriouronic acid having the structure Gal Al⟶2 Rha 1⟶(3 or 4) Gal in addition to at least one more acid having structure V or VI.

Methylation of the mixture of acids followed by reduction with lithium aluminium hydride and hydrolysis yielded the following methylated sugars.

2,3,4,6-Tetra-O-methylgalactose

2,3,4-tri-O-methylgalactose

2,3,6-tri-O-methylgalactose

2,4-di-O-methylgalactose (major)

2,3-di-O-methylgalactose (minor)

3,4-di-O-methylrhamnose.

The methylated sugars were identified by paper chromatography and by vapour phase chromatography of their methyl glycosides. Since no mono-O-methylrhamnose was detected, the branched structure VI can be eliminated from the possibilities. Aldotriouronic acids having structures IVa, Va and Vb would give rise to the methylated sugars shown in table 6 after methylation, reduction and hydrolysis.

	Methylated sugars arising from		
Aldotriouronic acid	Galactose residue	Rhamnose residue	Galacturonic acid residue
Gal A1⟶2Rha 1⟶4 Gal (IVa)	2,3,6-Me_3 Gal	3,4-Me_2Rha	2,3,4-Me_3Gal
Gal 1⟶3 Gal A1⟶2 Rha(Va)	2,3,4,6-Me_4 Gal	3,4-Me_2Rha	2,4-Me_2Gal
Gal 1⟶4 Gal A1⟶2 Rha(Vb)	2,3,4,6-Me_4 Gal	3,4-Me_2Rha	2,3-Me_2Gal

Table 6.

Thus if the aldotriouronic acids IVa and Va were present in the unknown mixture of acids, all the methylated sugars obtained after methylation, reduction and hydrolysis can be accounted for except the small amount of 2,3-di-O-methyl galactose. This dimethyl ether probably arises from a third aldotriouronic acid

(Vb) in the mixture, unless it was present as a result of undermethylation.

Gal A1⟶2 Rha 1⟶4 Gal IVa

Gal 1⟶3 Gal A1⟶2 Rha Va

Gal 1⟶4 Gal A1⟶2 Rha Vb

Thus, on the basis of the evidence available so far we can conclude that the mixture consists of the two aldotriouronic acids IVa and Va and probably a smaller amount of a third component Vb.

Methylation results.

A sample of the substantially arabinose free degraded gum I, which was prepared by autohydrolysis of the gum acid, was methylated with methyl sulphate and sodium hydroxide. The partially methylated polysaccharide was esterified by treatment with diazomethane and the methylation was completed by several treatments with methyl iodide and silver oxide. The fully methylated polysaccharide (OMe, 43%) was hydrolysed with sulphuric acid and the hydrolysate neutralised by treatment with barium hydroxide and barium carbonate. In order to separate the neutral and acidic methylated sugars, the syrup containing the acidic sugars as barium salts and obtained by concentration of the hydrolysate, was placed on a cellulose column and eluted with butan-1-ol half saturated with water to give fractions A and B, followed by water to give fractions C and D.

Fraction A consisting almost entirely of neutral sugars

was further fractionated on cellulose giving the following methylated sugars.

	Approximate weight
2,3,4,6-Tetra-O-methyl-D-galactose	0·115 g.
2,3,4-tri-O-methyl-D-galactose	0·263 g.
2,3-di-O-methyl-D-galactose	0·050 g.
3,4-di-O-methyl-L-rhamnose	0·020 g.
3-mono-O-methyl-L-rhamnose	0·020 g.

The rhamnose derivatives were characterised by their crystallinity and the tetra and tri methyl galactoses were characterised by formation of crystalline aniline derivatives. The aniline derivative of the dimethylgalactose failed to crystallise and hence its identity is based only on its optical rotation, the chromatographic behaviour of the sugar and its periodate oxidation products, and paper ionophoresis of the sugar. In addition to the above major components, traces of the following were detected chromatographically:- 2,3,4-tri-O-methylarabinose; 2,3,6-tri, 2,4-di-, 3,4-di- and 2-mono-O-methylgalactose; 2,3,4-tri-O-methylglucuronic acid and also unmethylated rhamnose and galactose.

Fraction B was found to be a mixture of acidic sugars, 2,3,4,6-tetra- and 2,3,4-tri-O-methylgalactose, chromatographically.

Fractions C and D consisted entirely of acidic sugars. In the latter the acidic sugars were present as barium salts. Paper chromatographic examination of fraction D after removal

of barium ions showed that it consisted of four components. A sample of the main component (referred to as subfraction D) was obtained pure by chromatography of the barium ion free fraction D on thick filter sheets. Fractions C, D(sub.) and D(main) were separately converted to the methyl ester methyl glycosides, reduced with lithium aluminium hydride and hydrolysed. Paper chromatographic examination of the products and vapour phase chromatography of the derived methyl glycoside samples showed that all three fractions contained the same partially methylated sugars (given below), but in differing proportions.

3,4,-Di-O-methylrhamnose,
3-mono-O-methylrhamnose,
2,3,4-tri-O-methylglucose,
2,3,4-tri-O-methylgalactose,
2,3,6-tri-O-methylgalactose (trace),
2,3-di-O-methylgalactose.

Subfraction D, however, had in addition appreciable amounts of an unidentified component (T 1·45 in system c).

The rest of the products, obtained after reduction and hydrolysis of the fractions C, D(main) and D(sub), were combined and fractionated on cellulose giving the following methylated sugars.

	Approximate Weight
2,3,4-Tri-O-methyl-D-galactose	0·032 g.
2,3-di-O-methyl-D-galactose	0·090 g.

	Approximate Weight
3,4-di-O-methyl-L-rhamnose	0·024 g.
3-mono-O-methyl-L-rhamnose	0·054 g.
2,3,4-tri-O-methyl-D-glucose	0·014 g.

The rhamnose derivatives were characterised by their crystallinity and the trimethyl galactose by formation of its crystalline aniline derivative. The 2,3-di-O-methyl galactose and 2,3,4-tri-O-methyl glucose were identified on the basis of optical rotation, and by paper chromatography of the sugars and of the products obtained on periodate oxidation.

In addition to the above methylated sugars traces of 2-O-methyl galactose and rhamnose were also detected chromatographically.

The undegraded gum was methylated with methyl sulphate and sodium hydroxide, followed by treatment with methyl iodide and silver oxide. The methylated polysaccharide was fractionally precipitated with light petroleum-chloroform and the fraction having OMe, 40·8% was hydrolysed with sulphuric acid. A good separation of the neutral and acidic (as barium salts) methylated sugars was achieved by preliminary fractionation on a cellulose column.

The mixture of neutral sugars was fractionated by partition chromatography on cellulose and the following sugars were characterised by formation of crystalline derivatives and/or by crystallisation of the sugars.

	Approximate Weight
2,3,5-Tri-O-methyl-L-arabinose	0·550 g.
2,3-di-O-methyl-L-arabinose	0·105 g.
2,5-di-O-methyl-L-arabinose	0·072 g.
2,3,4-tri-O-methyl-D-galactose	0.625 g.
2,3,6-tri-O-methyl-D-galactose	0.155 g.
2,3-di-O-methyl-D-galactose	0.104 g.
2,6-di-O-methyl-D-galactose	0.085 g.
2,4-di-O-methyl-D-galactose	0.577 g.
2-mono-O-methyl-D-galactose	0.539 g.
3-mono-O-methyl-L-rhamnose	0.040 g.
L-rhamnose	0.030 g.

In addition to the above major components, small amounts of 2,3,4-tri-O-methyl arabinose (optical rotation, paper chromatography) and 3-O-methyl galactose (paper chromatography of the sugar and its periodate oxidation products) were also detected. Traces of the following sugars were detected paper chromatographically and/or paper ionophoretically: 2,3,4,6-tetra-O-methyl galactose; 3,5-di-O-methyl arabinose; 3,4-di-O-methyl rhamnose; 2,3,4-tri-O-methyl galacturonic acid; arabinose and galactose.

The acidic fraction, which was uncontaminated with neutral methylated sugars, was converted into the corresponding mixture of methyl ester methyl glycosides, reduced with lithium aluminium hydride and hydrolysed to give a mixture of neutral sugars.

This mixture of sugars was fractionated on cellulose and the following sugars were characterised by crystallisation of the sugars and/or by the formation of crystalline derivatives.

	Approximate Weight
2,3,4-Tri-O-methyl-D-galactose	0·020 g.
2,3-di-O-methyl-D-galactose	0·230 g.
2,4-di-O-methyl-D-galactose	0·022 g.
3-mono-O-methyl-L-rhamnose	0·110 g.
L-rhamnose	0·028 g.
2,3-di-O-methyl-D-glucose	0·050 g.

In addition, a fraction containing small amounts of 2-O-methyl galactose and an unidentified sugar (probably a mono-methyl glucose) was also present.

The methylation results on both the undegraded and degraded gums are tabulated below. The proportions of the methylated sugars given are only very approximate, since many fractions were mixtures and several refractionations had to be performed to separate the components. The error is even more in the case of sugars from acidic fractions of the methylated gum, since hydrolysis of acidic polysaccharides are usually accompanied by some decomposition.

Hydrolysis products of methylated Combretum leonense gum and of methylated degraded Combretum leonense gum.

	Approximate molecular proportions			
	Undegraded gum		Degraded gum	
	Neutral	Acidic	Neutral	Acidic
2,3,4,6-Tetra-O-methyl-D-galactose	trace	-	6	-
2,3,4-tri-O-methyl-D-galactose	28	1	13	1·5
2,3,6-tri-O-methyl-D-galactose	7	-	trace	-
2,4-di-O-methyl-D-galactose	28	1	trace	-
2,3-di-O-methyl-D-galactose	5	11*	2.5	4*
2,6-di-O-methyl-D-galactose	4	-	-	-
3,4-di-O-methyl-D-galactose	-	-	trace	-
2-mono-O-methyl-D-galactose	27.5	1	trace	trace
3-mono-O-methyl-D-galactose	2	-	-	-
D-galactose	trace	-	trace	-
2,3,5-tri-O-methyl-L-arabinose	27	-	-	-
2,3,4-tri-O-methyl-L-arabinose	1	-	trace	-
2,3-di-O-methyl-L-arabinose	5.5	-	-	-
2,5-di-O-methyl-L-arabinose	4	-	-	-
3,5-di-O-methyl-L-arabinose	trace	-	-	-
L-arabinose	trace	-	-	-
3,4-di-O-methyl-L-rhamnose	trace	-	1	1
3-mono-O-methyl-L-rhamnose	2	6	1	3
L-rhamnose	2·5	1·5	trace	trace
2,3,4-tri-O-methyl-D-glucuronic acid	-	-	trace	0·5
2,3-di-O-methyl-D-glucuronic acid	-	2	-	-
2,3,4-tri-O-methyl-D-galacturonic acid	trace	-	-	-

* Arises mostly from 2,3-di-O-methyl-D-galacturonic acid.

The yield of arabinose ethers is lower than that expected from the results of total hydrolysis, which gave an approximate molecular proportion of 5:4 for galactose:arabinose. This low yield is most probably due to the loss by evaporation of the highly volatile 2,3,5-tri-O-methyl-L-arabinose.

Structural features of the gum.

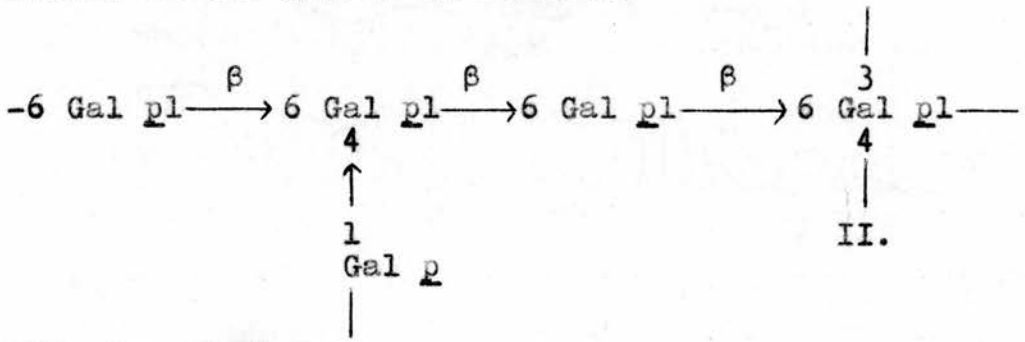
The structural features of the Combretum leonense gum, on the basis of the partial hydrolysis and methylation results, can be conveniently discussed by considering in turn the galactan framework, the acidic fragments and the acid-labile units.

The galactan framework.

The isolation of the homologous series of 1,6'-linked galactose oligosaccharides (I), indicates a main chain of 1,6'-linked β-D-galactopyranose residues in the gum. The methyl galactose from the methylated degraded gum consisted predominantly of 2,3,4-tri-O-methyl-D-galactose, thus confirming the evidence from partial hydrolysis.

Even though only small amounts of the disaccharide 4-O-β-D-galactopyranosyl-D-galactose were isolated it must be a true hydrolysis product and not an acid reversion product. This is so because the major acid reversion product of galactose is 6-O-α-D-galactopyranosyl-D-galactose (73) and it was not present in the partial acid hydrolysate of the gum. It is difficult to predict, on the present evidence, how this 1,4-galactobiose is incorporated in the molecular structure,

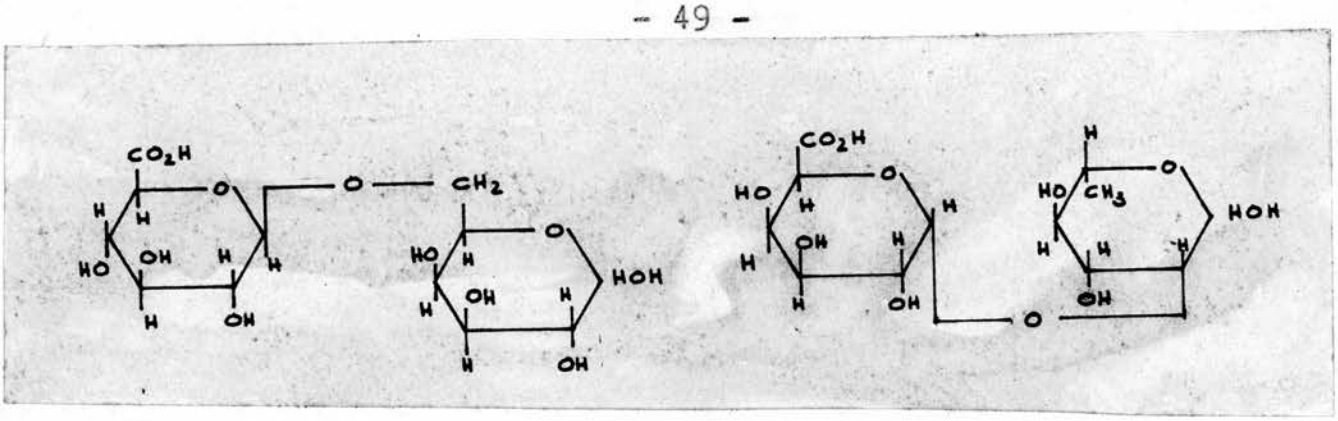
but it most probably arises from side chains attached to position 4 of the galactose residues on the main chain. The presence of branching points at C4 of galactose is also indicated by the isolation of 2,3-di-O-methyl-D-galactose from both methylated undegraded and degraded gums and of 2,3,6-tri-O-methyl-D-galactose from the methylated gum. The large amounts of 2-O-methyl-D-galactose isolated from the methylated gum must represent double branching points through positions 3 and 4 as shown in II. Hence the main chain of galactose residues in the gum is as follows:



II.

Acidic fragments.

The portion of the gum molecule most resistant to hydrolysis consists of two aldobiouronic acid residues, namely 6-O-(β-D-glucopyranosyluronic acid)-D-galactose (VII) which is the most common aldobiouronic acid residue encountered among gums and 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose (VIII) which is commonly encountered as a fragment from gums (61 & 62) and mucilages containing main chains of D-galacturonic acid and L-rhamnose residues.



VII VIII

Again, it is difficult to predict on the present evidence, how these aldobiouronic acid units are incorporated in the general molecular structure of the gum.

The tentative identification of the aldotriouronic acids (IVa, Va, Vb) suggests that the aldobiouronic acid (VIII) is flanked by galactose residues as shown in IX.

$$\text{Gal } \underline{p}\,1 \longrightarrow (3 \text{ or } 4)\ \text{Gal } \underline{p}\ \text{Al} \xrightarrow{\alpha} 2\ \text{Rha } \underset{\displaystyle 4}{\underline{p}}\,1 \longrightarrow 4\,\text{Gal } \underline{p}\,1 \longrightarrow \quad \text{IX.}$$

The major rhamnose derivative from the methylated gum is 3-O-methyl-L-rhamnose. It follows that almost all the rhamnose residues in the gum are branched at position 4 (IX). Also, the isolation of appreciable amounts of rhamnose from the methylated gum, all of which cannot be due to undermethylation, suggests the presence of some doubly branched rhamnose residues in the gum molecule as shown in X.

$$\text{Gal } \underline{p}\,1 \longrightarrow (3 \text{ or } 4)\ \text{Gal } \underline{p}\ \text{Al} \xrightarrow{\alpha} 2\ \text{Rha } \underset{\displaystyle 4}{\overset{\displaystyle 3}{\underline{p}}}\,1 \longrightarrow 4\ \text{Gal } \underline{p}\,1 \longrightarrow \quad \text{X}$$

The point of attachment of the galactose residue to the galacturonic acid residue in IX can be either 3 or 4. Since

the major galactose derivative isolated from the acidic portion of the methylated gum after reduction was 2,3-di-O-methyl-D-galactose the point of attachment must be C4. But the detection of the aldotriouronic acid Va among the hydrolysis products of the gum suggests that at least some of the galactose residues are attached to C3 of the galacturonic acid residue.

The main galactose derivative isolated from the acidic portion of the methylated degraded gum after reduction was also 2,3-di-O-methyl-D-galactose, hence the galactose residues attached to C4 of galacturonic acid are still intact after autohydrolysis.

The acid-labile periphery.

The main acid-labile units in the gum are arabinofuranose end groups, Ara f 1— . This follows from the fact that a large proportion of the arabinose is easily removed under the mild conditions of autohydrolysis and that the main arabinose derivative from the methylated gum is 2,3,5-tri-O-methyl-L-arabinose.

The presence of some arabinopyranose end groups is indicated by the isolation of 2,3,4-tri-O-methyl-L-arabinose from the methylated gum. Most of it must be attached to other arabinose units as shown in XI. But some of it is probably attached directly to galactose residues, this is indicated by the detection of an arabinopyranosyl-galactose (as yet unidentified) in the autohydrolysate. These are probably the few arabinose units which were not removed on autohydrolysis and were thus

present in the degraded gum giving rise to the only arabinose derivative, namely 2,3,4-tri-O-methylarabinose, on methylation and hydrolysis.

The presence of non-terminal arabinose in the periphery of the molecule is indicated by the isolation of arabinobioses and 3-O-galactosylarabinose. The main arabinobiose present was the 3-O-arabinopyranosylarabinose. This disaccharide was easily released on autohydrolysis and therefore the reducing end of the disaccharide probably exists in the furanose form in the polysaccharide. Thus the structural unit giving rise to this arabinobiose may be represented by

Ara p 1 $\xrightarrow{\beta}$ 3 Ara f 1— XI.

The disaccharide 3-O-galactosylarabinose was also liberated on mild acid hydrolysis and hence represents a peripheral unit in which the arabinose residue must be in the furanose form (XII).

Gal p 1 ⟶ 3 Ara f 1— XII.

The traces of 2,3,4,6-tetra-O-methyl-D-galactose detected in the methylated gum must arise from a few galactopyranose end groups present in the gum.

The -3Ara f 1— units (XI and XII) in the gum would give rise to the 2,5-di-O-methyl-L-arabinose, which was isolated from the methylated gum. On the other hand a unit of the type -3Ara p 1- would give rise to 2,4-di-O-methylarabinose, which was not detected among the hydrolysis products of the methylated gum and therefore is not present in the gum.

The isolation of substantial amounts of 2,3-di-O-methyl-

L-arabinose from the methylated gum shows the presence of doubly linked arabinose residues such as -5Ara *f*l- and/or -4Ara *p*l-. Such units were also indicated by the detection of other unidentified arabinobioses in the autohydrolysate of the gum. Since practically all the arabinose residues were removed by autohydrolysis, the arabinofuranase unit (-5Ara *f*l-) seems to be more likely to be present, rather than the arabinopyranose unit (-4Ara *p*l-).

Hence we may conclude that the main acid-labile group in the gum is Ara *f*l- and other present in small proportions are

Ara *p*l-

Ara *p*l⟶3Ara *f*l-

Gal *p*l⟶3Ara *f*l-

-(5)Ara *f*l-

and Gal *p*l-

The equimolecular proportions of 2,3,4-tri- and 2,4-di-O-methyl-D-galactose in the methylated gum and the presence of only a trace of 2,4-di-O-methyl-D-galactose in the methylated degraded gum shows that most of the acid-labile groups are attached to position 3 of the galactose residues in the gum. This is further indicated by the presence of only a trace of 2-O-methyl-D-galactose and an increased proportion of 2,3-di-O-methyl-D-galactose in the methylated degraded gum.

The acidic portions of the methylated undegraded and degraded gums after reduction gave rise entirely to 2,3-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose respectively.

This implies that all the D-glucuronic acid residues occupy a penultimate position in the gum with acid-labile groups attached to them through position 4.

The increased proportion of 3-O-methyl-L-rhamnose to rhamnose in the methylated degraded gum compared to the proportion in the methylated gum indicates that almost all the sugar residues attached to position 3 of rhamnose are acid-labile. The presence of only a trace of 3,4-di-O-methyl-L-rhamnose in the methylated gum and an increased proportion of it in the methylated degraded gum indicates that at least some of the residues attached to position 4 of rhamnose are acid-labile.

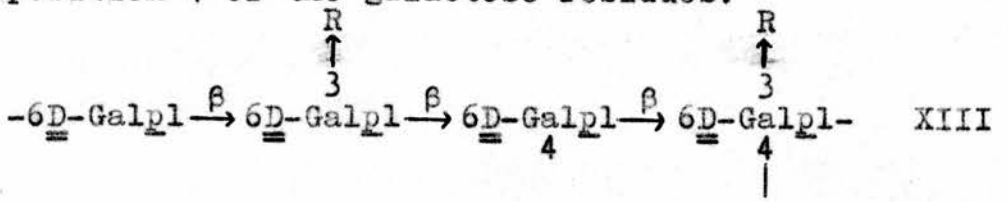
Thus on the evidence available so far, we can conclude that the acid-labile units present in the gum are attached mostly to positions C3 of galactose and C4 of glucuronic acid residues. Some of them are also attached to positions C3 and C4 of rhamnose residues.

General structure of the gum.

It is not possible to put forward a complete structure for Combretum leonense gum and it is doubtful if a discrete repeating unit exists. However the following structural features (XIII, XIV and XV) may be put forward for the gum, on the basis of the experimental evidence available so far.

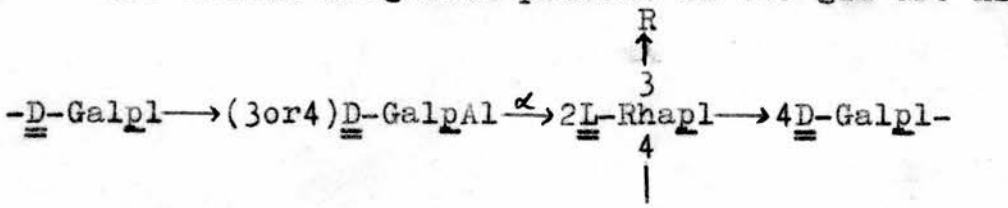
The galactan chain in the gum is composed of D-galactopyranose residues linked 1,6-β-, with acid-labile units attached to position 3 and acid resistant units attached to

position 4 of the galactose residues.

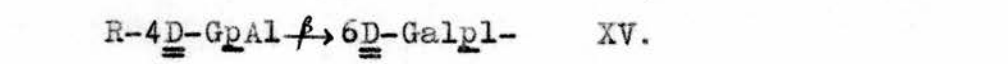


XIII

The acidic fragments present in the gum are XIV and XV.



XIV



XV.

The acid-labile units present in the gum, represented by R in XIII, XIV, and XV, are

L-araf1- (major)

L-Arap1 $\not\beta$ 3L-Araf1-

L-Aral $\longrightarrow$ (5)L-Araf1-

D-Galp1 $\not\beta$ 3L-Araf1-

L-Arap1-

D-Galp1-

On the present evidence it is difficult to say how these different structural units (XIII, XIV, and XV) are incorporated in the gum molecule.

Nature of heterogeneity of the gum.

Previous workers (39) analysing pure polysaccharides isolated from a number of different nodules of Combretum leonense gum found variations in composition, especially in uronic anhydride and acetyl contents. These variations were greater than could be ascribed to possible experimental errors.

Also, in the present investigation the purified polysaccharides obtained from two different batches of gum had different optical rotations and uronic anhydride contents.

These results indicated that some kind of heterogeneity exists in the gum polysaccharide. Since the polysaccharide has two different acidic residues (galacturonic and glucuronic), there is a possibility that these acidic units have their origin in different polysaccharides.

Chromatography of gum samples on columns of DEAE-cellulose in the phosphate form using phosphate buffers followed by sodium hydroxide for elution, gave rise to polysaccharide fractions having different uronic anhydride contents.

The first sample (A) of the gum polysaccharide examined by the above method gave two fractions (A_1 and A_2) having different uronic anhydride contents. Fractions A_1 and A_2 were eluted from the column with phosphate buffers, the sodium hydroxide eluate contained only traces of polysaccharide. The fractions A_1 and A_2, which were freed from inorganic ions by dialysis and treatment with ion exchange resins, were subjected to partial acid hydrolysis. Both the fractions gave the same neutral and acidic oligosaccharides, as shown in table 7.

A second sample (B) of the gum polysaccharide was fractionated on DEAE-cellulose column in a similar manner. In this case, elution with increasing concentrations of phosphate buffer gave small amounts of polysaccharide (fraction B_1) in

the early stages and later a major fraction (B_2). Elution with sodium hydroxide gave a third fraction (B_3) of higher uronic anhydride content. After preliminary examination of these three fractions (B_1, B_2 and B_3), it was thought desirable to obtain more of these fractions in order to study them in detail.

Polysaccharide fractions	% uronic anhydride	$[\alpha]_D$	Partial hydrolysis products		
			Monosaccharides	Neutral oligosaccharides	Acidic oligosaccharides
A_1	10·3	$-12\pm2^\circ$	Gal, Ara, Rha	1,6-Gal-Gal 1,3-Gal-Ara	1,2-GalA-Rha 1,6-GA-Gal
A_2	15·9	$-9\pm2^\circ$	Gal, Ara, Rha	1,6-Gal-Gal 1,3-Gal-Ara	1,2-GalA-Rha 1,6-GA-Gal
B_1	4·2	$+4\pm2^\circ$	Gal, Ara, Xy(trace)	1,6-Gal-Gal	-
B_2	15·4	$-8\pm2^\circ$	Gal, Ara, Rha	1,6-Gal-Gal 1,3-Gal-Ara	1,2-GalA-Rha 1,6-GA-Gal
B_3	20·3	$-3\pm2^\circ$	Gal, Ara, Rha	1,6-Gal-Gal 1,3-Gal-Ara	1,2-GalA-Rha 1,6-GA-Gal

Table 7.

Accordingly, two large scale fractionations (2g. polysaccharide each time) were carried out. By following the fractionation with the phenol-sulphuric acid colour test, it was found that a successful separation of the fractions was achieved. However, considerable difficulty and loss of material was encountered in isolating the polysaccharide fractions free from

the comparatively large amounts of inorganic ions. Fraction B_1, obtained from the phosphate buffer eluate, had a very low uronic anhydride content. On partial hydrolysis this fraction gave 6-O-β-galactosyl-galactose, galactose, arabinose and traces of xylose, but no rhamnose or acidic fragments could be detected in the hydrolysate (table 7). Fraction B_2, obtained from the phosphate buffer eluate, and fraction B_3 from the sodium hydroxide eluate had differing uronic anhydride contents. However, partial hydrolysis of these two fractions (B_2 and B_3) gave the same neutral and acidic oligosaccharides (see table 7).

Samples of fractions B_2 and B_3 were fully methylated and the mixtures of the methyl glycosides obtained by methanolysis were examined by vapour phase chromatography in three different systems. The vapour phase chromatographic patterns were qualitatively identical, showing that both fractions give rise to the same methyl glycosides on methylation and methanolysis. However, there was some variation in the proportions of the components in the two samples. These variations were not very great, except in the case of the methyl-2,3,5-tri-O-methyl-L-arabinoside which is highly volatile and hence could have been lost (to a greater extent in one of the samples) during the working up of the methanolysis product.

Due to the complexity of the vapour phase chromatograms all the methyl glycosides present in the mixture could not be identified. However, peaks corresponding to the methyl

glycosides of most of the methylated sugars previously isolated from the methylated gum were present in the vapour phase chromatograms.

Ultracentrifuge examination (kindly carried out by Dr. C. T. Greenwood) of the fractions B_2 and B_3 did not reveal any difference in them and their sedimentation coefficients were found to be the same.

From these results we could probably conclude, that the Combretum leonense gum nodules consist of varying amounts of structurally similar polysaccharides which differ in the proportions rather than in the nature of the constituent structural units.

At present there is no evidence available to suggest that the two acidic units (galacturonic and glucuronic) arise from different polysaccharides.

Comparison of the structural features of Combretum leonense gum with those of other gums.

For the above purpose, the gums may be conveniently divided into two main groups.

(a) Gums based on inner chains of D-galactose residues, and

(b) Gums based on inner chains of D-galacturonic acid and L-rhamnose residues.

The only gums which do not belong to either of these groups are those few gums based on inner chains of D-xylose residues [e.g.:- Sapota achras gum (111)]. Since Combretum leonense gum does not have any structural features in common with these gums, the above grouping is justified.

The Khaya, Sterculia and Cochlosperum gums belong to group (b). The sugar components of these gums and the acidic oligosaccharides isolated from them are summarised in table 8. The aldobiouronic acid 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose had been isolated from all the gums of this group, except the Sterculia caudata gum. It may be noted here, that this aldobiouronic acid had also been isolated from many acidic mucilage polysaccharides showing the close relationship of this group of gums to the mucilages.

The above aldobiouronic acid is the main acidic fragment from Combretum leonense gum. However, so far there is no evidence to show that this acidic fragment has the same significance in Combretum leonense gum as in the above group of gums where it arises from the main chain. The only other gum

which is known to have D-galacturonic acid residues linked to L-rhamnose and still does not belong to group (b) of gums is the Cholla gum (100 and 112).

The gums belonging to group (a), in general have a backbone of galactose residues which carry side chains containing aldobiouronic acid units and have acid-labile (mainly pentose) units on the periphery of the molecule. The gums of this group may be further divided into three sub groups on the basis of the nature of the linkages in the galactan backbone.

(i) Gums which have highly branched core of D-galactose residues which are involved in 1,3- and 1,6-linkages.

(ii) Gums having main chains composed of 1,3-linked D-galactopyranose residues, with 1,6-linked D-galactose incorporated in the side chains.

(iii) Gums which have main chains composed of 1,6-linked D-galactopyranose residues.

The Prunus gums and other gums such as Lemon, Cholla, Mesquite belong to sub group (i). The major structural features of this group of gums are summarised in table 9 in terms of the galactan framework, the acidic fragments and the acid-labile units.

The acid-labile periphery of this group of gums is composed mainly of pentose units. But in addition to L-arabinose residues, some of the gums of this group also contain appreciable quantities of D-xylose units in the periphery, and in this manner differ from the gums of sub groups (ii) and (iii).

The common acidic unit of this group of gums, with the exception of Cholla gum, is D-glucuronic acid which occurs sometimes wholly or partly as its 4-O-methyl ether. Partial hydrolysis of these gums gives rise to one or two of the following aldobiouronic acids, 4-O-α- and 6-O-β-[(4-O-methyl) D-glucopyranosyluronic acid]-D-galactoses, and 2-O-β-[D-glucopyranosyluronic acid]-D-mannose.

The Acacia gums and the Asofoetida gum belong to sub group (ii) and their main structural features are summarised in table 10.

In addition to acid-labile pentose units, some of the gums of this group have L-rhamnopyranose as end-groups in the periphery of the molecule. All the gums of this group so far investigated have only D-glucuronic acid as the acidic sugar residue. In Asofoetida gum part of the D-glucuronic acid is as its 4-O-methyl ether. The aldobiouronic acid, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose has been characterised as a fragment from all the gums of this sub group.

The Combretaceae gums and the Virgillia oroboides gum belong to sub group (iii) and the unknown structural features of these gums are summarised in table 11.

The majority of the acid-labile units in the gums of this sub group are made up of L-arabinose residues. In the Combretaceae gums the arabinose residues occur principally as single L-arabinofuranose end-groups. But Virgillia oroboides gum contains a large proportion of L-arabinopyranose, in

addition to L-arabinofuranose, end-groups. Evidence for the presence of two adjoining arabinose residues in the acid-labile periphery has been obtained for A.schimperi, C.leonense and V.oroboides gums by the isolation of arabinobioses. In the case of gum ghatti no arabinobioses were isolated from the hydrolysis. However, the presence of substituted arabinose residues (e.g.:- 3Araf1-) in the acid-labile periphery of gum ghatti is indicated by methylation studies on the undegraded and Smith-degraded gums (102 and 107). In addition to the above units, the V-oroboides gum has a small amount of D-xylopyranose residues in the acid-labile periphery linked (1→5) to L-arabinose.

Since methylation studies have not been carried out on A.schimperi gum it is not possible to predict the mode of attachment of the acid-labile units in this gum. In the case of the other three gums, the positions of attachment of the acid-labile units to the rest of the gum structure are given in the table below.

Gum	Positions to which acid-labile units are attached			
	D-galacto pyranose	D-glucuronic acid	D-mannose	L-rhamnose
A.latifolia	3	4	3 & 6	-
C.leonense	3	4	-	3
V.oroboides	3 & 4	?	3	-

All four gums of this sub-group give rise to the same homologous series (I) of galactose-containing oligosaccharides on partial hydrolysis, indicating the presence of chains of 1,6-β-linked D-galactopyranose residues in all of them. However, in the case of the two Anogeissus gums, that L-arabinose residues are incorporated in the main chain is shown by the isolation of a second homologous series of oligosaccharides (III).

O-β-D-galactopyranosyl-[(1→6)-β-D-galactopyranosyl]$_n$-(1→3)-L-arabinose. (n = 0-3) III.

The Anogeissus gums and the V.oroboides gum give rise to 3-O-β-D-galactopyranosyl-D-galactose (in varying proportions) indicating the presence of 1,3-linked D-galactose in the branches attached to the main chain. C.leonense gum differs from the above three gums in having 1,4-linked galactose in the branches, as shown by the isolation of 4-O-β-D-galactopyranosyl-D-galactose.

All the three Combretaceae gums give rise to 3-O-β-D-galactopyranosyl-L-arabinose on partial hydrolysis. In the case of the Anogeissus gums this unit is a fragment from the main chain where the arabinose residue is probably present in the pyranose form. Evidence for the presence of L-arabinopyranose residues in the main chain of gum ghatti has been obtained by the isolation of 2,4-di-O-methyl-arabinose from the hydrolysis products of the methylated Smith-degraded gum (107). In C.leonense gum the above disaccharide is liberated

on autohydrolysis and hence probably represents a peripheral unit in which the arabinose residue is present in the furanose form.

The Anogeissus gums and the V.oroboides gum contain D-glucuronic acid as their only acidic sugar residue. (In V.oroboides gum some of these acid residues occur as the 4-O-methyl ether). Whereas C.leonense gum has D-galacturonic acid as its main acidic sugar residue, in addition to smaller amounts of D-glucuronic acid.

All four gums have the aldobiouronic acid fragment 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, but in varying amounts. The Anogeissus gums and the V.oroboides gum have 2-O-(β-D-glucopyranosyluronic acid)-D-mannose as their main aldobiouronic acid. But the main aldobiouronic acid in C.leonense gum is 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, which, as mentioned earlier, is commonly encountered as a fragment from gums and mucilages containing interior chains of D-galacturonic acid and L-rhamnose residues.

It is thus seen that the greatest resemblances is between the Anogeissus and the V.oroboides gums. The C.leonense gum, although it has the same general pattern of molecular structure as the above gums, differs greatly in the detailed structural features. The V.oroboides and the C.leonense gums resemble each other and differ from the Anogeissus gums in not having L-arabinopyranose residues in the main chain.

Only preliminary investigation has been done on Combretum verticillatum gum (108), which belongsto the same family and genus as Combretum leonense gum. The soluble fraction (90%) of the C.verticillatum gum was found to be made up of galactose, arabinose and glucuronic acid by partial hydrolysis studies. It would be of interest to carry out a detailed investigation of this gum with a view to comparing its structural features with those of C.leonense gum.

Table 8.

Gum	Monosaccharides				Aldobiouronic acids	Refs.
	GalA	Rha	Gal	Other sugars		
Khaya grandifolia	+	+	+	4-MeGA & Ara (tr.)	GalpAl $\xrightarrow{\alpha}$ 2Rha 4MeGAl $\xrightarrow{\alpha}$ 4Gal GalpAl $\xrightarrow{\alpha}$ 2Rhal $\longrightarrow$ 4Gal	61
Khaya senegalensis (major component)	+	+	+	4-MeGA & Ara	GalpAl $\xrightarrow{\alpha}$ 2Rha 4MeGAl $\xrightarrow{\alpha}$ 4Gal	12
Sterculia setigera	+	+	+	Tagatose	GalpAl $\longrightarrow$ 2Rha GalpAl $\longrightarrow$ 4Gal	115 & 116
Sterculia urens (Karaya gum)	+	+	+	GA & Ara(tr.)	GalpAl $\xrightarrow{\alpha}$ 2Rha	117
Sterculia caudata (Brachychiton diversifolium)	?	+	+	GA	GpAl $\longrightarrow$ 2Rha	37
Cochlosperum gossypium (Kutira gum)	+	+	+	GA	GalpAl $\longrightarrow$ 2Rha GalpAl $\longrightarrow$ 4Gal(?)	17 & 118

Table 8. Sugar components and acidic oligosaccharides of gums based on inner chains of D-galacturonic acid and L-rhamnose residues.

Table 9.

Gum	Fragments from Periphery	Acidic fragments	Refs.
Damson	Araf1- -5Araf1-	GpA1 $\xrightarrow{\beta}$ 2Man-	119
Cherry	Araf1- Arap1 $\xrightarrow{\beta}$ 3Araf1-	GpA1 $\xrightarrow{\beta}$ 2Man- GpA1 $\longrightarrow$ 6Gal-	100 & 120
Egg plum	Araf1- -3Araf1- Xylp1-	GpA1 $\longrightarrow$ 6Gal-	121 & 122
Lemon	Araf1- Arap1 $\xrightarrow{\beta}$ 3Ara- 4-MeGpA1 $\xrightarrow{\alpha}$ 4Arap1-	(4-Me)GpA1 $\xrightarrow{\alpha}$ 4Gal-	74 123-125
Grape fruit	Araf1-	(4-Me)GpA1 $\longrightarrow$ 4Gal-	123 & 125
Golden apple	Xylp1 $\xrightarrow{\alpha}$ 3Ara- Arap1 $\xrightarrow{\beta}$ 3Ara- 4-MeGpA1 $\longrightarrow$ 3Ara-	4-MeGpA1 $\longrightarrow$ 6Gal-	126 & 127
Cholla	Xylp1 $\longrightarrow$ 5Araf1- ; Araf1-	GalpA1 $\longrightarrow$?Rha-	100 & 112

Table 9 (cont.)

Gum	Monosaccharides						Fragments from periphery	Acidic fragments	References
	Gal	Ara	Xyl	GA	4-Me GA	Other Sugars			
Mesquite	+	+	-	+	+	-	Araf1- -3Araf1-	4-MeGpA1→6Gal- (4-Me)GpA1→4Gal- (4-Me)GpA1→3Gal-	128-131
Khaya senegalensis (minor component)	+	+	-	+	+	-	Araf1-	4-MeGpA1→(?)Gal-	12, 28 & 118
Albizzia zygia	+	+	-	+	+	Rha(tr)	Galp1 $\xrightarrow{a}$ 3Ara-	4-MeGpA1→4Gal- GpA1→2Man-	9
Arabinogalactan from gum tragacanth	+	+	-	-	-	GalA (tr.)	Araf1- -5Araf1- -3Araf1- -2Araf1-	-	13 & 132

Table 9. Structural features of gums having a backbone of 1,3-β- and 1,6-β-linked D-galactopyranose residues.
[All the gums given in this table give rise to 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose on hydrolysis].

Table 10.

Gums	Proportions of monosaccharides					Fragments from periphery	Fragments from galactan framework	Acidic fragments	Refs.
	Gal	Ara	Rha	GA	Other sugars				
Asofoetida	5	3	tr.	1	4-MeGA	Araf1-	Galp1 $\xrightarrow{\beta}$ 3Gal. Galp1 $\xrightarrow{\beta}$ 6Gal.	GpA1 $\xrightarrow{\beta}$ 6Gal 4-MeGpA1 $\xrightarrow{\beta}$ 6Gal	135
Acacia senegal (gum arabic)	3	3	1	1	-	Araf1- Arap1 $\xrightarrow{\beta}$ 3Araf1- Galp1 $\xrightarrow{\alpha}$ 3Araf1- Rhap1-	Galp1 $\xrightarrow{\beta}$ 3Gal. Galp1 $\xrightarrow{\beta}$ 6Gal.	GpA1 $\xrightarrow{\beta}$ 6Gal	71, 72 103, 133
Acacia pycnantha	65	27	2	5	-	Araf1- Araf1 $\xrightarrow{\beta}$ 3Araf1- Rhap1-	Galp1 $\xrightarrow{\beta}$ 3Gal. Galp1 $\xrightarrow{\beta}$ 6Gal.	GpA1 $\xrightarrow{\beta}$ 6Gal.	38, 97, 134
Acacia mollissima (Black wattle	5	6	1	1	-	Arap1 $\xrightarrow{\beta}$ 3Ara		GpA1 $\xrightarrow{\beta}$ 6Gal.	136
Acacia cyanophylla	11	2	5	5	-	Galp1 $\xrightarrow{\alpha}$ 3Ara		GpA1 $\xrightarrow{\beta}$ 6Gal	104
Acacia karroo	25	18	1	6	-	Arap1 $\xrightarrow{\beta}$ 3Ara Galp1 $\xrightarrow{\alpha}$ 3Ara		GpA1 $\xrightarrow{\beta}$ 6Gal. GpA1 $\xrightarrow{\alpha}$ 4Gal.	137
Acacia catechu	9	4	3	3	-	Araf1-	-3Galp1- 2 1	GpA1 $\xrightarrow{\beta}$ 6Gal.	138
Acacia sundra	6	4	2	3	-	Araf1- Rhap1-		GpA1 $\xrightarrow{\beta}$ 6Gal	139, 140

Table 10. Structural features of gums having a backbone of 1,3-β-linked D-galactopyranose residues.

Table 11.

Gum	Monosaccharides Gal. Ara. Man. Rha. Xy. GA GalA.	Fragments from periphery
Anogeissus latifolia (gum ghatti)	+ + + Tr. + + -	Araf1- -2Araf1- -3Araf1- -5Araf1-
Anogeissus schimperi	+ + + Tr. + + -	Araf1- Araf1⟶3Araf1- Arap1⟶3Araf1-
Combretum leonense	+ + - + Tr. + +	Araf1- -5Araf1- Arap1⟶3Araf1- Galp1⟶3Araf1-
*Virgillia oroboides	+ + + Tr. + + +(4-Me) -	Araf1- Arap1- Arap1⟶5Araf1- Araf1⟶5Araf1- Xylp1⟶5Araf1-

* Except this gum the other three gums in this table belong to the family Combretaceae.

[Cont. on next page.]

Table 11 (cont.)

Gum	Fragments from galactan framework	Acidic Fragments	Refs.
Anogeissus latifolia (gum ghatti)	Galp1→6Galp1→$_n$6Galp. (n=0-2) Galp1→6Galp1→$_n$3Arap. (n=0-3) Galp1→3Galp.	GpA1→2Man. GpA1→6Gal. GpA1→2Manp1→2Man.	101, 102, 106 & 107
Anogeissus schimperi	Galp1→6Galp1→$_n$6Galp. (n=0-3) Galp1→6Galp1→3Ara. (n=0-3) Galp1→3Galp	GpA1→2Man. GpA1→6Gal. (traces) GpA1→6Galp1→(?)Ara.	40
Combretum leonense	Galp1→6Galp1→$_n$6Galp. (n=0-3) Galp1→4Galp	GpA1→6Gal. GalpA1→2Rha. GalpA1→2Rhap1→4Gal. Galp1→(3or4)GalpA1→2Rha.	
*Virgillia oroboides	Galp1→6Galp1→$_n$6Galp. (n=0-3) Galp1→3Galp.	GpA1→6Gal. GpA1→2Man.	113 114

Table 11. Structural features of gums having a backbone of 1,6-β-linked D-galactopyranose residues.

Smith degradation studies on the gum.

The gum was subjected to Smith degradation studies (94) in order to obtain information on the periodate resistant part of the molecule. It was hoped that these studies would also provide information as to the relationship between the structural features put forward for the galactan framework and the acidic fragments of the gum.

Since the periodate oxidised uronic acid residues are not completely cleaved during Smith degradation (107), it was thought desirable to reduce the gum to the corresponding neutral polysaccharide before subjecting it to Smith degradation.

Accordingly, the gum (uronic anhydride, 13·9%) was acetylated and the gum acetate reduced with diborane (82) to give the almost neutral polysaccharide (uronic anhydride, ca. 1·4%). This polysaccharide on hydrolysis with N-acid gave only traces of acidic sugars.

A trial experiment was carried out by oxidising the reduced gum (1 g.) with sodium periodate until no more oxidant was consumed (30 hr.). It was found that 0·76 mols of the oxidant was consumed per sugar unit. The polyaldehyde was reduced with potassium borohydride to the polyalcohol and the acetal linkages in the polyalcohol were cleaved with mineral acid at room temperature without affecting the glycosidic linkages. This procedure is referred to as Smith degradation (94).

The degraded polysaccharide (yield ca. 5%), obtained by precipitation with excess ethanol, on hydrolysis gave mainly 1,6-galactobiose and galactose together with smaller amounts of

arabinose and rhamnose. The main part of the carbohydrate material (non-reducing oligosaccharides), together with the low molecular weight material such as glycerol and threitol, was obtained by evaporation of the supernatant mother liquor. This mixture did not have any free reducing sugars, but after hydrolysis galactose, arabinose and rhamnose were detected among the products. This showed the presence of glycosides of mono- and oligosaccharides in the above mixture.

A larger sample of the reduced gum (14 g.) was subjected to Smith degradation and the degraded polysaccharide 'P' was precipitated with excess ethanol as before. Evaporation of the mother liquor gave the material 'A' in about 60% yield.

The ethanol soluble material 'A' was extracted with dry acetone to remove most of the glycerol and threitol. Attempts to effect fractionation of the acetone insoluble material by chromatography on Dowex resin X2 and charcoal-Celite columns were not very successful. However, seven fractions (A_{1-7}) were obtained by adding increasing amounts of acetone to a solution of the material in water.

A portion of fraction A_1 was methylated by Haworth and Purdie procedures. The methylated sample [OMe, 39·9%; $[\alpha]_D$ -31·9° (*c*, 0·86, chloroform)] was hydrolysed and among the products the following methylated sugars were detected, by paper chromatography and by vapour-phase chromatography of the derived methyl glycosides.

2,3,5-Tri-O-methyl arabinose

2,3,4,6-tetra-O-methyl galactose

2,3,4-tri-O-methyl galactose

-di-O-methyl galactoses (2,3- and 2,4-)

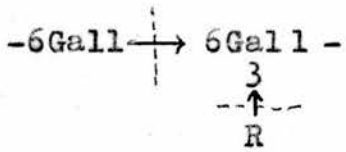
3,4-di-O-methyl rhamnose

3-mono-O-methyl rhamnose.

The detection of 2,3,5-tri-O-methyl arabinose indicates the presence of arbinofuranose end-groups in the degraded gum. In the original gum these arabinose residues must have been substituted on either 2- or the 3-positions in order to be unsusceptible to periodate oxidation.

R⟶ (2 or 3)L-Ara 1-

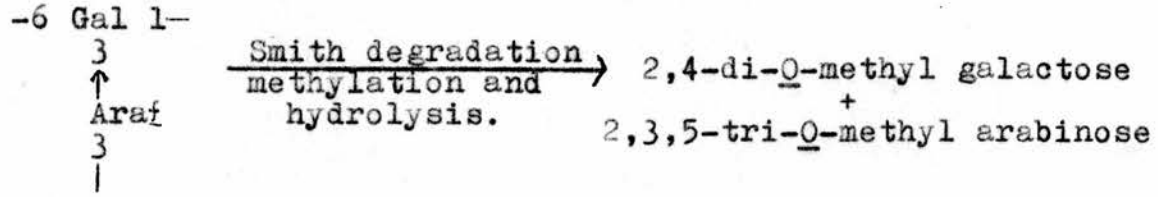
The presence of appreciable amounts of 2,3,4,6-tetra-O-methyl galactose indicates that a large proportion of non-reducing galactose end-groups have arisen on Smith degradation. It is probable that such groups arose by cleavage of the 1,6-linked galactose chain as shown below.



The unsubstituted galactose residue and the sugar unit R would be removed on Smith degradation.

Since 2,3,4-tri-O-methyl galactose was the main galactose derivative, a considerable proportion of the 1,6-linked galactose residues in the gum must have been unsusceptible to periodate oxidation, probably due to substituents at position 3.

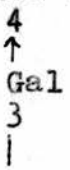
The dimethyl galactoses must have arisen from galactose residues carrying a periodate resistant sugar unit at position 3. For example,



The detection of 3,4-di-O-methyl rhamnose is evidence that rhamnose residues substituted at C4 by periodate cleavable sugar units

are present in the gum.

Since 3-O-methyl rhamnose was also present, at least some of the substitutents at C4 of rhamnose must be periodate resistant. For example,



Another portion of the fraction A_1 was subjected to partial acetolysis and the products were deacetylated by two treatments with barium methoxide. The products on chromatographic examination were found to consist of at least three non-reducing oligosaccharides. In order to investigate these oligosaccharides in detail, a large scale partial acetolysis was carried out on the combined fractions (A_{1-7}) and the degraded polysaccharide 'P'.

Attempts to fractionate the deacetylated products by adsorption chromatography on charcoal and by partition

chromatography on cellulose were unsuccessful. A trial experiment showed that the non-reducing sugar glycosides could be separated from the reducing sugars by adsorbing the latter on strongly basic resin, such as Amberlite resin IR400(OH).

Hence, the rest of the partial acetolysis products were placed on a column of De-Acidite F(OH) resin and eluted with water. Five fractions containing non-reducing oligosaccharides were obtained, which were further fractionated on thick filter sheets.

E X P E R I M E N T A L

EXPERIMENTAL.

General Methods.

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

Small scale hydrolyses were carried out by heating the sample with the given normality of acid at 100°. Where sulphuric acid was used, saturated 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 through a celite bed and the filtrate taken to dryness.

Hydrochloric acid hydrolysates were worked up by neutralising with excess silver carbonate, filtering, passing hydrogen sulphide filtering through celite and evaporating to dryness. Products of methanolysis were neutralised in the same manner.

Paper chromatography - Qualitative work was done on Whatman No.1 or 4 papers. Descending development with the following solvent systems (v/v) was employed.

(A) Ethyl Acetate - Pyridine - Water (10 : 4 : 3).

(B) Ethyl Acetate - Acetic Acid - Formic Acid - Water (18 : 3 : 1 : 4).

(C) Ethyl Acetate - Acetic Acid - Formic Acid - Water (18 : 8 : 3 : 9).

(D) Butan-1-ol - Ethanol - Water (4 : 1 : 5, Upper layer).

(E) Butan-1-ol - Acetic Acid - Water (4 : 1 : 5, Upper layer).

(F) Benzene - Ethanol - Water (11 : 3 : 1, Upper layer).

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

(H) Butan-1-ol - Ethanol - Water (1 : 1 : 1, Upper layer).

(I) Butan-2-one - Acetic Acid - Water (9 : 1 : 1, saturated with boric acid).

The chromatograms, after development for the required length of time, were air dried and the sugars located by using the following spray reagents.

I. Aniline oxalate.

Unless otherwise stated, reducing sugars were located by spraying with saturated aqueous solution of aniline oxalate and heating at 140-160° for about 5 minutes. The colours of the sugar spots quoted refer to that of this spray.

II. Silver nitrate (48).

Polyhydroxy compounds in general, whether reducing or not, were detected by dipping the chromatogram in the silver nitrate reagent (1 ml. of saturated aqueous silver nitrate solution added to 20 ml. of acetone), drying and then dipping in aqueous ethanolic sodium hydroxide (0·5N). These chromatograms were preserved by treating with an aqueous solution of sodium thiosulphate (10%), followed by washing in flowing water for half an hour and drying.

With this reagent the sugars show up as grey to black spots, the reducing sugars appear immediately, the more inert compounds take longer.

III. Hydroxylamine-ferric chloride (49).

Esters and lactones were detected by spraying the

chromatogram with a methanolic solution of hydroxylamine (made by mixing equal volumes of N-hydroxylamine hydrochloride in methanol and N-potassium hydroxide in methanol and filtering), followed after 10 minutes by a ferric chloride solution (1·6%) in hydrochloric acid (1%). Lactones and esters show up as mauve spots on a pale yellow background.

IV. Periodate-benzidine (50).

Glycitols were detected by spraying the chromatograms with an aqueous solution of sodium periodate (0·2%), air drying for 5 minutes and dipping in an ethanollic solution of benzidine (0·25 g. in 80 ml. of ethanol, and 20 ml. of acetic acid). Any sugar which reacts with periodate shows up as a white spot on a blue background, the blue colour fades after a few days.

V. Periodate-acetylacetone.

Compounds that will yield formaldehyde with periodate were detected on chromatogram by spraying with a sodium periodate solution (0·03M, buffered with acetate to pH 3·4). After 3 minutes the chromatogram is sprayed with potassium iodide solution (1%) followed by sodium thiosulphate (1%) to destroy periodate. Finally on spraying with a solution an acetylacetone (1 ml.) and ammonium acetate (15 g.) in methanol (50 ml.) compounds yielding formaldehyde give yellow spots, which appear more intense under ultraviolet light.

Unless otherwise stated, the R_G values of methylated sugars refer to the rate of movement relative to 2,3,4,6-tetra-O-methyl-D-glucose on No.1 paper in solvent system D.

Paper electrophoresis (51) was carried out in borate buffer (pH 10) at 500 volts for 5-6 hr., saturated aqueous aniline oxalate with little glacial acetic acid was used for spraying the dried ionophoretograms.

Gas-liquid partition chromatography (55).

This was carried out on a 'Pye Argon Chromatograph' using argon as the mobile gas phase. The stationary liquid phase was supported on Celite and consisted of

a) Apiezon M

b) Butanediolsuccinate polyester

c) Polyphenol.

Operating temperatures used were 150°C for a), 175°C for b), and 200°C for c).

The $\underline{T}$ (retention times) values of the methyl ether methyl glycoside is relative to that of methyl-2,3,4,6-tetra-$\underline{O}$-methyl-β-$\underline{D}$-glucopyranoside.

Optical rotations were observed for water solutions (unless otherwise stated) at ca.20° using sodium D-line as light source.

Quantitative separation of sugars.

Thick paper - Whatman 3MM and 17MM papers were used. 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 cases of neutral and acidic unmethylated and mono-methylated sugars. The higher methylated sugars were extracted in a soxhlet with hot methanol.

Cellulose columns - were packed dry and washed with water,

followed by the solvent to be used. The solvents were purified as below.

Butan-1-ol was refluxed for 1-2 hours with potassium hydroxide (1% w/v) and distilled.

Light petroleum was shaken overnight with concentrated sulphuric acid (10% v/v) washed free of acid with distilled water and distilled.

Pyridine was refluxed with potassium hydroxide (1% w/v) and distilled.

The sugar mixture was placed on the column either by freeze drying it on to cellulose powder or by dissolving in a small quantity of butanol in the case of barium salt free methylated sugars.

The sugars were then eluted from the column using the given eluants and the eluate was collected on an automatic turntable in fractions of a suitable volume. A small sample (ca. 5-8 ml.) from every fifth tube was taken 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 or by extracting with acetone and filtering in the case of acetone soluble sugars. The final filtrate was taken to dryness, dried over phosphorus pentoxide and weighed.

In the case of sugars which have been eluted with butan-1-ol containing solvents, evaporation gave a high boiling oil. Purification of this was effected by distribution between water

and benzene (or light petroleum), but considerable amounts of sugar are lost in this process. Another way of purification is by placing the mixture on thick paper and eluting with benzene. The movement of the oil can be seen as it colours the paper yellow. When the oil has run off, the paper is thoroughly dried and extracted with cold water.

Charcoal-Celite columns were used to separate oligosaccharides from monosaccharides and from one another (88). Celite was washed with concentrated hydrochloric acid: water (1 : 1) allowed to stand overnight, filtered, washed till free of chloride with tap water and finally washed with distilled water. Charcoal was washed thrice with boiling distilled water. The mixture (1 : 1) was packed as a slurry into columns and washed thoroughly with water.

The mixture of sugars in small quantity of water was allowed to soak into the column and the monosaccharides were eluted with water. The oligosaccharides were then separated by stepwise elution with aqueous ethanol. The eluate (ca. 2 l.) was evaporated to a small volume treated with Amberlite resin IR 4B(OH), taken to dryness and chromatographically examined.

DEAE-cellulose columns used for fractionation of polysaccharides (26). Diethyl aminoethyl cellulose (30 g.) was washed alternately with 0·5 $\underline{N}$ HCl and 0·5 $\underline{N}$ NaOH (500 ml. for each washing). The cellulose was stirred with the acid (or base) for 5-10 min., centrifuged and the turbid solution decanted off. The cellulose was then washed twice with distilled water. A

perforated disc was placed at the bottom of the column (2 cm. diameter), then a layer of glass wool, followed by a 1 cm. layer of silver sand and finally 1 cm. layer of acid washed Celite. The cellulose was poured in as a water slurry and slight air pressure applied at the top. The solvent level was always kept above cellulose to avoid air bubbles. A layer of glass wool was placed at the top as well. The initial generation and subsequent re-generations of the cellulose in the phosphate form was done by eluting the column with 2 litres of 0·5 M sodium dihydrogen phosphate solution (pH-6 adjusted by adding NaOH) followed by equilibration with 1 litre of 0·005 M sodium dihydrogen phosphate solution of the same pH. The rate of flow of the column during this operation was adjusted to ca. 40 ml./hr.

Methoxyl determinations were carried out by means of the semi-micro Zeisel method (59). A 10% solution of sodium antimony tartrate (1 ml.) was used as scrubber. In almost all cases the methylated compound dissolved in hydriodic acid and therefore no other solvent was required. In the case of methylated polysaccharides heating was carried on for 1½-2 hours.

Demethylations (69) were carried out by heating the sugar (ca. 5 mg.) with hydriodic acid (1 ml.) in a sealed tube on a boiling water bath for 8 min. The contents were immediately diluted with water (ca. 10 ml.) silver carbonate added until neutral, a small quantity of charcoal stirred in and filtered. The last trace of silver was removed by hydrogen sulphide and filtration through charcoal bed. The filtrate evaporated to

dryness and was examined chromatographically in solvent A.

Small scale periodate oxidation of methylated sugars was carried out by the method of Lemieux and Bauer (70). The sugar (1-2 mg.) was dissolved in 0·5N sodium metaperiodate solution (0·2 ml.)and kept at 0° for 1 hour. The excess of periodate was destroyed by the addition of ethylene glycol (1 drop) and the solution, after 5 min., was made alkaline to phenolphthalein with 0·5N sodium hydroxide solution. The solution was taken to dryness, extracted with acetone, taken to a syrup and examined chromatographically in solvent D. Results were obtained as follows for some standard sugars:-

Sugar	Oxidation Products	
	R_G	Colour
2-methyl galactose	0·24	yellow
2,4-dimethyl galactose (unaffected)	0·50	pink/brown
2,3-dimethyl galactose	0·82,0·93 and 1·05	grey, brown and grey
2,6-dimethyl galactose	0·24	yellow
3,4-dimethyl galactose	0·93	pink.

Aniline derivatives of methylated sugars were prepared by refluxing the sugar in ethanolic redistilled aniline (equimolar quantities) for 30 min., in the dark. In most cases the syrup crystallised on removal of the solvent in vacuum desiccator and was recrystallised from ethyl acetate.

Lactones of aldonic acids of methylated sugars were prepared by oxidation with bromine water. The sugar (100 mg.) was

dissolved in water (2-3 ml.) and bromine (1 ml.) was added and the mixture was kept in the dark at room temperature for 2 days. Excess bromine was removed by aeration and the solution evaporated to dryness. The residue was extracted with ether and the lactone was recrystallised from the given solvent.

Aldonamides. The lactone prepared as above was dried in a vacuum desiccator, dissolved in dry methanolic ammonia (prepared by bubbling ammonia gas into dry ice cooled methanol, until saturation) and left in the ice box for 2 days. Evaporation of the solvent gave the crystalline amide which was recrystallised from the given solvent.

Phenylosazones. Sugar (10 mg.) was heated for 30 min. on a boiling water-bath with 0·01 ml. each of phenylhydrazine (redistilled) and glacial acetic acid, water (0·25 ml.) and a drop of saturated sodium bisulphite solution. On cooling and adding water (ca. 1.5 ml.) the phenylosazone was precipitated and was recrystallised from boiling water.

Potassium borohydride reductions of sugar to sugar alcohols (65) 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 about 30 min. The filtrate was taken to dryness and the borate ions were removed by repeated evaporation with methanol.

Lithium aluminium hydride reductions (66) were carried out

in dry tetrahydrofuran. Tetrahydrofuran which had been standing over sodium wire was filtered and distilled off lithium aluminium hydride.

The acidic material was converted to the methyl ester methyl glycoside and was dissolved in the solvent. Lithium aluminium hydride was added and the mixture refluxed, with exclusion of moisture, on a boiling water bath for 1 hour, more lithium aluminium hydride was added and refluxing continued for a further hour. Excess hydride was destroyed by addition of ethyl acetate and water. The hydroxide precipitates were filtered and the residue extracted repeatedly with chloroform and acetone. The combined extracts and the filtrate were taken to dryness, extracted with dry chloroform and again concentrated to a syrup.

Diborane reduction (82). The diborane was prepared by the dropwise addition of boron trifluoride etherate (4·0 g.) in diglyme (10 ml.) to a stirred solution of lithium borohydride (0·5 g.) in diglyme (10 ml.) in an atmosphere of nitrogen, and by bubbling the evolved gas into tetrahydrofuran (40 ml.) over a period of 1-1½ hr. The diborane solution was then added to the sample dissolved in tetrahydrofuran and allowed to stand for a few hours. The excess diborane was destroyed by addition of ethanol, solvent removed under reduced pressure and the product treated with portions of methanol to remove boric acid and evaporated to dryness.

Alkaline degradation. Small scale alkaline degradation was carried out by dissolving the sugar (10 mg.) in saturated lime water (2 ml.), bubbling nitrogen to expel oxygen and allowing the

solution to stand in a stoppered flask at 25° for the required length of time (2-4 weeks). At the end of this time, the solution was treated with Amberlite resin IR-120 (H), evaporated to dryness and examined chromatographically in acidic solvent systems such as B. Any saccharinolactone formed could be detected by spray reagent III.

Purification of methyl iodide for use in Purdie methylation was done by refluxing with silver oxide and distilling in a dry system.

Preparation and drying of silver oxide used in Purdie methylation. The silver oxide which was prepared by addition of sodium hydroxide (40 g. in 500 ml.) to a solution of silver nitrate (170 g. in 500 ml.), was washed with cold water (4 l.), and hot water (1 l.). After filtering with suction and draining the water completely, the silver oxide was ground with acetone (1 l.), filtered and finally washed with ether to dry. The silver oxide was further dried in vacuum desiccator and stored in a dark bottle.

Small scale methylations. The sugar (2-4 mg.) was weighed out into a small conical flask and dissolved in water (1 ml.). Methyl sulphate (0·3 ml.) and sodium hydroxide (30%, 0·3 ml.) was added slowly over a period of one hour with magnetic stirring. Further quantities of methyl sulphate (0·3 ml.) and sodium hydroxide (0·6 ml.) were added and stirring continued overnight. The reaction flask was kept immersed in ice water to prevent excessive heating due to stirring. Two more additions of the

reagents were made on two successive days. The reaction mixture was then heated on a boiling water bath for 15 min. and extracted with chloroform in a liquid-liquid extractor for 10 hr. The chloroform extract was dried over anhydrous sodium sulphate and taken to dryness. The product was then dissolved in methyl iodide (5 ml.) and refluxed for six hours with additions of silver oxide (ca. 0·5 g. in all). The silver oxide was filtered and extracted with hot chloroform in a solid-liquid extractor. The combined filtrate and chloroform extract were taken to dryness, dried in a vacuum desiccator and methanolised. The products were examined by gas-liquid partition chromatography.

Methanolysis, was carried out by heating the sugars with dry methanollichydrochloride (2%) in a sealed tube for 12-15 hr. on a boiling water bath. The solution was neutralised with silver carbonate, filtered, silver carbonate washed with acetone and the combined filtrate and washing were taken to dryness under vacuum.

Anthrone method for estimation of sugars (46). The reagent was made by dissolving anthrone (20 mg.) in 100 ml. of AnalaR sulphuric acid (70% v/v). It was aged at least for 2 hours before use and discarded after 24 hours. The reagent was stored in an ice box when not in use.

Various volumes (0·05 to 1·00 ml.) of the polysaccharide solution (containing about 100 µg./1 ml.) were pipetted into Quickfit tubes and diluted to 1 ml. with distilled water. The tubes were cooled in a mixture of ice and salt and anthrone reagent (10 ml.) was added to each tube from a burette. The

tubes were immediately stoppered, shaken, heated on a boiling water-bath for 7 minutes and cooled in the dark for half an hour. The intensity of the green colour produced was measured using an Unicam spectrophotometer (at 620 mμ) against a blank prepared with water (1 ml.).

The standard curve was obtained by plotting μg. polysaccharide/ml. against the optical intensity (ϵ).

The polysaccharide content of the fractions from DEAE-cellulose columns was then estimated by treating 1 ml. (or 0·1 ml. diluted to 1 ml. if sugar concentration is high)of the fraction as above and measuring the optical intensity .

Phenol-sulphuric acid method for estimation of sugars (44). The reagent was made by dissolving AnalaR phenol (5 g.) in 100 ml. of copper free distilled water.

Various volumes (0·05-1·00 ml.) of the polysaccharide solution was pipetted into test-tubes (15 x 2 cm.) and diluted to 1 ml. with water. Phenol solution (1 ml.) was added to each, followed by concentrated H_2SO_4 (5 ml., AnalaR), which was added from a pipette, the stream of the acid being directed on to the solution surface so as to let the temperature rise to the maximum and to facilitate mixing. The tubes were allowed to stand for 10 minutes, shaken and placed for 10-20 minutes in a water-bath at 25-30°C. The optical intensity was now measured with a Unicam at 485 mμ against a blank prepared with water (1 ml.). The standard curve was obtained as in the case of the Anthrone method.

Uronic acid anhydride determinations.

(a) Carbazole method (67). The solution (2 ml.) was pipetted into Quickfit tubes and cooled in freezing mixture. Concentrated sulphuric acid (12 ml.) which was previously cooled to 3°C was added from a burette. The tube was immediately stoppered, contents mixed and cooled to about 5°C. The tube was then placed in a boiling water bath for 10 minutes, cooled to 20°C, 1 ml. of 0·15% carbazole reagent [prepared by dissolving 0·15 g. of reagent grade carbazole in 100 ml. of spectroscopic ethanol] added, shaken and allowed to stand at room temperature for 25 ± 5 minutes.

The optical intensity of the pinkish mauve colour developed was immediately measured on a spectrophotometer at 520 mμ, against a blank prepared with water (2 ml.). The uronic acid anhydride content was then calculated by referring to the standard curve made with galacturonic acid.

(b) Decarboxylation method (95). More accurate, but required more material.

Purification of Gum.

The crude gum (30 g.) was crushed to small pieces and was dissolved in water (600 ml.) with mechanical stirring. The gum dissolved with comparative ease and only mechanical impurities (wood particles) were left undissolved. The solution was filtered through muslin cloth and to the filtrate was poured slowly with stirring concentrated hydrochloric acid (18 ml.) followed by ethanol (2·5 litres). The precipitate was allowed to settle and the supernatant liquid decanted off. The gum acid was then washed repeatedly with ethanol till the washings were free of chloride ions. The purified gum acid was obtained by centrifugation and drying first in a vacuum oven at 40°-50° for 12 hours and then in a vacuum desiccator over phospherous pentoxide for two days, as a white powder. Yield - 23 g. (ca.77%).

$[\alpha]_D$ -15·9° (c, 3·98 N/10 sodium hydroxide).

Uronic acid anhydride 15·2% (by decarboxylation method).

A second lot of gum (40 g.) was purified in exactly the same way. Yield - 32 g. (80%).

$[\alpha]_D$ -9·8° (c, 1·01 N/10 sodium hydroxide).

Uronic acid anhydride 13·9% (by decarboxylation method).

Autohydrolysis of purified gum acid.

Trial experiment.

A solution of the gum acid (2 g. in 50 ml. water) was heated on a boiling water bath for 96 hours. Samples (10 ml.) were withdrawn at various times and optical rotation was measured.

Time in hours	0	4	12	24	48	96
$[\alpha]_D$	-10·5°	+10·3°	+30·5°	+43·0°	+49·0°	+48·5°

After measuring the rotation the samples were treated with Amberlite resin IR 4B (OH), concentrated and poured into ethanol (4 volumes) to precipitate the polysaccharide present. The precipitated polysaccharide was coagulated by addition of alcoholic potassium iodide, centrifuged and washed with ethanol. The filtrates and ethanol washings were concentrated and examined chromatographically in solvents A and B. Table below shows the sugars present and a rough idea of the amounts (visual estimation).

Time in hr.	Galactose	Arabinose	Rhamnose	Arabinobiose (later identified as 1,3-β-)	
4	1	8	1	tr.	Other
12	1	6	1	1	disac-
24	2	6	1	1	charides
48	3	6	1	2	were also
96	4	8	1	1	present.

An attempt to separate the mono and disaccharides by using a charcoal column did not give any additional information.

The degraded polysaccharides obtained after various times were hydrolysed in a sealed tube with N-sulphuric acid for four hours, worked up in the usual way and examined chromatographically in solvents A and B. [Iodine was interfering with the chromatography as a result of the addition of potassium iodide]. The results are tabulated below.

Time in hr.	Galactose	Arabinose	Rhamnose	1,2-GalA-Rha.
4	6	4	1	1
12	6	4	1	1
24	6	2	1	2
48	6	1	1	2
96	10	1	1	2

Large scale autohydrolysis.

A solution of gum acid (20 g. in 200 ml.) was heated on a boiling water bath for 36 hours till rotation became constant [+43·0°].

The solution was neutralised with saturated barium hydroxide solution followed by addition of solid barium carbonate, filtered and poured into ethanol (4 volumes). The precipitated polysaccharide (degraded gum I) was centrifuged, washed twice with ethanol, dissolved in water (200 ml.) and re-precipitated with

ethanol and finally dried (10.5 g.), $[\alpha]_D$ +51·3 (c, 1·13). Uronic acid anhydride 25.7% [Average of three determinations by the decarboxylation method].

The filtrate and ethanol washings were combined, concentrated (200 ml.), treated with Amberlite resin IR 120 (H) to remove barium ions and then with Amberlite resin IR 45 (OH) to remove acidic sugars. Concentration yielded a syrup (9·2 g.) which was found to be a mixture of mono and oligosaccharides by chromatographic examination.

The IR 45 resin column was eluted with 1% formic acid, the elute concentrated and examined chromatographically in solvent B and C showed galactose, arabinose, galactotriose and two unidentified acidic oligosaccharides.

Partial hydrolysis of degraded gum I.

The degraded gum was hydrolysed with N/2-sulphuric acid for 2 hr. at 100°. The product on chromatographic examination in solvent A was found to contain galactose (main), arabinose, rhamnose (traces) and 1,6-galactobiose, no 1,3-galactosylarabinose could be detected. The rest of the syrup was placed on a small charcoal column and eluted first with water (300 ml.) followed by ethanol (15%, 500 ml.). The eluates were taken to dryness and examined in different solvent systems. The water eluate contained galactose, arabinose, rhamnose and 1,2-galacturonosylrhamnose. The ethanol eluate contained 1,6-galactotriose, 1,6-galactobiose, 1,2-galacturonosylrhamnose and traces of 1,3-galactosylarabinose, galactose and arabinose. The rest of the

degraded gum I was subjected to methylation studies. See page(122).

Separation and examination of the sugars obtained by auto-hydrolysis.

The syrup (9·2 g.) was placed on a charcoal-Celite column (30 x 5·5 cm.) and eluted first with water, and later with gradually increasing concentrations of ethanol. Fractions of about 2000 ml. were collected, concentrated to dryness and examined chromatographically in solvents A and B. (Table 1).

Sixteen fractions were collected in all, but satisfactory separation was not obtained. Fraction A (7·9 g.) which in addition to monosaccharides contained some oligosaccharides was reseparated on the same column. The column was first eluted with water and then with 20% ethanol. Fractions of about 500 ml. were concentrated and examined. (Table 2).

Table 1 - (Initial separation).

Fraction	Eluant	Wt.in g.	Contents
A	water	7·9644	Monosaccharides and small amounts of disaccharides.
B	water	0·1610	Monosaccharides, galactobiose, traces of galactosyl arabinose and arabino-biose.
C	water	0·1799	Mixture of various mono and oligo-saccharides.
D	water	0·1706	-do-
E	water	0·0740	-do-
F	5% ethanol	0·1433	1,6-Galactotriose.
G	20% ethanol	0·6531	Mixture of various sugars.

Table 2 - (Reseparation of fraction A).

Fraction	Eluant	Wt.in g.	Contents
a	water	6·6438	Monosaccharides.
b	water	0·0714	Arabinobiose (Main), with traces of other sugars.
c	water	0·0684	Mixture of disaccharides.
d	20% ethanol	0·2722	-do-

Fractions B, C, D, E, c and d were combined (0·956 g.) and separated on a cellulose column (44 x 2 cm.) using butanol half saturated with water as eluant. Fractions (20 ml.) were collected every half hour and examined in the usual manner.

Fraction	Tubes	Wt. in mg.	Contents
i	1-38	310	Arabinose and rhamnose
ii	39-100	76	Galactose (main), arabinose and two disaccharides
iii	101-125	25	1,3-arabinobiose and traces of other sugars
iv	126-270	67	Mixture of disaccharides
v	271-470	16	1,4-galactobiose and traces of other sugars
vi	water	288	Mostly 1,6-galactobiose.

Examination of the fractions.

Fraction ii.

This fraction (76 mg.) consisted of galactose (main), arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose and a sugar having R_{Gal} 1·20 in solvent A and giving a pink stain with aniline oxalate. After two fractionations on thick paper using solvents B and A, a syrup (11 mg.) having $[\alpha]_D$ +28·0° (c, 0.36) was obtained from the mixture. This syrup was mainly the unidentified sugar (R_{Gal} 1·20) with a trace of galactose. Total hydrolysis of a sample gave almost entirely arabinose with only a trace of galactose. Another sample after reduction with potassium borohydride and hydrolysis gave arabitol, galactitol and arabinose.

The rest of the syrup (3 mg.) was methylated, methanolysed and the mixture of glycosides obtained, examined by vapour phase chromatography in systems a and b. The following methyl glycosides were detected:-

Methyl-2,3,5-tri-O-methyl-L-arabinoside (T 0·55 and 0·72-0·73 in system b)

Methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1·04 in system b)

Methyl-2,3-di-O-methyl-L-arabinoside (T 1·56 and 1·95 in system b)

Methyl-2,3,4,6-tetra-O-methyl-D-galactoside (T 1·80 in system b and 1·25 and 1·37 in system a)

Methyl-2,3,5,6-tetra-O-methyl-D-galactofuranoside (T 1·12 in system a, a very small peak).

Fraction iii was combined with fraction b and purified on thick paper using solvent B. The pure partially crystalline sugar (50 mg.), had R_{Gal} 0·82, 0·68 in solvents A and B respectively and $[\alpha]_D$ +164·0° (c, 1·0).

The disaccharide gave a phenyl osazone, which after two recrystallisations from water had m.p. and mixed m.p. (with authentic sample 230-232°) 225-228°, and $[\alpha]_D$ +42·0° (c, 0·07 acetone). The X-ray powder diagram of the phenylosazone was identical to that of the authentic sample. Periodate oxidation of the phenylosazone gave 1·12 and 1·45 molecules of formaldehyde after 30 and 300 minutes respectively, but no mesoxaldialdehyde-1,2-bisphenylhydrazone was formed. This result is consistant with that of Hough et al. (86), reported for a 1,3-linked arabinose disaccharide. From this evidence the disaccharide appears to be 3-O-β-L-arabinopyranosyl-L-arabinose.

Attempts to recrystallise the partially crystalline sugar from aqueous ethanol were unsuccessful.

The syrup (20 mg.) was methylated by four additions of methyl sulphate and 30% sodium hydroxide. The methylation was completed by a treatment with methyl iodide and silver oxide. A sample on methanolysis and vapour phase chromatographic examination showed methyl-2,3,4-tri-O-methylarabinoside (T 1·04 in system b), methyl-2,4-di-O-methylarabinoside (T 2·26 and 2·37 in system b) and only a trace of methyl-2,5-di-O-methylarabinoside (T 0·41-0·42 in system a), indicating that the methylation was complete. The rest of the methylated disaccharide was

hydrolysed with N-hydrochloric acid at 100° for 4 hr. and the products (19 mg.) fractionated on thick paper using solvent D.

Subfractions. The syrup (6·7 mg.) was chromatographically pure and identical to 2,4-di-O-methyl-L-arabinose. The aniline derivative was prepared which after recrystallisation from ethyl acetate had m.p. and mixed m.p. (with authentic sample 129-130°) 120-122°.

Subfraction 2. This fraction (8 mg.) was chromatographically pure and identical to 2,3,4-tri-O-methyl-L-arabinose. The amide of aldonic acid of the methylated sugar was prepared, but failed to crystallise.

Fraction iv.

The syrup (67 mg.), $[\alpha]_D$ +67·7° (c, 0·74) was fractionated on thick paper using solvent A.

Subfraction (iv)a.

This fraction was a mixture of two sugars having the same chromatographic mobilities as 3-O-β-galactosylarabinose and 3-O-β-arabinopyranosylarabinose. The sugar having the same speed as 3-O-β-galactosylarabinose was obtained pure (9 mg.) by separation on thick paper using solvent B, $[\alpha]_D$ +52·7° (c, 0·46), R_{Gal} 0·69 (solvent A). A sample (1·5 mg.) on hydrolysis with N-sulphuric acid for 2 hr. at 100° gave galactose and arabinose only. Another sample (1·5 mg.) after reduction with potassium borohydride and hydrolysis gave mainly arabinose and galactitol with traces of galactose and arabitol. A third sample (3 mg.) was methylated, methanolysed and the

products examined by vapour phase chromatography.

The main component (T 0·90 in system b and 0·85 in system c) was unidentified, other components which were present are methyl-2,3,5-tri-O-methyl-L-arabinoside (T 0·55 and 0·72 in system b), methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1·04 in system b), methyl-2,3,4,6-tetra-O-methyl-D-galactoside (T 1·80 in system b and 1·54, 1·62 in system c), methyl-2,4-di-O-methyl-L-arabinoside (T 2·34 in system b and 1·15 in system c) and methyl-2,3,6-tri-O-methyl-D-galactoside (T 3·30, 4·30 and 4·74 in system b).

Subfraction (iv)b.

This fraction gave the same chromatographic pattern as the original mixture.

Subfraction (iv)c.

This fraction (6 mg.) was a mixture of 3-O-β-L-arabinopyranosyl-L-arabinose and an unknown which had R_{Gal} 0·93 in solvent A and 0·53 (same as 3-O-β-galactosylarabinose) in solvent B.

On hydrolysis with N-sulphuric acid for 3 hr. it gave arabinose, galactose and a trace of xylose. On potassium borohydride reduction, followed by hydrolysis with N-sulphuric acid for 3 hr., it gave a product in which galactose and arabinose were still present; the glycitols in this mixture could not be identified due to heavy streaking. The rest of this fraction was methylated, methanolysed and the products examined by vapour phase chromatography. The pattern obtained was complicated, the following glycosides were the major components.

Methyl-2,3,5-tri-O-methyl-L-arabinoside (T 0·55 and 0·72 in system b), methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1·04 in system b), methyl-2,3,4-tri-O-methyl-D-xyloside (T 0·46 and 0·55 in solvent b), methyl-2,3,4,6-tetra-O-methyl-D-galactoside (T 1·53 and 1·62 in system c), methyl-2,3,4-tri-O-methyl-D-galactoside (T 2·64 and 29·2 in system c) and methyl-2,5-di-O-methyl-L-arabofuranoside (T 0·71 and 1·03 in system c).

Fraction v.

Purification of this fraction on thick paper gave a chromatographically pure sugar (5·7 mg.), $[\alpha]_D$ +93·0 +80·7° (c, 0·57), having the same chromatographic mobility as 4-O-β-D-galactopyranosyl-D-galactose in solvents A and B. However this sugar gave a pinkish brown stain with aniline oxalate, whereas the authentic sugar gives a brown stain.

Fraction vi.

This fraction (288 mg.) was chromatographically a bit streaky, but had the same mobility as 6-O-β-D-galactosyl-D-galactose in different solvent systems.

A sample (4 mg.) was methylated, methanolysed and the products examined by vapour phase chromatography. The major peaks were those of methyl-2,3,4,6-tetra-O-methyl-D-galactoside (T 1·80 in system b) and methyl-2,3,4-tri-O-methyl-D-galactoside (T 4·50 and 7·45 in system b), however minor peaks corresponding to methyl-2,3,5,6-tetra-O-methyl-D-galactofuranoside (T 1·41 and 2·04 in system b), methyl-2,3,6-tri-O-methyl-D-galactoside (T 3·26, 4·20 and 4·72 in system b) and methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1·07 in system b) were also present.

Partial hydrolysis I. Neutral Oligosaccharides.

Trial experiment.

The polysaccharide was dissolved in water and filtered through muslin. The solution was heated on a boiling water bath and the appropriate quantity of 4N-sulphuric acid was added to make the strength of the resulting solution 0·5N. Refluxing was continued and samples (equal volume) were removed after 0·5, 1, 2, 3 and 4 hours. Neutralisation and subsequent operations were carried out with care in order to minimize losses of the hydrolysate, which after concentration was spotted as one spot on Whatman 3MM paper and examined in solvent A. Visual inspection of the resulting chromatograms suggested that the best yield of neutral oligosaccharides could be obtained by hydrolysing the gum with 0·5N-sulphuric acid for 2 hours at 100°.

Large scale hydrolysis.

Accordingly, the gum (30 g.) was hydrolysed with 0·5N-sulphuric acid (450 ml.) for 2 hours at 100°. After cooling, neutralisation was effected, by first adjusting the pH to about 5 by addition of saturated solution of barium hydroxide and subsequently adding solid barium carbonate. The resulting solution was centrifuged, filtered through Celite, concentrated to about 200 ml. and poured into ethanol (800 ml.) to precipitate the unhydrolysed gum (degraded gum II). This precipitate was separated by centrifugation, washed twice with ethanol and dried (10.0 g.). The supernatant was concentrated to a syrup (16·5 g.).

Separation of the neutral oligosaccharides.

The above syrup (16.5 g.) was dissolved in water and placed on top of a charcoal-Celite column (50 x 7 cm.). The column was first eluted with water, till most of the monosaccharides had come off, and later with gradually increasing concentrations of aqueous ethanol. Fractions (ca. 2000 ml.) collected every 24 hours were evaporated to a small volume, treated with Amberlite resin IR 4B (OH), evaporated to dryness and examined chromatographically in solvents A, B and H. The latter solvent is suitable for the higher oligosaccharides which are very slow in solvent A. These fractions (36 in all) were combined according to their contents and the following eight final fractions shown in table 3 were obtained.

Fraction	Eluant	Wt. in g.	Contents
A	Water	11·569	Galactose, arabinose, rhamnose and traces of disaccharides
B	2·5% ethanol	0·500	1,6-galactobiose, 1,3-galactosylarabinose and traces of mono and trisaccharides
C	2·5% ethanol	0·130	1,6-galactotriose, 1,6-galactobiose and 1,3-galactosylarabinose
D	5% ethanol	0·080	1,6-galactotriose, 1,6-galactobiose and 1,4-galactobiose
E	5% ethanol	0·332	1,6-galactotriose (main) and 1,6-galactotetraose
F	10% ethanol	0·512	1,6-galactopentaose, 1,6-galactotetraose and 1,6-galactotriose
G	15% ethanol	0·116	Mixture of sugars, chromatograms not clear due to streaking
H	20% ethanol	0·050	Galactose, arabinose and other sugars.

Table 3.

Examination of the fractions and identification of the neutral oligosaccharides.

Fraction A. 11·569 g.

Since this fraction contained mainly monosaccharides, which had already been identified in the preliminary investigation (98), it was not examined further.

Fraction B. 0·500 g.

Chromatography in solvents A and B indicated this fraction

to be a mixture of sugars, the main component of which was 6-O-β-galactosylgalactose. The syrup was separated into four fractions on a cellulose column (45 x 2 cm.) using solvent A. The fractions bulked appropriately, evaporated to small volume, treated with charcoal and taken to dryness.

Subfraction B (i). 0·055 g.

This fraction was chromatographically found to contain 3-O-galactosylarabinose mixed with galactose and arabinose. It was fractionated on 3MM paper using solvent A and the pure 3-O-galactosylarabinose obtained was combined with the next fraction for further examination.

Subfraction B (ii). 0·080 g.

This fraction was mainly 3-O-galactosylarabinose with some 6-O-galactosylgalactose. It was separated on 3MM paper and combined accordingly.

Identification of 3-O-D-galactosyl-L-arabinose.

The syrupy sugar (87 mg.) had R_{Gal} 0·59 and 0·53 in solvent systems A and B respectively, $[\alpha]_D$ +22·4° (c, 1·25).

Total hydrolysis of a sample (5 mg.) with N-sulphuric acid for 4 hours and chromatographic examination of product in solvents A and B showed galactose and arabinose only.

The syrup crystallised after two months and was recrystallised from aqueous ethanol. m.p. and mixed m.p. 175-177° (with authentic sample of 3-O-β-D-galactosyl-L-arabinose melting at 202-203°). X-Ray powder photograph of the crystals was identical with that of the authentic sample.

Subfraction B (iii).

This fraction, 0·289 g., R_{Gal} 0·23 in solvent A, $[\alpha]_D$ +30·0° (c, 0·15), was chromatographically pure and identical to 6-O-galactosylgalactose in solvents A and B.

When a sample (5 mg.) was totally hydrolysed with N-sulphuric acid at 100° for 6 hours and examined chromatographically in solvent A, only galactose was detected.

All attempts to crystallise the sugar were unsuccessful. When methanol was tried as a solvent some crystals appeared but these lost their crystallinity on taking out of the solvent.

The phenylosazone of the sugar was prepared and recrystallised from water. m.p. and mixed m.p. 179-181°. X-Ray powder photograph of the osazone was identical with that of an authentic sample.

Two attempts to prepare the phenylosotriazole (96) from the phenylosazone were unsuccessful.

The syrup (100 mg.) was dissolved in water (5 ml.) and methylated by three additions of the reagents methylsulphate (12 ml.) and 30% sodium hydroxide solution (16 ml.) with the usual precautions. Extraction with boiling chloroform gave the methylated sugar (60 mg.). A sample (3 mg.) on hydrolysis with N-sulphuric acid for 4 hours at 100°, gave in addition to 2,3,4,6-tetra- and 2,3,4-tri-O-methylgalactoses small amounts of dimethyl galactoses due to incomplete methylation. Hence the syrup was dissolved in water and methylated by a further addition of the reagents. The fully methylated sugar was seeded with a

crystal of methyl hepta-O-methyl 6-O-β-D-galactosyl-D-galactoside and left in a desiccator over phosphorous pentoxide, crystals appeared after a long time. These crystals could not be separated from the adhering syrup for melting point determination.

The methylated disaccharide (42 mg.) was hydrolysed with N-hydrochloric acid for 4 hours at 100° and worked up in the usual manner. The product was fractionated into three fractions on thick paper using solvent D.

Fraction a. 3·2 mg. Chromatographically identical to 2,3-di-O-methyl-D-galactose in solvent systems D and F.

Fraction b. 12·0 mg. Chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose. It was identified by conversion to the aniline derivative, m.p. 165-166° and mixed m.p. 163-165° (with authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine).

Fraction c. 10·1 mg. Chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose. When heated with ethanolic aniline, afforded an aniline derivative, m.p. and mixed m.p. 189-190°, (with authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 198°).

Subfraction B (iv). 0·064 g.

This fraction was chromatographically pure and identical to 1,6-galactotriose and was hence combined with the same obtained from fraction E and examined further.

Fraction C. 0·130 g.

This fraction was found to be a mixture of 1,6-galactotriose,

1,6-galactobiose and 1,3-galactosylarabinose.

It was separated on 17MM paper using solvent system A into 1,6-galactotriose (30 mg.), 1,6-galactobiose (45 mg.) and 1,3-galactosylarabinose (12 mg.). These sugars were combined with the appropriate subfractions and examined further.

Fraction D. 0·080 g.

This fraction on paper chromatography separated into 1,6-galactobiose, 1,6-galactotriose and a third component having chromatographic mobility faster than 1,6-galactobiose and giving a brown stain fluorescent in u.v. light with spray reagent I. It was separated into three fractions on thick paper using solvent system A.

Subfraction D (i). 0·040 g.

This was chromatographically pure and identical to 1,6-galactotriose.

Subfraction D (ii). 0·013 g.

This fraction was found to be a mixture of 1,6-galactotriose and 1,6-galactobiose.

Subfraction D (iii).

This fraction (0·016 g.), $[\alpha]_D$ +35·5→ +27·6° (c, 0·82), had R_{Gal} 0·49 and 0·40 in solvent systems A and B respectively. It was chromatographically and ionophoretically different from 3-O-β-galactopyranosylgalactose and 4-O-α-galactopyranosylgalactose, but was identical to 4-O-β-galactopyranosylgalactose. Attempts to crystallise the syrup were unsuccessful.

The sugar (2 mg.) was dissolved in water (0·02 ml.) and the solution diluted to 0·2 ml. with glacial acetic acid. Lead tetra

acetate (10 mg.) in glacial acetic acid (0·8 ml.) was added and the mixture allowed to stand for two hours (97). Excess oxalic acid (10% in acetic acid) was added to destroy excess reagent and precipitate lead ions. The precipitate was filtered, the filtrate evaporated to dryness and the residue was taken up in a few drops of water and heated for 2 hours to effect hydrolysis. Chromatographic examination of the product in solvents A and B showed galactose, a trace of arabinose and a tetrose (slower than erythrose). Since no lyxose (which would arise from 3-O-D-galactosyl-D-galactose) was detected, the sugar is most probably 4-O-β-D-galactopyranosyl-D-galactose.

The syrup (4 mg.) was methylated, methanolysed and the products on examination by gas-liquid partition chromatography showed peaks corresponding to methyl-2,3,4,6-tetra-O-methyl-D-galactoside (T 1·80 in system b), methyl-2,3,5,6-tetra-O-methyl-D-galactofuranoside (T 1·44-1·45 in system b), methyl-2,3,6-tri-O-methyl-D-galactoside (T 3·28 and 4·76 in system b), a trace of methyl-2,3,4-tri-O-methyl-D-galactoside (T 7·46 in system b), methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1·04 in system b), and methyl-2,3,5-tri-O-methyl-L-arabinoside (T 0·55 and 0·74 in system b).

Fraction E.

This fraction (0·330 g.) had 1,6-galactotriose as its main component together with 1,6-galactotetraose and a trace of an unknown sugar.

It was fractionated on a cellulose column (45 x 2 cm.) using solvent A as eluant, into three fractions.

Subfraction E (i).

The sugar (0.011 g.) had a chromatographic mobility slightly slower than galactose and gave a pink stain with aniline oxalate spray. It was tentatively identified as 3-O-β-L-arabinopyranosyl-L-arabinose, larger quantities of which were obtained from the autohydrolysate of the gum acid.

Subfraction E (ii). 0.252 g.

R_{Gal} 0·68 in solvent H. $[\alpha]_D$ +12·6° [c, 1·5].

Chromatographic examination in solvents A, B and H showed this fraction to be pure and identical to O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose.

Total hydrolysis with N-sulphuric acid for four hours at 100°C gave only galactose. Partial hydrolysis with 0·5N-sulphuric acid for half an hour at 100° and chromatographic examination of the product showed galactose, 1,6-galactobiose and some unhydrolysed trisaccharide.

The syrup (150 mg.) was dissolved in water (5 ml.) and methylated by three additions of methyl sulphate (12 ml.) and 30% sodium hydroxide (18 ml.) on three successive days, the usual precautions being observed. The solution was heated on a water bath for one hour, cooled, and extracted with chloroform in the cold to give the methylated product (51 mg.). Continuous extraction in a Soxhlet with boiling chloroform gave another 52 mg.

A sample (3 mg.) of the product was hydrolysed with N-hydro chloric acid at 100° for three hours and examined chromatographically in solvent D. The main components were 2,3,4,6-

tetra-O-methylgalactose and 2,3,4-tri-O-methylgalactose, but considerable amounts of di and mono methyl galactoses were also present.

The partially methylated trisaccharide (100 mg.) was dissolved in water and treated to two more additions of the above reagents. Extraction with boiling chloroform in soxhlet gave the fully methylated product (102 mg.). Hydrolysis of a sample (3 mg.) and chromatographic examination showed only 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose.

The remainder of the methylated trisaccharide was hydrolysed with N-sulphuric acid for four hours at 100°, neutralised with Amberlite resin IR 4B (OH) and concentrated to dryness. The resulting syrup was separated on thick paper using solvent system D, into two fractions.

Fraction (a). 0·035 g., R_G 0·89.

Chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose. It was identified by conversion to the aniline derivative, which crystallised readily and was recrystallised twice from ethanol. m.p. and mixed m.p. 190-191° (with authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine melting at 195-196°).

Fraction (b). 0·059 g., R_G 0·73.

This was chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose. It was identified by conversion to the aniline derivative, which after recrystallisation from ethyl

acetate had m.p. 160-162° and mixed m.p. 165-166° (with authentic sample melting at 169°).

Subfraction E (iii) was chromatographically identical to 1,6-galactotetraose.

Fraction F. 0·512 g.

Chromatographic examination in solvents A and H showed this fraction to be a mixture of 1,6-galactopentaose, 1,6-galactotetraose and 1,6-galactotriose. These were chromatographically checked with the corresponding sugars which had been isolated from gum ghatti. Fractionation of the mixture on charcoal-Celite column (30 x 3 cm.), followed by separation on thick paper gave two pure fractions.

Subfraction F (i). 0·098 g. R_{Gal} 0·26 and R_F 0·11 in solvnnt H. $[\alpha]_D$ +11·1° (c, 2·7). Chromatographically indistinguishable from O-β-D-galactopyranosyl-[(1→6)-O-β-D-galactopyranosyl]$_2$-(1→6)-D-galactose. Total hydrolysis gave galactose only and partial hydrolysis yielded 1,6-galactobiose, a slight trace of sugar having R_{Gal} 0.63 in solvent B, and galactose.

Subfraction F (ii). 0·038 g. R_{Gal} 0·15 and R_F 0·04 in solvent H. $[\alpha]_D$ +7·1° (c, 1·12). Chromatographically indistinguishable from O-β-D-galactopyranosyl-[(1→6)-O-β-D-galactopyranosyl]$_3$-(1→6)-D-galactose. Total hydrolysis gave galactose only and partial hydrolysis yielded 1,6-galactobiose and galactose.

Partial Hydrolysis II - Acidic Oligosaccharides.

Trial Hydrolysis.

The gum acid (100 mg.) was dissolved in water (5 ml.), heated on a water bath and added 4N-sulphuric acid (15 ml.) to make the strength of the resulting solution normal. Refluxed on a boiling water bath and 1 ml. samples were removed after 2,3,4,6, 9 and 20 hours. These samples were neutralised with barium carbonate, deionised with Amberlite resin IR 120 (H), evaporated to dryness and spotted as one spot 3MM paper and examined in solvents B and C. Visual inspection of the resulting chromatograms suggested that the best yield of acidic oligosaccharides could be obtained by hydrolysing the gum acid with N-sulphuric acid for 9 hours at 100°.

In later trial hydrolysis experiments two-dimensional chromatographic development technique using solvents system A, followed by B, was used with a view to getting a better qualitative separation of the acidic and neutral oligosaccharides. But the results of these chromatograms were not very satisfactory; only traces of acidic oligosaccharides could be seen on final spraying. Hence the separation was effected by spotting the sugars on No.4 paper and first developing in solvent A for 4-5 days and when all the neutral sugars had run off the paper, it was dried and developed overnight in solvent system C. This method proved more useful.

Similar trial hydrolysis on the unhydrolysed portion of the gum (degraded gum II) obtained after initial hydrolysis (see

page 100) showed that the best yield of acidic oligosaccharides could be obtained by hydrolysing this degraded gum II with N-sulphuric acid for 3 hours at 100°C.

Large scale hydrolysis.

The degraded gum II (9·5 g.) was hydrolysed with N-sulphuric acid at 100° for 3 hours, after cooling neutralised with barium carbonate, deionised with Amberlite resin IR 120 (H) and taken to a syrup. (5·65 g.).

Gum acid (1 g.) was also hydrolysed with N-sulphuric acid at 100° for 9 hours and worked up as above. (0·80 g.).

Separation of the acidic oligosaccharides.

The combined syrup (6·45 g.) was placed on a column (60 x 2·5 cm.) of Amberlite resin CG 45 generated in the formate form. (Made by pouring 250 ml. of the resin into the column and washing thoroughly with distilled water). The column was first eluted with water till no more sugars (neutral) came off. The acidic sugars were now eluted with aqueous formic acid of increasing concentration (0·5%, 1%, 2%). The elution of the sugars was followed by collecting fractions of about 1 litre, concentrating to dryness and examining chromatographically in the solvents A, B and C. The acidic oligosaccharides which were very slow on chromatograms developed in solvent B, moved much faster and separated well from one another in solvent C.

Fractions	Eluant	Weight	Contents
1 to 6	water	2·9524	Monosaccharides
7 to 10	water	0·0770	Neutral oligosaccharides
11 to 17	0·5% HCOOH	1·9928	3 acidic oligosaccharides and traces of other unidentified sugars
18 and 19	1% HCOOH	0·5263	Traces of acidic oligosaccharides, contaminated with inorganic matter
20	2% HCOOH	0·0960	Mostly resin

The mixture of acidic oligosaccharides (Fr. 11 to 17; 1·9928 g.) was separated on 17MM paper using solvent system C into five subfractions.

Subfraction I. 2-O-α-D-galacturonosyl-L-rhamnose.

This sugar (0·635 g.), $[\alpha]_D$ +66·3° (c, 3·4), had $R_{Gal\ A}$ 0·79 and 0·82 in solvent systems B and C respectively, it gave a characteristic brown/yellow stain with aniline oxalate, which appears orange in ultra violet light.

A sample of the sugar was converted to the methyl ester methyl glycoside with methanolic hydrochloride, reduced with potassium borohydride and hydrolysed with N-sulphuric acid at 100° for 4 hours. The product on chromatographic examination, was found to contain galactose and rhamnose only.

The acid (0·515 g.) was dissolved in water (10 ml.) and 1 ml. each of methyl sulphate and 30% sodium hydroxide solution were added dropwise over a period of one hour with vigorous stirring.

The reaction flask was kept in ice and an atmosphere of nitrogen was maintained to prevent degradation by alkali. Methyl sulphate (30 ml.) and 30% sodium hydroxide (60 ml.) were added dropwise over a period of 3-4 hours and the mixture stirred overnight. Three more additions of the reagents were made during the next three consecutive days. Vigorous stirring was maintained during and between methylations. After the final methylation the solution which was alkaline was heated on a boiling water bath for 30 min., cooled and just acidified with dilute sulphuric acid. The sodium sulphate was precipitated by addition of ethanol. The precipitate was filtered and washed with ethanol, and the combined filtrate and washings were made slightly alkaline and concentrated to small volume (about 50 ml.). The solution was now again made acidic (just acidic, as any free sulphuric acid might cause hydrolysis) and extracted 4 times with chloroform in the cold. The combined extracts were concentrated to about 10 ml., filtered and the methylated acid was precipitated by addition of excess light petroleum (b.p. 60-80°). Yield, 0·459 g. (Found: OMe, 40·2%. Calc.: OMe, 44·0%).

A sample (10 mg.) was reduced with diborane and hydrolysed with N-hydrochloric acid at 100° for 4 hrs. After treatment with Amberlite resin IR 4B and concentration the product was examined chromatographically in solvent D. It was found to contain 2,3,4-tri-O-methyl-D-galactose, 3,4-di-O-methyl-L-rhamnose, a trace of sugar travelling between the two and a faint spot on the starting line. This spot on the starting line did

not move in solvent E and hence is not the unreduced acid.

A portion of the methylated acid was recrystallised from light-petroleum (b.p. - 100-120°). The colourless plates had m.p. 67-70° and mixed m.p. 70-71° with an authentic sample of methyl 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnoside penta-methyl ether dihydrate. This product had OMe, 42·3%, $[\alpha]_D$ +94·4° (c, 1·335 in chloroform), and gave an X-ray powder photograph identical to that obtained from the authentic sample.

The rest of the methylated acid was reduced with diborane. Yield - 0·342 g. A portion of this reduced methylated acid (150 mg.) was hydrolysed with N-hydrochloric acid at 100° for 4 hours, neutralised with Amberlite resin IR 4B and taken to dryness. Chromatographic examination of the product in solvents D, F and G showed 2,3,4-tri-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose, but none of these solvents were found suitable for separation. Hence the mixture was separated into three fractions on a cellulose column (50 x 2 cm.) using light petroleum (b.p. 100-120°):butanol (7:3) saturated with water as eluant.

Fraction (a).

This fraction (0·062 g.; R_G 0·90) was chromatographically pure and indistinguishable from 3,4-di-O-methyl-L-rhamnose. The syrup crystallised and was recrystallised from ether-light petroleum (b.p. 40-60°). The clusters of needles obtained had a m.p. 86° and mixed m.p. 88-90° (with authentic sample melting at 89-90°).

Fraction (b).

This fraction (0·008 g.) was found to be a mixture of 2,3,4-tri-O-methylgalactose and 3,4-di-O-methylrhamnose.

Fraction (c).

This fraction (0.085 g.; R_G 0·72) was chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose. It was identified by conversion to the aniline derivative, which crystallised and was recrystallised from acetone, m.p. 153°. After another recrystallisation from ethyl acetate it had m.p. 159-160° and mixed m.p. 165-166° (with authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine melting at 165-166°).

A second portion of the partially crystalline reduced methylated acid (50 mg.) was dissolved, in acetone-light petroleum (40-60°) and left to stand in an ice box. No crystallisation occurred even after months. However, on allowing the solution to stand at room temperature the solvent evaporated and the syrup crystallised spontaneously yielding long blackish needles which melted in the range of 99-118°. Attempts to purify these crystals by recrystallisation were unsuccessful.

The rest of the reduced methylated acid (80 mg.) was remethylated with methyl iodide and silver oxide. The resulting methyl ester methyl glycoside of 2-O-α-D-galacturonosyl-L-rhamnose pentamethyl ether was dissolved in warm n-hexane. Spontaneous evaporation gave long needles which after separation on porous tile had a melting point of 109°. A melting point of 93-94° (indefinite) is given in the literature. Several attempts

to recrystallise the product from n-hexane gave gelatinous crystals on the sides of the flask.

Subfraction II. D-Galacturonic acid.

This fraction (0·040 g.), had R_{GalA} 1·00 in solvent B and $[\alpha]_D$ +16·3° [c, 1·84].

A sample (5 mg.) was converted to the methyl ester methyl glycoside, reduced with potassium borohydride and the methyl glycoside obtained was hydrolysed for 2 hours with N-hydrochloric acid. The product on chromatographic examination was found to contain galactose only.

The sugar (20 mg.) was dissolved in water (1 ml.) and a solution of 2,5-dichlorophenylhydrazine (20 mg.) in hot methanol (1 ml.) was added to it. Evaporation of the solution on a boiling water bath gave a syrup which crystallised spontaneously. The product was washed with ether to remove excess reagent and recrystallised from dioxane (very difficultly soluble). The crystals obtained had a melting point of about 350°.

The 2,5-dichlorophenylhydrazone made with authentic galacturonic acid had a m.p. 180°.

Subfraction III. 6-O-β-D-Glucuronosyl-D-galactose.

This fraction (0·305 g.), had R_{Gal} 0·41 and 0·54 in solvent systems B and C respectively, $[\alpha]_D$ +21·4° (c, 2.1), and was chromatographically homogenous. A sample was subjected to methanolysis, reduction with potassium borohydride and hydrolysis. The product on chromatographic examination was found to contain galactose, glucose and traces of rhamnose. The rhamnose had

probably arisen from the triuronic acid (Subfraction IV) which travels just slower than this aldobiuronic acid.

The acid (154 mg.) was dissolved in water (5 ml.) and methylated with methylsulphate and sodium hydroxide following the same procedure as for 2-O-D-galacturonosyl-L-rhamnose (Subfraction I). Extraction with chloroform gave a syrup (139 mg.) OMe, 40·8%.

This syrup was dissolved in methyl iodide (4 ml.) and the mixture refluxed with four equal additions of silver oxide (29 g. in all). The silver oxide was filtered and repeatedly extracted with boiling chloroform. Evaporation of the combined filtrate and chloroform extracts gave the fully methylated acid (122 mg.). The syrup crystallised on standing in a desiccator and was recrystallised from acetone-light petroleum (b.p. 40-60°) mixture. The rosette-like crystals obtained had m.p. and mixed m.p. 81-83° with an authentic sample of the methyl ester methyl glycoside of 6-O-D-glucuronosyl-D-galactose hexamethyl ether, of m.p. 85°. The crystals had $[\alpha]_D$ -16·2° ([c, 0·802].

The X-ray powder photograph was identical with that of the authentic sample.

Subfraction IV. Triuronic acids.

This fraction (0·116 g.), had R_{Gal} 0·16 and 0·27 in solvent systems B and C respectively, $[\alpha]_D$ +62·9° (c, 0·64) and was chromatographically homogenous but streaky. Paper ionophorosis showed one homogenous spot about 12·7 cm. from the origin.

A sample (3.5 mg.) was hydrolysed with N-sulphuric acid at

100° for 4 hours. Chromatographic examination of product in solvent B showed galactose, 2-O-galacturonosylrhamnose and some rhamnose.

Chromatographic examination of the product after methanolysis, borohydride reduction and hydrolysis showed galactose and rhamnose only.

A sample (10 mg.) was dissolved in water and converted to the potassium salt by adding potassium carbonate (10 mg.). Potassium borohydride (15 mg.) was added to the solution and left to stand overnight. Next day the solution was deionised with Amberlite resin IR 120 (H), repeatedly evaporated with methanol and hydrolysed with N-sulphuric acid at 100° for 4 hours. After neutralisation with barium carbonate, deionisation and evaporation to dryness the product was examined chromatographically using solvent I and spray reagent IV.

The chromatogram showed the presence of galactitol and rhamnitol in equal amounts in addition to galactose, rhamnose and 2-O-galacturonosylrhamnose. Hence it appears that this fraction is a mixture of aldotriuronic acids.

Trial alkaline degradation on 2-O-galacturonosylrhamnose showed that it was not affected by alkali up to 3 weeks. Trial degradations done on maltose and 3,6-anhydrogalactose gave two different saccharinolactones travelling at different speeds on chromatograms.

A sample (6 mg.) of the triuronic acid was subjected to alkaline degradation. After 2 weeks half the solution was

worked up and spotted on two papers and developed in solvent A. One paper was sprayed with reagent III, no spots due to acid lactones were visible. The second paper was sprayed with reagent I showing unchanged triuronic acid and almost an equal amount (visible inspection) of 2-O-galacturonosylrhamnose.

The rest of the solution was worked up after about 2 weeks and examined as above. Still no saccharinolactones could be detected, however spray reagent I showed unchanged triuronic acid travelling a bit faster than the original mixture of acids, 2-O-galactouronosylrhamnose and a trace of a pink spot travelling slightly faster than arabinose.

The mixture of triuronic acids (25 mg.) was dissolved in water and methylated with four additions of methyl sulphate and sodium hydroxide following the same procedure as for 2-O-D-galacturonosyl-L-rhamnose. Extraction with chloroform gave a syrup (21 mg.).

Hydrolysis of a sample (1 mg.) with N-sulphuric acid for four hours followed by chromatography in solvent D showed that the methylation was incomplete.

The syrup was dissolved in water and remethylated by two more additions of the reagents. The fully methylated acid was converted to the methyl ester methyl glycoside by refluxing with methanolic hydrochloride, reduced with lithium aluminium hydride and hydrolysed with N-sulphuric acid for 6 hours at 100°. The product was examined chromatographically in solvents D, F and G, and found to contain the following sugars.

2,3,4,6-Tetra-O-methyl-galactose

-tri-O-methyl-galactose (one or more)

-di-O-methyl-galactose (one or more)

and 3,4-di-O-methyl-rhamnose.

No 3-O-methyl-rhamnose was detected.

The rest of the syrup was converted to the methyl glycosides and examined by vapour phase chromatography in all three systems. The methyl glycosides of the methylated sugars given in the table below were detected. T values in parentheses are those corresponding to more than one glycoside.

Methyl glycosides of	T in system b	T in system c
2,3,4,6-Tetra-O-methyl-galactose	1·80	1·52 & (1·62)
2,3,4-tri-O-methyl-galactose	4·76 & 7·50	2·66 & 2·94
2,3,6-tri-O-methyl-galactose	3·27, 4·33 & 4·87	(1·62) & (2·50)
2,3-di-O-methyl-galactose	2·23 & 2·62 (system a)	(2·50), 3·21, (3·74) & (4·36)
2,4-di-O-methyl-galactose	-	(3·74) & (4·36)
3,4-di-O-methyl-rhamnose	0·73 & 1·00	0·61

Subfraction V.

This fraction (0·032 g.) was found to be a mixture of 2-O-galacturonosylrhamnose and arabinose by chromatographic examination.

Methylation of degraded gum.

The degraded gum I (9 g.) was dissolved in water (50 ml.) and treated with methyl sulphate (72 ml.) and 30% (w/v) aqueous sodium hydroxide (144 ml.) below 20° in an atmosphere of nitrogen and with vigorous stirring. Six further additions of the same quantities of the reagents were made on six successive days. The final mixture was heated on a boiling water bath for 1 hr. and filtered. The filtrate was dialysed in running water until free of sulphate ions. The residue which was insoluble in water and most organic solvents (acetone, dioxan, ethanol and methanol), was shaken with water, dialysed, acidified and extracted with chloroform. The dialysed filtrate was also acidified and extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulphate and evaporated to dryness. (4 g.; OMe, 30·3%; Ash 1·9%). The combined aqueous solutions were concentrated to a small volume and treated with two more lots of methyl sulphate and sodium hydroxide. The reaction mixture was heated on a boiling water bath acidified until just neutral and extracted with chloroform, nothing came into chloroform.

The reaction mixture was therefore dialysed for about a week, concentrated to a small volume and freeze dried (1·5 g.; OMe, 32·5%; Ash, 3·2%).

The combined methylated degraded gum (5·5 g.) was dissolved in anhydrous ether and a little methanol. The solution was cooled in ice and ethereal solution of diazomethane (prepared by

treating nitrosomethyl urea with potassium hydroxide) was added in small portions until evolution of gas ceased. The presence of excess diazomethane was ascertained by removing a few drops into a test tube and introducing a glass rod moistened with glacial acetic acid when immediate evolution of gas occurred. The solvent was removed under reduced pressure to give the methylated degraded gum. (4·7 g.; OMe, 35·2%).

This was then methylated thrice with methyl iodide and silver oxide to give a product (2·98 g.; OMe, 42·8%) whose methoxyl content was not increased on further treatment with methyl iodide and silver oxide.

Hydrolysis of the methylated degraded gum.

The methylated degraded gum was suspended in 2N sulphuric acid (100 ml.) at room temperature for 5 days and at 40-50° for a further day. The resulting solution was diluted to 200 ml. heated on a boiling water bath for 10 hr. (constant rotation), cooled, neutralised with saturated barium hydroxide followed by barium carbonate, filtered and washed with precipitate thrice with water. The combined filtrate and washings were concentrated to a thick syrup (1.7 g.) which was placed on a cellulose column (45 x 2·5 cm.) and eluted with butan-1-ol half saturated with water to give fractions A to C, followed by elution with water to give fraction D.

Fraction	Wt. in mg.	Contents
A	820	Neutral methylated sugars (about 7)
B	83	Tetra-O-methyl galactose, tri-O-methyl galactose and acidic sugars
C	103	Acidic methylated sugars
D	637	Barium salts of acidic methylated sugars

Examinations of the fractions.

Fraction A: The syrup (820 mg.) was placed on a column (55 x 1·5 cm.) and eluted with light petroleum (b.p. - 100-120°) - butan-1-ol (7:3, later 1:1) saturated with water, followed by butan-1-ol half saturated with water to give 10 fractions. A further fraction was obtained by elution of the cellulose with water. The fractions were examined chromatographically in various solvent systems and the probable sugar content of the fractions are tabulated below.

Fraction	Tubes	Wt. in mg.	Colour	R_G	Contents
1	1-37	135	Pink/brown	0·86	2,3,4,6-tetra-O-methyl galactose and 3,4-O-dimethyl rhamnose
2	38-55	7	Brown and pink	0·84	2,3,6-tri-O-methyl galactose,
				0·73	2,3,4-tri-O-methyl arabinose
3	56-90	243	Pink	0·73	2,3,4-tri-O-methyl galactose
4	91-154	41	Pink	0·77	2,3,4-tri-O-methyl galactose and 3-O-methyl rhamnose (trace)
			Khaki	0·66	
5	155-170	8	Khaki	0·68	3-O-methyl rhamnose
6	171-200	12	Light pink	streak	Same as fraction C
7	201-300	46	Brown	0·50	2,3-O-dimethyl galactose
8	301-370	7	Light pink	0·48	2,4-O-dimethyl galactose and 2,3-O-dimethyl galactose
9	371-510	23	Khaki and pink	0·35	Rhamnose
				0·43	2,4-O-dimethyl galactose and 3,4-O-dimethyl galactose
10	510-717	30	Pink	streak	2,3,4-O-trimethyl glucuronic acid, 2-O-methyl galactose, galactose and unknown
11	Water	28	Pink	streak	Barium salts of Acidic methylated sugars.

Fraction 1. 2,3,4,6-Tetra-O-methyl-D-galactose, 3,4-di-O-methyl-L-rhamnose.

This fraction (0·135 g.), had R_G 0·86 and $[\alpha]_D$ +84·1° (c, 0·58).

Paper chromatography in solvents (D) and (G) showed a single component, but in solvent (F) it separated into two components corresponding to the above two sugars and a trace of an unknown sugar faster than tetra-O-methyl-glucose.

The syrups were separated into 4 fractions on thick paper using solvent system (F).

Subfraction 1a.

The syrup (0·015 g.), R_G 0·87, $[\alpha]_D$ +18·0° (c, 0·60) gave a khaki stain with aniline oxalate spray, and was chromatographically and ionophoretically pure and identical to 3,4-di-O-methyl-L-rhamnose. It crystallised on seeding as long white fibres of m.p. 93-94° and was recrystallised from ether-light petroleum (40-60°) m.p. and mixed M.P. 95-96° (with authentic sample of 3,4-di-O-methyl-L-rhamnose).

Subfraction 1b (0·005 g.).

Paper chromatography in solvent F showed this to be a mixture of 3,4-di-O-methyl-rhamnose and 2,3,4,6-tetra-O-methyl-galactose.

Subfraction 1c.

This fraction (0·094 g.), R_G 0·89, $[\alpha]_D$ +90·7° (c, 0·97) was chromatographically and ionophoretically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose. The aniline derivative was prepared and recrystallisation gave long white needles m.p. 191-192°

and mixed m.p. 195°; with authentic 2,3,4,6-tetra-O-methyl-D-galactosylamine of m.p. 198°.

Subfraction 1d.

This fraction (0·012 g.) had R_G 0·89 and 1·01 (trace).

Paper chromatography showed it to be mainly 2,3,4,6-tetra-O-methyl-D-galactose with a trace of an unknown which moves a bit faster than 2,3,4,6-tetra-O-methyl-D-glucose.

Fraction 2. 2,3,4-Tri-O-methyl-L-arabinose and 2,3,6-tri-O-methyl-D-galactose.

This fraction (0·007 g.) had $[\alpha]_D$ +95·2° (c, 0·40), R_G 0·84 (pink) and 0·73 (brown). Paper chromatography in solvents D, F, and G showed it to be a mixture of the above two sugars. Demethylation gave galactose, arabinose and traces of mono-O-methylgalactoses.

Fraction 3. 2,3,4-Tri-O-methyl-D-galactose.

This fraction (0·243 g.), had $[\alpha]_D$ +117·7° (equil.) (c, 0·97), R_G 0·73. Gas-liquid partition chromatography and paper chromatography in the three solvents D, F and G, showed it to be pure and identical to 2,3,4-tri-O-methyl-D-galactose.

The sugar was characterised by conversion to the aniline derivative m.p. 166-167° and mixed m.p. 168-169° (with authentic sample of 2,3,4-tri-O-methyl-D-galactosylamine of m.p. 161-162°).

Fraction 4. 2,3,4-Tri-O-methyl-D-galactose and 3-O-methyl-L-rhamnose.

This fraction (0·041 g.), had $[\alpha]_D$+67·1°(c, 0·30), R_G 0·77 (pink)

and 0·66 (khaki).

Paper chromatography in the three solvents D, F and G showed it to be a mixture of the above two sugars. The sugars were separated into two fractions on thick paper using solvent system F.

Subfraction 4a. 0·012 g.

This was chromatographically pure and identical to 3-O-methyl-L-rhamnose and was combined with fraction 5.

Subfraction 4b. 0·011 g.

This was chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose.

Fraction 5. 3-O-Methyl-L-rhamnose.

This fraction (0·008 g.), $[\alpha]_D$ +25·0° (c, 0·24), R_G 0·68 (khaki) was chromatographically pure and identical to 3-methyl-L-rhamnose in three different solvent systems.

The combined syrups of subfraction 4a and this fraction crystallised on seeding and leaving in the vacuum desiccator. m.p. and mixed m.p. (with authentic sample 115-116°) 115-118°. Attempts to recrystallise it from methanol-ether were unsuccessful.

Fraction 6. Methylated acidic sugars.

This fraction (0·012 g.) streaked in four different solvent systems tried (A, D, F and G). In solvent system E it separated into 4 components and was found to contain the same sugars as fr. C. Hence this fraction which consists of methylated acidic sugars was combined with fr. C for further examination.

Fraction 7. 2,3-Di-O-methyl-D-galactose.

This fraction (0·046 g.), $[\alpha]_D$ +80·6° (equil.) (c, 0·72), R_G 0·50, was chromatographically (3 solvents) and ionophoretically indistinguishable from 2,3-di-O-methyl-D-galactose. Chromatography of the periodate oxidised sugar showed three spots grey (R_G 0·82), brown (R_G 0·93) and grey (R_G 1·05) identical to those given by 2,3-di-O-methyl-D-galactose. The aniline derivative was prepared but failed to crystallise.

Fraction 8. 2,4-Di-O-methyl-D-galactose and 2,3-di-O-methyl-D-galactose.

Chromatographic examination of this fraction (0·007 g.), R_G 0·48 in solvents A, D and F showed it to be a mixture of the above two sugars. Ionophoresis gave a streak of about 5 cm. long starting from the origin line. Periodate oxidation followed by examination of the products by chromatography showed the three spots characteristic of 2,3-di-O-methyl-D-galactose and unchanged 2,4-di-O-methyl-D-galactose.

Fraction 9. Rhamnose (main), 2,4-di-O-methyl-D-galactose and 3,4-di-O-methyl-D-galactose.

Paper chromatography of this fraction (0·023 g.), R_G 0·43 (trace) and 0·35, in solvents D and F showed it to be a mixture of rhamnose, 2,4-di-O-methyl-D-galactose and a third component which gave a pink stain with aniline oxalate. In solvent B this fraction separated into four components corresponding to rhamnose, 2,4-di-O-methylgalactose (trace), an acidic sugar

(trace) and a sugar having the same chromatographic speed as 3,4-di-O-methylgalactose.

Paper ionophoresis gave three spots, a pink spot (1.2 cm. from origin) due to 2,4-di-O-methylgalactose, a khaki spot (4.4 cm. from origin) due to rhamnose and a brown spot (6.7 cm. from origin) probably due to 3,4-di-O-methylgalactose. Periodate oxidation followed by chromatographic examination of the product showed a pink spot due to unchanged 2,4-dimethylgalactose, a trace of slowing pink spot probably due to an acid and a pink spot (R_G 0.81) of a dimethyl pentose, which must have arisen from 3,4-di-O-methyl-D-galactose.

Fraction 10. 2,3,4-Tri-O-methyl-D-glucuronic acid traces of 2-O-methyl-D-galactose and galactose and an unknown.

This fraction (0.030 g.) streaked in solvents A, D and F and also indicated probable presence of galactose. However in solvent E it gave a discrete pink spot (R_G 0.88) most probably due to a tri-methyl hexauronic acid and two faint spots corresponding to galactose and 2-O-methylgalactose.

In solvent B it separated into a very fast moving pink spot due to the acid and three other spots due to galactose, 2-O-methylgalactose and an unknown.

Paper chromatographic pattern in solvents A, D and E of the syrup after treatment with Amberlite resin IR 120 (H) was not much different from that of the original syrup. The pattern also did not change on hydrolysis of the syrup with N-sulphuric

acid for two hours.

The rest of the syrup (ca, 10 mg.) was separated into two fractions on 3MM paper using solvent system B.

Subfraction a.

Chromatographic examination of this fraction (0.002 g.) in solvent B showed galactose, 2-O-methylgalactose and an unknown moving between the above two. Periodate oxidation and examination of product in solvent D showed two yellow spots of R_G 0·24 (methoxymalondialdehyde from 2-O-methylgalactose) and R_G 0·75 (?).

Subfraction b.

This fraction (0.003 g.), R_G 0·85 (solvent E) was chromatographically pure in solvents B and E. Chromatographic examination of the product after methanolysis, potassium borohydride reduction and hydrolysis showed 2,3,4-tri-O-methyl-D-glucose.

Fraction 11. Barium salts of acidic methylated sugars.

This fraction (0.028 g.) streaks in solvents D and E. When it was deionised with Amberlite resin IR 120 (H) and examined in the above two solvents it was found that this fraction had the same components as fraction C and was therefore combined with fraction C for further examination.

Fraction B.

Chromatographic examination of this fraction (0·083 g.) in solvents D, E and F showed it to be a mixture of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose and fraction C.

This fraction was not examined further.

Fraction C.

This fraction (0·103 g.), had R_G 0·19 (streaky) and gave a stain with aniline oxalate which appeared purplish pink in U.V. light.

Chromatography of this syrup in solvent A showed a streak at the origin, but no spots corresponding to neutral methylated sugars were detected.

In solvent E it separated into the following 4 components:-

R_G	Colour in daylight	Colour in U.V. light
0·07	pink	-
0·44	brownish pink	purplish pink
0·65	light khaki	orange
0·76	pink	purple

A few mg. of this fraction was deionised with Amberlite resin IR 120 (H) and examination in solvent E gave the same pattern as above, except that spot (R_G 0·07) was absent.

The combined Fraction C, 6 and 11 was dissolved in water, shaken up with Amberlite resin IR 120 (H) and taken to dryness. The dry syrup was converted to the methyl ester methyl glycoside by refluxing with methanolic hydrogen chloride, cooled, neutralised with silver carbonate and taken to dryness.

The dry syrup was dissolved in purified dry tetrahydrofuran and lithium aluminium hydride was added and the solution refluxed for 1 hr., a little more of lithium aluminium hydride was added and refluxed for a further hour. The excess hydride was destroyed by adding ethyl acetate and water alternately.

Filtered and the residue was extracted several times with acetone and chloroform. The combined filtrate and extracts were taken to dryness, extracted with dry chloroform and again concentrated. Yield - 110 mg.

Chromatographic examination of this syrup in solvents D and E showed a pink spot of R_G 0·19 (solvent D) and R_G 0·14 (solvent E).

This syrup was hydrolysed with N-sulphuric acid by refluxing at 100°C for 6 hours. The cooled solution was neutralised with barium hydroxide followed by barium carbonate, filtered through Celite, shaken with Amberlite resin IR 120 (H) and taken to dryness.

Paper chromatography in solvents (A, D and F) and visual examination showed the presence of the sugars shown in table 4.

The main bulk of this syrup was combined with the corresponding syrup from Fraction D for separation and examination.

Fraction D.

This fraction (0·637 g.) did not move on chromatograms developed in solvents D and E, hence it consists of barium salts of acidic sugars only. The syrup was deionised by treating with Amberlite resin IR 120 (H). Yield - 489 mg.

The deionised syrup streaked in solvent A, but in solvent E it separated into the following four components.

R_G	Colour in daylight	Colour in U.V. light
0·18	light grey	pinkish orange
0·39 (main)	brown	pinkish orange
0·55	pink	-
0·74	crimson	-

16 mg. of the sugar (R_G 0·39) was obtained pure by separating 50 mg. of the deionised syrup on thick paper using solvent E. This sugar (16 mg. R_G 0·39) was examined separately and is referred to as subfraction D.

This subfraction D and the main fraction D (468 mg.) were both separately subjected to methanolysis, reduction with lithium aluminium hydride and hydrolysis with N-sulphuric acid for 6 hours at 100°. Paper chromatographic examination of the resulting syrups in solvents A, D and F showed the presence of the sugars shown in table 4. An approximate proportion of the sugars (visual estimation) is also given in the table.

Sugars	Fraction C	Subfraction D	Main fraction D
3,4-di-O-methyl-L-rhamnose	++++	++++	++++
3-O-methyl-L-rhamnose	++	++++	++++
2,3,4-tri-O-methyl-D-glucose	+++	++	trace
2,3,4-tri-O-methyl-D-galactose	+	trace	+
2,3(?)-di-O-methyl-D-galactose	++	+++	++++

Table 4.

Subfraction D had an unidentified sugar, which gave a pink stain with aniline oxalate and had low R_G value.

Small samples of the above three fractions were treated with dry methanollic hydrochloride and the resulting mixtures of methyl glycosides were analysed by vapour phase chromatography.

The results are shown in the table below.

Methyl glycosides	T values in system ( )	Fraction C	Subfraction D	Main fraction D
Methyl-3,4-di-O-methyl-L-rhamnoside	0.61(c)	+	+	+
Methyl-3-O-methyl-L-rhamnoside	1.00(c)	+	+	+
Methyl-2,3,4-tri-O-methyl-D-glucoside	1.34 & 1.81(c)	+	+	+ (little)
Methyl-2,3,4-tri-O-methyl-D-galactoside	4.74 & 7.48(b)	+	+(little)	+
Methyl-2,3,6-tri-O-methyl-D-galactoside	1.27(a) & 1.66(c)	trace	trace	trace
Methyl-2,3-di-O-methyl-D-galactoside	2.46,3.69 & 4.20(c)	+	+	+
Unknown I	0.43(b)	-	+	trace
Unknown II	1.45(c)	-	+	+(little)

The combined syrup (285 mg.) from fractions C and D was placed on a cellulose column (45 x 2 cm.) and eluted with light petroleum (b.p. - 100-120°) - butan-1-ol (7:3, later 1:1) saturated with water and finally with water.

Fraction	Tubes	Wt. in mg.	Colour	R_G	Content
(i)	1-150	38·4	Khaki and pink	0·86	3,4-di-O-methyl-L-rhamnose 2,3,4-tri-O-methyl-D-glucose
(ii)	151-270	32·0	Pink	0·72	2,3,4-tri-O-methyl-D-galactose
(iii)	271-335	45·1	Khaki	0·67	3-O-methyl-L-rhamnose
(iv)	336-435	92·6	Brown and trace of khaki		2,3-di-O-methyl-D-galactose, 3-O-methyl-L-rhamnose and rhamnose
(v)	436-576	13·9	Brown	0·54	2,3-di-O-methyl-D-galactose and 2-O-methyl-D-galactose
(vi)	Water	12·3	Grey	Streak	

Fraction (i). 2,3,4-Tri-O-methyl-D-glucose and 3,4-di-O-methyl-L-rhamnose.

This fraction (0·038 g.), had R_G 0·86, $[\alpha]_D$ +45·5° (c, 0·72).

Paper chromatography in solvents D, F, G and I showed either a spot or streak with two overlapping colours, suggesting that it is a mixture of at least two sugars. On paper ionophoresis it separated into two distinct spots, a khaki spot 4·8 cm. below starting line corresponding to standard 3,4-di-O-methyl-L-

rhamnose and a pink spot 2·4 cm. above the starting line corresponding to standard 2,3,4-tri-O-methyl-D-glucose.

The syrup was separated into two fractions by ionophoresis on 3 MM paper, (borate buffer pH 10, 500v, 7 hrs.). The sugars were located by cutting out a centre strip and spraying with saturated aqueous aniline oxalate acidified with acetic acid. The borate complex was then eluted with cold water, treated with Amberlite resin IR 120 (H) to remove sodium ions, taken to dryness and repeatedly evaporated with methanol to remove borate.

Fraction (i)a.

This fraction (0·008 g.), R_G 0·91, $[\alpha]_D$ +46·1° → +73·0° (c, 0·26), was chromatographically and ionophoretically pure and identical to 2,3,4-tri-O-methyl-D-glucose. The aniline derivative was made, but failed to crystallise.

Fraction (i)b.

This fraction (0·014 g.), R_G 0·90, $[\alpha]_D$ +23·0° (c, 0·32), was chromatographically and ionophoretically pure and identical to 3,4-di-O-methyl-L-rhamnose. The syrup crystallised on standing in desiccator as a cluster of long needles and was recrystallised from ether - light petroleum (40-60°). m.p. 93-95° and mixed m.p. 91-92° with sample of authentic 3,4-di-O-methyl-L-rhamnose.

Fraction (ii). 2,3,4-Tri-O-methyl-D-galactose.

This fraction (0·032 g.), R_G 0·72, $[\alpha]_D$ +125·0° (c, 0·20),

was chromatographically pure and indistinguishable from standard 2,3,4-tri-O-methyl-D-galactose in solvents D and F. It was characterised by preparing the aniline derivative, m.p. and mixed m.p. 162-163°.

Fraction (iii). 3-O-Methyl-L-rhamnose.

This fraction (0·045 g.), R_G 0·67, $[\alpha]_D$ +38·3° (c, 0·47), was chromatographically pure and indistinguishable from 3-O-methyl-L-rhamnose in solvents D and F. The syrup crystallised on seeding and leaving the desiccator and was recrystallised from methanol ether. m.p. and mixed m.p. 115-117°.

Fraction (iv). 2,3-Di-O-methyl-D-galactose with traces of 3-O-methyl-L-rhamnose and rhamnose.

This fraction (0·093 g.), had R_G 0·52, and $[\alpha]_D$ +78·2° (c, 0·62).

Chromatography in solvent D showed a brown spot (R_G 0·52), with a trailing (khaki) probably due to 3-O-methyl-rhamnose. In solvent F the main component gave a brown streak corresponding to 2,3-di-O-methyl-galactose and there was a trace of a khaki spot just below starting line corresponding to rhamnose. In solvent G it separated into three spots corresponding to 2,3-di-O-methyl-galactose (Main), 3-O-methyl-L-rhamnose and rhamnose. Paper ionophoresis gave a brown spot 2 cm. from the line, with a khaki trail, standard 2,3-di-O-methylgalactose moved 2·1 cm. on the same paper.

Periodate oxidation and chromatography in solvent D gave a

pattern characteristic of 2,3-di-O-methyl-D-galactose (2 grey and one brown spot) and a trace of brown spot (R_G 1·00, pink in u.v. light) due to 3-O-methyl-rhamnose.

This fraction was separated into three fractions using solvent G.

Subfraction a. (0·009 g.).

Chromatography in solvents F and G showed mostly 2,3-di-O-methyl-galactose and some rhamnose.

Subfraction b. (0·041 g.).

Chromatographically pure and identical to 2,3-di-O-methyl-D-galactose. It was converted to the amide of the aldonic acid, but all attempts to crystallise it were unsuccessful.

Subfraction c. (0·008 g.).

Chromatography showed this fraction to be a mixture of 2,3-di-O-methyl-D-galactose and 3-O-methyl-L-rhamnose.

Fraction (v). 2,3-Di-O-methyl-D-galactose and 2-O-methyl-D-galactose.

Chromatography in solvents D, F and G and ionophoresis showed this fraction (0·014 g.), R_G 0·52 and 0·29, to be a mixture of the above two sugars. These two sugars had greatly differing mobilities in solvent system G. Periodate oxidation and chromatography showed a light pattern characteristic of 2,3-di-O-methyl-D-galactose and a bright yellow spot.

Fraction (vi).

This fraction (0·012 g.) streaked in solvents D, E and F,

the streak was greyish in daylight and violet in u.v. light (characteristic of inorganic ions). After treatment with Amberlite resin IR 120 (H) it still did not give any clear picture in solvent D, E and A. Among other things this fraction probably consists of traces of galactose, arabinose and rhamnose.

Methylation of whole gum.

The gum acid (20 g.) was dissolved in water (200 ml.) and methylated in an atmosphere of nitrogen below 20°C. Methyl sulphate (160 ml.) and sodium hydroxide solution (30%; 240 ml.) were added dropwise with stirring over a period of about 8 hours and continued stirring overnight. Four similar additions of methyl sulphate and sodium hydroxide were made on four successive days. Secondary-octyl alcohol was added occasionally to control frothing. The reaction mixture was heated on a boiling water bath for half an hour and the solid which separated was filtered while hot. The residue was dissolved in water, dialysed in running water till free of sulphate ions, concentrated under reduced pressure and freeze-dried. (10·0 g.; OMe, 35·6%).

The filtrate too was dialysed till free of sulphate ions, concentrated under reduced pressure and freeze-dried. (4·0 g.; OMe, 17·4%).

The methylated polysaccharide (10 g.) was dissolved in water (300 ml.) and passed through a column of Amberlite resin IR 120 (H) to remove sodium ions, treated with silver carbonate, filtered, concentrated and freeze dried. Yield of silver salt - 12·0 g.

The silver salt (12 g.) was dispersed in methyl iodide (125 ml.) and methanol (50 ml.). The mixture was refluxed in a dry flask fitted with a dry double walled condenser protected by a calcium chloride tube. Silver oxide (15 g.) was added in four batches one at the start and one every fourth hour. The residue was filtered and extracted repeatedly with hot chloroform. The combined filtrate and extract were concentrated to a small volume and the methylated gum was precipitated by addition of excess light-petroleum (b.p. 60-80°). [9·5 g., ash 2·4%, OMe, 38·0% (corrected for ash)]

Two further Purdie methylations on this product did not change the methoxyl content appreciably.

Attempted fractionation of the methylated gum.

The methylated gum was successively extracted by boiling for about an hour with mixtures of light petroleum (b.p. 60-80°) and chloroform, containing increasing amounts of chloroform.

Two main fractions of OMe contents 38·0% and 39·0% were obtained with mixtures containing (75:25 and 70:30 v/v of light-petroleum and chloroform respectively).

Another Purdie methylation of the fraction having OMe, 39·0% did not increase the methoxyl content further.

Two further portions of gum (20 g. and 10 g.) were methylated separately by Haworth's procedure as described above. Yield 7 g. (lost some by accident) and 9·5 g. respectively. OMe, 35·0% and 31·4% respectively.

These were separately converted to silver salts and methylated with methyl iodide and silver oxide as described earlier.

Yield 5 g. and 7·5 g.

OMe, 38·7% and 37·3%.

The above two methylated gums after another treatment with methyl iodide and silver oxide had methoxyl contents 39·3% and 39·5% respectively.

These were now combined (12 g.) and methylated with methyl iodide (180 ml.) and silver oxide (15 g.). The combined filtrate and chloroform extracts were concentrated under reduced pressure to about 100 ml. and the methylated polysaccharide was precipitated in two stages by addition of light petroleum (b.p. 60-80°) 300 ml. and then a further 100 ml.

Fraction I. 7.5 g., ash, 2·3%, OMe, 40·8% (corrected for ash).

Fraction II. 3·5 g., OMe, 38·8%.

A further Purdie methylation on fraction I did not increase its methoxyl value and hence it was taken to be the fully methylated gum.

Hydrolysis of the methylated gum.

A trial hydrolysis was done on 50 mg. The gum was refluxed with methanolic hydrochloric acid (2%, 5 ml.) for 6 hours, treated with Amberlite resin IR 4B (OH), evaporated to dryness and the hydrolysis completed by refluxing for a further 6 hours with 0·5N hydrochloric acid. The solution was neutralised with Amberlite resin IR 4B (OH) and taken to dryness. Chromatography in solvents D and E showed mixtures containing at least ten different methylated sugars.

The rest of the methylated gum was dissolved in sulphuric acid (2N, 200 ml.), left to stand at room temperature for 3 days, diluted to double the volume with water and heated on a water-bath, when slight precipitation occurred. Hence it was left to stand at 40° for another 3 days, then at 80° overnight and finally heated in a boiling water bath for 8 hours until rotation became constant ($[\alpha]_D$ ca +32°). Only very slight precipitation occurred. Neutralisation of the solution was effected by addition of saturated solution of barium hydroxide (0·44N) until the pH of the solution was about 6, followed by addition of solid barium carbonate. The solution was centrifuged, the precipitate washed with water (thrice) and the filtrate and washings evaporated to a small volume (100 ml.). The solution was again centrifuged to remove some barium sulphate which was still present, filtered and the filtrate was concentrated to a syrup (5.7 g.).

Separation of acidic and neutral methylated sugars.

The syrup was placed on a cellulose column (80 x 3·5 cm.) by freeze-drying it on to cellulose, and the column was eluted with butan-1-ol saturated with water.

Ten fractions each of about 400 ml. were collected, evaporated and examined chromatographically in solvents D and E, and were found to be entirely neutral methylated sugars. Since nothing more came off with this eluant, the column was washed with water. The first 500 ml. was evaporated and examined chromatographically in solvent D and E. No spots were visible on the chromatograms

developed in the solvent D, showing the absence of neutral sugars. A small amount of this syrup was deionised with Amberlite resin IR 120 (H) and examined in solvent E. It was streaky and seemed to contain at least four components, but nothing could be detected in the region corresponding to 2,3,4-tri-O-methyl-D-glucuronic acid. The elution with water was continued until no more came off.

Wt. of neutral methylated sugars = 3·40 g.

Wt. of acidic methylated sugars (as barium salts) = 0·98 g.

Examination of neutral methylated sugars.

The neutral methylated sugars (3·40 g.) were separated by chromatography on cellulose column (8·0 x 3·5 cm.). The column was eluted successively with the following solvents:

Light petroleum (b.p. - 100-120°): butan-1-ol (7:3, saturated with water)

" " : " " " (1:1 ")

" " : " " " (1:2 ").

Butan-1-ol half saturated with water and finally with water.

Twenty-two fractions were collected in all:-

Fraction	Tubes	R_G	Colour	Wt. in mg.	Contents
1	21-50	1·01	Yellow	31	Unknown
2	61-100	0·99	Black, red in u.v.	535	2,3,5-tri-O-methyl-L-arabinose
3	101-225	0·99 & 0·93	Black & brown	22	2,3,5-tri-O-methyl-L-arabinose (main) 2,3,4,6-tetra-O-methyl-D-galactose (?) and an unknown (trace)
4	311-410	0·86	Black, red in u.v.	128	2,5-di-O-methyl-L-arabinose, 3,5-di-O-methyl-L-arabinose (trace) & 3,4-di-O-methyl-L-rhamnose (trace)
5	411-500	0·87 & 0·78	Black, red in u.v. & brown/pink	35	Same sugars as in fraction 4 and 2,3,4-tri-O-methyl-L-arabinose
6	501-600	0·80 & 0·74	Black, red in u.v. & brown/pink	28	Same sugars as in fraction 5 and an unknown sugar (trace)
7	601-660	0·76	Brown	34	2,3,6-tri-O-methyl-D-galactose
8	661-765	0·73 & 0·61	Brown & black, red in u.v.	423	Tri-O-methyl-D-galactoses and 2,3-di-O-methyl-L-arabinose
9	766-875	0·68	Pink/brown	361	2,3,4-tri-O-methyl-D-galactose
10	876-975	0·74 & 0·62	Pink/brown & khaki	55	2,3,4-tri-O-methyl-D-galactose (main) 3-O-methyl-L-rhamnose and an unknown sugar
11 & 12	976-1100	0.62	Khaki & pink (trace)	44	3-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-galactose (trace)
13	1101-1260	0·54	Brown	94	2,6-di-O-methyl-D-galactose (main) & 2,3-di-O-methyl-D-galactose
14	1261-1355	0·54	Brown	105	2,3-di-O-methyl-D-galactose & 2,4-di-O-methyl-D-galactose

Fraction	Tubes	R_G	Colour	Wt. in mg.	Contents
15	1356-1408	0·50	Pink/brown	337	2,4-di-O-methyl-D-galactose
16	1409-1500	0·52& 0·42	Pink/brown & khaki	268	2,4-di-O-methyl-D-galactose and rhamnose
17	1501-1600	Streak	Pink	37	2-O-methyl-D-galactose, rhamnose (trace) and an unknown sugar (trace)
18	1601-1680	0·29	Brown	99	2-O-methyl-D-galactose and above unknown sugar (trace)
19 & 20	1681-1880	0·30	Black/brown	427	2-O-methyl-D-galactose
21	1881-2140	0·12 etc.	Brown etc.	97	(3)-O-methyl-D-galactose, galactose, arabinose and 2,3,4-tri-O-methyl-D-galacturonic acid
22	water	streak	Pink	45	Acidic methylated sugars

Total weight recovered 3·145 g. (92·5%).

Examination of the fractions.

Fraction 1.

This fraction (0·031 g.), had the same chromatographic mobility (R_G 1·04) as 2,3,4-tri-O-methyl-L-rhamnose, but it had $[\alpha]_D$ -22·2° (c, 0·45), whereas the above sugar has $[\alpha]_D$ +24·0°.

Chromatographic examination in solvent D of the products after treatment with 0·1N-sodium hydroxide for 0·5 hr. showed a brown spot (R_G 0·19) and a heavy pink spot (R_G 0·97). Hydrolysis of a further sample (5 mg.) with N-hydrochloric acid for 2 hr. and

chromatography in solvent E gave a similar pattern, the products of acid hydrolysis were streaking in solvent D.

This fraction is most probably a methylated disaccharide.

Fraction 2. 2,3,5-Tri-O-methyl-L-arabinose.

This fraction (0·535 g.), R_G 0·99, $[\alpha]_D$ -33·0°→ -41·0°(equil.) (c, 0·49) gave a characteristic black stain with aniline oxalate which appears red in u.v. light.

Paper chromatography in solvents D, F and G showed it to be pure and identical to 2,3,5-tri-O-methyl-L-arabinose. It was characterised by conversion to the crystalline amide of the aldonic acid, recrystallised from ethyl acetate. m.p. and mixed m.p. - 137-138° (with authentic sample melting at 129-130°).

Fraction 3. 2,3,5-Tri-O-methyl-L-arabinose (main)
2,3,4,6-tetra-O-methyl-D-galactose and unknown.

Chromatography of this fraction (0·022 g.) in solvent D showed two spots R_G 0·99 and R_G 0·93, touching each other. In solvent F it gave a black streak corresponding to the trimethyl-arabinose and the top of the streak was pink. But chromatography in solvent G showed two distinct spots, the pink spot corresponding to 2,3,4,6-tetra-O-methylgalactose separated well from the black streak.

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

Subfraction a.

This fraction (0·007 g.) was chromatographically identical to 2,3,4,6-tetra-O-methyl-D-galactose.

An attempt to prepare the aniline derivative was not successful, as the derivative failed to crystallise. The sugar was recovered by treating the aniline derivative with Amberlite resin IR 120 (H) for 0·5 hr., filtering and concentrating. Demethylation of this syrup gave galactose only.

Subfraction b.

Chromatography of this fraction (0·013 g.) in solvent D indicated mainly 2,3,5-tri-O-methylarabinose, but in solvent G a trace of an unidentified sugar separated from above sugar. This second component was not identical to 2,3,4-tri-O-methyl-, 2,4-di-O-methyl-, 2,5-di-O-methylarabinoses or to 3,4-di-O-methylrhamnose. Hydrolysis of a sample (3 mg.) with N-hydrochloric acid for 2 hours, did not change the chromatographic pattern in solvents D and G.

Fraction 4. 2,5-Di-O-methyl-L-arabinose (main) and small amounts of other sugars.

Chromatography of this fraction (0·128 g.) in solvents A, D, G and I showed only one component (R_G 0·86) which was black in daylight and red in u.v. light, characteristic of methylated arabinofuranoses. But chromatography in solvent F gave two spots, a long black spot which in addition to dimethylarabinose seemed to contain dimethylrhamnose and a brown spot which had the same speed as trimethylgalactose.

Paper ionophoresis gave two distinct spots one at the origin and a pink spot 15·4 cm. from the origin.

The syrup was separated into three fractions on thick paper

using solvent F.

Subfraction 4a and Subfraction 6a

0·004 g. and 0·006 g.

These fractions were chromatographically identical to 2,3,6-tri-O-methyl-D-galactose in different solvent systems.

Subfraction 4b and Subfraction 6b.

0·034 g. and 0·007 g.

$[\alpha]_D$ -19·0° → -6·0° [const.] [c, 0·33].

These fractions were chromatographically and ionophoretically homogeneous and corresponded to 2,5-di-O-methyl-L-arabinose. It was characterised by conversion to the amide of the aldonic acid. m.p. - 125-127°, mixed m.p. - 126-127° (with authentic sample of 2,5-di-O-methyl-L-arabinamide melting at 127-128°).

Subfraction 4c and subfraction 6c.

0·026 g. 0·016 g.

$[\alpha]_D$ -23·0 [equil.] [c, 0·61]

Paper chromatography suggested these fractions to be a mixture of 3,4-dimethylrhamnose and dimethylarabinose. However paper ionophoresis showed three spots corresponding to 2,5-di-O dimethyl-L-arabinose, 3,4-di-O-methyl-L-rhamnose and 3,5-di-O-methyl-L-arabinose. Demethylation of a sample gave arabinose and rhamnose.

Fraction 5. Same sugars as Fraction 4 and 2,3,4-tri-O-methyl-L-arabinose.

Paper chromatographic examination of this fraction (0·035 g.), R_G 0·87 and 0·78, showed this to be a mixture of the above sugars.

It was combined with fraction 6 for separation and further examination.

Fraction 6. Same sugars as Fraction 5 and an unknown.

Chromatographic examination in solvents A, D, F and G showed this fraction (0·028 g.), R_G 0·80 and 0·74 to contain the same sugars as the above fraction and in addition a component with same chromatographic speed as 2,3,5-tri-O-methylarabinose was also detected.

The combined syrup (63 mg.) of fractions 5 and 6 was separated into five fractions using solvent F.

Subfraction 6a	0·006 g.	These three were examined
Subfraction 6b	0·007 g.	with the corresponding
Subfraction 6c	0·016 g.	fractions of 4.

Subfraction 6d.

This fraction (0·011 g.) $[\alpha]_D$ +113·5° (c, 0·37) was chromatographically pure and identical to 2,3,4-tri-O-methyl-L-arabinose. The amide of the aldonic acid was prepared, but all attempts to crystallise it were unsuccessful.

Subfraction 6e.

This fraction (3 mg.) was chromatographically identical to 2,3,5-tri-O-methyl-L-arabinose in three solvent systems.

Fraction 7. 2,3,6-Tri-O-methyl-D-galactose.

This fraction (0·034 g.), R_G 0·76, $[\alpha]_D$ +10·0°→+83·3°(equil.) (c, 0·60) was chromatographically indistinguishable from 2,3,6-tri-O-methyl-D-galactose in four different (A, D, F and G) solvent systems. It was characterised by preparing the aldonolactone

which crystallised on evaporation of the solvent and was recrystallised from ether as rossettes of needles.
m.p. - 97-98°, mixed m.p. - 98-99° (with authentic sample melting at 98-99°).

Fraction 8. 2,3-Di-O-methyl-L-arabinose and tri-O-methyl-galactoses.

Paper chromatography of this fraction (0·423 g.) R_G 0·73 and 0·61, $[\alpha]_D$ +94·6°→+115·0° (equil.) (c, 0·30) in solvents A, B, D, F and G suggested it to be a mixture of 2,3-dimethyl-arabinose and trimethyl-galactose. But in none of these solvents did these sugars separate well enough for a proper quantitative fractionation. Paper ionophoresis gave no separation either, it gave a spot 3 cm. above the starting line.

Demethylation gave galactose and arabinose (in the approximate ratio of 2:1, visual estimation) and a trace of a monomethylgalactose.

Separation of the syrup (250 mg.) was attempted on a small cellulose column using light petroleum (b.p. - 100-120°): butan-1-ol (1:1) saturated with water as eluant. Two fractions were collected.

(a) 0·143 g. (lost by accident).

This gave the same chromatographic pattern as the original fraction and was therefore a mixture of the suspected sugars.

(b) This fraction (0·043 g.) was however chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose. It was characterised by preparing the aniline derivative m.p. and mixed m.p. -

162-164° (with an authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine).

The remaining syrup (160 mg.) was fractionated on 17MM papers using solvent G. In this solvent a long streak was obtained and this streak was divided into three by visual guess.

Fr. (i)

This fraction (0·012 g.) was chromatographically identical to 2,3,4-tri-O-methyl-D-galactose.

Fr. (ii)

This fraction (0·118 g.) was a mixture of both the components.

Fr. (iii)

This fraction (0·028 g.) $[\alpha]_D$ +86·4° (c, 0·55) was almost pure chromatographically and was taken to be 2,3-dimethyl-arabinose. The amide of the aldonic acid was prepared and recrystallised twice from ethyl acetate.
m.p. - 147-149°; mixed m.p. - 142-144° (with authentic sample of 2,3-di-O-methyl-L-arabonamide melting at 153-154°).

Vapour phase chromatographic examination of a sample of the original fraction after converting to the methyl glycosides showed methyl-2,3-di-O-methyl-L-arabinoside (T, 1·57, 1·78 and 1·96 in system b), methyl-2,3,4-tri-O-methyl-D-galactoside (T, 4·76 and 7·48 in system b) and methyl-2,3,6-tri-O-methyl-D-galactoside (T, 3·27, 43·1 in system b).

Since the m.p. and mixed m.p. of the amide of the aldonic acid of 2,3-dimethyl-arabinose, was not very satisfactory,

attempts were made to obtain a purer sample of this sugar.

Amides of the aldonic acids of 2,3-di-O-methyl-L-arabinose; 2,3,4-tri-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-galactose were spotted on chromatograms and developed in solvents B, D, E and I. The chromatograms after drying were sprayed with a 0·2% solution of ninhydrin and heated in the oven for 15 minutes. It was found that 2,3-dimethyl-arabonamide and 2,3,4-trimethyl-galactanamide had the same chromatographic speed in these solvents, whereas 2,3,6-trimethyl-galactanamide did not give a stain with ninhydrin and was therefore not located.

Trail chromatography of a synthetic mixture of the above three sugars on paper impregnated with dimethyl sulphoxide using dimethylsulphoxide in benzene (5% v/v) as the mobile phase (68), showed that 2,3-di-O-methylarabinose can be obtained pure by this method. Hence the syrup (30 mg.) was chromatographed on 3MM filter sheets impregnated with dimethyl sulphoxide and after development with the above solvent, side strips were cut, dried in the oven for 5 min. and sprayed with aniline oxalate. The section of the chromatogram corresponding to 2,3-di-O-methyl-arabinose was cut out and placed in a vacuum desiccator over water for about two days. The paper was then eluted with water and the eluate taken to dryness. A thick oil smelling of dimethylsulphoxide was obtained, which on chromatographic (paper and vapour phase) examination was found to consist almost entirely of 2,3-di-O-methylarabinose, with a trace of trimethylgalactose.

The oil was treated with bromine and the resulting aldono-lactone crystallised. The lactone was converted to the

aldonamide, which crystallised on leaving in the desiccator. m.p. 140-145°. After two recrystallisations from ethyl acetate it had m.p. 154-155° and mixed m.p. (with authentic sample 153-154°) 153-154°.

Fraction 9. 2,3,4-Tri-O-methyl-D-galactose.

This fraction (0.361 g.), R_G 0.68, $[\alpha]_D$ +136.0°→+109.0° (equil.) (c, 0.40), was chromatographically pure and indistinguishable from standard 2,3,4-tri-O-methyl-D-galactose, in solvent systems A, D, F and G. It was characterised by preparing the aniline derivative, which crystallised on taking to dryness and was recrystallised from ethyl acetate. m.p. 168-169°. Mixed m.p. 169-170° (with authentic sample melting at 161-162°).

Fraction 10. 2,3,4-Tri-O-methyl-D-galactose (main) 3-O-methyl-L-rhamnose and unknown.

This fraction (0.055 g.), R_G 0.74 and 0.62, $[\alpha]_D$ +57.0° (c, 0.32) on paper chromatography in solvents D and F appeared to be a mixture of 2,3,4-tri-O-methylgalactose and 3-O-methylrhamnose. But in solvent G a sugar having the same chromatographic speed as 2,6-di-O-methylgalactose, but moving faster than 2,4-di-O-methylgalactose was located. The stain which this sugar gave with aniline oxalate suggested it to be the 2,3-isomer. However, chromatography of the periodate oxidised product showed a yellow spot (methoxymalonaldehyde) in addition to unchanged trimethylgalactose and spots characteristic of 3-O-methylrhamnose. Only the 2,6-dimethylgalactose would give methoxymalonaldehyde on periodate oxidation.

A sample (3 mg.) was hydrolysed with N-sulphuric acid for 2 hours, chromatography of the hydrolysis product in solvent G showed traces of two slower moving sugars (pink stains with aniline oxalate) in addition to the three original sugars. These traces could have been some demethylation products.

Fraction 11 and 12. 3-O-Methyl-L-rhamnose and trace of 2,3,4-tri-O-methyl-D-galactose.

These two fractions were combined after chromatographic examination. 0·044 g., R_G 0·62, $[\alpha]_D$ +49·0°→+27·4° (equil.) (c, 0·29).

Chromatography in solvents D and G showed a khaki spot with a pink trail, but in solvent F these two separated well. Hence this fraction was separated on thick paper using solvent F into two compounds.

Subfraction a.

This fraction (0·006 g.) was chromatographically indistinguishable from 2,3,4-tri-O-methyl-D-galactose in solvents D and F.

Subfraction b.

This component (0·029 g.), $[\alpha]_D$ +27·4° (c, 0·32) was chromatographically (paper and vapour phase) pure and indistinguishable from 3-O-methyl-L-rhamnose.

The syrup crystallised on seeding and leaving in a desiccator m.p. - 114-118°. Recrystallised with difficulty from methanol ether. m.p. - 116-117°; mixed m.p. - 115-117°, (with authentic 3-O-methyl-L-rhamnose melting at 115-116°).

Fraction 13. 2,6-Di-O-methyl-D-galactose (main) and 2,3-di-O-methyl-D-galactose.

Chromatography of this fraction (0·094 g.), R_G 0·54, $[\alpha]_D$ +37·0° → +80·0° (equil.) (c, 0.38) in three solvent systems D, F and G suggested the presence of a sugar having the same chromatographic speed as 2,6- and 2,3-di-O-methylgalactoses. Paper ionophoresis however showed it to be a mixture of the above two sugars, the main one being 2,6-di-O-methylgalactose. Chromatography of the periodate oxidised product gave a bright yellow spot (R_G -0·24) and traces of 2 grey and one pink spot, this is in agreement with the ionophoresis results. The syrup crystallised on seeding with a crystal of 2,6-di-O-methyl-D-galactose and leaving in the desiccator, m.p. - 104-106°. Several attempts to recrystallise were unsuccessful. The syrup (30 mg.) was converted to the aniline derivative which crystallised on seeding with a crystal of 2,6-di-O-methyl-N-phenyl-D-galactosylamine. Recrystallised from ethyl acetate m.p. and mixed m.p. - 115-117° (with authentic sample of 2,6-di-O-methyl-N-phenyl-D-galactosylamine). X-ray powder diagram of this sample was found to differ from that of 2,3-di-O-methyl-N-phenyl-D-galactosylamine.

Fraction 14. 2,3-Di-O-methyl-D-galactose (main) and 2,4-di-O-methyl-D-galactose.

This fraction (0·105 g.), had R_G 0·54 and $[\alpha]_D$ +50·0° → +76·0° (equil.) (c, 0·40).

Demethylation gave only galactose. Chromatography in solvents D and I gave no separation of the components in solvents

F and G it separated into a long brown streak corresponding to 2,3- (or 2,6)-di-O-methylgalactose and a pink spot above this streak corresponding to 2,4-di-O-methylgalactose.

Paper ionophoresis gave only one spot 2·2 cm. from the origin, 2,6-di-O-methylgalactose moved 5·3 cm. on the same paper.

Chromatography of the periodate oxidised product gave the three spots characteristic of 2,3-di-O-methylgalactose, but unchanged 2,4-di-O-methylgalactose could not be detected.

The syrup was separated into two fractions on thick paper using solvent system F.

Subfraction 14a.

This fraction (0·007 g.), $[\alpha]_D$ +62·5° (c, 0·16) was chromatographically indistinguishable from 2,4-di-O-methyl-D-galactose in solvents D and F.

The syrup crystallised and was recrystallised from ethyl acetate, m.p. and mixed m.p. 89-91° (with authentic sample of 2,4-di-O-methyl-D-galactose).

Subfraction 14b.

This fraction (0·072 g.), $[\alpha]_D$ +70·6° (c, 0·64) was chromatographically and ionophoretically pure and identical to standard 2,3-di-O-methyl-D-galactose. It was finally characterised by conversion to the aniline derivative which after recrystallisation from ethyl acetate was coloured brown had m.p. - 122-124°. After a second recrystallisation it had m.p. and mixed m.p. - 124-126° (with authentic sample of 2,3-di-O-methyl-N-phenyl-D-galactosylamine melting at 142-144°). X-Ray

powder photograph of the aniline derivative was identical to that of an authentic sample.

Fraction 15. 2,4-Di-O-methyl-D-galactose.

This fraction (0·337 g.), R_G 0·50 was crystalline and had $[\alpha]_D$ +104.5 → +90.6° (equil.) [c, 0.29].

It was chromatographically pure and indistinguishable from standard 2,4-di-O-methyl-D-galactose, in three different solvent systems.

After recrystallisation from aqueous acetone it had an m.p. 88-94°. A small quantity of this was recrystallised from ethyl acetate and had m.p. and mixed m.p. 103° (with authentic 2,4-di-O-methyl-D-galactose).

It was finally characterised by preparing the aniline derivative which crystallised on evaporation of solvent. After recrystallisation from ethyl acetate it had m.p. - mixed m.p. 215-216·5° (with authentic 2,4-di-O-methyl-N-phenyl-D-galactosylamine melting at 212-214°).

Fraction 16. 2,4-Di-O-methyl-D-galactose and rhamnose.

Chromatography of this fraction (0·268 g.), R_G 0·52 and 0·42, in solvents D and F showed it to be a mixture of the above two sugars.

This was fractionated on thick paper using solvent F.

Subfraction 16a. Crystalline (0·205 g.).

Chromatographically pure and identical to 2,4-di-O-methyl-D-galactose. Recrystallised from ethyl acetate it had m.p. - mixed m.p. - 90-91° (with authentic sample of 2,4-di-O-methyl-D-galactose).

Subfraction 16b.

This fraction (0·026 g.) $[\alpha]_D$ +6·35 → +12·7° [c, 0·63] was chromatographically pure and identical to rhamnose in solvents A and D. Crystallised on seeding and was recrystallised from aqueous acetone. m.p. 86-88°.

Fraction 17. 2-O-Methyl-D-galactose, traces of rhamnose and an unknown.

Chromatography in solvents D, F and G showed this fraction (0·037 g.) to be a mixture of 2-O-methylgalactose, rhamnose and a third component which gave a pink stain with aniline oxalate and was chromatographically faster than 2-methylgalactose in solvent D and moved at the same rate as 2,4-dimethylgalactose in solvent F.

The syrup was separated into two subfractions on thick paper using solvent F, irrigated for 88 hours.

Subfraction 17a.

This fraction (0·010 g.) was suspected to be a monomethylarabinose. In solvent D it showed a component moving faster than 2-O-methyl-L-arabinose. In solvents A and D it moved faster than arabinose, ribose, xylose and lyxose.

Periodate oxidised 3-O-methyl-D-xylose gave a brown spot (R_G 0·74) and a yellow spot (R_G 0·22), 4-O-methyl-D-xylose gave a brown spot (R_G 0·83) on chromatographic examination in solvent D. Since this fraction on periodate oxidation gave a grey spot (R_G 0·74), the possibilities of it being a 3-O-methyl- or a 4-O-methyl pentose can be ruled out.

Subfraction 17b. Crystalline (0·017 g.).

Chromatography in different solvents showed this fraction to contain mostly 2-O-methyl-D-galactose with a trace of rhamnose.

Fraction 18. 2-O-Methyl-D-galactose and a trace of the above unknown (Fraction 17a).

This fraction (0·099 g.), R_G 0·29, was crystalline and had $[\alpha]_D$ +78·2° [c, 0·42].

Chromatography in solvents D and F showed it to be mainly 2-O-methyl-D-galactose with a trace of the unknown (pink colour with aniline oxalate) encountered in the previous fraction.

After two recrystallisations from aqueous acetone it had m.p. and mixed m.p. - 153-155° (with authentic sample of 2-O-methyl-D-galactose melting at 157°).

Fraction 19 and 20. 2-O-Methyl-D-galactose.

Combined after preliminary chromatographic examination.

This fraction (0·427 g.), R_G 0·30, was crystalline and had $[\alpha]_D$ +40·5→ +85·0° (equil.) [c, 0·49]. It gave a brown stain with aniline oxalate which was flourescent in u.v. light.

It was chromatographically pure and indistinguishable from 2-O-methyl-D-galactose, in three different solvent systems. After recrystallisation from aqueous acetone it had m.p. - 154-155° and mixed m.p. - 155-156° (with authentic sample of 2-O-methyl-D-galactose melting at 157°).

Fraction 21. 2,3,4-Tri-O-methyl-D-galacturonic acid (3?)-O-methyl-D-galactose, galactose and arabinose.

This fraction (0·097 g.), R_G 0·12, was partially crystalline,

but the greasy crystals could not be separated from the adhering oil.

Paper chromatographic examination in solvents A, D, F and G was not very satisfactory due to streaking, but gave indications for the presence of at least the last three mentioned sugars. In solvent E a component (pink stain with aniline oxalate) separated well from the others, but the three neutral sugars were overlapping. However chromatography in solvent B gave a clear picture, it showed four well separated spots, the acidic component was very much faster than the other three.

The syrup was separated into four subfractions on thick paper using solvent B.

Subfraction 21b.

Chromatography of this fraction (0·012 g.) showed arabinose and a trace of 3-O-methyl-D-galactose.

Subfraction 21a. (0·007 g.).

Chromatographically indistinguishable from galactose.

Subfraction 21c. (0·013 g.)

Chromatographically indistinguishable from 3-O-methyl-D-galactose. Chromatography of the periodate oxidised product gave a pink spot (R_G 0·40), [mono-methyl pentose]. The syrup was seeded and left in a desiccator, but failed to crystallise. All attempts to crystallise it from a solvent were also unsuccessful.

Subfraction 21d.

This fraction (0·010 g.) which was obviously an acidic component, was converted to the methyl ester methyl glycosides

with dry methanolic hydrochloric acid, reduced with lithium aluminium hydride in tetrahydrofuran and hydrolysed with hydrochloric acid. Chromatographic examination of the product in solvent D showed 2 spots, a heavy pink/brown spot (R_G 0·66) corresponding to 2,3,4-tri-O-methyl-D-galactose and a weak pink spot (R_G 0·38). This second spot did not show up in solvents E and B, hence this is probably due to unreduced 2,3,4-tri-O-methyl-D-galacturonic acid which would move at the same speed as 2,3,4-tri-O-methylgalactose in these two solvents.

Fraction 22. Acidic methylated sugars.

This fraction (0·045 g.) obtained by water elution was completely free of neutral methylated sugars and was therefore combined with the bulk of the acidic methylated sugars for further examination.

Examination of the acidic methylated sugars.

The barium salts of the acidic methylated sugars obtained from the preliminary separation together with fraction 22 (1·025 g.) was dissolved in water and treated with Amberlite resin IR 120 (H). The brown syrup obtained on concentration was refluxed with 2% methanolic hydrochloric acid (150 ml.) for 6 hr., neutralised with silver carbonate and concentrated to a syrup. The product was dissolved in anhydrous tetrahydrofuran, lithium aluminium hydride added, the mixture refluxed for one hour, more lithium aluminium hydride added and refluxed for a further hour. Excess hydride was destroyed by addition of ethyl acetate and water alternatively. The solution was /filtered and the residue ~~was~~ repeatedly

extracted with hot acetone and chloroform. The combined extracts and filtrate were taken to dryness and the product was extracted with dry chloroform and again concentrated to a syrup (0·870 g.). The reduced material was hydrolysed with N-hydrochloric acid (100 ml.) at 100° for 6 hr., neutralised with silver carbonate and concentrated to a syrup (0·760 g.).

This mixture of methylated sugars was separated into seven fractions by chromatography on a cellulose column (45 x 2·5 cm.) using light petroleum (b.p. - 100-120°)-butan-1-ol (7:3, then 1:1) both saturated with water and finally butan-1-ol half saturated with water as eluants.

Fraction	Tubes	R_G	Colour	Wt. in mg.	Contents
1	71-100	0·75	Pink/brown	17·4	2,3,4-tri-O-methyl-D-galactose
2	101-139	0·75	Pink and khaki	103·6	2,3-di-O-methyl-D-glucose, 3-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-galactose (trace)
3	140-156	0·66	Khaki	36·7	3-O-methyl-L-rhamnose
4	157-177	0·51 0·60	Pink/brown Khaki	30·3	2,3-di-O-methyl-D-galactose and 3-O-methyl-L-rhamnose
5	178-228	0·52	Pink/brown	180·2	2,3-di-O-methyl-D-galactose
6	229-290	0·51 0·40	Pink/brown Khaki	96·6	2,3-di-O-methyl-D-galactose 2,4-di-O-methyl-D-galactose and rhamnose (trace)
7	291-350	0·50 0·35	Pink Brown	58·8	Unknown and 2-O-methyl-D-galactose.

Fraction 1. 2,3,4-Tri-O-methyl-D-galactose

This fraction (0·017 g.), R_G 0·75, $[\alpha]_D$ +68·0⟶+100·4° (equil.) [c, 0·26], was paper chromatographically pure and indistinguishable from 2,3,4-tri-O-methyl-D-galactose in solvents D, F and G. It was characterised by conversion to its aniline derivative which after recrystallisation from ethyl acetate had m.p. and mixed m.p. - 168° (with authentic sample melting at 161-2°).

Fraction 2. 2,3-Di-O-methyl-D-glucose, 3-O-methyl-L-rhamnose and a trace of 2,3,4-tri-O-methyl-D-galactose.

This fraction (0·104 g.), R_G 0·75 and 0·66, had $[\alpha]_D$ +33·5°⟶+39·4° (equil.) [c, 1·01].

Paper chromatography in solvents D and G showed only two components with chromatographic speeds the same as trimethylgalactose and mono-methylrhamnose. In solvent F it separated into three components, two of them close to each other and having the same speed as mono-methylrhamnose and di-O-methylglucose, the third, which was very light, corresponded to 2,3,4-trimethylgalactose and separated well from the above two. Paper ionophoresis showed two components having the same mobility as 3-O-methylrhamnose and 2,3-di-O-methylglucose.

Chromatographic examination of the products obtained on periodate oxidation of the original syrup and on periodate oxidation of the borohydride reduced syrup, gave indications that this fraction consists of the above mentioned sugars. The

chromatographic patterns were compared with those of periodate oxidised authentic samples of these sugars and other suspected sugars such as 3,4-di-O-methyl-D-mannose (which would give the same products as 3,4-di-O-methyl-D-glucose).

This fraction was separated on thick paper using solvent F into 3 components. No attempts were made to collect the trimethylgalactose which was a minor component as the paper had to be irrigated for a long time to get a good separation of the major components and by that time the trimethylgalactose had run off the paper.

Subfraction a.

This fraction (0·036 g.), $[\alpha]_D$ +32·2° (c, 0·30), was chromatographically pure and identical to 3-O-methyl-L-rhamnose, crystallised after long time in the desiccator m.p. 108-114°.

Subfraction b.

Paper chromatography showed this fraction (0·021 g.) to be a mixture of 2,3-di-O-methyl-D-glucose and 3-O-methyl-L-rhamnose.

Subfraction c.

This fraction (0·031 g.) was partially crystalline and had $[\alpha]_D$ +13·0 → +17·3° (equil.) [c, 0·69].

Periodate oxidation gave the same product as authentic 2,3-dimethylgalactose. It was finally characterised by preparing the phenyl hydrazide of the aldonic acid.

The lactone of the aldonic acid of the methylated sugar was prepared in the usual manner. To the partially crystalline lactone an equimolar portion of redistilled phenylhydrazine was

added and the mixture heated on boiling water bath for one hour. Excess phenylhydrazine was removed by ether extraction and the residue was recrystallised from ethyl alcohol. m.p. and mixed m.p. - 173-175° (with derivative prepared from an authentic sample of 2,3-di-O-methyl-D-glucose which had a m.p. of 174-175°).

Fraction 3. 3-O-Methyl-L-rhamnose.

This fraction (0·037 g.), R_G 0·66, $[\alpha]_D$ +10·0 → +34·6° (equil.) (c, 0·41), was indistinguishable from 3-O-methylrhamnose in three different solvent systems. The syrup crystallised on standing in an ice box m.p. - 108-114°. Recrystallised from methanol-ether with difficulty it had m.p. and mixed m.p. - 106-108° (with authentic sample of 3-O-methyl-L-rhamnose melting at 115-116°). The X-ray powder diagram was identical with that of the authentic sample.

Fraction 4. 3-O-Methyl-L-rhamnose (main) and 2,3-di-O-methyl-D-galactose.

This fraction (0·030 g.), had R_G 0·60 and 0·51 and $[\alpha]_D$ +41·5° [c, 0·39].

Paper chromatography in solvents D and F gave a poor separation, but in solvent G the separation was better and the components corresponded to 3-O-methylrhamnose and 2,3- or 2,6-dimethylgalactose.

Demethylation gave rhamnose, galactose and traces of 2- and 3-monomethylgalactoses.

Periodate oxidation and chromatographic examination gave evidence for the presence of 3-O-methyl-L-rhamnose and 2,3-di-O-methyl-D-galactose. No methoxymalondialdehyde was detected,

thus showing the absence of 2,6-di-O-methyl-D-galactose.

Fraction 5. 2,3-Di-O-methyl-D-galactose.

This fraction (0.180 g.), had R_G 0·52 and $[\alpha]_D$ +85·0° [c, 0·57].

Paper chromatography in solvent system D, F and G showed it to be either 2,3- or 2,6-di-methylgalactose. Paper ionophoresis showed a single spot 3·4 cm. from the origin, standard 2,3-di-O-methylgalactose moved 3·6 cm. on the same paper. Periodate oxidation and chromatographic examination of the products confirmed the above observation, giving the characteristic pattern of 2,3-di-O-methylgalactose. It was finally characterised by preparing the aniline derivative, which on recrystallisation from ethyl acetate gave colourless short needles of m.p. - 133-135° and mixed m.p. - 131-132° (with authentic sample of 2,3-di-O-methyl-N-phenyl-D-galactosylamine of m.p. - 135-137°).

Fraction 6. 2,3-Di-O-methyl-D-galactose,
2,4-di-O-methyl-D-galactose and rhamnose.

This fraction (0.097 g.), had R_G 0·51 and 0·40 and $[\alpha]_D$ +35·7° $\rightarrow$ +55·7° (equil.) [c, 0·50].

Paper chromatography in solvents D and G showed two components corresponding to rhamnose and dimethylgalactose. In solvent F it separated into a khaki spot (rhamnose) and two other spots close to each other and of different colour (pink and brown); these had the same speed as 2,4- and 2,3-di-O-methylgalactoses respectively.

The mixture was separated on thick paper using solvent F into three fractions.

Subfraction a.

This was fraction (0·018 g.), chromatographically pure and identical to rhamnose in solvents A and D. The syrup crystallised and was recrystallised from aqueous acetone. m.p. - 90-92°.

Subfraction b. Crystalline.

This fraction (0·014 g.) was chromatographically pure and identical to 2,4-di-O-methyl-D-galactose. The crystals melted in the range 82-85°.

Subfraction c.

Paper chromatography showed this fraction (0·028 g.) to be mainly 2,3-di-O-methyl-D-galactose with a trace of 2,4-dimethylgalactose.

Chromatographic examination of the periodate oxidised product gave the pattern characteristic of 2,3-dimethylgalactose. It was finally characterised by preparing the aniline derivative, which crystallised after two months and was recrystallised from ethyl acetate m.p. 110-112°(?).

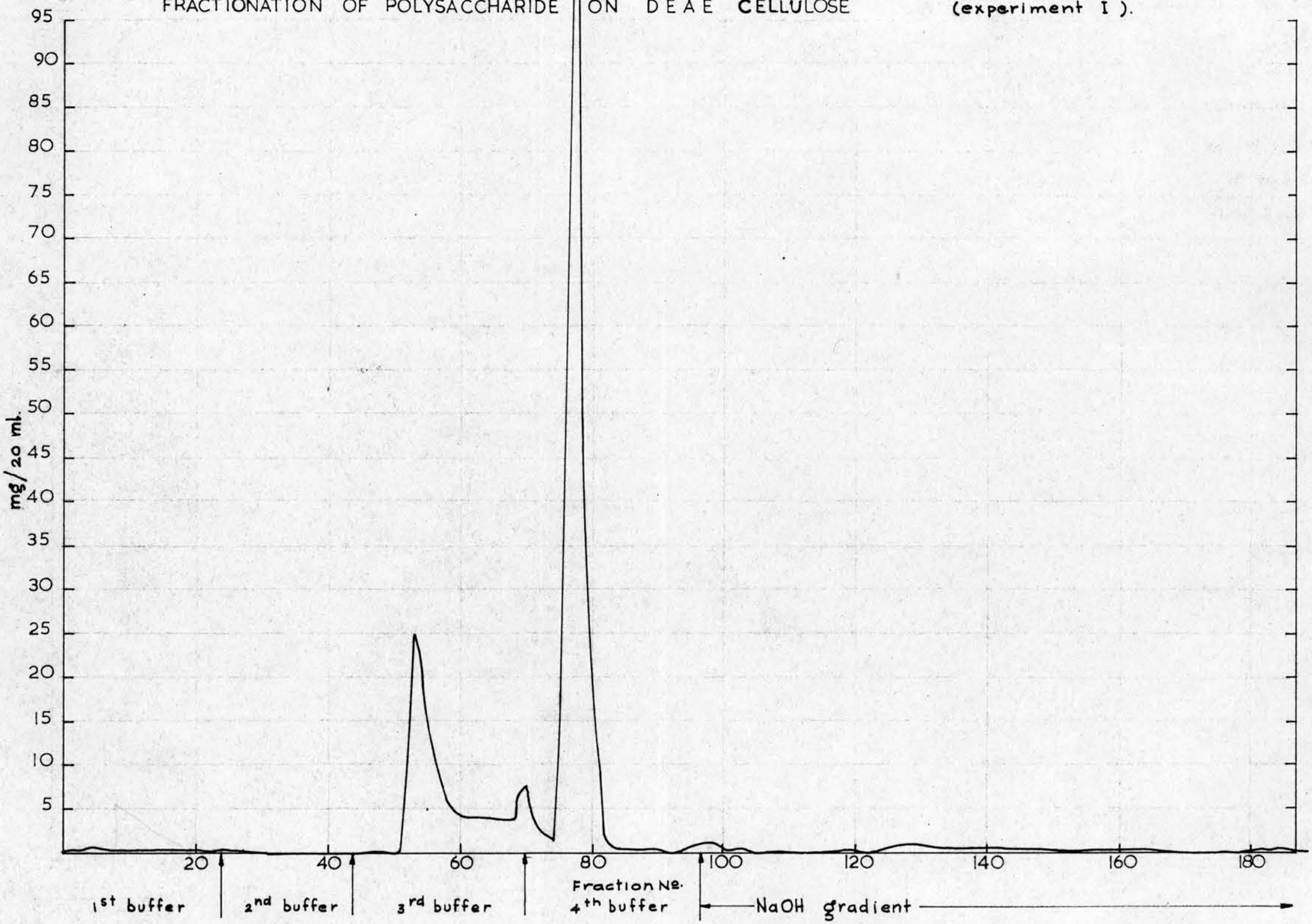
Fraction 7. An unknown and 2-O-methyl-D-galactose.

This fraction (0·059 g.), R_G 0·50 and 0·35, had $[\alpha]_D$ +76·0° [c, 0·20].

Paper chromatography in solvent D showed a component moving slightly faster than 2-O-methylgalactose and a pink unidentified

spot. In solvents F and G the pink spot had the same speed as 2,4-dimethylgalactose. This spot did not correspond to either 4- or 3-O-methylglucose. Periodate oxidation gave a yellow spot (methoxymalondialdehyde). Demethylation gave a trace of glucose, galactose and 2-O-methylgalactose.

FRACTIONATION OF POLYSACCHARIDE ON DEAE CELLULOSE (experiment I).



DEAE-cellulose column chromatography.

Experiment I. (With polysaccharide uronic anhydride 13.9%).

The purified polysaccharide (600 mg.) was dissolved in water (20 ml.), run on to the DEAE-cellulose column (40 x 2 cm.) and left to stand overnight.

The column was first eluted successively with

a) 0·025M (500 ml.) b) 0·05M (500 ml.)

c) ~~0·25M~~ 0·10M (500 ml.) d) 0·25M (500 ml.)

of sodium dihydrogen phosphate buffer pH 6. Any remaining polysaccharide was finally eluted by gradient elution with sodium hydroxide (0-0·3M, 2 litres).

Fractions (20 ml.) were collected every half hour and the amount of polysaccharide present in the fractions was determined by the phenol-sulphuric acid method, using a calibration curve based on the polysaccharide.

The curve obtained by plotting the polysaccharide concentration in each fraction against the fraction number is shown in fig.1.

The uronic acid content of the fractions (given below) were determined by the carbazole method using a calibration curve based on galacturonic acid.

Fraction No.	53	59	70	76	77	79
% Uronic anhydride	11·0	11·1	12·4	16·3	17·9	16·8

Fractions were bulked as indicated in table 5, reduced in

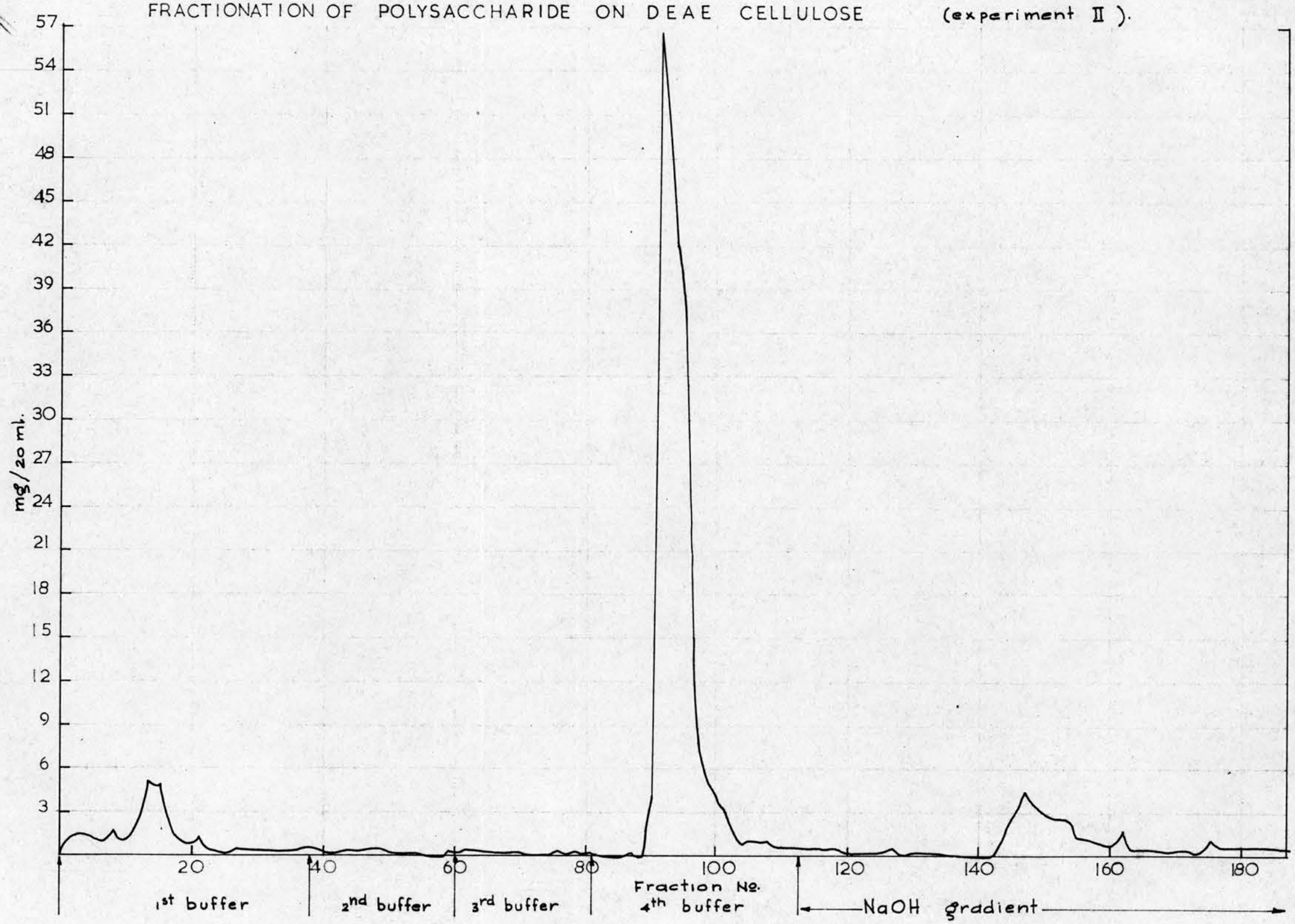
volume, dialysed, treated with Amberlite resins IR 120 (H) and IR 4 B(OH), and freeze dried. The yield estimated by the phenol-sulphuric acid method and actually isolated, specific rotation and uronic anhydride content of the fractions are given in table 5. Values for the original polysaccharide are also included.

Fraction	Tubes	Yield in mg.		% Uronic anhydride		$[\alpha]_D$ in 0·1N-sodium hydroxide
		Estimated	Actually isolated	Carbazole	decarboxylation	
I	52-74	173	156	11·5	10·3	-12±2 (c,0·92)
II	75-84	390	354	17.0	15·9	-9±2 (c,1·03)
Polysaccharide	-	-	-	16.5	13·9	-9·8°(c,1·01)

Table 5.

These fractions (I and II) were hydrolysed with 0·5N-sulphuric acid for 2 hr. and 4 hr., worked up in the usual way and examined chromatographically. Both fractions gave galactose, arabinose, rhamnose and 1,6-galactobiose, no other oligosaccharides could be detected. The syrups were placed on charcoal in a sintered glass crucible and eluted first with water (300 ml.) and then with aqueous ethanol (10%, 300 ml.). The eluates were concentrated and examined chromatographically in solvents A and B. The water eluate in both cases had galactose, arabinose, rhamnose, 1,3-galactosylarabinose (trace) and traces of two unidentified sugars (R_{Gal} 0·19 and 0·28) both

FRACTIONATION OF POLYSACCHARIDE ON DEAE CELLULOSE (experiment II).



of which gave pink stains with aniline oxalate. The ethanol eluates in both cases had mainly 1,6-galactobiose together with some 1,3-galactosylarabinose.

Hydrolysis with N-sulphuric acid for 8 hr. at 100° followed by chromatography of the products in solvent A (5 days) and then in solvent B (2 days) showed the presence of 2-O-glucuronosylgalactose in both the fractions.

Experiment II. (With polysaccharide uronic anhydride 15·8%).

The polysaccharide (300 mg.) was used in this experiment. The procedure was exactly the same as in experiment I, except that the anthrone colorimetric method was used to determine the amounts of polysaccharide in the fractions.

The curve obtained is shown in Fig.2 and the results are tabulated below.

Fraction No.	13	15	91	92	95	97	145	147
% Uronic anhydride (carbazide method)	0	0	13·2	13·6	13·8	13·4	16·5	17·2

Fraction	Tubes	Yield in mg.		% uronic anhydride		$[\alpha]_D$ in 0·1N-sodium hydroxide
		Estimated	Actually isolated	Carbazole	decarboxylation	
I	7-21	19·7	20·0	0	n.d	+12±2 (c,0·68)
II	89-108	203·2	160·2	13·5	15·3	-17±1 (c,0·96)
III	143-162	32·9	28·0	16·9	n.d	-20±5 (c,0·40)
Polysaccharide	-	-	-	13·6	15·2	-15·9° (c,3·98)

Hydrolysis of fraction I with 0·5N-sulphuric acid for 2 hr. and with 2N-sulphuric acid for 4 hr. gave galactose and arabinose only.

Hydrolysis of fraction II with 2N-sulphuric acid for 4 hr. gave galactose, arabinose, rhamnose, 1,6-galactobiose, 2-O-galacturonosylrhamnose and 6-O-glucuronosylgalactose.

Hydrolysis of fraction III with N-sulphuric acid for 6 hr. and with 2N-sulphuric acid for 4 hr. gave galactose, arabinose, 1,6-galactobiose, 2-O-galacturonosylrhamnose and 6-O-glucuronosyl-galactose (slight trace).

Experiment III. (Large scale fractionation of the polysaccharide used in experiment II).

The polysaccharide (2 g.) dissolved in water (25 ml.) was run on to a DEAE-cellulose column (70 x 3·5 cm.), and allowed to soak in overnight.

The column was eluted successively with the following:
a) 0·025M sodium dihydrogen phosphate buffer pH 6 (1·7 l.)
b) 0·25M sodium dihydrogen phosphate buffer pH 6 (2 l.)
c) 0·30M sodium hydroxide (1 l.).

The eluant b was changed after making sure, by testing a few ml. of the eluate with phenol-sulphuric acid, that no more polysaccharide was coming off with the preceding eluant.

The eluates were collected separately and an estimate of the amount of polysaccharide in each eluate was obtained by the phenol-sulphuric acid method.

To isolate the polysaccharides, the eluates were evaporated

to a small volume and dialysed against tap water for ten days.

A precipitate was formed in the dialysis bag, which on removal by centrifuging and hydrolysing gave traces of glucose and amino acid (detected on paper chromatogram using a solution of ninhydrin for spraying). It dissolved easily in dilute nitric acid and gave a bright yellow precipitate with ammonium molybdate indicating that most of it was inorganic phosphate.

The centrifugates were passed through columns of Amberlite resins IR 45 and IR 120, concentrated to a small volume and freeze dried. The polysaccharides thus obtained were found to contain a lot of extraneous matter, mostly inorganic phosphate. Hence these fractions were again dissolved in water and dialysed against distilled water (changed every 24 hr. and kept stirred all the time) for five days. The dialysates, which now gave a negative test for phosphates, were then freeze dried. However, nothing was obtained from eluate (a).

A second lot (2 g.) of the same polysaccharide was fractionated in a similar manner, on the same DEAE-cellulose column after regenerating it to the phosphate form.

The results of these two fractionations are tabulated below.

Eluant	Fraction	Fractions of the first lot(2g.)					Fractions of the second lot (2g.)				
		Wt. in mg.		% Uronic anhydride		$[\alpha]_D$	Wt. in mg.		% Uronic anhydride		$[\alpha]_D$
		Estimated	Isolated	Carbazole	decarboxylation		Estimated	Isolated	Carbazole	Decarboxylation	
0·025M phosphate buffer	I	114	-	-	-	-	n.d	31	4·2%	n.d	+4±2
0·25M phosphate buffer	II	1415	720	15·3	14·7	-5±2	n.d	890	16·0	15·4	-8±2
0·30M sodium hydroxide	III	540	250	20·6	n.d	-10±2	n.d	100	20·3	n.d	-3±2

Examination of the fractions.

Fraction I.

A sample was hydrolysed with 0·5N-sulphuric acid for 1 hr. and products examined chromatographically in solvents A and B. Galactose, arabinose and a small amount of xylose were detected in both solvent systems. In the acid solvent a sugar moving between galactose and arabinose and giving a yellow stain with spray reagent I was also detected.

Hydrolysis with N-sulphuric acid for 3 hr. gave 1,6-galactobiose in addition to the above mentioned sugars, but no aldobiouronic acids were detected.

Fraction II.

On hydrolysis with N-sulphuric acid for 8 hr. followed by chromatography in solvent A, galactose, arabinose, rhamnose, 1,6-galactobiose, and 1,3-galactosylarabinose (trace) were detected. Chromatography of the products in solvent A (5 days) followed by solvent B (2 days) showed the presence of 2-O-galacturonosylrhamnose, 6-O-glucuronosylgalactose and a trace of an unidentified sugar (R_{GalA} 0·60 in solvent B) which gives a brown stain appearing black in u.v. light with spray reagent I.

The polysaccharide (300 mg.) was methylated by six additions of methyl sulphate (2 ml.) and sodium hydroxide (30%, 5 ml.) in an atmosphere of nitrogen and at low temperatures. After heating the reaction mixture for half an hour on a boiling water bath, it was extracted with chloroform. The chloroform extract was dried, concentrated to a small volume and the

methylated polysaccharide (302 mg.) precipitated by addition of light petroleum (b.p. 100-120°). The partially methylated polysaccharide was subjected to three treatments with methyl iodide and silver oxide. The methylated polysaccharide (102 mg.) recovered had OMe, 39·6% and showed a very small hydroxyl peak in the I.R absorption spectra.

Another treatment with the above reagents gave the fully methylated polysaccharide (72 mg., OMe, 40·6%) which was methanolysed and the products were examined by vapour phase chromatography. The results are compared (see below) with those obtained from fraction III.

Fraction III.

Hydrolysis of this fraction with N-sulphuric acid for 8 hr. gave the same sugars (neutral and acidic) as fraction II, except that the unidentified sugar (R_{GalA} 0·60 solvent B) was absent in this case.

The polysaccharide (250 mg.) was methylated as above. After six treatments with methyl sulphate and sodium hydroxide, the partially methylated polysaccharide (259 mg.) was recovered and treated thrice with methyl iodide and silver oxide. The methylated polysaccharide (15 mg.) which gave negligible hydroxyl peak in the I.R absorption spectra, was methanolysed and examined by vapour phase chromatography.

Vapour phase chromatographic examination of the methanolysed products of methylated fractions II and III.

These products examined in all three systems gave identical patterns, indicating that the components present in both cases

were the same. However, there were slight differences in the proportions of the components present as indicated by the height of the peaks. The most obvious difference in proportion was in that of 2,3,5-tri-O-methyl-L-arabinose which was present in a comparatively small amount in fraction III. It is very probable that this difference in proportions is mainly due to the loss of the very volatile methyl-2,3,5-tri-O-methyl-L-arabinoside during the working up of the methanolysis products.

It is not possible to interpret unambiguously the complicated patterns obtained. Since we already have an idea of the possible methylated sugars present, from studies on methylated whole gum, we can assign the major peaks with fair accuracy. The following table gives the T values of the methyl glycosides of the methylated sugars present. When a T value corresponds to more than one methyl glycoside it is given within parenthesis.

Methyl glycosides of	T in system b	T in system c
2,3,5-Tri-O-methyl-L-arabinose	0·55 & (0·72)	0·47 & (0·59)
2,3,4-tri-O-methyl-L-arabinose	(1·02)	(0·84)
2,3-di-O-methyl-L-arabinose	1·56 & 1·78	0·64-0·65 & (0·84)
2,5-di-O-methyl-L-arabinose	-	0·71 & (1·03)
3,4-di-O-methyl-L-rhamnose	(0·72)&(1·02)	(0·59)
3-O-methyl-L-rhamnose	3·63 & 5·38	(1·03)
2,3,4,6-tetra-O-methyl-D-galactose	1·80(S)	1·52-1·53 & (1·62)
2,3,4-tri-O-methyl-D-galactose	(4·71) & 7·50	2·64 & 2·94
2,3,6-tri-O-methyl-D-galactose	(3·26),4·13 & (4·71)	(1·62) & 2·52(S)
2,4-di-O-methyl-D-galactose	-	(3·74) & 4·40
2,3-di-O-methyl-D-galacturonic acid	5·36	2·25
Unknown I	2·53 & (3·26)	-
Unknown II	0·46	-
Unknown III	-	2·52,3·24 & (3·74)

When T is not of a peak, but that of a shoulder on a peak it is denoted by a '(S)' after the value.

All the methylated sugars except those asterisked, were isolated and identified from the hydrolysis products of

methylated whole gum. The T's of the unknowns I and II corresponds to those of methyl-2,3,4-tri-O-methyl-D-glucuronic acid and of methyl-2,3,4-tri-O-methyl-L-rhamnoside (and/or) methyl-2,3,4-tri-O-methyl-D-xyloside respectively. None of these sugars were detected in the hydrolysis products of methylated whole gum. While unknown III is most probably methyl-2,3-di-O-methyl-D-galactoside, which however has been isolated and characterised from the methylated whole gum.

Smith degradation on the reduced gum.

Acetylation of the gum acid.

The gum acid (20 g.) dried in a vacuum oven at 40-50° over sodium hydroxide was dispersed in formamide (250 ml.) by stirring at 45-50°. Pyridine (135 ml.) was added slowly over a period of half an hour, with vigorous stirring. The reaction mixture was cooled to 30° and acetic anhydride (50 ml.) was added with stirring over a period of one and a half hours. A second portion of acetic anhydride (50 ml.) was added likewise and the reaction mixture was allowed to stand overnight at room temperature. Next day a further portion of acetic anhydride (20 ml.) was added and stirring continued for one hour. The mixture was poured into water (1·5 l.) containing concentrated hydrochloric acid (20 ml.) and crushed ice. After stirring for half an hour, the precipitate was filtered and washed with hydrochloric acid (0·5%) followed by water. The precipitate was resuspended in water, allowed to stand overnight, filtered and dried in a vacuum oven at 30-50° over sodium hydroxide for four days (31·0 g.).

Reduction of the gum acetate with diborane.

The gum acetate (31 g.) was completely dissolved in 1,2-dimethoxyethane (400 ml.) which had been dried over sodium and then distilled from lithium aluminium hydride. Sodium borohydride (8 g.) was added with stirring, it did not dissolve completely. To the mixture, boron trifluoride etherate (60 g. in 100 ml. of 1,2-dimethoxymethane) was added in portions

(15 ml.) every 10 min. After each addition the flask was stoppered with a rubber bung and shaken by hand, gently at first and then vigorously to break up any gel formed. On the first addition of boron trifluoride the whole mixture solidified, hence more 1,2-dimethoxyethane (200 ml.) was added. At the end of the additions, the flask was stoppered and allowed to stand overnight. The mixture was then poured into ice and water (1 l.) and neutralised with dilute sodium hydroxide. Chloroform was added and the resulting emulsion was concentrated to a thick syrup. The syrup was taken up in sodium hydroxide (300 ml., 0·1N), the pH of the solution adjusted to 9 by addition of strong alkali and warmed for half an hour at 50-60°. The reaction mixture was dialysed first against running tap water for three days, followed by changes of distilled water for two days. The dialysed solution was filtered, to remove some precipitate formed in the dialysis bag, and the filtrate was concentrated to a thick syrup. This syrup was poured into a stirred mixture of ethanol (750 ml.) and ether (250 ml.) and the white precipitate was filtered, washed with acetone and dried. The gum was redissolved in water, treated with Amberlite resins IR 120 (H) and IR 45 (OH) concentrated to a small volume and freeze dried. The reduced gum (15 g.) had a uronic anhydride content of only 1·4%, whereas the original gum acid had a uronic anhydride content of 13·9%.

A small sample of the reduced gum was hydrolysed with N-sulphuric acid for 8 hr. at 100°. Paper chromatography of the

products in different solvent systems showed the presence of only a trace of acidic material (identical to 2-O-D-galacturonosyl-L-rhamnose). However, it was not possible to detect any glucose among the hydrolysis products. This is not surprising, since on heavy spotting the galactose was streaking and would have masked the small amount of glucose present.

Trial Smith degradation.

The reduced gum (1 g.) was dissolved in water (20 ml.) freshly prepared sodium metaperiodate solution (75 ml., 0·1884N) was added and the solution was made up to 100 ml. In another flask 75 ml. of the same periodate solution was diluted to 100 ml. with distilled water. In a third flask 75 ml. of the periodate solution was treated with excess ethylene glycol and the mixture diluted to 100 ml. to give a sodium iodate solution of the same molarity as the above two solutions. All the three solutions were shaken and kept in the dark at room temperature.

After certain intervals of time a 1 ml. aliquot was removed from each solution, diluted 2500 times and the optical density of the resulting solution measured in the Unicam spectrophotometer at 222·5 mμ (99). From these results the consumption of periodate per sugar unit after various intervals of times were calculated. The results are tabulated below.

Time (hr.)	4	11	24
Moles of periodate consumed/sugar unit	0·73	0·75	0·76

After 30 hr. the oxidation was stopped by addition of ethylene glycol (2 ml.) and the solution was dialysed against tap water for three days. The dialysate was concentrated to a small volume (25 ml.), potassium borohydride (200 mg.) was added and the solution was allowed to stand for six hours. At the end of this period, a further portion (200 mg.) of potassium borohydride was added and the solution was allowed to stand overnight at room temperature. The solution was treated with Amberlite resins IR 120 (H) and IR 45 (OH), concentrated to a syrup, methanol was added and the solution taken to dryness.

The residue was dissolved in N-sulphuric acid and allowed to stand at room temperature. After 3 hr. the solution was neutralised by addition of barium hydroxide followed by barium carbonate. The neutralised solution was treated with Amberlite resin IR 120, and concentrated to a small volume. The degraded gum (50 mg.), precipitated by pouring the concentrated solution into excess ethanol, was centrifuged off and washed repeatedly with ethanol and finally with methanol. The combined centrifugate and washings were taken to dryness to give the low molecular weight material (331 mg.).

On chromatographic examination in solvent A and B, using spray reagents I and II this material was found to contain glycerol, threitol(and/or erythritol) and other glycitols, but no free reducing sugars were detected on paper. However, on hydrolysis with N-sulphuric acid for 6 hr. this material yielded galactose, arabinose and rhamnose, in addition to the above

sugar alcohols.

A sample of the degraded gum (see above) on partial hydrolysis with 0·5N-sulphuric acid for half an hour at 100° gave galactose, arabinose, rhamnose, 1,6-galactobiose and an unidentified non-reducing sugar (R_{Gly} 0·91, in solvent B), but no glycerol, threitol or other sugar alcohols were detected in this partial hydrolysate.

Large Scale Smith degradation.

The reduced gum (14 g.) was dissolved in water (500 ml.), sodium periodate solution (45 g. in 500 ml.) was added and the mixture was shaken and allowed to stand in the dark at room temperature. The change in periodate concentration was followed spectrophotometrically, and no further uptake of the oxidant was observed after 26 hours. At the end of 36 hr. excess periodate was destroyed by addition of ethylene glycol and the reaction mixture was dialysed against tap water for three days.

The dialysate was concentrated to a small volume and reduction to the polyalcohol effected by addition of potassium borohydride (3 g.) and leaving the mixture at room temperature for two days. The solution was treated with Amberlite resins IR 120 and IR 45, concentrated to a small volume and poured into excess ethanol containing 5% light petroleum. The polyalcohol separated in a colloidal form and could not be isolated. Hence the solution was taken to dryness and the residue was repeatedly evaporated with methanol to remove borate ions.

The polyalcohol (10·5 g.) thus obtained was dissolved in

N-sulphuric acid (150 ml.) and allowed to stand at room temperature. The hydrolysis was followed by observing the change of optical rotation (α).

Time (hr.)	0	1	2	3
α (in a 0·5 dm. tube)	-0·50°	-0·45°	-0·41°	-0·40°

The hydrolysis was stopped after 3 hr. by neutralisation with barium hydroxide and barium carbonate. The neutralised solution was filtered, treated with Amberlite resin IR 120, concentrated to a small volume and poured into excess ethanol. The degraded polysaccharide separated on adding a few crystals of ammonium acetate. The degraded polysaccharide III, was removed on the centrifuge, washed with ethanol and dried (0·818 g.).

A small sample of this degraded polysaccharide on hydrolysis with 0·5N-sulphuric acid gave galactose, arabinose, 1,6-galactobiose and an unidentified reducing sugar (R_{Rha} 1·10 in solvent A), but no sugar alcohols were detected in the acid hydrolysate.

The centrifugate and alcohol washings were taken to dryness to give the low molecular weight materials (8·7 g.).

Examination of the low molecular weight materials.

Chromatographic examination revealed it to have exactly the same components as the corresponding mixture obtained in the trial experiment described earlier.

An attempt was made to remove most of the glycerol and

threitol by extracting the mixture with dry acetone.

The acetone soluble fraction (1·7 g.) by chromatographic examination in solvents A, B, H and I was found to contain almost entirely glycerol, threitol and glycollic aldehyde, with traces of rhamnose and two unidentified reducing sugars. Hydrolysis of a small sample gave traces of galactose and arabinose. Another sample on reduction with potassium borohydride followed by hydrolysis gave galactose, arabinose, rhamnose, threitol and two unidentified components.

The rest of the acetone soluble fraction was placed on a charcoal-Celite column (45 x 2 cm.) and eluted with water, followed by 20% ethanol and finally with 40% ethanol. The following fractions were collected.

Fraction	Eluant	Wt. in mg.	Contents
S_1	Water	1253	Glycerol, threitol, glycollic aldehyde and traces of arabinose and rhamnose
S_2	water	43	Two non-reducing oligosaccharides (R_{Gal} 0·88 and 1·08 in solvent A)
S_3	20% ethanol	33	Three non-reducing oligosaccharides (above two and third R_{Gal} 0·53 in solvent A).
S_4	40% ethanol	46	A non-reducing oligosaccharide (R_{Gal} 1·08 in solvent A) and a streak from origin.

The acetone insoluble fraction (7·0 g.) on chromatographic examination in solvents B, H and I, using spray reagent II showed

a heavy black streak and a trace of glycerol, whereas spray reagent I failed to give any stains indicating the absence of free reducing sugars.

On hydrolysis with 0·5N-sulphuric acid, this fraction gave galactose, arabinose, rhamnose, 1,6-galactobiose, glycerol, threitol and an unidentified reducing sugar (R_{Rha} 1·10 in solvent A).

A sample (800 mg.) of the acetone insoluble fraction was placed on a Dowex resin X2 (lithium form) column (70 x 2 cm.) and eluted with water. Fractions (1·5-2 ml.) were collected every 12 min. and every fifth fraction was evaporated and examined chromatographically. These chromatograms were streaky and hence did not reveal any fractionation. Therefore, every tenth fraction was hydrolysed by heating at 100° for 1 hr. with Amberlite resin IR 120 (H) and examined chromatographically in solvent A using spray reagents I and II. The fractions were bulked according to the hydrolysis products.

Fraction	Tubes	Wt.in mg.	Chromatography in solvents A & H	Chromatography of hydrolysis products
D_1	1-50	260	Sugar stays on line	Galactose, arabinose, rhamnose & threitol
D_2	51-80	75	Streak. Yellow spots with spray reagent V	Galactose, arabinose, glycerol & threitol
D_3	81-125	154	Glycerol, threitol glycollic aldehyde & streak	Glycerol, threitol, & traces of galactose & arabinose
D_4	126-200	41	Streak	Galactose, arabinose, & traces of glycerol & threitol

The rest of the acetone insoluble fraction (6·2 g.) was fractionated into seven fractions (table below) by dissolving in water and adding increasing amounts of acetone.

Fraction	A_1	A_2	A_3	A_4	A_5	A_6	A_7
Volumes of acetone	2	3	4	5	6	8	Mother liquor
Wt.in mg.	0·231	1·557	0·756	0·380	0·201	0·249	2·411

These fractions were chromatographically examined in solvents A, B, C and H using spray reagents I, II and V, no free reducing sugars were detected in any of the fractions. Fraction A_7 was found to be composed mainly of glycerol and threitol, with small amounts of other slow moving components. Fractions A_1-A_3 did not move to any appreciable extent on chromatograms and thus were composed of high molecular weight materials. Fractions A_4-A_6 did not give a clear pattern on chromatograms due to streaking.

Small samples of the fractions A_1-A_6 were hydrolysed with 0·5N-sulphuric acid for 1 hr. and the products examined chromatographically in solvent using spray reagents I and II. All the fractions gave the following components, but in varying amounts:- galactose, arabinose, rhamnose, threitol, 1,6-galactobiose and at least four unidentified reducing sugars (R_{Rha} 9·90, 1·10, 1·31 and 1·59 in solvent A).

Fraction A (2·411 g.) was placed on a charcoal-Celite column (47 x 2 cm.) and eluted with water followed by increasing

concentrations of ethanol. The following fractions were collected and evaporated to dryness and examined chromatographically.

Fraction	Eluant	Wt. in mg.	Chromatography of fractions
B_1	Water	892	Glycerol and threitol
B_2	water	63	Glycerol, threitol and two non-reducing oligosaccharides
B_3	10% ethanol	84	
B_4	15% ethanol	97	Not clear
B_5	20% ethanol	185	
B_6	40% ethanol	283	
B_7	50% ethanol	78	

Examination of Fraction A_1.

Methylation of A_1. A sample (100 mg.) was methylated by four additions of methyl sulphate and sodium hydroxide solution (30%), followed by two treatments with methyl iodide and silver oxide. The fully methylated product (62 mg.) OMe, 39·9%, $[\alpha]_D$ -31·9° (c, 0·86 in chloroform) was hydrolysed and the products examined by paper chromatography in solvents D and F. The following sugars were detected 2,3,4,6-tetra-O-methylgalactose; 2,3,4-tri-O-methylgalactose; 2,4-di-O-methylgalactose; 2,3,5-tri-O-methylarabinose; 3,4-di-O-methylrhamnose; 2,3-di-O-methylrhamnose; 3-O-methylrhamnose and a trace of mono-O-methylgalactose.

A small sample of the hydrolysis product was methanolysed

and examined by vapour phase chromatography. The presence of the methyl glycosides of the following methylated sugars (table below) were indicated.

Methyl glycosides of	T in system(a)	T in system(b)	T in system(c)
2,3,5-Tri-O-methyl-L-arabinose	0·39	0·55 & 0·72	0·46-0·47 & 0·60
2,3,4,6-tetra-O-methyl-D-galactose	1·29 & 1·41 -1·42	1·80	1·49 & 1·61
2,3,4-tri-O-methyl-D-galactose	1·76 & 1·90	4·74 & 7·50	2·65 & 2·94
2,4-di-O-methyl-D-galactose	-	-	3·75 & 4·44
2,3-di-O-methyl-D-galactose	1·62 & 2·20	-	2·49, (3·75 & 4·44)
3,4-di-O-methyl-L-rhamnose	0·53	1·01	0·60
3-O-methyl-L-rhamnose	0·70	3·63	1·01-1·02

In addition to these, a peak (T 0·60 in system (a) and 1·48 in system (b)) which could be due to methyl 2,3-di-O-methyl-L-rhamnoside and a peak (T 0·45 in system b) which could be due to either methyl-2,3,4-tri-O-methyl-L-rhamnoside or methyl-2,3,4-tri-O-methyl-D-xyloside, were also present.

Partial Acetolysis on fraction A_1.

Another portion (97 mg.) of fraction A_1 was added to a chilled mixture of acetic anhydride (5 ml.) and concentrated sulphuric acid (0·15 ml.). After standing in the ice bath for half an hour, the mixture was mechanically shaken for 6 hr. and

allowed to stand at room temperature. A sample (2·5 ml.) was pipetted out after 18 hr. and the rest was allowed to stand for a further 24 hr. These two samples were separately worked out as described below.

Each sample was poured into ice water (100 ml.), pH of the solution adjusted to 4 with sodium bicarbonate and the mixture was extracted with chloroform (5 times). The chloroform extracts were dried over sodium sulphate, taken to a syrup and dried in vacuum desiccator over sodium hydroxide. The dried syrups were dissolved in dry methanol (2 ml.), chilled and barium methoxide in methanol (5 ml., 0·5N) was added and the mixture was left to stand overnight in the ice box. The basic solutions were poured into water (50 ml.), treated with Amberlite resins IR 120, IR 45, again IR 120 and taken to dryness. Paper chromatographic examination of these products suggested that the deactylation was incomplete. Hence the syrups were again treated with barium methoxide, as described above.

Chromatographic examination in solvent A showed monosaccharides and at least three non-reducing oligosaccharides which gave positive reactions with spray reagents II and V. The product obtained after 24 hr. partial acetolysis had a higher proportion of these oligosaccharides.

Large scale partial acetolysis.

The fractions D_1, D_2, A_1-A_6, B_4-B_6 and the degraded polysaccharide III were all combined (4·86 g.) and dried thoroughly in a vacuum desiccator. The dried material was dissolved

completely in a chilled mixture of acetic anhydride (85 ml.) and concentrated sulphuric acid (2·5 ml.) by mechanical shaking for 7 hr., and the solution was allowed to stand at room temperature for 19 hours. The dark solution was poured into ice water (1 l.), stirred for 15 min. and extracted with chloroform (6 times). Trouble was encountered due to emulsions, which were broken up by centrifuging. The chloroform extract was washed with water, sodium bicarbonate and finally with water. The extract was then dried over anhydrous sodium sulphate, evaporated to a syrup and dried in a vacuum desiccator over sodium hydroxide.

The syrup was dissolved in dry methanol (50 ml.) and deacetylated with methanollic solution of barium methoxide (50 ml., 0·5N). The syrup (3·08 g.) which was isolated as described above still contained acetyl sugars. Hence, the syrup was dissolved in dry methanol (50 ml.) and barium methoxide (1 g.) was added, when a white precipitate was formed. The suspension was allowed to stand for 24 hr. in an ice box and methanol was evaporated off. The residue was taken up in water (200 ml.), and treated with Amberlite resins IR 120, IR 45 and again IR 120. Concentration gave a syrup (2·32 g.), chromatographic examination of which showed that deactylation was almost complete.

The syrup (2·32 g.) was placed on a charcoal column (27 x 3 cm.) and eluted with water followed by increasing concentrations of ethanol. Six fractions were collected, but the chromatographic pattern of these were not clear due to streaking.

One of the fractions (T_2, 157 mg.) was dissolved in water

and extracted with ether in a soxhlet for 18 hr., to remove any polymerised aldehydes which might be the cause of streaking. The ether phase was found to contain mainly glycerol, and threitol with traces of galactose, arabinose and reducing oligosaccharides. The sugars present in the water phase were still streaking on chromatograms.

A sample of the fraction T_2 was reduced with potassium borohydride and examined chromatographically. Chromatograms sprayed with reagent I gave no stains, indicating that reduction was complete and the chromatograms sprayed with reagent II showed several stains but there was streaking, even though less than before reduction.

Another sample of T_2 was heated with N-sodium hydroxide at 100° for 1 hr. and neutralised with Amberlite resin IR 120 (H). On concentration and chromatographic examination no reducing sugars were detected, but at least three non-reducing oligosaccharides giving a positive reaction with spray reagents II and V were found to be present. The alkali treated sample, on acid hydrolysis gave galactose, rhamnose and traces of other sugars.

Yet another sample of T_2 was placed on a small column of the strongly basic Amberlite resin IR 400 (OH) and eluated with water. Chromatographic examination, of the water eluate and of the acid hydrolysis product of this eluate gave similar results as above, but the yields of the oligosaccharides were better in this case.

Bibliography.

1) J. V. Kubal and N. Gralen. J. Colloid. Sci., 1948, 3, 457.

2) F. Smith and R. Montgomery. "The Chemistry of Plant Gums and Mucilages." Reinhold Publishing Corp., New York, N.Y., 1959.

3) E. L. Hirst. "Proceedings of the IVth International Congress of Biochemistry." Vienna, Pergamon Press., 1959, I, 31.

4) J. K. N. Jones and F. Smith. Advances in Carbohydrate Chemistry, 1949, 4, 243.

5) E. L. Hirst. Endeavour. 1951, 10, 106.

6) E. L. Hirst and J. K. N. Jones. "Modern Methods of Plant Analysis." Springer-Verlag., Berlin (1955). Vol.II; P.275.

7) C. T. Greenwood. Advances in Carbohydrate Chem., 1952, 7, 289.

8) D. M. W. Anderson and N. J. King. Talanta, 1961, 8, 497.

9) D. W. Drummond and E. E. Percival. J. Chem. Soc., 1961, 3908.

10) E. Hoaglund, B. Lindberg and J. McPherson, Acta Chem. Scand., 1956, 10, 1160.

11) H. El. Khaden and M. M. Megahed. J. Chem. Soc., 1956, 3953.

12) G. O. Aspinall, M. J. Johnston and A. M. Stephen. J. Chem. Soc., 1960, 4918.

13) S. P. James and F. Smith. J. Chem. Soc., 1945, 739, & 749.

14) R. Montgomery and F. Smith. J. Amer. Chem. Soc., 1957, 79, 695.

15) F. Smith and H. C. Srivastava. J. Amer. Chem. Soc., 1959, 81, 1715.

16) D. G. Easterby and J. K.M. Jones. Nature, 1950, 165, 614.

17) E. L. Hirst and S. J. Dunstan. J. Chem. Soc., 1953, 2332.

18) B. C. Bera, A. B. Foster and M. Stacey. J. Chem. Soc., 1955, 3788.

19) S. A. Barker, M. Stacey and G. Zweifel. Chem. & Ind., 1957, 330.

20) K. C. B. Wilkie, J. K. N. Jones, B. J. Excell and R. E. Semple. Canad. J. Chem., 1957, 35, 795.

21) M. Heidelberger, J. Adams and Z. Dische. J. Amer. Chem. Soc., 1956, 78, 2853.

22) W. W. Binkley. Advances in Carbohydrate Chem., 1955, 10, 55.

23) S. Gardell. Acta Chem. Scand., 1957, 11, 668.

24) A. E. Goodban, and H. S. Owens. J. Polymer Sci., 1957, 23, 825.

25) B. Siegel, G. A. Candela and R. M. Howard. J. Amer. Chem. Soc., 1954, 76, 1311.

26) H. Neukom, H. Deuel, W. J. Heri and W. Kündig. Helv. Chim. Acta, 1960, 43, 64.

27) W. Heri, H. Neukom and H. Deuel. Helv. Chim. Acta, 1961, 44, 1939.

28) G. O. Aspinall and R. Young. Unpublished results.

29) D. H. Northcote. Biochem. J., 1954, 58, 353.

30) A. B. Foster, P. A. Newton-Hearn and M. Stacey. J. Chem. Soc., 1956, 30.

31) I. A. Preece and R. Hobkirk. Chem. & Ind., 1955, 257.

32) D. R. Briggs, E. F. Garner, F. Smith. Nature, 1956, 178, 154.

33) E. J. Bourne, A. B. Foster and P. M. Grant. J. Chem. Soc., 1956, 4311.

34) B. A. Lewis and F. Smith. J. Amer. Chem. Soc., 1957, 79, 3929.

35) B. J. Hocevar and D. H. Northcote. Nature, 1957, 179, 488.

36) F. G. Torto. Nature, 1957, 180, 864.

37) E. L. Hirst, E. E. Percival and R. S. Williams. J. Chem. Soc., 1958, 1942.

38) G. O. Aspinall, E. L. Hirst and A. Nicolson. J. Chem. Soc., 1959, 1697.

39) D. M. W. Anderson, E. L. Hirst and N. J. King. Talanta, 1959, 3, 118.

40) G. O. Aspinall and T. B. Christensen. J. Chem. Soc., 1961, 3461.

41) A. C. Flood, E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1948, 1679.

42) M. Somogyi. J. Biol. Chem., 1952, 195, 19.

43) E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1949, 1659.

44) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Reben and F. Smith. Analyt. Chem., 1956, 28, 350.

45) J. K. Barlett, L. Hough and J. K. N. Jones. Chem. & Ind., 1951, 29, 76.

46) R. Dreywood. Ind. Eng. Chem.; Analyt. Ed., 1946, 18, 499.

47) R. W. Bailey. Biochem. J., 1958, 68, 669.

47) W. N. Howarth, etc. J. Chem. Soc., 1915, 107, 8.

48) W. E. Trevelyan, D. P. Procter, J. S. Harrison. Nature, 1950, 166, 444.

49) D. J. Bell. "Modern Methods of Plant Analysis." Springer-Verlag., Berlin. Vol.II P.13.

50) J. A. Cifonelli and F. Smith. Analyt. Chem., 1954, 26, 1132.

51) R. Consden and W. M. Stanier. Nature, 1952, 169, 783.

52) T. Purdie and J. C. Irvine. J. Chem. Soc., 1903, 83, 1021.

53) R. Kuhn, H. Trischmann, I. Löw. Angew. Chemie, 1955, 67, 32.

54) R. Kuhn, H, H. Baer and A. Seeliger. Annalen, 1958, 611, 236.

55) C. T. Bishop and F. P. Cooper. Canad. J. Chem., 1960, 38, 388.

56) C. M. Fear and R. C. Menzies. J. Chem. Soc., 1926, 937.
E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1938, 496.

57) L. Hough and J. K. N. Jones. Chem. & Ind., 1952, 30, 380.

58) I. Mukat. J. Amer. Chem. Soc., 1934, 56, 2449.
K. Freudenberg and H. Boppel. Ber., 1938, 71, 2505.

59) R. Belcher [illegible]. "Semi-micro Quantitative Organic Analysis." Longmans, Green & Co., 1954.

60) H. S. Isbell, H. L. Frush, B. H. Bruchner, G. N. Kowkabany and G. Wampler. Analyt. Chem., 1957, 29, 1523.

61) G. O. Aspinall, E. L. Hirst and N. K. Matheson. J. Chem. Soc., 1956, 989.

62) I. Croon and B. Lindberg. Acta Chem. Scand., 1957, 11, 192.

63) H. O. Bouveng and B. Lindberg. Advances in Carbohydrate Chem., 1960, 15, 66.

64) L. Hough, J. K. N. Jones and W. H. Wadman. J. Chem. Soc., 1949, 2511.

65) W. J. Whelan and K. Morgan. Chem. & Ind., 1955, 33, 1449.

66) B. Lythgoe, and S. Trippett. J. Chem. Soc., 1950, 1983.

67) E. A. McComb and R. McCready. Analyt. Chem., 1952, 24, 1630.

68) B. Wickberg. Acta Chem. Scand., 1958, 12, 615.
B. Lindberg and B. Wickberg. Arkiv. för Kemi, 1959, 13, 447.

69) L. Hough, J. K. N. Jones and W. H. Wadman. J. Chem. Soc., 1950, 1702.

70) R. U. Lemieux and H. F. Bauer. Canad. J. Chem., 1953, 31, 814.

71) F. Smith. J. Chem. Soc., 1939, 1724.

72) F. Smith. J. Chem. Soc., 1940, 1035.

73) C. N. Turton, A. Bebbington, S. Dixon and E. Pacsu. J. Amer. Chem. Soc., 1955, 77, 2565.

74) P. Andrews and J. K. N. Jones. J. Chem. Soc., 1955, 583.

75) D. J. Manners. Lectures, Monographs and Reports of "The Royal Institute of Chemistry." 1959, No.2.

76) A. J. Charlson. Unpublished results.

77) T. J. Painter. Chem. & Ind., 1959, 47, 1588.

78) T. J. Painter. Chem. & Ind., 1960, 48, 1214.

79) T. J. Painter and W. T. J. Morgen. Nature, 1961, 191, 39.

80) G. O. Aspinall, I. M. Cairncross and A. Nicolson. Proc. of Chem. Soc., 1959, 270.

81) G. O. Aspinall, A. Canaz-Rodriguez. J. Chem. Soc., 1958, 4020.

82) F. Smith and A. M. Stephen. Tetrahedron letters, 1960, No.7, 17.

83) J. M. Bobbit. Advances in Carbohydrate Chem., 1956, 11, 1.

84) A. S. Perlin. Advances in Carbohydrate Chem., 1959, 14, 9.

85) A. J. Charlson, P. A. J. Gorin and A. S. Perlin. Canad. J. Chem., 1956, 34, 1811; 1957, 35, 365.

86) L. Hough, D. B. Powel and B.M. Woods. J. Chem. Soc., 1956, 4799.

87) M. J. Clancy and W. J. Whelan. Chem. & Ind., 1959, 673.

88) R. L. Whistler and D. F. Durso. J. Amer. Chem. Soc., 1950, 72, 677.

89) J. K. N. Jones, R. A. Wall and A. O. Pittet. Canad. J. Chem., 1960, 38, 2285.

90) R. Derungs and H. Deuel. Helv. Chim. Acta, 1954, 37, 657.

91) L. Hough and M. B. Perry. Chem. & Ind., 1956, 768.

92) T. G. Halsall, E. L. Hirst and J. K. N. Jones, J. Chem. Soc., 1947, 1427.

93) V. C. Barry. Nature, 1943, 152, 537.
V. C. Barry and P. W. D. Mitchell. J. Chem. Soc., 1954, 4020.

94) A. M. Unrau. Chem. & Ind., 1960, 1419.
Also I. J. Goldstein, G. W. Hay, B. A. Lewis [redacted] F. Smith. Abstracts of 135th Amer. Chem. Soc. meeting. Boston, Mass., April, 1959, p.3D.

95) R. L. Whistler and J. N. Bemiller. Advances in Carbohydrate Chem., 1958, 13, 289.

95a) D. M. W. Anderson. Talanta, 1959, 2, 73.

96) R. M. Hann and C. S. Hudson. J. Amer. Chem. Soc., 1944, 66, 735.

96a) M. Heidelberger. "Proceedings of the IVth International Congress of Biochemistry." Vienna, Pergamon Press. 1959, Vol.I, 52.

97) A. S. Perlin. Analyt. Chem., 1955, 27, 396.

98) G. O. Aspinall, T. B. Christensen and F. I. Philips. Unpublished results.

99) G. O. Aspinall and R. J. Ferrier. Chem. & Ind., 1957, 1216.

100) P. Andrews, D. H. Ball and J. K. N. Jones. J. Chem. Soc., 1953, 4090.

101) G. O. Aspinall, B. J. Auret and E. L. Hirst. J. Chem. Soc., 1958, 221.

102) G. O. Aspinall, B. J. Auret and E. L. Hirst. J. Chem. Soc., 1958, 4408.

103) F. Smith. J. Chem. Soc., 1939, 744.

104) A. J. Charlson, J. R. Nunn and A. M. Stephen. J. Chem. Soc., 1955, 269.

105) G. O. Aspinall and R. S. Fanshaw. J. Chem. Soc., 1961, 4215.

106) G. O. Aspinall, E. L. Hirst and A. Wickstrøm. J. Chem. Soc., 1955, 1160.

107) G. O. Aspinall and T. B. Christensen. Unpublished results.

108) R. J. McIlroy. J. Chem. Soc., 1957, 4147.

109) N. J. King. Ph.D. Thesis. Edinburgh, 1960.

110) H. O. Bouveng. Acta Chem. Scand., 1961, 15, 78.

111) E. V. White. J. Amer. Chem. Soc., 1953, 75, 4692; 1954, 76, 4906.

112) F. Brown, E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1949, 1761.

113) F. Smith and A. M. Stephen. J. Chem. Soc., 1961, 4892.

114) A. M. Stephen. Unpublished results.

115) E. L. Hirst, L. Hough and J.K. N. Jones. J. Chem. Soc., 1949, 3145.

116) L. Hough and J. K. N. Jones. J. Chem. Soc., 1950, 1199.

117) G. O. Aspinall and Nasir-ud-din. Unpublished results.

118) G. O. Aspinall and M. J. Johnston. Unpublished results.

119) E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1938, 1174; 1939, 1482; 1946, 506.

120) J. K. N. Jones. J. Chem. Soc., 1939, 558; 1947, 1055; 1949, 3141.

121) E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1947, 1064; 1948, 120.

122) F. Brown, E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1949, 1757.

123) J. J. Connell, R. M. Hainsworth, E. L. Hirst and J. K. N. Jones. J. Chem. Soc., 1950, 1696.

124) P. Andrews and J. K. N. Jones. J. Chem. Soc., 1954, 1724.

125) E. L. Hirst and J. K. N. Jones. Research, 1951, 4, 411.

126) P. Andrews and J. K. N. Jones. J. Chem. Soc., 1954, 4134.

127) B. O. Lindgren. Acta Chem. Scand., 1957, 11, 1365.

128) E. V. White. J. Amer. Chem. Soc., 1946, 68, 272; 1947, 69, 622 and 2264; 1948, 70, 367.

129) J. I. Cunneen and F. Smith. J. Chem. Soc., 1948, 1141 and 1146.

130) F. Smith. J. Chem. Soc., 1951, 2246.

131) M. A. Akher, F. Smith and D. Spriestersbach. J. Chem. Soc., 1952, 3637.

132) G. O. Aspinall and J. Baillie. Unpublished results.

133) J. Jackson and F. Smith. J. Chem. Soc., 1940, 79.

134) E. L. Hirst and A. S. Perlin. J. Chem. Soc., 1954, 2622.

135) J. K. N. Jones and G. H. S. Thomas. Canad. J. Chem., 1961, 39, 192.

136) A. M. Stephen. J. Chem. Soc., 1951, 646.

137) A. J. Charlson, J. R. Nunn and A. M. Stephen. J. Chem. Soc., 1955, 1428.

138) R. K. Hulyalker, T. R. Ingle and B. V. Bhide. J. Indian Chem. Soc., 1956, 33, 861; 1959, 36, 31.

139) S. Mukherjee and A. N. Shrivastava. J. Amer. Chem. Soc., 1958, 80, 2536.

140) S. Mukherjee and A. N. Shrivastava. Proc. Indian Acad. Sci., 1959, 50A, 374.

Also Chemical Abstracts, 1960, 22386b.