THE MOLECULAR STRUCTURE OF PLANT GUMS, WITH SPECIAL
REFERENCE TO GUM GHATTI

- by -

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INTRODUCTION.
INTRODUCTION

Plant gums are exudates from the bark, leaves or fruit of certain trees and shrubs of a variety of widely distributed genera. The viscid secretions harden, on drying, into nodules which consist of polysaccharide material together with various impurities.

The chemical and physical properties of the polysaccharide material of gums show a general resemblance to those of the mucilages (1 - 7). Both gums and mucilages are high molecular weight acidic polysaccharides which swell in water to form gels or viscous solutions but are, however, differentiated by their biological environment and function.

In contrast to the exuded gums, mucilages are obtained by aqueous extraction of various plant organs, such as seeds, bark or leaves. In some instances they constitute a protective layer, probably for the retention of moisture, as for example the mucilaginous coating on cress seeds, while in other cases they may provide a food reserve.

The reason for gum formation is uncertain, although gums are known to be produced after mechanical injury of the plant or after invasion by bacteria or fungi. They are also exuded after a period of drought or by a tree in poor health. It is now considered unlikely that micro-organisms are responsible for the actual synthesis of gums, although bacterial infection may stimulate the exudation of material which effectively seals any wound in the tree.

It /
It has been found that gum formation occurs simultaneously with the disappearance of starch granules caused by breakdown of cellular tissue and it therefore appears that gums are synthesized from glucose units. Nothing is known of the mechanism by which the conversion takes place, although this is a problem of fundamental biochemical interest. It has been suggested (8, 9) that pentose sugars could be formed by a process involving oxidation of a hexose at C6 and subsequent decarboxylation:

\[
\begin{align*}
D\text{-glucose} & \rightarrow D\text{-glucuronic acid} \rightarrow D\text{-xylose} \\
D\text{-galactose} & \rightarrow D\text{-galacturonic acid} \rightarrow L\text{-arabinose}
\end{align*}
\]

While it is possible that such a process may take place at the monosaccharide level, it has been shown that there is no direct conversion of polysaccharides. For example, the pectin of *Lupinus albus* contains a furanose 1:5- linked araban and a pyranose 1:4- linked galactan (8). The latter could not, by oxidation and decarboxylation, give rise to a furanose araban, without intermediate hydrolysis and resynthesis.

Gums consist of residues of uronic acid, hexose, pentose and methyl pentose joined by a complicated variety of linkages. The uronic acid is generally present as the calcium or magnesium salt and acids commonly found are D-glucuronic acid and its monomethyl ether and D-galacturonic acid, while neutral sugars most frequently encountered are D-galactose, D-mannose, L-arabinose, D-xylose and L-rhamnose. Hexoses and uronic acids occur in pyranose form, while of the pentoses L-arabinose is generally furanose, D-xylose is generally pyranose and L-rhamnose has only been found in pyranose form.

PURIFICATION
PURIFICATION

Crude nodules of gum contain polysaccharide material together with varying amounts of terpenoid resins, lignin and enzymes and other proteins which may be closely associated or combined with the carbohydrate. In addition, fragments of bark, dust and insects may be trapped in the gum. Purification is effected by solution in water followed by filtration and precipitation with organic solvents, while the free gum acid is obtained by pouring an aqueous solution into acidified ethanol or methanol (87) or by precipitation in glacial acetic acid (11).

FRACTIONATION

The purified material may contain more than one polysaccharide and the establishment of homogeneity is a most difficult problem. Fractionation has been attempted by a variety of methods, but so far only gum tragacanth (12) and the gum component of Olibamum (13) have been separated into more than one polysaccharide. Fractional precipitation from an aqueous solution with organic solvents may be facilitated by adding metal ions (calcium, barium or lead) or by varying the pH; alternatively precipitation may be effected by complexing agents such as copper (14). Precipitation of acidic polysaccharides with cetyltrimethylammonium bromide (Cetavlon) has been described (15, 16) and use of this reagent has recently been extended to the separation of neutral polysaccharides which form complexes with boric acid (17).
Immunological fractionation utilises the fact that anti-pneumococcus sera give specific precipitates with certain polysaccharides. Gum arabic, recovered from its specific precipitate with Type II antipneumococcus serum, contains only one third to one fifth as much rhamnose as does the original gum (18).

Electrophoresis in a Tiselius apparatus (19) has been widely applied to polysaccharides, including gum arabic which has been separated from Acacia cyanophylla gum (20) and from a plum gum (21). Gum arabic itself appeared to be electrophoretically homogeneous, although some degree of heterogeneity has been demonstrated by immunological procedure (18). Electrophoresis in a column of powdered glass has been described (22) and may be applicable to the fractionation of plant gums.

The possibility of adsorption on cellulose makes paper ionophoresis unsuitable for polysaccharides, but ionophoresis on glass fibre paper has recently been suggested (23, 24). Using this technique Smith (25) has examined several gums in solutions of 2N sodium hydroxide and has suggested that gum ghatti, gum tragacanth and the gums of Acacia pycnantha, Acacia senegal and Acacia arabica are heterogeneous.

Partition by the carrier technique involves separation of acidic polysaccharides between an aqueous phase and an organic phase containing fatty amines and has been used for a mixture of hyaluronic acid and chondroitin sulphuric acid on a column of siliconized Celite (26).
Gradient elution on a cellulose column has been applied to the separation of mucopolysaccharides by Gardell (27). The mixture is precipitated at the top of the column and the different substances are brought selectively into solution by elution with decreasing concentrations of aqueous ethanol containing barium acetate.

There is at present no general criterion of homogeneity and much work remains to be done on the whole question of fractionation. Moreover, when fractionation is achieved it does not necessarily mean that different molecular species are involved, since many of the methods employed will differentiate between molecules of varying size.

An estimation of molecular weight is obtained from the rate of sedimentation in the ultracentrifuge, which also gives an indication of degree of purity. Chemical determination of molecular weight depends on estimation of reducing end group (28), but this method is inaccurate for high polymers.

Preliminary Investigation

Preliminary investigation of the gum includes determination of optical rotation, ash content and methoxyl and acetyl estimations (29). Uronic anhydride content is given by estimation of carbon dioxide liberated on heating with 12% hydrochloric acid (30), while equivalent weight is obtained by direct titration with dilute alkali. In certain cases, infra red analysis may /
Identification and estimation of monosaccharide residues present

Acid hydrolysis causes partial or complete breakdown of polysaccharides with liberation of their component sugars. Many gum acids consist of a backbone, or nucleus, of galactose and uronic acid residues with an outer structure of pentose units. The glycosidic link of a furanoside is particularly acid-labile and as L-arabinose generally occurs in furanose form, it can often be removed under mild hydrolytic conditions leaving a more resistant or degraded gum acid. Since this mild hydrolysis usually occurs on heating an aqueous solution of the free gum acid alone, it is termed autohydrolysis (33).

The degraded gum is broken down by heating with dilute mineral acid, but the glycosidic linkage of a uronic acid is sufficiently resistant to hydrolysis to require more drastic conditions for cleavage. Hydrolysis with N hydrochloric or sulphuric acid at 100° for four to six hours therefore liberates neutral monosaccharides together with acidic disaccharides (aldobiouronic acids) or higher acidic fragments. The drastic conditions necessary for hydrolysis of a glycuronosyl linkage result in decomposition of sugars, particularly of uronic acids which undergo decarboxylation. Consequently, gums with a high uronic acid content present the most difficult problem. Acidic fragments are best investigated by reduction of the methyl esters.
esters with lithium aluminium hydride (34) or potassium borohydride (35) followed by hydrolysis.

In early work, the sugars liberated on hydrolysis of a gum were identified by the use of specific precipitants; for example, xylose was identified as the cadmium bromide - cadmium xylonate double salt by means of oxidation with bromine in the presence of cadmium carbonate. The method, however, was not quantitative and trace amounts of sugars escaped detection.

The advent of partition chromatography brought a new approach to carbohydrate chemistry. Column chromatography was first used for the separation of amino-acids by Martin and Synge (36) and later Consden, Gordon and Martin developed the technique of paper chromatography (37) which was applied to sugars by Partridge (38, 39). Chromatography is now universally used in carbohydrate chemistry and details of techniques, solvents and sprays are given in reviews (7, 40). In general, neutral solvent mixtures based on n-butanol or butan-2-one separate neutral sugars but give streaking with uronic acids which may, however, be separated in acidic solvents such as ethyl acetate-acetic acid-water. Basic solvents containing pyridine are of wide application, any uronic acid present remaining on the starting line, presumably as a pyridine salt. Selection of solvent proportions is to some extent arbitrary, although recently a selection procedure has been described (44). A solvent containing a higher proportion of water is needed for the separation of oligosaccharides than for that of monosaccharides, while a very low water content /
content is required for chromatography of methylated sugars.

While a preliminary indication of sugars present may be obtained by paper chromatography, final identification by the preparation of derivatives is necessary for conclusive proof. Moreover, the paper chromatogram will not differentiate D- and L- sugars. Separation of a mixture of sugars is achieved by column chromatography on cellulose, as first described by Hough, Jones and Wadman (42), and is followed by the identification of crystalline derivatives (43).

Chromatography has been extended to the quantitative estimation of component sugars, which is carried out by the method of Flood, Hirst and Jones (44). The mixture is separated on a paper chromatogram and the positions of sugars detected by spraying only guide-strips from the sides. Sugars are then eluted with water and estimated by any suitable micro-method, such as the Somogyi procedure (45, 46).

MODE OF LINKAGE OF MONOSACCHARIDES

Once the monosaccharide residues present in the gum have been identified and estimated, the more difficult problem of mode of linkage remains to be solved. This problem may be investigated by methylation studies, partial hydrolysis and degradation by periodate oxidation.

Methylation is achieved by treatment with dimethyl sulphate and 30% aqueous /
aqueous sodium hydroxide solution (47) followed by methyl iodide and silver oxide (48) until the methoxyl content is constant. Gums which are resistant to the aqueous method of methylation may be treated with thallium hydroxide and methyl iodide (49) or with sodium in liquid ammonia and methyl iodide (50). As a further variation of methylation technique, the polysaccharide may be dissolved in dimethyl formamide and then treated with methyl iodide and silver oxide (51). The fully methylated polysaccharide is insoluble in hot aqueous acid but it may be possible to effect a partial hydrolysis in cold aqueous acid, followed by hydrolysis at 100°C, or the methylated polysaccharide may be subjected to methanolyis followed by hydrolysis.

In early work, the mixture of methylated sugars was separated by fractional distillation of methyl glycosides - a difficult procedure requiring reasonably large amounts of material. Column chromatography was first applied to methylated sugars by Hough, Jones and Wadman (52) and is now a standard practice, requiring very small amounts of material. Cellulose columns are generally used, but chromatography on Celite (53) has been described and methylated sugars may also be separated by gradient elution of columns of charcoal:Celite (54, 55).

If chromatography fails to separate the mixture, it may be possible to utilise borate complexes which are formed only by sugars with suitably disposed hydroxyl groups (56), of which adjacent cis hydroxyl groups are the most important. The negatively charged complex ions may be adsorbed on an anion /
anion exchange resin and later eluted with boric acid (57, 58). Variations of the technique include gradient elution from a charcoal column with boric acid-butan-2-one (59), paper chromatography using solvents saturated with boric acid and paper ionophoresis in borate buffer (60, 61).

The positions of free hydroxyl groups on the partially methylated sugars then indicate points of linkage to other sugar residues or positions engaged in ring formation. From methylation studies alone it may not be possible to decide whether ring structures are pyranose or furanose. For example, the detection of 2:3-di-O-methyl-L-arabinose could indicate a 4-linked L-arabopyranose or a 5-linked L-arabofuranose, but the additional evidence required may be obtainable by the isolation of disaccharides or by measurement of optical rotations or rates of hydrolysis. In general, the mode of linkage becomes apparent but not the order in which the sugars are joined. Some light is thrown on the latter problem by a comparison of the fragments obtained from the methylated gum with the methylated degraded (usually arabinose-free) gum, while still further information is available from a study of partial hydrolysis products.

During autohydrolysis, free L-arabofuranose is liberated in quantity, and often pentose-containing di- and trisaccharides are split off from the more resistant part of the polymer, the break usually occurring at an acid-labile furanoside linkage. For example, 3-O-β-L-arabopyranosyl-L-arabinose has been isolated by autohydrolysis of *Acacia karroo* (62), peach, cherry (63), lemon /
lemon (64) and golden apple gums (65).

Mild hydrolysis with dilute sulphuric or hydrochloric acid liberates oligosaccharides, including polymers of galactose, as well as monosaccharides from the backbone of the gum, while aldobiouronic acids will survive prolonged hydrolysis with H acid.

It is known that oligosaccharides are synthesised from monosaccharides in dilute acid solution (66) and consequently oligosaccharides obtained in small yield from partial hydrolysies are of uncertain structural significance. Nevertheless, a synthetic or reversion product can often be differentiated from a true hydrolysis product since the latter will break down on prolonged hydrolysis, whereas the concentration of a reversion product reaches an equilibrium value (64).

Acidic fragments are separated by precipitation as barium salts with ethanol, by adsorption on anion exchange resins (68) and by chromatography on cellulose columns with acidic eluants. A crude fractionation of neutral oligosaccharides may be achieved by adsorption on columns of charcoal-Celite, followed by elution with aqueous ethanol (69), while a complete separation is given by chromatography on cellulose columns using butan-1-ol-water (52) or ethyl-acetate-pyridine-water as eluant.

Neutral oligosaccharides are identified by methylation, hydrolysis and separation of the partially methylated sugars, while aldobiouronic acids are best /
best reduced (34, 35) before hydrolysis.

Partial degradation by periodate oxidation (70) provides additional structural information. A structure with hydroxyl groups on adjacent carbon atoms is oxidised by salts of metaperiodic acid:

\[
\text{CHOH} + \text{IO}_4^- \rightarrow \text{CHO} + \text{IO}_3^-
\]

or if there are more than two adjacent hydroxyl groups, then formic acid is produced:

\[
\text{CHOH} + 2 \text{IO}_4^- \rightarrow \text{HOOH} + 2 \text{IO}_3^-
\]

Measurement of periodate consumed, formic acid produced and identification and estimation of sugars unattacked give information on structure which may be compared with that derived from methylation studies. The oxidised polysaccharide may be subjected to partial hydrolysis either before or after reduction with potassium borohydride. Barry has evolved a degradative technique which involves osazone formation by heating the oxypolysaccharide with phenylhydrazine acetate (71). Osazone fragments are readily removed from the polymer under the mild acid conditions employed. This oxidation and degradation can be repeated successively until either the gum is completely degraded.
degraded or only a resistant nucleus remains.

From a comparison of the results of these various studies it is possible to decide the main structural features and types of linkage predominating in the gum. The polymer is so complicated in all cases that no unique formula can be put forward and indeed it is probable that there is no discrete repeating unit, but rather a structure in which certain chains and types of branching recur.

STRUCTURE OF GUM ARABIC

Gum arabic has been studied in greater detail than any other plant gum and may be considered as an example of the application of the methods described.

Various investigations (33, 72, 75-77) have shown that the gum acid contains residues of D-glucuronic acid (1), D-galactose (3), L-rhamnose (1) and L-arabinose (3) in the molar proportions indicated by figures in brackets. Autohydrolysis of arabic acid liberates L-arabinose, L-rhamnose and 3-O-D-galactopyranosyl-L-arabinose (33), leaving a degraded gum acid containing only D-galactose and D-glucuronic acid residues.

The disaccharide, 3-O-D-galactopyranosyl-D-galactose (72), has been isolated by prolonged autohydrolysis of the degraded gum acid and the aldobiouronic /
aldobiouronic acid, 6-O-β-D-glucuronosyl-D-galactose (73, 74), has been obtained by acid hydrolysis of gum arabic.

Gum arabic (arabic acid)  
\[ \rightarrow \text{(autohydrolysis)} \]
Degraded arabic acid  +  L-arabinose  +  L-rhamnose  +  
3-0-D-galactopyranosyl-L-arabinose.

(Hydrolysis, (prolonged autohydrolysis) 
mineral acid)

D-galactose  +  3-0-D-galactopyranosyl-D-galactose

6-O-β-D-glucuronosyl-D-galactose

From the hydrolysis products of the methylated degraded gum, Smith (75) obtained glycosides of 2;3;4;6-tetra-O-methyl-D-galactose (1), 2;3;4-tri-O-methyl-D-galactose (5), 2;4-di-O-methyl-D-galactose (3) and 2;3;4-tri-O-methyl-D-glucuronic acid (3), indicating that D-glucuronic acid occurs as end group while D-galactose residues are joined by 1:3- and 1:6- links.

By partial hydrolysis of the methylated degraded gum, Jackson and Smith isolated a hexamethyl aldobiouronic acid (76) which gave, on hydrolysis, methyl glycosides of 2;3;4-tri-O-methyl-D-glucuronic acid and 2;3;4-tri-O-methyl-D-galactose, showing that side chains do not consist of a single D-glucuronic acid residue, but rather that each terminal acid unit is linked through at least one galactose residue to the main chain.
The evidence so far obtained suggests that the degraded gum could have one of the following types of structure, or the structure could be of an intermediate type.

(a) \[ \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 3 \, \text{Gal} \, 1 - \, 3 \, \text{Gal} \, 1 - \, \text{R} \]

(b) \[ \text{Gal} \, 1 - \, 3 \, \text{Gal} \, 1 - \, 3 \, \text{Gal} \, 1 - \, 3 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, 6 \, \text{Gal} \, 1 - \, \text{R} \]

\[ R = \text{1 Gal6 - 1 GA} \text{ or } \text{1 Gal6 - 1 Gal6 - 1 GA} \]

\[ \text{Gal} = \text{D-galactose} \]

\[ \text{GA} = \text{D-glucuronic acid} \]


From a comparison of all his results Smith was able to suggest a repeating /
repeating unit for arabic acid, although several variations of this structure would also fit the evidence:-

\[
\begin{array}{cccccc}
\text{Gal} & \text{ Gal} & \text{ 6 Gal} & \text{ Gal} & \text{ 6 Gal} & \text{ Gal} \\
3 & & 3 & & & \\
\text{R} & & \text{R} & & & \\
\text{R} & \text{4GM-Gal} & \text{R} & \text{4GM-Gal} & \text{R} & \text{4GM-Gal} \\
\text{3} & & 3 & & & \\
\text{R} & & \text{R} & & & \\
\end{array}
\]

\[ R = \text{L-arabofuranose or} \]
\[ \text{L-rhamnopyranose or} \]
\[ 3\text{-D-galactosyl-L-arabofuranose} \]

While methylation studies show clearly that galactose residues must be linked through positions 1, 3 and 6, the problem to be decided is whether they are mutually 1:6- linked with side chains on position 3 or the converse. In this connection Dillon, O'Callachain and O'Colla (78) showed that after three stages of the Barry degradation they obtained a polysaccharide containing only galactose residues, which was resistant to further attack by periodate, indicating a high proportion of 1:3- links. The same conclusion was reached after a study of the fragment of gum remaining after periodate oxidation and partial hydrolysis to remove the cleaved aldobiouronic acid side chains (79). Gum arabic must therefore contain a backbone of 1:3- linked galactose residues bearing side chains on position 6. 1:6-Linked galactose residues are present in the side chains.
MOLECULAR STRUCTURE OF PLANT GUMS

Recent investigations indicate that gums obtained from plants of different species within one genus show similarities in their molecular structure, although precise conclusions can be drawn only when the gum examined is an authentic sample from a given botanical source. Some confusion inevitably arises when gum collection is carried out indiscriminately, and often a gum marketed under a commercial trade name may come from a variety of plants. Nevertheless, it is now possible to trace relationships and to make a broad classification of gums which, however, does not include galactomannans such as gum guar and carob bean gum, but is confined to acidic polysaccharide exudates.

GUMS CONTAINING D-GLUCURONIC ACID [Table I, p. 24]

Early work on gums showed that, with the exception of gum tragacanth, acidity was due to the presence of D-glucuronic acid. It is now known that the uronic acid present may be monomethyl D-glucuronic acid or D-galacturonic acid, but D-glucuronic acid remains the most frequently encountered. Gums containing this uronic acid often exhibit a typical structure consisting of a resistant nucleus of residues of D-galactose, D-glucuronic acid and, in some cases, D-mannose, surrounded by easily split chains of L-arabinose and D-xylose units; L-arabinose occurs most frequently in /
in the acid-labile furanose form but has recently been found in pyranose form as well. It is generally observed that D-galactose is linked through positions 1, 3 and 6.

**Genus Acacia (Family Leguminoseae)**

All gums of the genus *Acacia* contain the same sugar residues (D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid) in different proportions [Table I, p. 24] and undergo autohydrolysis with liberation of arabinose. Some rhamnose may also be liberated, but is generally less readily removed than arabinose. The degraded gums consist of D-galactose and D-glucuronic acid residues, but acid units are not necessarily confined to the resistant part of the polymer. They may also form an integral part of the easily split side chains since, in *Acacia karroo* (62) and *Acacia cyanophylla* (80) gums, acid is liberated during autohydrolysis and, in the case of the latter gum, the aldobiouronic acid so produced is the same as that obtained by hydrolysis of the degraded gum.

Disaccharides isolated from the products of both autohydrolyses and acid hydrolyses are shown in Table II [p. 27].

The same aldobiouronic acid, 6-O-D-glucuronoasyl-D-galactose, has been detected in all members so far examined, while in *Acacia karroo* gum a second aldobiouronic acid, 4-O-α-D-glucuronoasyl-D-galactose, has been found (62). *Acacia* /
Acacia karroo gum may either be heterogenous, or it may be different from other Acacia gums in containing two aldobiouronic acid residues.

Of this genus, only gum arabic has been fully methylated and extensively investigated [p. 13 - 16], but work now in progress shows that methylated Acacia pyroanthes gum (85) contains a high proportion of 2:4-di-O-methyl-D-galactose residues, indicating a branched structure with 1:3- and 1:6-linked galactose. This may be compared with the 1:3- and 1:6-linked galactose known to exist in gum arabic.

Genus Prunus (Family Rosaceae)

A number of different Prunus gums have been examined in detail [Table I, p. 24]. Qualitative analyses are generally similar but proportions of monosaccharides vary with botanical species. For example, results obtained for gum from wild cherry trees from Indiana (90) differ from results for English cherry trees (89), although samples from different cherry trees of the same botanical origin appear to be identical. A complication arises in that cherry trees can be grown as grafts on different stocks.

Sugar residues detected are D-galactose, D-mannose, L-arabinose, D-xylose and D-glucuronic acid, although D-mannose does not occur in all Prunus gums and in some D-xylose exists only as a trace which may be derived from
from a secondary component.

All these polysaccharides undergo autohydrolysis with ready liberation of pentose sugars leaving a degraded gum, consisting of D-galactose, D-glucuronic acid and, sometimes, D-mannose. On prolonged autohydrolysis D-galactose is split off, but there has as yet been no report of liberation of acidic fragments, a fact which may indicate that the acid is confined to the resistant part of the framework. 3-O-β-L-Arabopyranosyl-L-arabinose has been obtained from peach and cherry gums by mild hydrolysis (63) and (4- or) 5-O-β-D-xylopyranosyl-L-arabinose from peach gum (63) under similar conditions [Table II, p. 27].

One aldobiouronic acid has been isolated from each gum. In the gums containing mannose, it seems likely that all the mannose and uronic acid are mutually linked as 2-O-β-D-glucuronosyl-D-mannose, while the mannose-free gums contain 6-O-glucuronosyl-D-galactose and so resemble those of the genus Acacia.

The results of methylation studies [Table III, p. 28] show that galactose is involved in 1:3- and 1:6- linkages. While it is not possible from the information available to suggest precise structures, the general pattern is that of a main backbone consisting of D-galactose residues, with side chains containing aldobiouronic acid and pentose units. Arabinose occurs in furanose form as end groups and in 1:3- and 1:5- linkages.

The
The aldobiouronic acid isolated from both methylated egg plum gum (97) and methylated degraded egg plum gum (98) gave, on hydrolysis, 2:4-di-O-methyl-D-galactose and 2:3-di-O-methyl-D-glucuronic acid, indicating that the aldobiouronic acid is surrounded by galactose residues:

\[ \text{Gal} - 4 \ \text{GA} \ 1 - 6 \ \text{Gal} \ 1 - \text{Gal} \]

There is no end group glucuronic acid in egg plum gum, whereas damson (95) and cherry (93) gums contain both terminal and 1:4-linked D-glucuronic acid.

No methylated derivatives of D-mannose have been isolated.

**Genus Anogeissus (Family Combretaceae)**

Gum ghatti (*Anogeissus latifolia*) (101) contains residues of D-glucuronic acid, D-galactose, D-mannose, L-arabinose, D-xylose and traces of L-rhamnose. It bears general resemblances to the Prunus gums with the important difference that it contains both the aldobiouronic acids, 2-O-β-D-glucuronosyl-D-mannose and 6-O-β-D-glucuronosyl-D-galactose.

The further investigation of gum ghatti forms the subject of the present work.
The gum of *Anogeissus schimpcri* (102, 103) contains the same monosaccharides as gum ghatti. The aldobiouronic acids have not been fully investigated but one acid, probably 2-0-β-D-glucuronosyl-α-D-mannose, has been detected on a paper chromatogram (103).

**Melia Azadirachta (Family Meliaceae)**

Neem gum (106), an exudate from the tree *Melia azadirachta*, is interesting in that it contains L-fucose residues which can be removed with L-arabinose by autohydrolysis. Other residues present are D-galactose and D-glucuronic acid and the aldobiouronic acid isolated is 4-0-D-glucuronosyl-D-galactose.

**Puya Chilensis (Family Bromeliaceae)**

A few of the gums containing D-glucuronic acid are markedly dissimilar from those already considered. Chagual gum (107), obtained from *Puya chilensis*, contains residues of the monosaccharides commonly found, but the proportions are unusual and the aldobiouronic acid is 2-0-α-D-glucuronosyl-D-xylose. Residues present are L-arabinose (7%), D-xylose (31%), D-galactose (36%) and 2-0-D-glucuronosyl-D-xylose (27%).

This gum is unique in containing a relatively small amount of arabinose and a large amount of xylose and, furthermore, there is no preferential /
preferential liberation of arabinose on hydrolysis.

Achras Sapota (Family Sapotaceae)

Sapote gum from Achras sapota bears little or no resemblance to other glucuronic acid-containing gums. It contains residues of D-glucuronic acid (some of which may be present as the monomethyl ether), D-xylose and L-arabinose (108), but no hexose sugar has yet been identified.

The aldobiouronic acid 2-O-D-glucuronosyl-D-xylose has been detected (111) and methylated Sapote gum (109-111) has been shown to contain residues of 3-O-methyl-D-xylose (3), 3:4-di-O-methyl-D-glucuronic acid (1), 2:3:4-tri-O-methyl-D-glucuronic acid (1), 2:3:4-tri-O-methyl-L-arabinose (1) and 2:3:4-tri-O-methyl-D-xylose (1). The main chain is, therefore, composed largely of D-xylose residues which also constitute branch centres. Sapote gum possesses the further novel feature of L-arabopyranose end units.
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>GA</th>
<th>Gal</th>
<th>Ar</th>
<th>Rh</th>
<th>Aldobiouronic Acid</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leguminosae</td>
<td>Acacia</td>
<td>Senegal (Arabic)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>GA 1 $\beta$-6 Gal</td>
<td>72-77</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Cyanophylia</td>
<td>5</td>
<td>11</td>
<td>2</td>
<td>5</td>
<td>GA 1 $\beta$-6 Gal</td>
<td>80</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Mollissima</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>GA 1-6 Gal</td>
<td>81</td>
</tr>
<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Catechu</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>GA 1 $\beta$-6 Gal</td>
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<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Pycnantha</td>
<td>3</td>
<td>40</td>
<td>20</td>
<td>1</td>
<td>GA 1 $\beta$-6 Gal</td>
<td>83</td>
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<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Karroo</td>
<td>6</td>
<td>28</td>
<td>24</td>
<td>1</td>
<td>GA 1 $\beta$-6 Gal</td>
<td>62</td>
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<tr>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Gleditschia</td>
<td>1</td>
<td>1.8</td>
<td>3.2</td>
<td>-</td>
<td>GA 1-6 Gal</td>
<td>84</td>
</tr>
</tbody>
</table>

Key: -

- GA = D-Glucuronic Acid
- Gal = D-Galactose
- Ma = D-Mannose
- Ar = L-Arabinose
- Xy = D-Xylose
- Rh = L-Rhamnose
- Fu = L-Fucose
- tr = trace

Figures refer to molar proportions of sugar residues.
<table>
<thead>
<tr>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>Ref.</th>
<th>Other Sugars</th>
<th>Aldolaccharonic Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg plant (English)</td>
<td>Dosemion</td>
<td>Rosaceae</td>
<td>87</td>
<td>2</td>
<td>GA 1 β, 2 Ma</td>
</tr>
<tr>
<td>Wild cherry (U.S.A.)</td>
<td>Avicululus</td>
<td>Rosaceae</td>
<td>88</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Almond (Peach)</td>
<td>Ferrid (Almond)</td>
<td>Rosaceae</td>
<td>89</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Silk oak</td>
<td>Ledolites</td>
<td>Rosaceae</td>
<td>90</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Dhalit (Silk oak)</td>
<td>Ledolites</td>
<td>Rosaceae</td>
<td>91</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Drum stick</td>
<td>Foringa</td>
<td>Combretaceae</td>
<td>92</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Combretu®</td>
<td>Melia</td>
<td>Meliaceae</td>
<td>93</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Ptery,® Ster! (leer)</td>
<td>Melia</td>
<td>Meliaceae</td>
<td>94</td>
<td>2</td>
<td>Gal 1 – 2 Ma</td>
</tr>
<tr>
<td>Family</td>
<td>Genus</td>
<td>Species</td>
<td>Ref.</td>
<td>Other Sugars</td>
<td>Aldobiouroncels</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------</td>
<td>--------------------------</td>
<td>----------</td>
<td>--------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GA Gal</td>
<td>Ar Ma Xy</td>
<td>GA 1 2 Xy</td>
</tr>
<tr>
<td>Sapotaceae</td>
<td>Bixa</td>
<td>Chilensia Chilensis</td>
<td>107</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Adinas</td>
<td>Sapotes Sapotes</td>
<td>108</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Phoradendron</td>
<td>Terax</td>
<td>110</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Feronia</td>
<td>Elephantum Elephantum</td>
<td>111</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Exocarposseae</td>
<td>Exocarposseae</td>
<td>112</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Miliaceae</td>
<td>Miliaceae</td>
<td>113</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td></td>
<td>Rutaceae</td>
<td>Rutaceae</td>
<td>114</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>
### Table II
**Disaccharides Isolated from Plant Gums**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Disaccharides isolated by autohydrolysis</th>
<th>Disaccharides isolated by acid hydrolysis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Gums containing D-glucuronic acid.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Acacia</td>
<td>Senegal (arabic)</td>
<td>Gal 1 - 3 Ar</td>
<td>Gal 1 β - 3 Gal</td>
<td>33, 72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyanophylla</td>
<td>Gal 1 α - 3 Ar</td>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mollissima</td>
<td>Ar 1 β - 3 Ar (tr)</td>
<td></td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Karoo</td>
<td>Ar 1 β - 3 Ar</td>
<td>Gal 1 β - 3 Gal</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pycoanthia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virgilia</td>
<td>Gal 1 - 3 Ar</td>
<td></td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oroboides</td>
<td>Ar 1 α - 5 Ar</td>
<td></td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Divaricata</td>
<td>Ar 1 α - 5 Ar</td>
<td></td>
<td>86</td>
</tr>
<tr>
<td>Rosaceae</td>
<td>Prunus</td>
<td>Peach</td>
<td>Ar 1 β - 3 Ar</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cherry</td>
<td>Ar 1 β - 3 Ar</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>(b) Gums containing 4-O-methyl-D-glucuronic acid.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Citrus</td>
<td>Lemon</td>
<td>Ar 1 β - 3 Ar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anacardiaceae</td>
<td>Spondias</td>
<td>Cytheria (Golden apple)</td>
<td>Ar 1 β - 3 Ar</td>
<td>Gal 1 β - 3 Gal</td>
<td>65</td>
</tr>
<tr>
<td>(c) Gums containing D-galacturonic acid.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>Cactaceae</td>
<td>Opuntia</td>
<td>Fulgida (Cholla)</td>
<td>Xy 1 β (4 or) 5 Ar</td>
<td></td>
<td>63</td>
</tr>
</tbody>
</table>
**TABLE III**

**HYDROLYSIS PRODUCTS OF METHYLATED GUMS CONTAINING D-GLUCURONIC ACID**

(Figures refer to the positions at which methyl groups are attached to sugars)

<table>
<thead>
<tr>
<th>Methylated Gum</th>
<th>GA</th>
<th>Gel</th>
<th>Ar</th>
<th>Xy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry</td>
<td>2:3:4</td>
<td>2:4:6</td>
<td>2:3:5</td>
<td>-</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>2:4</td>
<td>2:5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>2:4:6</td>
<td>2:4</td>
<td>2:4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:4:6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damson</td>
<td>2:3:4</td>
<td>2:4:6</td>
<td>2:3:5</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>2:4</td>
<td>2:3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>2:4:6</td>
<td>2:3:4</td>
<td>2:3:4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:4:6</td>
<td>2:4</td>
<td>2:4</td>
<td></td>
</tr>
<tr>
<td>Egg Plum</td>
<td>2:3</td>
<td>2:4:6</td>
<td>2:3:5</td>
<td>2:3:4</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>2:4</td>
<td>2:5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Degraded Egg Plum</td>
<td>2:3</td>
<td>2:3:4:6</td>
<td>-</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>2:3:4</td>
<td>2:4:6</td>
<td>2:3:4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:4:6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sapote</td>
<td>2:3:4</td>
<td>-</td>
<td>2:3:4</td>
<td>2:3:4:1</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>3:4</td>
<td></td>
<td></td>
<td>3</td>
<td>111</td>
</tr>
</tbody>
</table>
GUMS CONTAINING 4-O-METHYL-D-GLUCURONIC ACID [Table IV, p. 34]

Recently a number of gums have been found to contain monomethyl D-glucuronic acid residues and in all investigations so far the acid has proved to be the 4-methyl-ether. It is probable that monomethyl uronic acids and monosaccharides occur more commonly in nature than was originally assumed. 2-O-(4-O-Methyl-D-glucuronosyl)-D-xylose has been found in a number of hemicelluloses, including those of corn cob (115), wheat straw (116), western hemlock (117), loblolly pine (118), and flax straw (119), while 2-O-methyl-D-xylose has been isolated from plum leaf hemicellulose (120).

**Boswellia Carterii and Commiphora Myrrha (Family Burseraceae)**

Frankincense (123) and myrrh (121, 122) are typical oleo-gum-resins, exuded from cracks in the bark of *Boswellia carterii* and *Commiphora myrrha* respectively. The carbohydrate material is heavily contaminated with resins and essential oils; in the case of gum myrrh, there is also protein present, some of which may be combined with the polysaccharide. Gum myrrh has been separated into fractions with varying protein content but the polysaccharide appears to be homogeneous.

Residues of D-galactose, L-arabinose and 4-O-methyl-D-glucuronic acid are present in gum myrrh and gum frankincense. Both contain a relatively high proportion of uronic acid and a low proportion of arabinose which /
which is, nevertheless, liberated on autohydrolysis.

Gum myrrh contains residues of two aldobiouronic acids, (122), 6-0-(4-O-methyl-β-D-glucuronosyl)-D-galactose and 4-0-(4-O-methyl-α-D-glucuronosyl)-D-galactose, which often occur, either singly or together, in gums containing 4-O-methyl-D-glucuronic acid. Comparison may be made with the unmethylated analogues found in Acacia karroo gum (62). It is common to find D-glucuronic acid joined to C_4 of D-galactose by an α-glycosidic link and D-glucuronic acid joined to C_6 of D-galactose by a β-link.

The gums of frankincense and myrrh are closely related, but so far 4-0-(4-O-methyl-α-D-glucuronosyl)-D-galactose has not been detected in frankincense.

**Genus Citrus (Family Rutaceae)**

Grapefruit resembles lemon gum, both of which contain residues of D-galactose, L-arabinose and 4-O-methyl-D-glucuronic acid (124, 125). As in the case of gum frankincense and gum myrrh, the uronic acid content is fairly high and the arabinose relatively low and readily removed. Autohydrolysis of lemon gum yields the disaccharide 3-O-β-L-arabopyranosyl-L-arabinose (64) [Table II, p. 27], which has also been isolated from peach, cherry (63) golden apple (65) and Acacia karroo gums (62) and from larch β-galactan (67), while from the products of very mild acid hydrolysis the aldobiouronic acid 4-0-(4-O-methyl-α-D-glucuronosyl)-L-arabinose has been obtained (125).
In the original work on these two gums the presence of 4-O-D-glucuronosyl-D-galactose was reported (124), but the glucuronic acid was later found to be the 4-methyl-ether (125). This aldobiouronic acid does not occur among the products of very mild hydrolysis of lemon gum and so must form part of the degraded gum.

*Spondias Cytheria* (Family Anacardiaceae)

Golden apple gum, from the tree *Spondias cytheria*, contains residues of D-galactose, L-arabinose and, probably, 4-O-methyl-D-glucuronic acid, although the position of the methyl group has not been fully established. D-Xylose and traces of L-rhamnose and L-fucose have also been reported (68).

Autohydrolysis yields arabinose and galactose and the disaccharides 3-O-β-L-arabopyranosyl-L-arabinose, 3-O-α-D-xylopyranosyl-L-arabinose (65) and 3-O-(4-O-methyl-α-D-glucuronosyl)-L-arabinose (68) and leaves a degraded gum composed of D-glucuronic acid, D-galactose and L-arabinose, the latter probably in pyranose form. The hydrolysed degraded gum yields a second aldobiouronic acid, 6-O-(4-O-methyl-β-D-glucuronosyl)-D-galactose, and two disaccharides, 3-O-β-D-galactosyl-D-galactose and 6-O-β-D-galactosyl-D-galactose (68) [Table II, p. 27]. The presence of the latter two disaccharides indicates that galactose residues are mutually engaged in both 1:3- and 1:6- linkages.

Golden
Golden apple gum may be compared with lemon gum in possessing two aldobiouronic acid residues, one of which contains L-arabinose and must form part of an easily broken side chain.

**Prosopis Juliflora (Family Leguminosae)**

Mesquite gum, from the tree *Prosopis juliflora* contains residues of D-galactose, L-arabinose and 4-O-methyl-D-glucuronic acid (129-136). The proportion of uronic acid to arabinose is lower than in gum myrrh, gum frankincense and the Citrus gums and bears more resemblance to the ratio obtained for *Prunus* gums.

Methylation studies have been carried out independently by White (129-132) and Smith (133-136). With minor quantitative variations, both have identified the following sugars after hydrolysis of the methylated gum:

- 2:3:4-tri-O-methyl-D-glucuronic acid,
- 2:3:5-tri-O-methyl-L-arabinose,
- 3:5-di-O-methyl-L-arabinose and 2:4-di-O-methyl-D-galactose.

Smith points out that, as in gum arabic, galactose residues could be 1:3-linked with side chains on position 6, or they might be both 1:3- and 1:6-linked.

From the acidic fraction of methylated mesquite gum and from the methylated degraded gum, White (131) obtained a heptamethyl disaccharide uronic acid ester which he proved to be the methyl ester methyl glycoside of 3-O-(2:3:4-tri-O-methyl-D-glucuronosyl)-2:4-di-O-methyl-D-galactose. He concluded that
the original gum must contain the fragment:

\[ \text{4-mo-GA} \rightarrow 3 \text{Gal} \rightarrow 6 \]

Cuneen and Smith isolated two aldobiouronic acids, 6-O-\(\beta\)-D-glucuronosyl-D-galactose and 4-O-D-glucuronosyl-D-galactose, from mesquite gum (133) and Smith later found that the acid in fact existed as the 4-methyl-ether (135). From the methylated gum (134) they obtained a partially methylated aldobiouronic acid in which tri-O-methyl-D-glucuronic acid was linked to 2:4-di-O-methyl-D-galactose and suggested that, in view of the aldobiouronic acids already identified, this indicated the presence of the fragment:

\[ \text{GA} \rightarrow 6 \text{Gal} \rightarrow 3 \]

It is probable that three aldobiouronic acids are present in mesquite gum.
### TABLE IV

**GUMS CONTAINING 4-O-METHYL-D-GLUCURONIC ACID**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>4-me-GA</th>
<th>Gal</th>
<th>Ar</th>
<th>Other Sugars</th>
<th>Aldobiouronic Acids</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burseraceae</td>
<td>Commiphora</td>
<td>Myrrha</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>-</td>
<td>4-me-GA 1 6 Gal</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Boswellia</td>
<td>Carterii</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>Fu</td>
<td>4-me-GA 1 6 Gal</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>123</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Citrus</td>
<td>Grapefruit</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>4-me-GA 1 4 Ar</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lemon</td>
<td>12</td>
<td>3.5</td>
<td>17</td>
<td></td>
<td>4-me-GA 1 4 Gal</td>
<td>125</td>
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<tr>
<td></td>
<td>Fagara</td>
<td>Xanthoxyloides</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>126</td>
</tr>
<tr>
<td>Anacardiaceae</td>
<td>Spondias</td>
<td>Cytheria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Xy</td>
<td>4-me-GA 1 6 Gal</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Golden apple)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rh</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fu</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lannea</td>
<td>Grandis (Modal)</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>4-me-GA 1 6 Gal</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Prosopis</td>
<td>Juliflora</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>4-me-GA 1 6 Gal</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Mesquite)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:**

- 4-me-GA = 4-O-Methyl-D-glucuronic acid
- Gal = D-Galactose
- Ar = L-Arabinose
- Fu = L-Fucose
- Rh = L-Rhamnose
- Xy = D-Xylose
GUMS CONTAINING D-GALACTURONIC ACID [Table V, p. 39]

Cochlospermum gossypium (Family Hixaceae), Genus Khaya (Family Heliaceae) and Genus Sterculia (Family Sterculiaceae).

While it is generally true that D-glucuronic acid is characteristic of gums and D-galacturonic acid a feature of mucilages, a certain type of plant gum contains a high proportion of D-galacturonic acid residues and does not follow the usual pattern of a galactose backbone surrounded by acid-labile pentose units. These polysaccharides are notable for their resistance to hydrolysis, due to stable galacturonosyl linkages and the absence of L-arabofuranose residues. Included in this category are the Sterculia gums, (S. setigera, S. urce, S. tormentosa) (137, 138), Karaya gum (Cochlospermum gossypium) (139) and the Khaya gums (K. grandifolia, K. senegalensis) (141), all of which are partly acetylated and contain D-galactose, D-galacturonic acid and L-rhamnose in varying proportions. The gum of S. setigera was the first natural product found to contain D-tagatose (142). Quantitative estimations are extremely difficult to carry out, since the drastic conditions necessary for complete hydrolysis cause considerable decomposition.

The Khaya gums contain 4-O-methyl-D-glucuronic acid and traces of L-arabinose in addition to the residues mentioned above. The occurrence of two different uronic acid residues in a single molecule is a feature peculiar to gums of this genus. Two aldobiouronic acids have been isolated in numerous other /
other cases, but these have always involved the same uronic acid residue.

The same aldobiouronic acids, 2-O-D-galacturonosyl-L-rhamnose and 4-O-D-galacturonosyl-D-galactose, occur in the gums of *Cochlospermum gossypium* and *Sterculia setigera* and the latter also contains a D-galacturonosyl-D-galacturonic acid. It is probable that adjacent D-galacturonic acid residues occur in *Khaya grandifolia* gum which further resembles *Sterculia setigera* gum in containing 2-O-D-galacturonosyl-L-rhamnose. This aldobiouronic acid has been found in mucilages, for example linseed mucilage (144) and slippery elm mucilage (145).

From methylation studies [Table VI, p. 41] it is evident that commonly occurring features are terminal D-galactopyranose residues and branched rhamnose units. The 1:4- linked galactose present in each case is in contrast to the 1:3- and 1:6- linked galactose found in *Acacia* and *Prunus* gums.

Mention must be made of the gum exudate of *Brachychiton diversifolium* (146), previously known as *Sterculia caudata*. This has been found to contain residues of L-rhamnose (23%), D-galactose (27%) and uronic acid (50%). The acid has been characterised as D-glucuronic acid and, so far, attempts to detect D-galacturonic acid have failed. Apart from this one remarkable fact, the gum closely resembles other *Sterculia* gums in being partly acetylated and resistant to hydrolysis and containing branch points at rhamnose residues.
Opuntia Fulcida (Family Cactaceae)

Cholla gum (10) is an exudate of the white cactus, Opuntia fulcida, and bears resemblances both to Acacia and Prunus gums and to some mucilages. The galacturonic acid content is low and pentose content high, this being in direct contrast to the Sterculia and Khaya gums. Cholla gum is consequently readily hydrolysable, dilute acid liberating L-arabinose, D-xylose and D-galactose and leaving a D-galactose and D-galacturonic acid nucleus. The disaccharide (4- or 5-0-β-D-xylopyranosyl-L-arabinose has also been isolated from the products of mild hydrolysis (63). [Table II, p. 27].

Methylation (Table VI) has shown that most of the arabinose occurs as arabofuranose end units, but in addition some is present as

\[
\begin{array}{c}
5 \\
\text{Ar} \\
1 \\
2
\end{array}
\]

This type of linkage has not so far been encountered in other gum acids, but is known to exist in the galactoaraban of tragacanth (151), and in slippery elm mucilage (149).

Cholla gum, like Acacia and Prunus gums, contains 1:3- and 1:6-linked galactose residues.

Astragalus (Family Leguminoseae)

Gum /
Gum tragacanth from a species of *Astragalus* was one of the earliest gums investigated. It has been shown to be a mixture (12) which has been methylated and fractionated giving

A. An acidic methylated polysaccharide, tragacanthic acid.
B. A neutral methylated polysaccharide.
C. A glycoside.

The differing solubilities of A, B and C in hot and cold water facilitated fractionation.

The acid polysaccharide A (12, 150) was found to contain residues of 2:3:4-tri-O-methyl-L-fucose, 2:3:4-tri-O-methyl-D-xylose, 3:4-di-O-methyl-D-xylose, 2:3-di-O-methyl-D-galacturonic acid and monomethyl-D-galacturonic acid.

The fully methylated neutral polysaccharide B (151) was shown to contain residues of 2:3:5-tri-O-methyl-L-arabinose, 2:3-di-O-methyl-L-arabinose, L-arabinose and di-O-methyl-D-galactose. The presence of di-O-methyl-D-galactose indicates branching at galactose residues and shows that the polysaccharide is not a simple araban, while the existence of L-arabinose residues indicates that L-arabinose is linked to four other sugars. Here a comparison may be made with cholla gum (10) and slippery elm mucilage (149).

The glycoside fraction C appeared on further examination to be steroid in nature.
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>GaLA</th>
<th>Gal</th>
<th>Rh</th>
<th>Other Sugars</th>
<th>Acidic Oligosaccharides</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterculiaceae</td>
<td>Sterculia</td>
<td>Setigera</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>Ta (1)</td>
<td>GalA 1 —— 2 Rh</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Xy ?</td>
<td>GalA 1 —— GalA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rh—</td>
<td>GalA 1 —— 4 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ketose?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urens</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tormentosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>Meliaceae</td>
<td>Khaya</td>
<td>Grandifolia</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4-me-GA (1)</td>
<td>GalA 1 —— 2 Rh</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ar</td>
<td>4-me-GA 1 —— 4 Gal</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Senegalensis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4-me-GA</td>
<td>GalA 1 —— 2 Rh1 —— 4 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bixaceae</td>
<td>Cochlospernum</td>
<td>Gossypium</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>keto-hexose</td>
<td>GalA 1 —— 2 Rh</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ar</td>
<td>GalA 1 —— 4 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Odina</td>
<td>Wodier</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td></td>
<td>GalA 1 —— Gal</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Jeol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>148</td>
</tr>
<tr>
<td>Castaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE V**

**GUMS CONTAINING D-GALACTURONIC ACID**
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Gal A</th>
<th>Gal</th>
<th>Rh</th>
<th>Other Sugars</th>
<th>Acidic Oligosaccharides</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cactaceae</td>
<td>Opuntia</td>
<td>Fulcida (Cholla)</td>
<td>1</td>
<td>3</td>
<td>tr</td>
<td>Ar (6)</td>
<td>Xy (2)</td>
<td>10</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Astragalus</td>
<td>Tragacanth</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Xy</td>
<td>Fu</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Ar</td>
<td></td>
<td>151</td>
</tr>
</tbody>
</table>

Key:—

GalA = D-Galacturonic acid
Gal = D-Galactose
Rh = L-Rhamnose
Ta = D-Tagatose
Xy = D-Xylose
Ar = L-Arabinose
Fu = L-Fucose

4-me-GA = 4-O-Methyl-D-glucuronic acid
TABLE VI

HYDROLYSIS PRODUCTS OF METHYLATED GUMS CONTAINING D-GALACTURONIC ACID
(Figures refer to the positions at which methyl groups are attached to sugars)

<table>
<thead>
<tr>
<th>Methylated Gum</th>
<th>Gala</th>
<th>Gal</th>
<th>Rh</th>
<th>4-mo-GA</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochlospermum gossypium</td>
<td>-</td>
<td>2:3:4:6</td>
<td>2:3:4</td>
<td>-</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:3:6</td>
<td>3:4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterculia setigera</td>
<td>2</td>
<td>2:3:4:6</td>
<td>3:4</td>
<td>-</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:3:6</td>
<td>2 and/or 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khaya grandifolia</td>
<td>2:3</td>
<td>2:3:4:6</td>
<td>3</td>
<td>2:3:4</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2:3:6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methylated Gum</th>
<th>Gala</th>
<th>Gal</th>
<th>Ar</th>
<th>Xy</th>
<th>Fu</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholla</td>
<td>2</td>
<td>2:4</td>
<td>2:3:5</td>
<td>2:3:4</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2:3</td>
<td>unmethyalted</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2:3:4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tragacanth A. Acid</td>
<td>2:3</td>
<td>monomethyl</td>
<td>-</td>
<td>2:3:4</td>
<td>2:3:4</td>
<td>12,150</td>
</tr>
<tr>
<td>B. Neutral</td>
<td>-</td>
<td>dimethyl</td>
<td>2:3:5</td>
<td>2:3</td>
<td>2:3</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>unmethyalted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
OBJECT OF THE PRESENT INVESTIGATION

A preliminary investigation of gum ghatti from Anogeissus latifolia, Wall (family, Combretaceae), carried out by Aspinall, Hirst and Wickström (101) revealed some similarities to gums of both the Prunus and Acacia genera. Hydrolysis of gum ghatti yielded two aldobiouronic acids, one of which, 2-O-β-D-glucuronosyl-D-mannose, had also been obtained from damson and cherry gums, and the other aldobiouronic acid, 6-O-β-D-glucuronosyl-D-galactose, had been isolated from the mannose-free Prunus gums and from the Acacia gums [Table I, p. 24].

Of all the plant gums, only gum arabic has been examined in great detail and it was considered that it would be of interest to carry out an extensive investigation of the highly complicated structure of gum ghatti. No other gum of the genus Anogeissus had been fully examined, but it was hoped to make a fuller comparison of gum ghatti with the gums of the Prunus and Acacia genera, in particular with gum arabic.
EXPERIMENTAL.
EXPERIMENTAL

SECTION I. GENERAL METHODS.

Paper Chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (v/v):

A. Butan-1-ol-ethanol-water (4 : 1 : 5, upper layer).
C. Ethyl acetate-acetic acid-water (3 : 1 : 3, upper layer).
D. Butan-1-ol-acetic acid-water (4 : 1 : 5, upper layer).
E. Benzene-ethanol-water (169 : 47 : 15, upper layer).
F. Ethyl acetate-acetic acid-formic acid-water (18 : 3 : 1 : 4).
G. Butan-2-one, half saturated with water containing 1% ammonia.
H. Ethyl acetate-acetic acid-water (3 : 3 : 1).
J. Ethyl acetate-pyridine-water (10 : 4 : 3).
K. Butan-1-ol-ethanol-water (1 : 1 : 1).

Papers were sprayed with a saturated aqueous solution of aniline oxalate and spots were developed at 120° for ten minutes. Unless otherwise stated, chromatography of methylated sugars was carried out in solvent A, and R<sub>G</sub> values refer to rate of movement relative to 2:3:4:6-tetra-O-methyl-D-glucose in that solvent.

Thick paper sheets (Whatman 3 MM) for chromatographic separation of sugars were
were first extracted with methanol in a Soxhlet apparatus. 

**Cellulose Columns** were packed dry and then washed with water, followed by the solvent mixture. Solvents used for column chromatography were purified as follows:— Light petroleum (b.p. 100°-120°) (2 litres) was shaken overnight with concentrated sulphuric acid (200 ml.), washed free of acid and distilled. Butan-1-ol (1 litre) was refluxed with potassium hydroxide (10 g.) for one hour and then distilled.

Fractions of 15 - 20 ml. were collected from columns. A sample (6 ml.) was taken from every fifth fraction, evaporated to dryness and examined chromatographically. Fractions were bulked accordingly, evaporated under reduced pressure and cleaned by dissolving the sugar in a little water and treating with charcoal.

Evaporations were carried out at 40° under reduced pressure.

Optical rotations were observed at 18° ± 2°.

Small scale hydrolysates were carried out at 100° on 5 mg. samples with acid (1 ml.) of the specified normality. In the case of sulphuric acid hydrolysates, neutralisation was effected with solid barium carbonate, followed by filtration and evaporation. Hydrochloric acid hydrolysates were neutralised with silver carbonate, filtered, treated with hydrogen sulphide, evaporated to dryness to coagulate silver salts, redissolved, filtered and evaporated. Hydrolysates were then ready for chromatographic examination.
Methoxyl determinations were carried out by the Zeisel semi-micro procedure (152-154).

Demethylations of methylated sugars were carried out by the procedure of Hough, Jones and Wadman (155), using sugar (5 mg.) and hydrobromic acid (1 ml.). The solution was kept at 100° for eight minutes, neutralised with silver carbonate, filtered, treated with hydrogen sulphide and evaporated. The products were redissolved, filtered and identified chromatographically.

Paper Ionophoresis (60, 61) was carried out in borate buffer (pH 10) at a potential of 500 volts. Papers were run for five hours and then sprayed with a saturated aqueous solution of aniline oxalate acidified with glacial acetic acid.

Aniline derivatives of methylated sugars were prepared by refluxing equimolar amounts of aniline and the sugar in dry ethanol (2 - 3 ml.) for 30 - 40 minutes with exclusion of light. Evaporation of the solvent gave the anilide.

Lactones of aldonic acids of methylated sugars were prepared by oxidation of the sugar (10 - 100 mg.) in water (2 - 3 ml.) with bromine (5 - 20 drops). The mixture was kept in the dark for three days, excess bromine was removed by aeration and the solution was neutralised with silver carbonate, filtered, treated with hydrogen sulphide and evaporated to dryness. The residue was extracted with ethyl acetate or ethanol. When possible, the product was distilled under reduced pressure (0.05 mm. mercury) but this step was omitted when less than 50 mg. sugar was available. The lactone was recrystallised, generally /
generally from ethyl acetate.

Amides of aldonic acids of methylated sugars were prepared by treating the lactone (5 - 100 mg.) with methanolic ammonia (5 - 10 ml.). The solution was kept at 0° for two days, evaporated and the product recrystallised from ethyl acetate or ethanol.

Periodate oxidation of methylated sugars was carried out by the method of Lemieux and Bauer (156). The syrup (1 mg.) was dissolved in 0.5 N sodium metaperiodate solution (0.12 ml.) and kept at 0° for one hour. Ethylene glycol (2 - 3 mg.) was added, the solution was warmed to room temperature and, after five minutes, made alkaline to phenolphthalein with 0.5 N sodium hydroxide. The product was examined chromatographically in solvent A. Results obtained for some standard sugars were as follows:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Oxidation Product</th>
<th>R_f</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3-di-O-methylgalactose</td>
<td></td>
<td>0.66</td>
<td>Grey</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.78</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.87</td>
<td>Grey</td>
</tr>
<tr>
<td>2:4-di-O-methylgalactose</td>
<td></td>
<td>0.37</td>
<td>Pink</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Unattacked 2:4-di-O-methylgalactose)</td>
</tr>
<tr>
<td>2:6-di-O-methylgalactose</td>
<td></td>
<td>0.15</td>
<td>Brilliant yellow</td>
</tr>
<tr>
<td>2-O-methylaldoses</td>
<td></td>
<td>0.15</td>
<td>Brilliant yellow</td>
</tr>
<tr>
<td>3-O-methylglucose</td>
<td></td>
<td>0.35</td>
<td>Wine red</td>
</tr>
<tr>
<td>4-O-methylmannose</td>
<td></td>
<td>0.60</td>
<td>Brown</td>
</tr>
<tr>
<td>6-O-methylgalactose</td>
<td></td>
<td>0.65</td>
<td>Diffuse yellow</td>
</tr>
</tbody>
</table>

Reduction /
Reduction of methylated acidic material was carried out with lithium aluminium hydride (34) in dry tetrahydrofuran or methylal. Tetrahydrofuran was first treated with sodium and then distilled off lithium aluminium hydride. Methylal (157) was fractionally distilled, using a Widmer column, treated with sodium for 24 hours and then refluxed for four hours with freshly cut sodium. Finally the methylal was distilled off lithium aluminium hydride.

The acidic material was converted to the methyl ester methyl glycoside and was dissolved in the solvent. An excess of lithium aluminium hydride was added and the mixture was heated under reflux for two hours. Excess hydride was destroyed by water, the organic layer was separated and the aqueous layer concentrated to dryness. The residue was extracted with chloroform or acetone and the reduced material was recovered from the combined organic extracts.

Reduction of neutral oligosaccharides was effected with potassium borohydride (158). The sugar (10 mg.) was dissolved in water (2 ml.) and treated with potassium borohydride (10 mg.) for 20 hours. Excess hydride was destroyed by shaking the solution with Amberlite resin IR 120 (H) (0.5 g.) for 30 minutes, followed by filtration and evaporation. Removal of borate ions was effected by the addition of methanol (3 x 15 ml.), followed by evaporation and, finally, treatment with Amberlite resin IR 4B (OH).
SYNTHESES OF METHYLATED SUGARS

In the course of methylation studies it was found necessary to synthesise the following methylated sugars as reference compounds.

Synthesis of L-0-methyl-D-mannose (159)

1:6-Anhydro-2:3-O-isopropylidene-β-D-mannopyranose (0.5 g.) was dissolved in dry acetone (3 ml.) and methyl iodide (3 ml.), in the presence of Drierite (0.5 g.). Silver oxide (1 g.) was added in small portions to the refluxing mixture over a period of nine hours. The solution was filtered, the silver residues were extracted with acetone and the combined organic extracts were evaporated, yielding a yellow syrup which was dissolved in a little ether. The addition of light petroleum (b.p. 40° - 60°) gave crystals with an indefinite melting point of about 100°, indicating the presence of much unchanged material. (The methylated product should melt at 52° - 53°).

The methylation was repeated, using methyl iodide (15 ml.) in which the syrup was readily soluble, Drierite (1 g.) and silver oxide (1 g.). The latter was added to the refluxing mixture over a period of six hours. Extraction of the silver residues as before gave a yellow syrup (0.41 g.).

The product was purified by chromatography on a small column of alumina, which had been shaken with N acetic acid, washed free of acid and dried at 100°. Elution of the column with ether-petroleum ether gave a syrup (0.4 g.) with methoxyl content 14.5% (Calc. 14.3%).
The methylated material was hydrolysed with N hydrochloric acid (4 ml.) at 100° for five hours. After neutralisation with silver carbonate, filtration and treatment with hydrogen sulphide, the solution was concentrated and the residue was extracted with ethanol, yielding a yellow syrup which failed to crystallise. Chromatographic examination in solvent A showed mainly 4-O-methylmannose with a trace of mannose.

Pure 4-O-methyl-D-mannose was obtained by chromatography on cellulose, using butan-1-ol, 66% saturated with water, as eluant. The sugar crystallised from dioxane.

**Synthesis of 2:3-di-O-methyl-D-glucose (160)**

4:6-Benzylidene-α-methyl-glucoside (3 g.) was dissolved in methyl iodide (100 ml.) and acetone (5 ml.), in the presence of Drierite (1 g.). Silver oxide (6 g.) was added in small portions to the refluxing mixture over a period of six hours. The solution was filtered and the silver residues were extracted with acetone. Evaporation of the combined organic extracts gave white crystals, which were recrystallised from light petroleum (b.p. 60° - 80°) and had an indefinite melting point of about 100° and methoxyl content 26% (Calc. 30%).

The methylation was repeated, using methyl iodide (50 ml.), Drierite (1 g.) and silver oxide (6 g.). Extraction of the silver residues as before gave white crystals (2.4 g.).
The methylated product was hydrolysed with N hydrochloric acid (25 ml.) at 100° for one hour in an atmosphere of carbon dioxide. The benzaldehyde formed was removed by extraction with ether and the aqueous layer was neutralised with silver carbonate, filtered, treated with hydrogen sulphide and evaporated. Extraction of the residue with acetone gave a pale yellow syrup which was chromatographically pure and crystallised slowly.
SECTION II. PREPARATION AND EXAMINATION OF METHYLATED DEGRADED GUM Ghatti

PURIFICATION OF GUM

Crude gum ghatti, which consisted of hard yellowish nodules, was purified by the method described by Hirst and Jones for damson gum (87). The gum (10 g.) was dissolved in water (200 ml.), filtered through a muslin and the filtrate was poured, with stirring, into ethanol (700 ml.) containing concentrated hydrochloric acid (5 ml.). The precipitated gum acid was ground once with ethanol (25 ml.) containing concentrated hydrochloric acid (1 ml.). As it proved difficult to remove all chloride ions from the gum acid by washing with alcohol, the solid was again dissolved in water and reprecipitated with ethanol. The gum acid was obtained by centrifugation, triturated with ethanol until free of chloride ions and dried over phosphorus pentoxide, giving a pale cream hygroscopic powder.

AUTOHYDROLYSIS OF THE PURIFIED GUM ACID

A solution of the gum acid (1%) in water was heated on a boiling water bath for 48 hours. Samples (1 ml.) were withdrawn at approximately six hourly intervals and were poured into ethanol (4 ml.) to precipitate the polysaccharide present. The filtrates were concentrated and examined chromatographically.
chromatographically in solvent B. No hydrolysis was apparent after four hours, but from nine hours onwards a considerable amount of arabinose was liberated with some galactose, a small amount of xylose and traces of mannose. There was some evidence of an oligosaccharide after 24 hours. As it was not possible to estimate the time at which optimum arabinose production was reached, the autohydrolysis was repeated using the Somogyi method to estimate increasing reducing power.

A solution of gum acid (0.1254 g.) in water (250 ml.) was estimated to contain about 1 mg. of reducing sugar in 5 ml. of solution, assuming that up to 40% of the gum acid undergoes autohydrolysis. The solution was heated on a boiling water bath, duplicate samples (5 ml.) were withdrawn every four hours and reducing power was estimated with Somogyi's reagent (45):

<table>
<thead>
<tr>
<th>Hours of hydrolysis</th>
<th>Reducing sugar, expressed as % of original gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td>8.5</td>
<td>7.3</td>
</tr>
<tr>
<td>12.5</td>
<td>10.2</td>
</tr>
<tr>
<td>16</td>
<td>13.2</td>
</tr>
<tr>
<td>20</td>
<td>15.5</td>
</tr>
<tr>
<td>24</td>
<td>17.4</td>
</tr>
<tr>
<td>36.5</td>
<td>22.54</td>
</tr>
<tr>
<td>40.5</td>
<td>26.3</td>
</tr>
<tr>
<td>44.5</td>
<td>27.6</td>
</tr>
<tr>
<td>49</td>
<td>29</td>
</tr>
<tr>
<td>64.5</td>
<td>33.8</td>
</tr>
<tr>
<td>76</td>
<td>37.1</td>
</tr>
<tr>
<td>90</td>
<td>38.9</td>
</tr>
<tr>
<td>94.75</td>
<td>39.7</td>
</tr>
<tr>
<td>115</td>
<td>41.0</td>
</tr>
</tbody>
</table>

Results /
Results show a constant rate of production of arabinose up to 80 hours, but between 80 and 90 hours the rate begins to decrease. The sugars liberated were examined chromatographically in solvent B after 46 and 90 hours and in both cases mainly arabinose was detected with some galactose, xylose and traces of rhamnose.

After 115 hours heating, the solution was filtered, concentrated to a small volume and poured into ethanol. The precipitated degraded gum, obtained in poor yield, was hydrolysed with N sulphuric acid at 100° for six hours. Chromatographic examination in solvent B showed mainly galactose, with some mannose, arabinose and a trace of xylose, indicating that not all the arabinose is readily removable.

PREPARATION OF THE DEGRADED GUM ACID

With a view to obtaining a good yield of degraded gum, investigations were carried out on the gum acid (assuming that its behaviour is similar). Solutions of the gum acid were neutralised in turn with 5% solutions of barium hydroxide, sodium hydroxide and potassium hydroxide. A comparison of the yields of gum obtained by precipitation with ethanol showed that the barium salt was obtained in 77% yield, as compared with 60 - 63% yields for the sodium and potassium salts.
The gum acid (2 g.) was dissolved in water (200 ml.), heated at 100° for 92 hours, filtered and concentrated (20 ml.). The solution was brought to pH 6 with barium hydroxide, using a pH meter, and then poured into ethanol (4 volumes). The precipitated degraded gum was dissolved in water, treated with Amberlite resin IR 120 (H) and freeze dried. Yield 0.4 g.

The degraded gum was prepared in quantity as follows. Treating two litres at a time, the gum acid (76 g.) in 1% solution was heated at 100° for 92 hours. The solutions were filtered, concentrated, bulked (1500 ml.) and brought to pH 6 with barium hydroxide solution. The degraded gum was precipitated in three fractions by the addition of one, two and three volumes of ethanol. Each fraction was dissolved in water, treated with Amberlite resin IR 120 (H), filtered and freeze dried, giving pale cream coloured solids (6.6 g., 4.8 g. and 4.6 g.) having $[\alpha]_D = +9.6^\circ$, $+5.0^\circ$ and $+2.0^\circ$ respectively. A sample of each fraction was hydrolysed with $N$ sulphuric acid (1 ml.) at 100° for four hours. Chromatography of the products in solvents B and C showed much galactose, some mannose and arabinose and traces of xylose. There was no significant difference between the fractions, which were combined for methylation.

**METHYLATION OF THE DEGRADED GUM ACID**

The degraded gum (15 g.) was dissolved in water (180 ml.) and methylated /
methylated (10) in an atmosphere of nitrogen, keeping the temperature of
the solution below 20°. Dimethyl sulphate (120 ml.) and 30% sodium hydro-
oxide solution (240 ml.) were added dropwise, with stirring, over a period
of six hours and stirring was continued overnight. After five further
similar additions of dimethyl sulphate and sodium hydroxide, the solution
was heated on a boiling water bath, cooled, neutralised (pH 6) with glacial
acetic acid and again heated to 100°. Some material which separated was
removed by filtration, dissolved in chloroform, filtered and precipitated
with light petroleum, giving a brown solid (4 g.). Repeated extraction of
the aqueous solution with chloroform yielded a white solid (4 g.); the
combined products were dissolved in chloroform and precipitated with light
petroleum, giving a solid (8.2 g.) with methoxyl content 37%.

The partially methylated polysaccharide, which was insoluble in
methyl iodide, was dissolved in water (200 ml.), treated with Amberlite resin
IR 120 (H) at 0° and freeze dried to a powdery white solid which was soluble
in methyl iodide - acetone. The gum was divided into two batches (3.5 g.
each) and methylated with methyl iodide (200 ml.) and silver oxide (10 g.).
Each batch was isolated and subjected to a second methylation, giving yellow
syrups (3.2 g. and 3.5 g.) which were dissolved in chloroform. Addition of
light petroleum precipitated a white polysaccharide in each case (fraction (a)
- 1.8 g. and 1.9 g.), while evaporation of the remaining solutions yielded
yellow /
yellow syrups (fraction (b) = 1.4 g. and 1.6 g.). The four samples of methylated gum were examined:

<table>
<thead>
<tr>
<th>Fraction (a)</th>
<th>Fraction (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Batch</td>
<td></td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>-29.4°</td>
</tr>
<tr>
<td>OMe</td>
<td>44.6%</td>
</tr>
<tr>
<td>2nd Batch</td>
<td></td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>-27.4°</td>
</tr>
<tr>
<td>OMe</td>
<td>44.2%</td>
</tr>
</tbody>
</table>

(Rotations were observed in chloroform, $c = 1.0$).

The (a) fractions appeared identical but differed from the (b) fractions, which were assumed to be more highly degraded. (The fully methylated undegraded gum has $[\alpha]_D -72^\circ$).

**HYDROLYSIS OF THE METHYLATED DEGRADED GUM ACID**

A sample (0.05 g.) of the combined fractions (a) was insoluble in water but dissolved in 2 N hydrochloric acid after 18 hours at $34^\circ$C. The solution was diluted with water (1 volume), heated on a boiling water bath for six hours and then neutralised with silver carbonate. Chromatography in solvent A indicated at least five methylated sugars, but no unmethylated material.

The remainder of the combined fractions (a) (3.22 g.) dissolved in N hydrochloric acid (500 ml.) after standing for two days at $34^\circ$C. Heating caused /
caused coagulation of solid, but after five days at 34° it was possible to heat the solution to 100° without much precipitation of solid. After 12½ hours on a boiling water bath, rotation became constant at approximately $[\alpha]_D + 90°$. The solution was neutralised with silver carbonate, filtered, treated with hydrogen sulphide, filtered and concentrated to a syrup, which was finally dissolved in water and neutralised with barium carbonate. Filtration and concentration gave a dark brown syrup (3.0 g.), which was repeatedly extracted with dry ether in an attempt to separate neutral sugars from barium salts of acids.

EXAMINATION OF THE ETHER-SOLUBLE MATERIAL

Evaporation of the ether extracts gave a yellow syrup (1.9172 g.) which was separated by chromatography (52) on a cellulose column (60 x 3.4 cm.). The column was eluted with light petroleum-butan-1-ol (7:3) saturated with water, followed by light petroleum-butan-1-ol (1:1) saturated with water, and finally water.

Fraction 1. 0.023 g.  $R_g$ 0.93 - 0.97 and 0.72

Chromatography in solvent A indicated a mixture of sugars, $R_g$ 0.93 - 0.97, and a trace of tri-O-methylgalactose, $R_g$ 0.72

Chromatography in solvent E showed 2:3:4-tri-O-methylxylose (pink spot, $R_p$ 0.72) and two other sugars which corresponded with 2:3:4-tri-O-methylrhamnose.
methylrhamnose (yellow, $R_p$ 0.79) and 2:3:5-tri-O-methylarabinose (black spot, pink under ultraviolet light, $R_p$ 0.32).

Demethylation gave galactose and traces of arabinose and xylose.

The remainder of the fraction was hydrolysed with $N$ hydrochloric acid at 100° for six hours. The hydrolysate was shown chromatographically to contain much di-O-methylgalactose of unknown origin.

**Fraction 2.**

$0.097 \text{ g.} \quad R_g 0.90$

$[\alpha]_D = +100^\circ (c, 0.34)$.

$\text{O Me} = 49.0\%$ (Calc. 52.5% for tetra-O-methylhexose).

Chromatography in solvent A showed a pink spot identical with 2:3:4:6-tetra-O-methylgalactose and a trace of tri-O-methylgalactose.

The syrup ($0.039 \text{ g.}$) was identified as 2:3:4:6-tetra-O-methyl-D-galactose by conversion to the aniline derivative, m.p. and mixed m.p. 189° - 190°.

**Fraction 3.**

$0.092 \text{ g.} \quad R_g 0.89, 0.82 \text{ and } 0.72$

$\text{O Me} 38.9\%$ (Calc. 41.8% for tri-O-methylhexose).

Chromatography in solvents A and E showed three components corresponding with 2:3:4:6-tetra-O-methylgalactose, 3:4:6-tri-O-methylmannose and 2:3:4-tri-O-methylgalactose, $R_g 0.89, 0.82$, and 0.72 respectively. Demethylation /
Demethylation gave galactose and mannose.

Paper ionophoresis showed material which remained on the starting line (probably 2:3:4-tri-O-methylgalactose and 2:3:4:6-tetra-O-methylgalactose) and a component having the same mobility as 3:4:6-tri-O-methylmannose.

The syrup (1 mg.) was oxidised with periodate and the product shown chromatographically in solvent E to contain 2:3:4-tri-O-methylgalactose, 2:3:4:6-tetra-O-methylgalactose and 2:3:5-tri-O-methylarabinose. A sample of 2:3:4-tri-O-methylgalactose was also oxidised with periodate and shown to be unattacked. It follows that 2:3:5-tri-O-methylarabinose can only have arisen by oxidation of 3:4:6-tri-O-methylmannose, while the tri- and tetra-O-methylgalactose remained unattacked.

Separation of this fraction was attempted in various solvents, including butan-1-ol saturated with boric acid : water saturated with boric acid (1 : 1), on paper impregnated with boric acid (161). All attempts were unsuccessful.

The remainder of the syrup was converted into the corresponding mixture of aldohomides and was subjected to a Weerman reaction. Sodium hypochlorite solution was prepared by passing chlorine (5.5 g.) into a solution of sodium hydroxide (10 g.) in water (15 ml.). The solution was then made up to 100 ml. The amide mixture was dissolved in water (1 ml.), sodium hypochlorite /
hypochlorite added (1 ml.) and the solution kept at $0^\circ$ for 3 hours. Excess hypochlorite was destroyed with sodium thiosulphate. Sodium acetate and semicarbazide hydrochloride (saturated solution) were added and the mixture was left at $0^\circ$ for three days. White crystals of hydrazodicarbonamide were separated, washed and dried, m.p. 268$^\circ$ and mixed m.p. (with sample of m.p. 263$^\circ$ - 264$^\circ$) 264$^\circ$. The Weerman reaction further indicates the presence of 3:4:6-tri-O-methylmannose.

**Fraction 4.** 0.904 g. \[ \text{R} \text{G} \ 0.72 \]

\[
[a]_D = +118^\circ \quad (c, 0.42) 
\]

$$\text{OMe} = 41.7\% \quad (\text{Calc. 41.8}\% \text{ for a tri-O-methylhexose})$$

Chromatography in solvent A showed a pink spot identical with 2:3:4-tri-O-methylgalactose. Demethylation gave galactose only.

The sugar crystallised from acetone-ether as the monohydrate and was identified as 2:3:4-tri-O-methyl-D-galactose, m.p. 71$^\circ$ and mixed m.p. (with sample m.p. 75$^\circ$ - 76$^\circ$) 72$^\circ$ - 75$^\circ$. The sugar was also characterised as the aniline derivative, m.p. 165$^\circ$ - 167$^\circ$ and mixed m.p. (with sample of m.p. 159$^\circ$ - 161$^\circ$) 159$^\circ$ - 162$^\circ$.

**Fraction 5.** 0.112 g. \[ \text{R} \text{G} \ 0.72 \text{ and } 0.62. \]

Chromatographic examination in solvent A showed a mixture of 2:3:4-tri-O-methylgalactose, R$_G$ 0.72, and a substance, R$_G$ 0.62 (brown), corresponding /
corresponding with 3:4-di-O-methylmannose. Ionophoresis also showed a
component having the same mobility as 3:4-di-O-methylmannose.

Demethylation gave galactose, mannose and possibly a trace of
arabinose.

An attempt was made to separate the mixture in butan-1-ol saturated
with boric acid : water saturated with boric acid (1:1), on paper saturated
with boric acid (1:1), but with no success.

Another separation was attempted using Amberlite Resin IRA 400
(OH) (12 g.) in borate form (57, 58). The resin was packed in a small
column and washed with a solution of glucose, followed by water, and then boric
acid to elute the glucose. Finally the column was washed with water until
free of boric acid. The syrup was taken up in water and put on the column
which was eluted with water. No separation was obtained.

The whole fraction was converted to a mixture of lactones of
aldonic acids and then to the mixed amides. No crystals were obtained and
an attempted paper separation of the amides in solvent D was unsuccessful.

Fraction 6. 0.203 g.  Rg 0.49 and 0.75.

This fraction was a mixture of two components, one of which,
Rg 0.49, corresponded with 2:3-di-O-methylgalactose. A further chromatogram
run in solvent A was sprayed with p-anisidine hydrochloride (155) and also
indicated the presence of 2:3-di-O-methylgalactose. Demethylation gave only
galactose.
galactose.

Separation of the syrup (0.190 g.) on filter sheets, using solvent A, gave fractions 6 (a) (0.070 g.) and 6 (b) (0.054 g.)

**Fraction 6 (a)**

\[ R_g = 0.49 \]

\[ [\alpha]_D = +69^\circ \rightarrow +80^\circ \ (c, 0.37). \]

Paper ionophoresis showed 2:3-di-0-methylgalactose and possibly a trace of 2:4-di-0-methylgalactose. The presence of 2:3-di-0-methylgalactose was confirmed by periodate oxidation followed by chromatographic examination. The sugar was characterised as the aniline derivative of 2:3-di-0-methyl-D-galactose, which crystallised after one month and was then recrystallised from acetone - light petroleum, m.p. 125° - 128° and mixed m.p. 124° - 127°. An X-ray powder photograph confirmed the identity of the anilide.

**Fraction 6 (b)**

\[ R_g = 0.75. \]

The syrup (3 mg.) was hydrolysed with N hydrochloric acid at 100° for six hours. Chromatography of the product in solvent D indicated 2:3:4-tri-0-methylgalactose and possibly 2:3:4-tri-0-methylglucuronic acid.

The whole fraction (0.05 g.) was heated under reflux for six hours with 1.2% dry methanolic hydrochloric acid (4 ml.) and the product was neutralised with silver carbonate. The methyl ester methyl glycoside so formed (0.04 g.) was reduced with lithium aluminium hydride (0.04 g.) in methylal (15 ml.) and the reduced material was hydrolysed with N hydrochloric acid /
acid at 100° for six hours. Chromatography of the product in solvent A showed only 2:3:4-tri-O-methylgalactose. It is probable that this fraction contained a polymer of 2:3:4-tri-O-methyl-D-galactose, arising from incomplete hydrolysis of the methylated polysaccharide.

Fraction 7. 0.091 g.  \( R_g 0.48 \) and \( 0.75 \).

Chromatograms run in solvent A were sprayed with p-anisidine hydrochloride (155) and with aniline oxalate, and showed one component, \( R_g 0.75 \), similar to fraction 6 (b), and a binary mixture, \( R_g 0.48 \), probably consisting of 2:3-di-O-methyl-D-galactose and 2:4-di-O-methyl-D-galactose. Demethylation gave galactose only.

Separation of the syrup (0.088 g.) on filter sheets using solvent A gave fractions 7 (a) (0.054 g.) and 7 (b) (0.017 g.).

Fraction 7 (a) \( R_g 0.48 \).

Ionophoresis indicated 2:3-di-O-methylgalactose and 2:4-di-O-methylgalactose. The presence of 2:3-di-O-methylgalactose was confirmed by periodate oxidation followed by chromatographic examination. The oxidation products included some unaltered di-O-methylgalactose; this could be due to the presence of 2:4-di-O-methylgalactose which is unattacked by periodate.

The syrup (0.05 g.) was converted to the aniline derivative and crystals of 2:4-di-O-methyl-N-phenyl-D-galactosylamine were obtained, m.p. and /
and mixed m.p. 202° - 204°. This derivative crystallises much more readily than the aniline derivative of 2:3-di-O-methyl-D-galactose. The latter was probably present but was not obtained crystalline.

**Fraction 7 (b)**  \( R_g 0.75 \).

Chromatography showed fraction 7 (b) to be identical with 6 (b).

Hydrolysis with \( N \) hydrochloric acid at 100° for six hours gave 2:3:4-tri-O-methylgalactose.

**Fraction 8.**  \( 0.031 \) g.  \( R_g 0.36, 0.45 \) and 0.65.

Chromatography showed that this fraction contained a small amount of 4-O-methylmannose, \( R_g 0.36 \), a di-O-methylgalactose, \( R_g 0.45 \), and an unidentified sugar, \( R_g 0.65 \).

**Fraction 9.**  \( 0.043 \) g.

This fraction was obtained by water elution of the column and contained a complex mixture of acidic and neutral sugars, including 2:3:4-tri-O-methylglucuronic acid and 2:3:4-tri-O-methylgalactose. It was not examined further.

**EXAMINATION OF THE ETHER-INSOLUBLE RESIDUE**

The material remaining after ether extraction of neutral methylated sugars
sugars was dissolved in water and treated with Amberlite resin IR 120 (H) to remove barium ions. Evaporation gave a syrup (0.714 g.) which was shown by chromatography in solvent D to contain a mixture of substances, probably including 2:3:4-tri-O-methylgalactose.

A sample of the syrup (25 mg.) was converted to the methyl ester methyl glycosides by heating under reflux with 1% methanolic hydrochloric acid (4 ml.) for six hours. After neutralisation, the product was reduced with lithium aluminium hydride (14 mg.) in tetrahydrofuran (5 ml.) and was then hydrolysed with 0.8 N hydrochloric acid at 100° for six hours. Chromatography in solvents A and D showed a complicated mixture of at least five major components, none clearly separated. 2:3:4-tri-O-methylgalactose was present in large amount, also possibly di-O-methylgalactose and di-O-methylmannose.

The equivalent weight of the acidic material, obtained by titration with 0.01 N sodium hydroxide, was 597. This indicated the presence of either free neutral sugar together with aldobiouronic acid or aldotriouronic acid. If a third sugar unit were attached to an aldobiouronic acid, it might be expected to split off in the early stages of hydrolysis before cleavage of the aldobiouronic acid. Two samples (5 mg.) of the acidic syrup were hydrolysed with N hydrochloric acid (1 ml.) at 100° for 1 hour and 6 hours respectively. Chromatography in solvent D showed the products to be similar; both /
both included 2:3:4-tri-O-methylgalactose and 2:3:4-tri-O-methylglucuronic acid.

It appeared that free neutral sugar was present, together with aldobiouronic acids, and this was confirmed by neutralisation of the syrup (3 mg.) with barium carbonate, followed by examination in solvent A. A di-O-methylgalactose and 2:3:4-tri-O-methylgalactose were detected, while barium salts of acids remained on the starting line.

The remainder of the fraction (0.63 g.) was dissolved in water and neutralised with barium carbonate. The product (0.66 g.) was separated on filter sheets using solvent A to give:

- Fraction (a) 0.365 g. Barium salts of acids.
- Fraction (b) 0.092 g. Di-O-methylgalactose.
- Fraction (c) 0.190 g. Tri-O-methylgalactose.

Fractions (b) and (c) were contaminated with acids.

**Fraction (a)**

A sample (25 mg.) was treated with Amberlite resin IR 120 (H) to give a mixture of free acids. Titration with 0.01 N sodium hydroxide solution gave an acid equivalent of 370, indicating mainly aldobiouronic acid with some free uronic acid.

The barium-free syrup (5 mg.) was hydrolysed with N hydrochloric acid /
acid at 100° for six hours. The solution was neutralised with silver carbonate but was not treated with hydrogen sulphide. Chromatography in solvent A showed tri- and di-O-methylgalactose and tri- and di-O-methylmannose, while silver salts of acids remained on the starting line.

The remainder of fraction (a) (0.33 g.) was treated with Amberlite resin IR 120 (H) and then hydrolysed with N hydrochloric acid at 100° for seven hours. The solution was neutralised with silver carbonate but treatment with hydrogen sulphide was omitted, leaving the acids as silver salts. Separation on filter sheets in solvent A gave five fractions:

**Fraction (i) 0.143 g.**

This fraction contained silver salts of acids which were converted into free acids and separated on filter sheets in solvent D, giving chromatographically pure 2:3:4-tri-O-methylglucuronic acid (0.043 g.) and a mixture of at least three components (0.036 g.). Attempts to characterise the tri-O-methylglucuronic acid by conversion into the crystalline amide of methyl 2:3:4-tri-O-methyl-a-D-glucuronoside failed. Methanolysis of the syrupy amide was carried out in 2% methanolic hydrochloric acid (2 ml.) and the methyl ester methyl glycoside so formed was dissolved in methylal and reduced with lithium aluminium hydride. The product was hydrolysed with N hydrochloric acid at 100° for six hours. Chromatography in solvent A then showed only
2:3:4-tri-O-methylglucose, \( R_G \) 0.85.

The three component mixture was converted into the methyl ester methyl glycosides by refluxing with dry 1.3% methanolic hydrochloric acid for six hours. Reduction of the neutralised product with lithium aluminium hydride in methyalal was followed by hydrolysis with N hydrochloric acid at 100° for six hours. Chromatography in solvent A showed 2:3-di-O-methylgalactose to be the major component, with small amounts of tri- and di-O-methylgalactose and a trace of 2:3:4-tri-O-methylglucose.

**Fraction (ii)** 0.029 g.

Chromatography in solvent A showed this fraction to contain di-O-methylgalactose, \( R_G \) 0.49, probably the 2:3-dimethyl ether. Ionophoresis and chromatography of the periodate oxidation products confirmed the presence of 2:3-di-O-methylgalactose.

**Fraction (iii)** 0.026 g.

Chromatography showed 2:3:4-tri-O-methylgalactose, \( R_G \) 0.72, and a trace of a second component, \( R_G \) 0.60, probably di-O-methylmannose.

**Fraction (iv)** 0.035 g.

2:3:4-Tri-O-methylgalactose, \( R_G \) 0.72, was indicated by chromatography.

The syrup was characterised as 2:3:4-tri-O-methyl-D-galactose by conversion /
conversion to the aniline derivative, m.p. 159° - 162° and mixed m.p. 156° - 159°.

Fraction (y) 0.009 g.

Chromatography showed 3:4:6-tri-O-methylmannose, Rg 0.82.

The sugar failed to crystallise. It was converted to the lactone of the aldonic acid and then to the aldonamide, neither of which were obtained crystalline.

Fraction (b) 0.092 g.

This fraction was shown chromatographically to contain di-O-methylgalactose but acidic substances were also present.

The aniline derivative failed to crystallise, so the sugar was regenerated by the addition of water (5 ml.) and Amberlite resin IR 120 (H) (0.5 g.) (162). After heating under reflux for one hour, the recovered syrup was chromatographically identical with 2:3-di-O-methylgalactose, but contained acid as well. Periodate oxidation products indicated 2:3-di-O-methylgalactose.

Fraction (c) 0.190 g.

Chromatographic examination indicated 2:3:4-tri-O-methylgalactose, Rg 0.72, and acidic substances. The aniline derivative failed to crystallise, probably due to the acidic material present.
SECTION III. PREPARATION AND EXAMINATION OF METHYLATED GUM CHATTI

METHYLATION OF THE GUM ACID

The gum acid (25 g.) was dissolved in water (300 ml.) and methylated by the method used for the degraded gum acid. Five additions of dimethyl sulphate (200 ml.) and 30% sodium hydroxide solution (400 ml.) were made, with constant stirring.

The solution was heated on a boiling water bath, cooled, neutralised (pH 6) with glacial acetic acid and again heated to 100°C. The polysaccharide separated as a finely divided solid which was filtered from the hot solution. The solid was dissolved in chloroform and the polysaccharide was reprecipitated from the chloroform solution with light petroleum. The product (18 g.) had methoxyl content 35% and ash content 5.8%.

A sample of the polysaccharide (2 g.) dissolved in methyl iodide (55 ml.) with the addition of methanol (5 ml.); silver oxide (27 g.) was added to the refluxing mixture over a period of eight hours. The solution was filtered and the silver residues were extracted with chloroform. The combined organic extracts gave a syrup (about 2 g.), which was again treated with methyl iodide and silver oxide, yielding eventually a pale yellow syrup. This was dissolved in chloroform and precipitated with light petroleum, giving a white solid (1.2 g.).
The remainder of the partially methylated gum was divided into two further batches (7.5 g. each) and treated similarly with methyl iodide and silver oxide, giving products with $\left[\alpha\right]_D = -72^\circ$ (CHCl$_3$, c = 1) and OMe 38%.

Samples (10 mg.) of batches 2 and 3 were subjected to methanolysis and the products were examined chromatographically in solvent A. The two samples were identical, each containing at least eight or nine methylated sugars.

Each of the three batches of methylated gum was treated once more with methyl iodide and silver oxide, yielding white solids (0.9 g., 3.9 g. and 3.4 g.) with methoxyl contents 40.4%, 43.0% and 42.5% respectively.

A sample of the methylated gum acid was suspended in N hydrochloric acid. The mixture was kept at 40° for two weeks, but the gum proved insoluble.

Batches 2 and 3 of the methylated gum were combined (7.32 g.), dissolved in 2% methanolic hydrochloric acid (500 ml.) and heated for 12 hours until rotation became constant ($\left[\alpha\right]_D = -7^\circ$). The solution was concentrated (100 ml.), methanol (100 ml.) was added and the solution again concentrated (100 ml.). After the addition of water (500 ml.), all the methanol was evaporated and hydrochloric acid was added until titration showed the normality to be 0.55. The solution was then heated on a boiling water bath for 12 hours until rotation again became constant. The solution was neutralised with /
with silver carbonate, filtered, treated with hydrogen sulphide and concentrated. Extraction of the residue with methanol gave a syrup which was dissolved in water, neutralised with barium carbonate, filtered and concentrated to a syrup (7.3 g.).

SEPARATION OF ACIDIC FROM NEUTRAL METHYLATED SUGARS.

The mixture of methylated sugars (7.3 g.) was repeatedly extracted with dry ether and the extracts were bulked and evaporated, giving a pale yellow syrup (4.765 g.). Chromatography in solvent A showed the ether extract to be free of acids, but some of the less highly methylated neutral material was left with barium salts in the ether-insoluble residue. The latter (2.099 g.) was separated by chromatography on a cellulose column (50 x 2.5 cm.) into five fractions, using as eluant butan-1-ol, 80% saturated with water containing a trace of ammonia. Fractions of about 200 ml. were collected, evaporated and examined chromatographically in solvents A and D. When all neutral sugar had been removed, the column was eluted with water.

Fraction A. 0.803 g.

The syrup contained a mixture of neutral methylated sugars, free of acid. It was combined with the ether-soluble fraction.
Fraction B.  0.224 g.

Chromatographically pure 2-O-methyl-D-galactose crystallised immediately and was recrystallised from water-acetone, m.p. 157° - 158°.

\[[\alpha]_D^0 = +55° (5\text{ min.}) \rightarrow +39° (120\text{ mins. const.})\ (c, 1.91)\].

Fraction C.  0.142 g.

Chromatography showed a mixture of acidic and neutral sugars, including 2-O-methylgalactose. This fraction was not examined further.

Fraction D.  0.030 g.

The syrup contained acids and a trace of 2-O-methylgalactose.

Fraction E.  0.368 g.

Water elution of the column gave a mixture of acids.

Fractions D and E were retained for later examination.

EXAMINATION OF NEUTRAL METHYLATED SUGARS

The ether-soluble sugars and fraction A were combined (5.568 g.) and separated by chromatography on a cellulose column (76 x 3.5 cm.). The column was eluted with light petroleum (b.p. 100° - 120°)-butan-1-ol (7 : 3) followed by (1 : 1) both saturated with water. After elution of monomethylmannose, the solvent was changed to butan-1-ol half saturated with water and finally /
finally the column was washed through with water.

Twenty-one fractions were collected:

Fraction 1. 0.119 g.  $R_G 1.03$

$[a]_D = +15° (c, 0.39)$

Chromatography in solvent A showed mainly 2:3:4-tri-O-methylrhamnose, $R_G 1.03$ (yellow), while in solvent E a second and faster component was detected.

A sample of the syrup (15 mg.) was hydrolysed with N sulphuric acid (1 ml.) at 100° for four hours. Chromatography of the product in solvent A showed 2:3:4-tri-O-methylrhamnose, 2:3:5-tri-O-methylarabinose and the barium salt of an acid at the starting line. Hydrolysis of a further sample (10 mg.) was carried out with cold 5% barium hydroxide solution (2 ml.) for 30 minutes. After neutralisation with carbon dioxide, chromatography in solvent A gave a similar result, indicating cleavage of an ester linkage.

The remaining syrup (30 mg.) was then treated with cold barium hydroxide solution for 30 minutes, neutralised with carbon dioxide, filtered and evaporated. The product was separated on filter sheets in solvent A into fractions 1(a) and 1(b).

Fraction 1(a)  $R_G 1.03$ and 0.97

The syrup contained mainly 2:3:4-tri-O-methylrhamnose, $R_G 1.03$, and
and a trace of 2:3:5-tri-O-methyalarabinose, $R_g$ 0.97.

The aniline derivative of 2:3:4-tri-O-methyl-L-rhamnose was obtained as white needles, which were recrystallised from light petroleum, m.p. and mixed m.p. $98° - 100°$.

**Fraction 1(b). Barium salt.**

Treatment with Amberlite resin IR 120 (H) removed barium ions, and chromatography in solvent D showed 2:3:4-tri-O-methylglucuronic acid. The syrup was converted to the methyl ester methyl glycoside with dry methanolic hydrochloric acid, reduced with lithium aluminium hydride in methylal and hydrolysed with N hydrochloric acid. Chromatographic examination in solvent A showed 2:3:4-tri-O-methylglucose.

It is concluded that fraction 1 contained 2:3:4-tri-O-methyl-L-rhamnose and an ester of 2:3:4-tri-O-methyl-D-glucuronic acid.

**Fraction 2.**

$0.150 \text{ g.}$

$R_g$ 0.97 and 1.03

$[\alpha]_D = -44° \ (c, 0.59)$

Chromatography in solvent A showed 2:3:5-tri-O-methyalarabinose, $R_g$ 0.97 (black), with a trace of 2:3:4-tri-O-methylrhamnose, $R_g$ 1.03, but in solvent E only 2:3:5-tri-O-methyalarabinose was detected. The optical rotation indicated almost pure 2:3:5-tri-O-methyl-L-arabinose ($[\alpha]_D = 39.5°$).

**Fraction /**
Fraction 3.

1.835 g. \( R_G \) 0.97

\([\alpha]_D = -42.5^\circ \) (c, 0.68)

OMe = 46.2\% (Calc. 48.4\% for a tri-O-methylpentose)


2:3:5-Tri-O-methyl-L-arabinose was identified by conversion to the crystalline amide of the aldonic acid, m.p. 132° - 133° and mixed m.p. (with a sample of m.p. 129° - 130°) 129°.

Fraction 4.

0.034 g. \( R_G \) 0.97

\([\alpha]_D = +66^\circ \) (c, 0.48).

Chromatography in solvent E indicated mainly 2:3:4-tri-O-methylxylose, with some 2:3:5-tri-O-methylarabinose and an unidentified faster component. Demethylation gave xylose, galactose and a little arabinose.

Fraction 5.

0.034 g. \( R_G \) 0.90.

\([\alpha]_D = +30.5^\circ \) (c, 0.59).


2:3:4:6-Tetra-O-methyl-D-galactose was identified as the aniline derivative.
derivative, m.p. and mixed m.p. 175° - 180°.

**Fraction 6.**

0.017 g. \( R_G \) 0.98 and 0.83.

\([\alpha]_D = -35^\circ (c, 0.29)\).

Chromatography showed a main component, \( R_G \) 0.83, giving a brown spot fluorescing yellow in ultraviolet light. This was identical with both 3:5-di-O-methylarabinose and 3:4:6-tri-O-methylmannose, but differed from 2:5-di-O-methylarabinose which gives a black spot, \( R_G \) 0.85. A minor component, \( R_G \) 0.98 (black), was also present. Demethylation gave arabinose and small amounts of galactose and mannose.

It was found that a standard mixture of 3:4:6-tri-O-methylmannose, 2:5- and 3:5-di-O-methylarabinose could be clearly separated by ionophoresis. Fraction 6 was shown by this means to consist mainly of 3:5-di-O-methylarabinose with small amounts of the other two sugars.

**Fraction 7.**

0.230 g. \( R_G \) 0.83 and 0.85.

\([\alpha]_D = -24^\circ (c, 0.42)\).

\( OMe = 37.8\% \) (Calc. 34.8% for a di-O-methylpentose)

Chromatography and ionophoresis indicated approximately equal amounts of 2:5-di-O-methylarabinose, \( R_G \) 0.85 (black), and 3:5-di-O-methylarabinose, \( R_G \) 0.83 (brown).
An attempt was made to separate the mixture (40 mg.) by gradient elution on a charcoal column, using borate buffer (pH 10) and butan-2-one (59). This proved unsuccessful.

The remainder of the syrup (0.160 g.) was separated on an anion exchange resin in borate form (57, 58). Amberlite resin IRA 400 (OH) (15 g.), mesh 20 - 50, was stirred with 0.1 molar sodium tetraborate (1 litre), washed with water and packed in a small column. The syrup was taken up in a little water and put on to the column, which was eluted with water (500 ml.), yielding fraction 7(a) (0.040 g.). This was shown by ionophoresis to be pure 2:5-di-O-methylarabinose. Elution of the column with 0.5 molar boric acid (500 ml.) gave fraction 7(b) (0.010 g.), which was shown by ionophoresis to be pure 3:5-di-O-methylarabinose. Further elution with more concentrated acids failed to increase the yield. This method of separation afforded a pure sample of each component but much sugar was irreversibly adsorbed on the resin.

Fraction 7(a) was identified as 2:5-di-O-methyl-L-arabinose by conversion to the aldonic acid and then to the amide which crystallised from ethyl acetate, m.p. 122° and mixed m.p. (with a sample of m.p. 124° - 125°) 123° - 124°.

Fraction 7(b) was identified as 3:5-di-O-methyl-L-arabinose by conversion to the crystalline lactone of the aldonic acid, m.p. 65° and mixed m.p. (with a sample of m.p. 69° - 71°) 67° - 69°.

Fraction /
Fraction 8. (0.030 g.). $R_g$ 0.33 - 0.85 and 0.75.

Chromatography and ionophoresis showed this fraction to contain 2:5- and 3:5-di-O-methylarabinose, 3:4:6-tri-O-methylmannose and traces of 2:3:4-tri-O-methylgalactose and an unidentified sugar.

An attempt was made to separate the mixture by gradient elution on a charcoal column, using borate buffer (pH 10) with gradually increasing proportions of methyl ethyl ketone (59). This was unsuccessful.

Fraction 9. 0.174 g. $R_g$ 0.65 and 0.70.

$[\alpha]_D^\circ = + 71.5^\circ \rightarrow + 119.3^\circ$ (c, 0.42).

Chromatography in solvents A and F indicated a mixture of 2:3:4-tri-O-methylgalactose, $R_g$ 0.7, and 2:3-di-O-methylarabinose, $R_g$ 0.65, in about equal proportions.

Demethylation gave galactose and arabinose.

Separations in solvents A, F and G were not clear enough for fractionation on filter sheets.

A sample of the syrup (0.01 g.) was converted to a mixture of lactones of aldonic acids and examined chromatographically in solvent D, using hydroxylamine hydrochloride and ferric chloride sprays (163). Only one unresolved spot was visible, $R_p$ 0.78. Conversion of the lactones to amides gave a white crystalline substance which was examined chromatographically in solvent D. The paper was sprayed with ninhydrin and two well separated spots were detected.
detected, the faster one being identical with 2:3-di-O-methylarabonamide.

With a view to obtaining a separation on filter sheets, the remainder of the fraction was converted to the mixed aldonamides. The product, however, crystallised immediately and was twice recrystallised from ethanol-ethyl acetate giving white needles of 2:3-di-O-methyl-L-arabonamide, m.p. 154° and mixed m.p. 153° - 154°.

Fraction 10. 0.299 g. Rf 0.70

\[ [\alpha]_D^{134°} \rightarrow +109° \] (c, 0.51).

OMe = 37.4\% (Calc. 41.9\% for tri-O-methylhexose, 38.7\% for the monohydrate).

This fraction contained chromatographically pure 2:3:4-tri-O-methylgalactose.

The syrup crystallised slowly. Recrystallisation from acetone-ether gave 2:3:4-tri-O-methyl-D-galactose monohydrate, m.p. and mixed m.p. 66° - 67°.

Conversion to the aniline derivative gave 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine (white plates, recrystallised from ethanol), m.p. and mixed m.p. 161° - 162°.

Fraction 11. 0.059 g. Rf 0.70 and 0.52 (trace)

\[ [\alpha]_D^{134°} = +88.4° \] (c, 0.33).

Chromatography /
Chromatography showed 2:3:4-tri-O-methylgalactose, R<sub>G</sub> 0.70, and a trace of an unidentified sugar.

2:3:4-Tri-O-methyl-D-galactose was identified by conversion to the aniline derivative, which was recrystallized from ethanol as white plates, m.p. and mixed m.p. 164° - 165°.

**Fraction 12.** 0.166 g.  R<sub>G</sub> 0.65 and 0.70.

\[[\alpha]_D^0 + 126° \rightarrow + 118° \text{ (c. 0.38)}.\]

Chromatography showed a main component, R<sub>G</sub> 0.65 (brown), and a much smaller amount of 2:3:4-tri-O-methylgalactose.

Demethylation gave arabinose and a smaller amount of galactose. This result, together with the optical rotation, indicated a mixture of a di-O-methyl-L-arabopyranose and 2:3:4-tri-O-methyl-D-galactose.

Further chromatographic examination in solvent P showed that the major component did not correspond with either 2:3- or 3:4-di-O-methylarabinose and must therefore be 2:4-di-O-methylarabinose. A trace of 2:3:4-tri-O-methylgalactose was also detected. Ionophoresis confirmed the absence of 3:4-di-O-methylarabinose; both components of fraction 12 remained on the starting line, further suggesting that 2:4-di-O-methylarabinose was present.

The syrup (0.06 g.) was converted to the aniline derivative which crystallized slowly. It was recrystallized as white plates from butan-1-ol and /
and was identified as 2:4-di-O-methyl-N-phenyl-L-arabinosylamine, m.p. 129° - 130° and mixed m.p. (with a sample of m.p. 139° - 140°) 125° - 126°. X-Ray powder photographs proved the derived anilide and the authentic sample to be identical.

**Fraction 13.**

0.0305 g.  \( R^* \) 0.52 and 0.68.  
\([\alpha]_D = +55^\circ \) (c, 0.29).

Chromatography showed di-O-methylgalactose, \( R^* \) 0.52, as the main component, with some 2:3:4-tri-O-methylgalactose, \( R^* \) 0.68. Demethylation gave galactose and a trace of arabinose.

Chromatography of the products of periodate oxidation showed mainly unaltered 2:4-di-O-methylgalactose. Also present in small amounts were oxidation products with \( R^* \) 0.66 (grey), 0.73 (brown) and 0.15 (bright yellow). The first two come from 2:3-di-O-methylgalactose and the last is formed from 2-O-methylaldoses and 2:6-di-O-methylhexoses. In this case, it probably indicates a trace of 2-O-methyl-L-arabinose.

**Fraction 14.**

0.250 g.  \( R^* \) 0.49 and 0.68 (trace).  

Chromatography in solvent A showed mainly di-O-methylgalactose, \( R^* \) 0.49, probably the 2:4-di-O-methyl ether. Also present were traces of 2:3:4-tri-O-methylgalactose, \( R^* \) 0.68, and a substance, \( R^* \) 0.32 (black), which was suspected to be 2-O-methylarabinose.
Denethylation gave galactose and a small amount of arabinose.

Periodate oxidation followed by chromatography gave results similar to fraction 13. A large amount of 2:4-di-O-methylgalactose was indicated, with traces of 2:3-di-O-methylgalactose and a substance giving, on oxidation, a bright yellow spot, \( R_f 0.15 \), (probably 2-O-methylarabinose).

Ionophoresis showed that the major component resembled 2:4-di-O-methylgalactose which remained on the starting line, while a much smaller component corresponded with 2:3- or 2:6-di-O-methylgalactose, both of which moved 5 cm in 5 hours and were indistinguishable. An unidentified trace component moved about 3 cm in 5 hours (? 2-O-methylarabinose).

A sample of the syrup (0.005 g.) was converted to a mixture of lactones of aldonic acids and examined chromatographically in solvent D. The paper was heated in the oven to ensure lactonisation, and then sprayed with hydroxylamine hydrochloride followed by ferric chloride (163), showing a main component, \( R_f 0.64 \), corresponding with 2:4-di-O-methylgalactonolactone, a second component, \( R_f 0.37 \), and a trace, \( R_f 0.5 \).

A larger sample of the syrup (0.15 g.) was then converted to the aldonolactones and separated on filter sheets in solvent D into fraction 14 (a), \( R_f 0.37 \), and fraction 14 (b), \( R_f 0.64 \). The latter contained 2:4-di-O-methylgalactonolactone, but both fractions were impure and failed to crystallise. The lactones were converted to the amides but again no crystals were obtained.

The /
The main component of fraction 14 was identified as 2:4-di-O-methyl-D-galactose by conversion of the remainder of the syrup (0.06 g.) to the aniline derivative, which crystallised from ethanol-ethyl acetate, m.p. and mixed m.p. 206° - 208°.

Fraction 15. 0.393 g.  R_{G} 0.47.

\[
[a]_{D} = + 133^\circ \rightarrow + 89^\circ \quad (c, 0.54)
\]

\[
\text{OMe} = 27.8\% \quad (\text{calc.} 27.4\% \text{ for a di-O-methylhexose monohydrate})
\]

This fraction consisted of white crystalline material, which was chromatographically pure and identical with 2:4-di-O-methylgalactose. The sugar crystallised from aqueous acetone as large white plates, m.p. and mixed m.p. (with 2:4-di-O-methyl-D-galactose monohydrate) 99°.

The derived anilide crystallised readily from ethanol and had m.p. 213° - 214° both alone and mixed with a sample of 2:4-di-O-methyl-N-phenyl-D-galactosylamine.

Fraction 16. 0.109 g.  R_{G} 0.36 and 0.47.

Chromatography showed 2:4-di-O-methylgalactose, R_{G} 0.47, a substance giving a brown spot, R_{G} 0.36, and a trace of a slightly slower moving sugar, thought to be 2-O-methylarabinose.

Demethylation gave galactose, mannose and a trace of arabinose.

Ionophoresis showed 2:4-di-O-methylgalactose on the starting line, a sugar
which moved faster than either 2- or 3-0-methylmannose and a trace of a third component.

The sugar having $R_g \, 0.36$ (brown) was present in fraction 17 as well as fraction 16, and investigations on both fractions indicated 4-0-methylmannose. The latter was synthesised for use as a standard.

The remainder of fraction 16 ($0.1 \, g.$) was separated by chromatography on cellulose ($50 \times 2.5 \, cm.$), using solvent G as eluant. Two fractions were obtained:

**Fraction 16 (a)** \quad $R_g \, 0.32$ and $0.47$.

Chromatography indicated 2:4-di-0-methylgalactose, $R_g \, 0.47$, and a trace of a substance, $R_g \, 0.32$ (pink), thought to be 2-0-methylarabinose.

Demethylation gave galactose and a trace of arabinose.

Chromatography of the products of periodate oxidation showed unaltered 2:4-di-0-methylgalactose and a trace of an oxidation product, $R_f \, 0.15$ (bright yellow), arising from a 2-0-methylaldose or 2:6-di-0-methylhexose and probably indicating 2-0-methylarabinose.

**Fraction 16 (b)** \quad $R_g \, 0.36$.

The sugar was chromatographically pure and identical with 4-0-methylmannose, but failed to crystallise from dioxane.

The
The sugar was converted to the lactone of the aldonic acid, which crystallised from ethanol-ethyl acetate as white needles, m.p. 159° - 160°, both alone and mixed with 4-O-methyl-D-mannolactone.

Fraction 17. 0.047 g.  R₉ 0.36.

\[ [\alpha]_D = +43^\circ \rightarrow +37^\circ \quad (\text{cf. 4-O-methyl-D-mannose,}) \]
\[ [\alpha]_D = +32^\circ \rightarrow +22^\circ \].

Ome = 17.3% (Calc. 18.9% for a mono-O-methylpentose 16.0% for a mono-O-methylhexose).

Chromatography showed 4-O-methylmannose, R₉ 0.36 (brown), with a trace of a second component, R₉ 0.32.

The optical rotation and methoxyl content suggested a mixture of 4-O-methyl-D-mannose and a mono-O-methyl-L-arabinose.

Chromatography of the products of periodate oxidation showed a brown spot, Rₚ 0.60, identical with that produced by oxidised 4-O-methylmannose, and a trace of a bright yellow spot, Rₚ 0.15, probably arising from oxidised 2-O-methylarabinose.

The syrup (0.045 g.) was dissolved in water (1 ml.) and converted to the osazone by the following method. Phenylhydrazine hydrochloride (0.1 g.), sodium acetate (0.17 g.) and a trace of sodium bisulphite were added to the solution, which was heated on a boiling water bath for one hour. Dilution with /
with water (2 ml.) caused precipitation of a yellow solid which was filtered off, washed with dilute acetic acid and then with water. The osazone was recrystallised from aqueous ethanol, and had m.p. 135° - 136°. Further re-crystallisation failed to raise the melting point.

The osazone was examined by circular chromatography and appeared to be identical with 4-O-methylglucosazone, although a second component was present as well.

An X-ray powder photograph of the osazone did not correspond exactly with photographs of either 3-, 4- or 6-O-methylglucosazone, possibly due to the presence of a second component.

Fraction 18. 0.084 g. R₉ 0.25, 0.36 and 0.47.

Chromatography showed a mixture of 2-O-methylgalactose, R₉ 0.25, 4-O-methylmannose, R₉ 0.36, and 2:4-di-O-methylgalactose, R₉ 0.47.

Fraction 19. 0.43 g. R₉ 0.25.

\[ [\alpha]_D = +64° \longrightarrow +91° \] (c, 0.37)

OMe = 14.1% (Calc. 16% for a mono-O-methylhexose).

The crystalline sugar was chromatographically pure and identical with 2-O-methylgalactose. Recrystallisation from glacial acetic acid gave a product with m.p. 146° - 147°, alone and mixed with 2-O-methyl-D-galactose. After recrystallisation from aqueous acetone the sugar had m.p. 154°.
Preparation of the aniline derivative was attempted, but crystalline 2-O-methyl-D-galactose was recovered. Crassone formation was also unsuccessful. The aqueous mixture of sugar (0.05 g.), phenylhydrazine hydrochloride (0.1 g.), sodium acetate (0.17 g.) and bisulphite was heated for one hour at 100°, cooled and diluted, but no solid precipitated. Stronger conditions are required to replace the methoxyl group at carbon 2.

Fraction 20. 0.032 g.

Chromatography showed 2-O-methylgalactose and traces of un-methylated arabinose.

Fraction 21. 0.064 g.

Water elution of the column gave a mixture of acids. The syrup (3 mg.) was converted to the barium salt and examined chromatographically in solvent A, showing the absence of neutral sugars.

EXAMINATION OF ACIDIC METHYLATED SUGARS.

Fraction D.

A small sample (3 mg.) was converted to the barium salt and examined chromatographically in solvent A. The fraction contained acids and a faint trace of 2-O-methylgalactose.
Fraction E.

A sample (5 mg.) was converted to the barium salt and examined chromatographically in solvent A, showing the absence of neutral sugars. Another small sample was treated with Amberlite resin IR 120 (H) and shown by chromatography in solvent F to be a complicated mixture of seven or eight acidic substances, one of which appeared identical with 2:3:4-tri-0-methyl-D-glucuronic acid.

A trial hydrolysis of the syrup (5 mg.) was carried out with N hydrochloric acid at 100° for eight hours. The solution was neutralised with silver carbonate and, before treatment with hydrogen sulphide, was examined chromatographically in solvent A. The silver salts of acids remained on the starting line and at least three neutral sugars were detected, corresponding with mono-0-methylgalactose, mono-0-methylmannose and di-0-methylgalactose. After treatment with hydrogen sulphide, the hydrolysate was examined in solvent D and shown to contain mono-0-methylmannose and a mixture of several acids, including 2:3:4-tri-0-methylglucuronic acid. Hydrolysis was obviously incomplete.

A more drastic trial hydrolysis of the acidic syrup (10 mg.) was carried out with 2 N hydrochloric acid at 100° for 18 hours. The solution was neutralised with silver carbonate and, before treatment with hydrogen sulphide, was separated on a filter sheet in solvent A. Spraying of the side strips showed three neutral sugars - mono-0-methylgalactose, mono-0-methylmannose /
mono-O-methylmannose and di-O-methylgalactose. The silver salts of acids, which remained on the starting line, were eluted with water and treated with hydrogen sulphide. Chromatography of the product in solvent D showed one major component, probably a di-O-methylglucuronic acid. No tri-O-methylglucuronic acid was detected, although this was present after milder hydrolysis.

REDUCTION AND HYDROLYSIS OF ACIDIC METHYLATED MATERIAL

Fractions D, E and 21 were combined, dissolved in water and barium ions removed with Amberlite resin IR 120 (II). Concentration gave a brown syrup (0.376 g.) which was refluxed with dry 1.3% methanolic hydrochloric acid (50 ml.) for six hours, neutralised with silver carbonate and concentrated again to a syrup (0.364 g.). The product was dissolved in anhydrous methylal (40 ml.), lithium aluminium hydride was added (0.2 g.) and the mixture refluxed for one hour; a little extra lithium aluminium hydride was added and refluxing continued for a further hour. Excess hydride was destroyed by water, the methylal layer was separated, the aqueous layer taken to dryness and the residue repeatedly extracted with acetone and chloroform. The combined organic extracts were concentrated and the product was extracted with dry chloroform and again evaporated, yielding a syrup (0.33 g.)
The reduced material (5 mg.) was hydrolysed with N hydrochloric acid at 100°C for six hours and examined chromatographically in solvents A and D. Reduction was complete and sugars detected were:

Mono-O-methylgalactose
Mono-O-methylmannose (major component)
Di-O-methylgalactose
Di-O-methylglucose (major component)
Tri-O-methylgalactose (trace only)
Tri-O-methylglucose.

The remainder of the reduced material (0.33 g.) was hydrolysed with N hydrochloric acid (30 ml.) at 100°C for six hours, neutralised with silver carbonate and concentrated to a syrup (0.23 g.).

EXAMINATION OF REDUCED METHYLATED MATERIAL

The mixture of methylated sugars (0.23 g.) was separated into nine fractions by chromatography on a cellulose column (50 x 2.5 cm.), using as eluant light petroleum (b.p. 100°-120°) -butan-1-ol (1:1), saturated with water.

Fraction (a). 0.021 g.  \( R_g 0.85 \)

Chromatography indicated 2:3:4-tri-O-methylglucose.

The
The syrup was identified by conversion to 2:3:4-tri-O-methyl-N-phenyl-D-glucosylamine, which crystallised from light petroleum (b.p. 60° - 80°) as white needles, m.p. and mixed m.p. 134° - 135°.

**Fraction (b)**  0.019 g.  \( R_g 0.72 \) and 0.58.

The fraction contained 2:3-di-O-methylglucose and a smaller amount of 2:3:4-tri-O-methylgalactose.

**Fraction (c)**  0.047 g.  \( R_g 0.58 \).

Chromatography and ionophoresis showed 2:3-di-O-methylglucose, while chromatography of periodate oxidation products gave two spots, \( R_f 0.63 \) (brown) and \( R_f 0.72 \) (bright yellow), identical with those produced by oxidised 2:3-di-O-methylglucose.

The sugar (0.045 g.) was converted to the lactone of the aldonic acid and the partly crystalline syrup obtained was dissolved in dry ether (8 ml.) by refluxing for two hours. Freshly distilled phenylhydrazine (0.05 g.) was added; a white precipitate formed and increased as refluxing was continued for four hours. The mixture was left at 0° overnight. The precipitate was then filtered off and recrystallised from ethanol, m.p. 173° - 174° and mixed m.p. (with 2:3-di-O-methyl-D-glucosphenylhydrazide of m.p. 166° - 169°) 169° - 171°.

**Fraction (d)**  0.011 g.  \( R_g 0.58 \) and 0.54.

Chromatography /
Chromatography and ionophoresis showed 2:3-di-O-methylglucose, 
R<sub>G</sub> 0.58, with a trace of an unidentified sugar, R<sub>G</sub> 0.54.

**Fraction (e)** 0.009 g,  R<sub>G</sub> 0.49.

Chromatography and ionophoresis showed 2:4-di-O-methylgalactose.

The sugar was identified by conversion to 2:4-di-O-methyl-N-phenyl-D-galactosylamine, m.p. and mixed m.p. 212° - 214°.

**Fraction (f)** 0.010 g,  R<sub>G</sub> 0.49 and 0.36.

The mixture was found by chromatography to contain 2:4-di-O-methylgalactose and 4-O-methylmannose.

**Fraction (g)** 0.20 g,  R<sub>G</sub> 0.36.

4-O-Methylmannose was indicated by chromatography and ionophoresis. Chromatography of the products of periodate oxidation gave a brown spot, R<sub>p</sub> 0.60, identical with that produced by oxidised 4-O-methylmannose.

The sugar was identified by conversion to 4-O-methyl-D-mannono- lactone, which crystallised from ethanol - ethyl acetate and had m.p. and mixed m.p. (with sample of m.p. 161° - 162°) 150° - 151°.

**Fraction (h)** 0.013 g,  R<sub>G</sub> 0.32.

This fraction contained an unidentified sugar, possibly mono-O- methylglucose.
mono-O-methylglucose, with a trace of a second component.

Chromatography of periodate oxidation products gave two spots, $R_f$ 0.60 (brown) and $R_f$ 0.15 (bright yellow), similar to those produced by 4-O-methylglucose and a 2-O-methylaldehyde.

Fraction (j) 0.003 g. $R_g$ 0.25.

Chromatography of the sugar and its periodate oxidation product, $R_f$ 0.20 (bright yellow), indicated 2-O-methylgalactose.

The crystalline sugar had m.p. $139^\circ - 140^\circ$, both alone and mixed with 2-O-methyl-D-galactose.
SECTION IV. INVESTIGATION OF THE PARTIAL HYDROLYSIS PRODUCTS OF GUM GHATTI.

PARTIAL HYDROLYSIS OF GUM GHATTI

Trial hydrolysates were carried out on solutions of crude gum ghatti (5 g.) in dilute sulphuric acid (100 ml.). In each case the crude gum was dissolved in water, strained through muslin and heated to the boiling point on an electric multimantle. Sufficient 4 N sulphuric acid was added to bring the solution to the desired normality and heating was continued for the specified time. The solution was cooled, neutralised immediately with barium carbonate and filtered. The addition of four volumes of ethanol caused precipitation of polysaccharide material, which was removed on the centrifuge. The solution was evaporated and examined chromatographically in solvent J, with the following results:

<table>
<thead>
<tr>
<th>Conditions of hydrolysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>N sulphuric acid at 100° for one hour</td>
<td>Large quantities of monosaccharides were detected, but no oligosaccharides.</td>
</tr>
<tr>
<td>0.5 N sulphuric acid at 100° for 30 minutes</td>
<td>Chromatography showed monosaccharides and four disaccharides, two of which were present</td>
</tr>
</tbody>
</table>
Conditions of hydrolysis

Results

0.1 N sulphuric acid at 100° for four hours. Samples were examined at half hourly intervals.

present only as traces. Of the other two, the faster one appeared to be pentose-containing and the other was probably a galactobiose.

Chromatography showed monosaccharides and the same four disaccharides as in the previous case. The yield of disaccharides reached a maximum after 90 minutes and then decreased.

Crude gum ghatti (50 g.) was dissolved in water (975 ml.) and the solution was heated to the boiling point on an electric multimantle. Sufficient 4 N sulphuric acid was added to bring the solution to 0.1 N and heating was continued for 90 minutes. The solution was cooled, brought to approximately pH 4 with barium hydroxide solution, neutralised (pH 7) with barium carbonate, filtered and concentrated (200 ml.).

The hydrolysis was repeated, using a further 50 g. of crude gum. The final concentrates from both hydrolyses were bulked (400 ml.), poured into ethanol (2 litres) and the precipitated polysaccharide material, A(i), was removed on the centrifuge. The solution was concentrated (200 ml.), passed /
passed through a small column of Amberlite resin IR 120 (H) to remove barium ions and then through a column of Amberlite resin IR 4B (OH) to remove free uronic acids and acidic oligosaccharides. The eluted neutral solution was evaporated to dryness and yielded a semi-crystalline syrup, B (i) (about 35 g.). Chromatography of the product in solvent J showed monosaccharides (mainly arabinose and galactose) with small amounts of the oligosaccharides detected after trial hydrolyses.

The anion exchange resin column was eluted with 0.5 N sulphuric acid (250 ml.) and the eluate was neutralised with barium carbonate, filtered and concentrated to a syrup, C (i). Chromatography in solvent J showed mainly barium salts of acids with some arabinose and galactose. After treatment of the acidic material with Amberlite resin IR 120 (H), chromatography in solvent J indicated galactose, arabinose and (probably) an aldobiouronic acid.

The precipitated polysaccharide material, A (i) (about 50 g.), was dissolved in water (1 litre) and heated to the boiling point on an electric multimantle. Sufficient 4 N sulphuric acid was added to bring the solution to 0.5 N and heating was continued for 30 minutes. Neutralisation was effected with barium hydroxide solution followed by barium carbonate. After concentration of the solution and precipitation of polysaccharide material, A (ii), with ethanol, the solution was passed through cation and anion exchange resin columns as before. Evaporation of the neutral eluate gave /
gave a syrup, B (ii) (15 g.), which was shown by chromatography to contain the same mono- and oligosaccharides as the syrup, B (i), obtained from the first hydrolysis, but with a considerably higher proportion of oligo- to monosaccharides. The syrups B (i) and B (ii) were combined (50 g.) for separation on charcoal.

Elution as before of the anion exchange resin column with dilute sulphuric acid gave, after neutralisation and evaporation, a second acidic fraction, C (ii). This was shown by chromatography in solvent J to contain mainly barium salts of acids, with only a small amount of galactose and arabinose. After treatment with Amberlite resin IR 120 (H), chromatography of the syrup in solvent H revealed galactose, arabinose and at least one aldobiouronic acid.

SEPARATION OF THE NEUTRAL PRODUCTS OF PARTIAL HYDROLYSIS

Charcoal (400 g.) was washed thoroughly with water and mixed with Celite (400 g.) which had been washed successively with hot concentrated hydrochloric acid-water (1:1), water, a 1% aqueous solution of sodium bicarbonate and finally water.

The charcoal and Celite mixture was packed as an aqueous slurry into a column (6.8 x 55 cm.) which was then washed with water (10 litres). The
The column flowed at approximately 1800 ml. in 24 hours.

The mixture of neutral mono- and oligosaccharides, B (i) and B (ii) (50 g.), was separated on the charcoal column (69) which was eluted first with water and later with gradually increasing concentrations of aqueous ethanol. The eluate was found to be slightly acid and fractions were therefore treated with Amberlite resin IR 4 B (OH), evaporated to a small volume, again treated with the resin and finally evaporated to dryness and examined chromatographically in solvents J or K. It was found that the latter solvent was suitable for galactobiose and slower moving oligosaccharides which remained on the starting line in solvent J. R\textsubscript{Gal} values refer to the rate of movement of an oligosaccharide relative to that of galactose in solvent J.

The following fractions were obtained:

**Eluted with water**

1. A mixture of monosaccharides, identified chromatographically as arabinose (main component), galactose, xylose and traces of rhamnose. 36.0 g.

2. Chromatography showed rhamnose with a trace of arabinose. 0.800 g.

**Eluted with 2.5% ethanol**

3. Chromatography in solvent J showed 6-O-galactosyl-galactose, 3-O-arabinosylarabinose and a slower pentose- /
pentose-containing disaccharide. 0.230 g.

4. A mixture of fractions 3 and 5 with traces of other (probably pentose-containing) disaccharides. The main component was 6-0-galactosylgalactose. 0.550 g.

5. Identified as 6-0-5-D-galactosyl-D-galactose with a trace of galactose. 1.100 g.

Eluted with 5% ethanol

6. Contained 6-0-galactosylgalactose with traces of another hexose-containing disaccharide and three or four fast moving components, probably pentose-containing disaccharides. 0.260 g.

7. Equal amounts of 6-0-galactosylgalactose and another hexose-containing disaccharide, with traces of 3-0-arabinosylarabinose and other fast moving substances. 0.205 g.

8. Crystalline substance identified as 3-0-5-D-galactosyl-L-arabinose. 0.155 g.

9. Chromatography showed 3-0-galactosylarabinose with traces of monosaccharides and other substances. 0.250 g.

Eluted /
Eluted with 7.5% ethanol

10. A mixture of 6-0-galactosylgalactose, another hexose-containing disaccharide and traces of three other substances, probably pentose-containing. 0.080 g.

11. Shown to contain 1:6-linked galactotriose with traces of 6-0-galactosylgalactose, galactose and arabinose 0.830 g.

12. A mixture of fractions 11 and 13. 0.560 g.

13. Identified as digalactosylarabinose 0.265 g.

Eluted with 10% ethanol

14. A mixture of fractions 13 and 15 and unidentified traces. 0.235 g.

15. 1:6-linked galactotetraose. 0.470 g.

Eluted with 12.5% ethanol

16. A mixture of fractions 15 and 17 and unidentified traces. 0.200 g.

17. Pure crystalline substance identified as trigalactosylarabinose. 0.100 g.

Eluted with 15% ethanol

18. /
18. A mixture of fractions 17 and 19. 0.530 g.

19. Pure crystalline substance, identified as tetragalactosylarabinose. 0.115 g.

PURIFICATION AND IDENTIFICATION OF OLIGOSACCHARIDES

Fraction 5. 1.1 g.

Chromatography in solvent J indicated 6-O-galactosylgalactose with a trace of galactose.

A sample (5 mg.) was hydrolysed with N sulphuric acid at 100° for four hours. Chromatographic examination of the product in solvent J showed galactose, with a smaller amount of arabinose of unknown origin.

Ionophoresis indicated a major and a minor component, while chromatography in solvents G and K indicated 6-O-galactosylgalactose, galactose and a trace of arabinose, the latter being in insufficient amount to account for the arabinose produced on hydrolysis.

A sample (10 mg.) was partially hydrolysed with 0.5 N sulphuric acid at 100° for 20 minutes and examined chromatographically in solvent J. Only 6-O-galactosylgalactose, galactose and arabinose were detected. The galactobiose /
galactobiose was separated from the hydrolysate on a filter sheet and was hydrolysed with N sulphuric acid at 100° for three hours. Chromatography of the product then showed only galactose, indicating that all the arabinose was removed by mild hydrolysis.

Reduction of a sample (10 mg.) was effected with potassium borohydride (158) and the product was hydrolysed with N sulphuric acid at 100° for three hours. Chromatography in solvent J gave galactose and arabinose, showing that some, at least, of the arabinose was present in non-reducing form.

The original mixture was examined by chromatography in solvent J, followed by treatment of the paper with silver nitrate and sodium hydroxide solutions to reveal polyhydroxy compounds (164). Galactobiose, galactose and traces of other substances were detected.

A sample (10 mg.) was hydrolysed with N sulphuric acid at 100° for three hours and separated on two filter strips in solvent J. Sugars were eluted by the method of Flood, Hirst and Jones (44) and estimated by Somogyi's method (45). Results showed the molar ratio of galactose to arabinose to be 8.3 : 1.

The remainder of the syrup (1 g.) was separated into two fractions by chromatography on a cellulose column (45 x 2 cm.), using solvent J as eluant:—

Fraction /
Fraction (i) 0.15 g.

The syrup contained a mixture of arabinose, galactose and at least three pentose-containing disaccharides.

Fraction (ii) 0.56 g.

\([\alpha]_D = +31^\circ (c, 0.99)\).

The chromatographically pure syrup was identical with 6-O-galactosylgalactose and had \(R_f 0.31\) in solvent K and \(R_{Gal} 0.29\) in solvent J. Hydrolysis with \(N\) sulphuric acid at 100\(^\circ\) for four hours gave only galactose.

The syrup (0.2 g.) was dissolved in water (5 ml.); dimethyl sulphate (1 ml.) and 30\% sodium hydroxide solution (1 ml.) were added drop-wise over a period of one hour. This was followed by the addition of dimethyl sulphate (11 ml.) and 30\% sodium hydroxide solution (16 ml.) over a further three hours. The methylation was carried out in air, with constant stirring, and at a temperature of less than 20\(^\circ\). Two further additions of dimethyl sulphate (12 ml.) and sodium hydroxide solution (17 ml.) were made on two successive days and the mixture was then heated to 100\(^\circ\) for one hour, causing precipitation of sodium sulphate. The solution was extracted with chloroform in a continuous extractor for 16 hours and yielded the methylated disaccharide (0.202 g.). Trial hydrolysis of the product (3 mg.) with \(N\) sulphuric acid at 100\(^\circ\) for four hours showed, on chromatography in solvent A, 2:3:4:6-tetra-O-methylgalactose, 2:3:4-tri-O-methylgalactose, a trace of di-O-methylgalactose /
di-O-methylgalactose and a trace of a component giving a black spot,

\[ R_G = 0.82. \]

The methylated galactobiose (0.15 g.) was hydrolysed with \( \text{N} \) hydrochloric acid (7 ml.) at 100° for four hours and the mixture of methylated sugars (0.14 g.) was separated by chromatography on a cellulose column (35 x 1.6 cm.), using light petroleum-butan-1-ol (7:3), saturated with water, as eluant.

Three fractions were obtained:

<table>
<thead>
<tr>
<th>Fraction (a)</th>
<th>0.040 g.</th>
<th>( R_G = 0.90. )</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\alpha]_D)</td>
<td>+105° (c, 0.78).</td>
<td></td>
</tr>
</tbody>
</table>

The sugar was chromatographically pure and identical with 2:3:4:6-tetra-O-methylgalactose. It was identified by conversion to the aniline derivative which had m.p. 198° and mixed m.p. (with a sample of 2:3:4:6-tetra-O-methyl-\( \text{N} \)phenyl-\( \text{D} \)-galactosylamine of m.p. 189° - 190°) 190° - 192°.

<table>
<thead>
<tr>
<th>Fraction (b)</th>
<th>0.035 g.</th>
<th>( R_G = 0.90, 0.82 ) and ( 0.70. )</th>
</tr>
</thead>
</table>

Chromatography showed a mixture of 2:3:4:6-tetra-O-methylgalactose and 2:3:4-tri-O-methylgalactose, \( R_G = 0.90 \) and \( 0.70. \) with a trace of a substance having \( R_G = 0.82 \) (black). The latter was assumed to be a tri-O-methylgalactofuranose.
Fraction (c) 0.039 g.  R<sub>G</sub> 0.70.

\[ \alpha \] = +106° (c, 0.77).

Chromatography in solvent A showed pure 2:3:4-tri-O-methyl-galactose, R<sub>G</sub> 0.70. The sugar was identified by conversion to the aniline derivative, m.p. 159° - 160° and mixed m.p. (with a sample of 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamide of m.p. 164° - 165°) 161° - 162°.

Fraction 7.  0.205 g.

Chromatography in solvent J showed a mixture of 6-O-galactosyl-galactose, another hexose-containing disaccharide and traces of at least three pentose-containing disaccharides. A sample (3 mg.) of the mixture was hydrolysed with N sulphuric acid at 100° for four hours and chromatography of the product showed galactose, arabinose and possibly glucose. No mannose was detected.

The remainder of the syrup (0.20 g.) was separated by chromatography on a cellulose column (4.5 x 2 cm.), using solvent J as eluant. The following fractions were obtained:

Fraction (i)  0.065 g.
Chromatographic examination in solvent J showed a mixture of arabinose, galactose and four pentose-containing disaccharides.

**Fraction (ii) 0.01 g.**

Chromatography showed 3-O-arabinosylarabinose.

**Fraction (iii) 0.03 g.**

\[
\left[\alpha\right]_D^\circ = + 125^\circ (c, 1.095).
\]

This fraction contained one oligosaccharide, apparently a hexose-containing disaccharide, which had \(R_{\text{Gal}} \) 0.35 in solvent J.

Hydrolysis of the syrup (3 mg.) with N sulphuric acid for four hours at 100\(^\circ\) gave galactose and glucose in approximately equal proportions.

The syrup (5 mg.) was reduced with potassium borohydride (5 mg.) and the product was hydrolysed with N sulphuric acid at 100\(^\circ\) for three hours; chromatography then showed only galactose. The fraction therefore contained a galactosylglucose.

**Fraction (iv) 0.05 g.**

Chromatography in solvent J showed 6-O-galactosylgalactose.
Fraction 8. 0.155 g.

\[ [\alpha]_D = +80^\circ (5 \text{ mins.}) \rightarrow +62^\circ (20 \text{ mins.}} \text{ equilibrium}) (c, 0.775). \]

The chromatographically pure crystalline material had \( R_{Gal} 0.6 \) (pink spot) in solvent J and \( R_P 0.58 \) in solvent K. The sugar was re-
crystallised from aqueous ethanol and had m.p. 202°-203°.

A sample (3 mg.) was hydrolysed with N sulphuric acid at 100°
for four hours. Chromatography of the product in solvent J showed
arabinose and galactose.

Reduction of the sugar (10 mg.) was effected with potassium
borohydride (10 mg.) and the product was hydrolysed with N sulphuric acid
at 100° for four hours. Chromatography in solvent J then showed only
galactose, indicating that the substance was a galactosylarabinose.

The disaccharide (0.1 g.) was dissolved in water (5 ml.) and
methylated by the method used for galactobiose. Three additions of di-
methyl sulphate (9 ml.) and 30% sodium hydroxide solution (13 ml.) were
made on three successive days. The solution was heated on a boiling water
bath for one hour, cooled and extracted with chloroform for 16 hours in a
continuous extractor, giving a crystalline substance (0.12 g.).

The methylated product (3 mg.) was hydrolysed with N sulphuric
acid /
acid at 100° for four hours and examined chromatographically in solvent A. The main components of the mixture were 2:3:4:6-tetra-O-methylgalactose and 2:4-di-O-methylarabinose, but incomplete methylation was indicated by traces of tri-O-methylgalactose and mono-O-methylarabinose. A substance giving a black spot, Rg 0.85, was also detected and was assumed to be 2:5-di-O-methylarabinose.

The partially methylated disaccharide was again dissolved in water (5 ml.) and treated with two further additions of dimethyl sulphate (9 ml.) and 30% sodium hydroxide solution (13 ml.). The solution was heated on a boiling water bath for one hour and extracted as before with chloroform, giving white crystals (0.1 g.). Hydrolysis of a sample (3 mg.) with N sulphuric acid was followed by chromatographic examination, which showed that methylation was nearly complete.

The methylated disaccharide (0.1 g.) was hydrolysed with N hydrochloric acid (5 ml.) at 100° for four hours. The neutralised product (0.085 g.) was separated by chromatography on a cellulose column (40 x 2 cm.), using light petroleum-butan-1-ol (7:3) saturated with water as eluant. The following fractions were obtained:

<table>
<thead>
<tr>
<th>Fraction (a)</th>
<th>0.023 g.</th>
<th>Rg 0.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>[a]D</td>
<td>+ 76°</td>
<td>(c, 0.52).</td>
</tr>
</tbody>
</table>

Chromatography /
Chromatography in solvent A showed 2:3:4:6-tetra-O-methyl-galactose with a trace of 2:5-di-O-methylarabinose.

The main component was converted to the aniline derivative and identified as 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine, m.p. and mixed m.p. 193°.

Fraction (b) 0.005 g.  Rg 0.85

\[ [\alpha]_D = -20^\circ \text{ approximately } (c, 0.108). \]

Chromatography showed 2:5-di-O-methylarabinose.

Fraction (c) 0.01 g.  Rg 0.70 and 0.60.

The syrup was shown by chromatography to contain tri-O-methyl-galactose and 2:4-di-O-methylarabinose.

Fraction (d) 0.02 g.  Rg 0.60

\[ [\alpha]_D = +120^\circ (c, 0.30). \]

The syrup was chromatographically pure and identical with 2:4-di-O-methylarabinose in solvents A and F. It was clearly differentiated from 2:5- and 3:4-di-O-methylarabinose by chromatography in both these solvents.

The sugar was converted to the aniline derivative which crystallised as brown plates from butan-1-ol and had m.p. 114° - 115° and mixed m.p. (with a /
The derived anilide was obviously impure but its X-ray powder photograph proved identical with that of 2:4-di-O-methyl-N-phenyl-L-arabinosylemine.

Fraction 10. 0.08 g.

Chromatography in solvent J showed a mixture of at least six components, including 6-O-galactosylgalactose and another hexose-containing disaccharide which had $R_{Gal}$ 0.35 in solvent J. The mixture was separated by chromatography on a cellulose column (45 x 2 cm.) using solvent J as eluant. The following fractions were obtained:

Fraction (i) 0.025 g.

Chromatography in solvent J showed a mixture of pentose-containing disaccharides.

Fraction (ii) 0.005 g.

Chromatography in solvent J showed 3-O-galactosylarabinose.

Fraction (iii) 0.025 g.

$[a]_D$ /
\[
[a]_D = +69^\circ \text{ (5 mins.)} \rightarrow +55^\circ \text{ (equilibrium)}
\]

c, 1.415.

The crystalline substance was chromatographically pure and had \( R_{Gal} 0.35 \) in solvent J. A sample (3 mg.) was hydrolysed with N sulphuric acid at 100° for three hours and chromatography of the product in solvent J showed only galactose.

Further chromatography of the original material in solvent J, using Whatman No. 4 paper, showed the substance to be identical with 3-O-\( \beta \)-D-galactosyl-D-galactose.

Lead tetraacetate oxidation (165) was carried out as follows on the galactobiose isolated from fraction 10 and on authentic samples of 3-O-\( \beta \)-D-galactosyl-D-galactose and cellobiose.

The disaccharide (1 mg.) was dissolved in water (0.01 ml.) and the solution was made up to 0.1 ml. with acetic acid. Lead tetraacetate (5 mg.) in acetic acid (0.4 ml.) was added. After two hours, the solution was treated with excess oxalic acid, diluted with water and heated on a boiling water bath with Amberlite resin IR 120 (H). The solution was filtered, treated with Amberlite resin IR 4 B (OH), filtered and evaporated to dryness. The product was examined chromatographically in solvent J.

The galactobiose isolated from fraction 10 and 3-O-\( \beta \)-D-galactosyl-D-galactose /
D-galactose gave the same oxidation products, including lyxose, while the 1:4-linked disaccharide, cellobiose, gave completely different oxidation products which did not include lyxose.

The galactobiose was recrystallised from aqueous acetone and had m.p. 151° - 152°. Its X-ray powder photograph proved identical with that of 3-O-β-D-galactosyl-D-galactose.

**Fraction (iv)**  0.003 g.

The syrup was chromatographically identical with 6-O-galactosyl-galactose.

**Fraction 11.**  0.830 g.

The syrup consisted mainly of galactotriose, but traces of 6-O-galactosylgalactose and monosaccharides were also present. The mixture (0.58 g.) was separated by chromatography on a cellulose column (45 x 2 cm.), using solvent J as eluant. Three fractions were obtained:

**Fraction (i)**  0.04 g.

The syrup consisted of galactose and arabinose.

**Fraction (i)
Fraction (ii) 0.06 g.

The syrup was identified chromatographically as 6-O-galactosylgalactose.

Fraction (iii) 0.45 g.

\[[\alpha]_D^\circ = +20^\circ \text{ (c, 0.992).}\]

The syrup was chromatographically pure and had \(R_F 0.20\) in solvent K. Hydrolysis of a sample (3 mg.) with N sulphuric acid at 100\(^{\circ}\) for four hours gave only galactose. Partial hydrolysis of the syrup (3 mg.) was effected with 0.5 N sulphuric acid at 100\(^{\circ}\) for 30 minutes and chromatography of the product in solvent J showed galactose, 6-O-galactosylgalactose and unaltered starting material.

Galactotriose (0.2 g.) was dissolved in water (5 ml.) and methylated by the method used for galactobiose. Three additions of dimethyl sulphate (12 ml.) and 30\% sodium hydroxide solution (17 ml.) were made on three successive days. The solution was heated for one hour on a boiling water bath and extracted with chloroform in a continuous extractor for 16 hours, giving a syrup (0.22 g.).

A sample (3 mg.) of the methylated product was hydrolysed with N sulphuric acid at 100\(^{\circ}\) for three hours and chromatography of the product in solvent A showed 2;3;4-tri-O-methylgalactose, 2;3;4;6-tetra-O-methylgalactose and /
and a trace of a substance, $R_g 0.32$ (black), thought to be tri-O-methylgalactofuranose.

The methylated trisaccharide (0.22 g.) was hydrolysed with $N$ sulphuric acid at $100^\circ$ for four hours. The neutralised product (0.185 g.) was separated on filter sheets into two fractions, using solvent A:—

Fraction (a) 0.036 g. $R_g 0.09$  
$[\alpha]_D = +100^\circ$ (c, 0.523).

Chromatography showed 2:3:4:6-tetra-O-methylgalactose with a trace of a substance, $R_g 0.82$ (black), presumably tri-O-methylgalactofuranose.

The main component was identified as 2:3:4:6-tetra-O-methyl-D-galactose by conversion to the aniline derivative, m.p. and mixed m.p. $189^\circ - 190^\circ$.

Fraction (b) 0.08 g. $R_g 0.70$  
$[\alpha]_D = +104^\circ$ (c, 0.773).

The syrup consisted of 2:3:4-tri-O-methylgalactose with a trace of a substance giving a black spot, $R_g 0.82$.

The main component was identified as 2:3:4-tri-O-methyl-D-galactose by conversion to the aniline derivative, m.p. and mixed m.p. $159^\circ$
Fraction 13. 0.265 g.

\[ [\alpha]_D = +39^\circ \text{ (c, 0.915).} \]

The chromatographically pure crystalline substance had \( R_{\text{Gal}} 0.18 \)
in solvent J and \( R_f 0.25 \) in solvent K. The sugar was recrystallised from
aqueous ethanol and had m.p. 191\(^\circ\).

A sample (3 mg.) was hydrolysed with N sulphuric acid at 100\(^\circ\) for
four hours and the product was shown by chromatography to contain galactose
and arabinose. Partial hydrolysis of another sample (3 mg.) was effected
with 0.5 N sulphuric acid at 100\(^\circ\) for 30 minutes and chromatography of the
product showed arabinose, galactose, 3-0-galactosylarabinose, 6-0-galactosyl-
galactose and unaltered starting material.

The oligosaccharide (10 mg.) was dissolved in water (2 ml.) and
reduced with potassium borohydride (10 mg.). Partial hydrolysis of the
product with 0.5 N sulphuric acid at 100\(^\circ\) for 30 minutes was followed by
chromatographic examination in solvent J. Galactose and 6-0-galactosyl-
galactose /
6-0-galactosylgalactose were detected, but no arabinose. The remainder of the reduced material was completely hydrolysed with N sulphuric acid at 100° for four hours and shown to contain only galactose.

Fraction 13 therefore contained a digalactosylarabinose, O-β-D-galactopyranosyl-(1→ 6)-O-β-D-galactopyranosyl-(1→ 3)-L-arabinose.

The digalactosylarabinose (0.2 g.) was dissolved in water (5 ml.) and methylated by the method used for galactobiose. Three additions of dimethyl sulphate (12 ml.) and 30% sodium hydroxide solution (17 ml.) were made on three successive days. The solution was heated on a boiling water bath for one hour, cooled and extracted with chloroform in a continuous extractor for 16 hours, giving a partly crystalline product (0.21 g.).

A sample (3 mg.) of the product was hydrolysed with N hydrochloric acid at 100° for three hours and examined chromatographically in solvent A. The main components of the mixture were 2:3:4:6-tetra-0-methylgalactose, 2:3:4-tri-0-methylgalactose and 2:4-di-0-methylarabinose, but traces of 2:5-di-0-methylarabinose, di-0-methylgalactose and mono-0-methylarabinose were also present.

The partially methylated trisaccharide (0.21 g.) was treated with methyl iodide (40 ml.) and silver oxide (0.2 g.). Extraction of the silver residues gave a crystalline substance (0.203 g.).

A sample (3 mg.) of the product was hydrolysed with N hydrochloric acid /
acid at 100° for three hours. Chromatography in solvent A showed that methylation was nearly complete.

The remainder of the methylated material was hydrolysed with N hydrochloric acid at 100° for four hours. The neutralised product (0.180 g.) was separated by chromatography on a cellulose column (50 x 1.6 cm.), using light petroleum-butan-1-ol (7 : 3), saturated with water, as eluant. The following fractions were obtained:

Fraction (a) 0.047 g.  \( R_G \) 0.90

\([\alpha]_D^0 = +97° \) (c, 2.11).

The sugar was identified as 2:3:4:6-tetra-O-methyl-D-galactose by conversion to the aniline derivative, m.p. and mixed m.p. 190° - 191°.

Fraction (b) 0.005 g.  \( R_G \) 0.85.

Chromatography showed 2:5-di-O-methylarabinose. The sugar was identified by conversion to 2:5-di-O-methyl-L-arabonamide, m.p. and mixed m.p. 122°.

Fraction (c) 0.04 g.  \( R_G \) 0.70.

\([\alpha]_D^0 = +109° \) (c, 0.608).

The sugar was identified as 2:5:4-tri-O-methyl-D-galactose by chromatography and by conversion to the aniline derivative, m.p. 167° - 168°.
168° and mixed m.p. (with a sample of m.p. 164° - 165°) 164° - 165°.

Fraction (d) 0.032 g. Rg 0.60

\[ [\alpha]_D = +107° (c, 0.515). \]

Chromatography in solvents A and F showed 2:4-di-O-methyl-arabinose, which was readily differentiated from 2:3- and 3:4-di-O-methyl-arabinose.

The sugar was converted to 2:4-di-O-methyl-N-phenyl-L-arabinosylamine, m.p. and mixed m.p. 122°. The X-ray powder photograph of the aniline derivative proved identical with that of 2:4-di-O-methyl-N-phenyl-L-arabinosylamine.

Fraction 15 0.47 g.

Chromatography in solvent K showed one oligosaccharide, Rf 0.12, which had \([\alpha]_D +14° (c, 1.948).\)

The syrup (3 mg.) was hydrolysed with N sulphuric acid at 100° for four hours and chromatography of the product showed galactose only. Partial hydrolysis of a sample (3 mg.) was effected with 0.5 N sulphuric acid at 100° for 30 minutes and chromatographic examination in solvent J then /
then showed galactose, 6-O-galactosylgalactose and unaltered oligosaccharide, indicating a 1:6-linked galactose polymer, probably galactotetraose.

**Fraction 17.** 0.1 g.

The chromatographically pure crystalline substance had \([\alpha]_D + 26^\circ\) (c, 0.896) and \(R_p 0.16\) in solvent K. The sugar was recrystallised from aqueous ethanol and had m.p. 171°.

A sample (3 mg.) was hydrolysed with \(N\) sulphuric acid at 100° for four hours and chromatography of the product showed galactose and arabinose. After partial hydrolysis of a further sample (3 mg.) with 0.5 \(N\) sulphuric acid at 100° for 30 minutes, chromatography showed arabinose, galactose, 3-O-galactosylarabinose, 6-O-galactosylgalactose and unaltered starting material.

The oligosaccharide (5 mg.) was reduced with potassium borohydride (5 mg.) and partial hydrolysis of the product was effected with 0.5 \(N\) sulphuric acid at 100° for 30 minutes. Chromatography then indicated only galactose and 6-O-galactosylgalactose.

The oligosaccharide (10 mg.) was hydrolysed with \(N\) sulphuric acid at /
at 100° for four hours, neutralised and separated on two filter strips in solvent J. Sugars were eluted by the method of Flood, Hirst and Jones (44) and estimated by Somogyi’s method (45). Results indicated that the molar ratio of galactose to arabinose was 2.8 : 1.

This fraction contained a trigalactosylarabinose, 0-β-D-galactopyranosyl-[\((1 \rightarrow 6)\)-0-β-D-galactopyranosyl-]_2(1 \rightarrow 3)-L-arabinose.

**Fraction 19**

0.115 g.

The chromatographically pure crystalline substance had $R_p$ 0.10 in solvent K and $[\alpha]_D + 19^\circ$ ($c$, 1.052). The oligosaccharide was re-crystallised from aqueous ethanol and had m.p. 177° - 179° (with decomposition).

A sample (3 mg.) was hydrolysed with N sulphuric acid at 100° for four hours and chromatography of the product showed galactose and arabinose. Partial hydrolysis of another sample (3 mg.) with N sulphuric acid at 100° for 30 minutes was followed by chromatographic examination. Arabinose, galactose, 3-O-galactosylarabinose, 6-O-galactosylgalactose and unaltered oligosaccharide were detected.

The
The oligosaccharide (5 mg.) was reduced with potassium borohydride (5 mg.) and the product was partially hydrolysed with 0.5 N sulphuric acid at 100° for 30 minutes. Chromatography then showed only galactose and 6-0-galactosylgalactose.

The oligosaccharide (10 mg.) was hydrolysed with N sulphuric acid at 100° for four hours, neutralised and separated on two filter strips in solvent J. Elution of the sugars by the method of Flood, Hirst and Jones (44) and estimation by Somogyi's method (45) gave a molar ratio of galactose to arabinose of 3.7 : 1.

The fraction therefore contained a tetragalactosylarabinose, 0-β-D-galactopyranosyl-[((1 → 6)-0-β-D-galactopyranosyl-3)-β-D-galactopyranosyl-]_3(1 → 3)-L-arabinose.

\[ R_p \text{ VALUES OF HOMOLOGOUS OLIGOSACCHARIDES.} \]

**Fractions 5, 11 and 15.**

Using solvent K, \( R_p \) values were obtained for the homologous series of 1:6-linked galactose polymers isolated from fractions 5, 11 and 15.

Values of \( \log \frac{R_p}{1 - R_p} \) are as follows:—

\[ R_p / \]
<table>
<thead>
<tr>
<th>Fraction 5</th>
<th>(Galactobiose)</th>
<th>( R_F )</th>
<th>( \log \frac{R_F}{1 - R_F} )</th>
<th>Number of hexose units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.31</td>
<td>1.6526</td>
<td>2</td>
</tr>
<tr>
<td>Fraction 11</td>
<td>(Galactotriose)</td>
<td>0.20</td>
<td>1.3979</td>
<td>3</td>
</tr>
<tr>
<td>Fraction 15</td>
<td>(Probably galactotetraose)</td>
<td>0.12</td>
<td>1.1347</td>
<td>4</td>
</tr>
</tbody>
</table>

Plotting \( \log \frac{R_F}{1 - R_F} \) against the number of hexose units [Graph 1], a linear relationship was obtained, confirming that fraction 15 contained the galactotetraose of this series (166).

Fractions 8, 13, 17 and 19.

Fractions 8, 13, 17 and 19 have been shown to contain the first four members of the homologous series \( 0-\beta-D\text{-galactopyranosyl-}[(1 \rightarrow 6)- \quad 0-\beta-D\text{-galactopyranosyl-}]_n(1 \rightarrow 3)-L\text{-arabinose.} \)

Using solvent K, \( R_F \) values were obtained for these oligosaccharides and values of \( \log \frac{R_F}{1 - R_F} \) are as follows:
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Monosaccharide Structure</th>
<th>$R_F$</th>
<th>$\log \frac{R_F}{1 - R_F}$</th>
<th>Number of sugar residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>(Galactosylarabinose)</td>
<td>0.38</td>
<td>7.7874</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>(Digalactosylarabinose)</td>
<td>0.25</td>
<td>7.5228</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>(Trigalactosylarabinose)</td>
<td>0.16</td>
<td>7.2798</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>(Tetragalactosylarabinose)</td>
<td>0.10</td>
<td>7.0458</td>
<td>5</td>
</tr>
</tbody>
</table>

Plotting $\log \frac{R_F}{1 - R_F}$ against the number of monosaccharide units [Graph 2], the anticipated linear relationship was obtained (166).
DISCUSSION.
DISCUSSION

Gum ghatti, or Indian gum, is an exudate from the bark of Anogeissus latifolia, Wall (family, Combretaceae). The chemistry of the gum was first investigated by Hanna and Shaw (167), who reported the presence of pentosan (50%) and galactose or galacturonic acid (12%). After hydrolysis of the gum, they isolated L-arabinose and an aldobiouronic acid of equivalent weight 352.

A further examination of gum ghatti was carried out by Aspinall, Hirst and Wickström (101), who showed that the gum had an equivalent weight of 1600 and consisted of residues of D-glucuronic acid (1 mol.), D-mannose (1 mol.), D-galactose (3 mols.), L-arabinose (5 mols.), D-xylose (0.5 mol.) and traces of methylpentose (probably L-rhamnose). They found that autohydrolysis of the gum acid resulted in the ready liberation of 4 mols. of L-arabinose per equivalent, leaving a degraded gum containing residues of D-glucuronic acid, D-galactose and D-mannose, together with some arabinose. It therefore appeared that approximately four fifths of the arabinose residues were present in the acid-labile furanosic form and were situated in the outer part of the polymer. Partial hydrolysis of gum ghatti with mineral acid yielded two aldobiouronic acids, 6-O-D-glucurono-2-galactose and 2-O-D-glucurono-2-mannose, which must constitute the most resistant part of the molecule.
In the present work, methylation studies (168) have been carried out on gum ghatti and on the degraded gum acid, and neutral oligosaccharides produced by partial hydrolysis have been investigated.

The sample of gum ghatti examined was supplied by Messrs. Eimer and Amend, New York, and consisted of hard nodules which were almost completely soluble in water. The purified gum acid was obtained by dissolution of the nodules in water, followed by precipitation with acidified ethanol.

An aqueous solution of the purified gum acid was heated to 100° and autohydrolysis was followed, both chromatographically and using Somogyi's reagent (45) to estimate increasing reducing power. It was found that from nine hours onwards much arabinose, some galactose and traces of xylose and rhamnose were liberated, and that the rate of production of reducing sugar (mainly arabinose) decreased after about 90 hours.

The degraded gum acid was prepared by heating an aqueous solution of the purified gum for 92 hours; the addition of ethanol then caused precipitation of the polysaccharide. The latter still contained some arabinose, which was to be expected in view of the previous finding by Aspinall, Hirst and Wickström (101) that only four fifths of the arabinose is readily removable.

The degraded gum acid was methylated extensively with dimethyl sulphate and sodium hydroxide and was finally treated with methyl iodide and silver /
silver oxide, giving a fully methylated polysaccharide with methoxyl content 44%. The fraction insoluble in light petroleum-chloroform (10:1) was hydrolysed, converted to the barium salts and separated into an ether-soluble fraction containing neutral methylated sugars and an ether-insoluble fraction containing barium salts of acids, together with some neutral methylated sugars. The ether-soluble fraction was separated by chromatography on cellulose (52), giving 2:3:4:6-tetra-, 2:3:4-tri-, 2:3- and 2:4-di-O-methyl-D-galactose, all of which were characterised as crystalline anilides. 2:3-Di-O-methyl-D-galactose was present in much greater amount and is therefore of greater structural significance than 2:4-di-O-methyl-D-galactose.

No methyl ethers of D-mannose were isolated in a pure condition, but 3:4:6-tri-O-methyl-D-mannose was shown to be present by chromatography, paper ionophoresis, the detection of 2:3:5-tri-O-methylarabinose after periodate oxidation (156) and by conversion of a mixture of the sugar together with 2:3:4:6-tetra- and 2:3:4-tri-O-methylgalactose to the aldonamides, which gave a positive Weeman test. 3:4:6-Tri-O-methyl-D-mannose was the most important mannose derivative present, but traces of 4-0-methyl- and a di-O-methylmannose (probably 3:4-di-O-methylmannose) were also present.

In addition to the major components, traces of 2:3:5-tri-O-methylarabinose, 2:3:4-tri-O-methylxylose and 2:3:4-tri-O-methylrhamnose were detected.
detected chromatographically, but in insufficient quantity to be of structural significance.

The ether-insoluble residue was found to contain some neutral sugars, 2:3:4-tri- and a di-O-methylgalactose, which were separated from the barium salts on filter sheets. After removal of barium ions, the acidic material was shown to consist largely of aldobiuronic acids which were hydrolysed under strong conditions and the silver salts of acids were separated on filter sheets from neutral sugars. The latter consisted of 2:3:4-tri-O-methyl-D-galactose, which was identified as the aniline derivative, and 3:4:6-tri-O-methylmannose and 2:3-di-O-methylgalactose which were identified chromatographically. The mixture of acids was separated into two fractions: the major fraction was chromatographically pure and identical with 2:3:4-tri-O-methylglucuronic acid and, after conversion to the methyl ester methyl glycoside followed by reduction and hydrolysis, was identified chromatographically as 2:3:4-tri-O-methylglucose. The minor fraction was also converted to the methyl ester methyl glycosides, reduced and hydrolysed. The product was shown chromatographically to contain 2:3-di-O-methylglucose with small amounts of tri-O-methylglucose, di- and tri-O-methylgalactose.

These results show that the degraded gum consists of a backbone of 1:6-linked D-galactopyranose residues. Since 2:3-di-O-methyl-D-galactose is present in much greater amount than 2:4-di-O-methyl-D-galactose, it follows that /
that most of the side chains in the degraded gum are attached through position 4 of the galactose residues, although there may also be some branching through position 3.

Since the methylated aldobiouronic acids gave, on hydrolysis, mainly 2:3:4-tri-O-methylglucuronic acid, together with 2:3:4-tri-O-methylgalactose, 3:4:6-tri-O-methylmannose and some 2:3-di-O-methylgalactose, it follows that most D-glucuronic acid residues form the terminal groups of side chains, although a much smaller proportion of acid residues are engaged in 1:4-linkages. There is, so far, insufficient evidence to decide whether aldobiouronic acid units are directly attached to the main chain or whether one or more galactose residues are interposed.

Some non-reducing terminal galactopyranose residues are present in the degraded gum and these may also terminate side chains, but it is more probable that they arise by cleavage of the main chain during autohydrolysis.

Only a trace of 2:3:5-tri-O-methylarabinose was detected, although the unmethylated degraded gum contained an appreciable proportion of arabinose.

Some structural features of the degraded gum are as follows (I):—
Gum ghatti was methylated with dimethyl sulphate and sodium hydroxide, followed by treatment with methyl iodide and silver oxide, and the fully methylated polysaccharide was subjected to methanolation, followed by hydrolysis. A good separation of acidic from neutral methylated sugars was achieved by ether extraction of the hydrolysate, followed by further separation of the ether-insoluble material on a cellulose column.

The mixture of neutral methylated sugars was fractionated by chromatography on cellulose and the following methylated sugars were identified as crystalline derivatives:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Approximate weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4:6-Tetra-O-methyl-D-galactose</td>
<td>0.05 g.</td>
</tr>
<tr>
<td>2:3:4-Tri-O-methyl-D-galactose</td>
<td>0.45 g.</td>
</tr>
<tr>
<td>2:4-Di-O-methyl-D-galactose</td>
<td>0.70 g.</td>
</tr>
<tr>
<td>2-O-Methyl-D-galactose</td>
<td></td>
</tr>
</tbody>
</table>
2-0-Methyl-D-galactose  0.67 g.
2:3:5-Tri-O-methyl-L-arabinose  2.00 g.
3:5-Di-O-methyl-L-arabinose  0.15 g.
2:5-Di-O-methyl-L-arabinose  0.15 g.
2:3-Di-O-methyl-L-arabinose  0.10 g.
2:4-Di-O-methyl-L-arabinose  0.11 g.
4-O-Methyl-D-mannose  0.10 g.
2:3:4-Tri-O-methyl-L-rhamnose  0.05 g.

In addition, chromatographic evidence was obtained for traces of 2:3:4-tri-O-methylxylene and 3:4:6-tri-O-methylmannose. The presence of a small amount of 2:3-di-O-methylgalactose and a mono-O-methylpentose, possibly 2-O-methylarabinose, was indicated by chromatography, ionophoresis and periodate oxidation. The 2:3-di-O-methylgalactose is, however, of insignificant structural importance compared with 2:4-di-O-methylgalactose.

The acidic material was shown to be contaminated with a trace of 2-O-methylgalactose. A preliminary hydrolysis under drastic conditions indicated that the most important acidic component was a di-O-methylglucuronic acid, with perhaps some tri-O-methylglucuronic acid, and that these acids were linked with three neutral methylated sugars, namely mono-O-methylgalactose, mono-O-methylmannose and di-O-methylgalactose. All the acidic material was converted to the methyl ester methyl glycosides, reduced and
and hydrolysed. The product was separated by chromatography on cellulose into five major components which were characterised as crystalline sugars or derivatives:

<table>
<thead>
<tr>
<th>Component</th>
<th>Approximate weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4-Tri-O-methyl-D-glucose</td>
<td>0.021 g.</td>
</tr>
<tr>
<td>2:3-Di-O-methyl-D-glucose</td>
<td>0.050 g.</td>
</tr>
<tr>
<td>2:4-Di-O-methyl-D-galactose</td>
<td>0.009 g.</td>
</tr>
<tr>
<td>4-O-Methyl-D-mannose</td>
<td>0.025 g.</td>
</tr>
<tr>
<td>2-O-Methyl-D-galactose</td>
<td>0.008 g.</td>
</tr>
</tbody>
</table>

It is possible that all or part of the 2-O-methyl-D-galactose arose from the contaminating neutral sugar and not from hydrolysis of a partially methylated aldobiouronic acid.

Proportions of methylated sugars obtained from the hydrolysed methylated gum are given as approximate estimates only, since many fractions were impure and several stages were involved in the separation and identification of sugars. This applies particularly to the reduced methylated material and, moreover, it is known that hydrolysis of acidic polysaccharides is accompanied by some decomposition.

In the case of the methylated degraded gum, an even greater amount of refractionation was necessary and it is not possible to give a useful quantitative /
quantitative estimate. However, the proportion of 2:3:4-tri-O-methyl-D-galactose to di-O-methyl-D-galactose was relatively high, indicating chains of 1:6-linked galactose residues with comparatively few branch points.
TABLE VII
HYDROLYSIS PRODUCTS OF METHYLATED GUM GHATTI AND METHYLATED DEGRADED GUM GHATTI.

(Figures refer to the positions at which methyl groups are attached to sugars).

<table>
<thead>
<tr>
<th></th>
<th>Methylated Degraded Gum</th>
<th>Methylated Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrolysis Products</td>
<td>Approx. Proportion (Mols.)</td>
</tr>
<tr>
<td>Galactose</td>
<td>2:3:4:6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2:3:4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2:4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>3:4:6</td>
<td>Major component</td>
</tr>
<tr>
<td></td>
<td>Di-O-methyl</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Trace</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2:3:5</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>2:3:4</td>
<td>Trace</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>2:3:4</td>
<td>Trace</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>2:3:4</td>
<td>Major component</td>
</tr>
<tr>
<td></td>
<td>2:3</td>
<td>Minor component</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minor component</td>
</tr>
</tbody>
</table>
From a comparison of results obtained from the methylated gum with those obtained from the methylated degraded gum [Table VII], it can be seen that arabinose residues are preferentially attached to position 3 of the main chain galactose units. This is indicated by the presence of 2-O-methyl-D-galactose residues in the methylated gum and 2:3-di-O-methyl-D-galactose residues in the methylated degraded polymer, arabinose having been lost from position 3 in the latter case. A side chain containing an aldobiouronic acid unit is presumably attached to position 4 of both of these residues. The attachment of arabinose to position 3 of galactose is further indicated by the existence of a high proportion of 2:3:4-tri-O-methyl-D-galactose and a very small amount of 2:4-di-O-methyl-D-galactose in the degraded gum, while the undegraded gum has a comparatively low proportion of 2:3:4-tri-O-methyl-D-galactose and a large amount of 2:4-di-O-methyl-D-galactose.

The quantity of 2:3:5-tri-O-methyl-L-arabinose isolated from the methylated gum accounts for four of the five parts of arabinose present in gum ghatti. Nearly all the non-reducing end groups in the gum are L-arabofuranose residues, although very small amounts of 2:3:4-tri-O-methyl-L-rhamnose, 2:3:4:6-tetra-O-methyl-D-galactose and 2:3:4-tri-O-methyl-D-gluconic acid also arise from terminal residues. The remaining part of arabinose is made up of approximately equal proportions of 2:3-, 2:4-, 2:5- and
and 3:5-di-O-methyl-L-arabinose, of which only 2:3-di-O-methyl-L-arabinose represents a residue which would be susceptible to periodate oxidation. These results are in agreement with those of Aspinall, Hirst and Wickström (101), who found that about 20% of the arabinose residues were unattacked by periodate and it follows that most of the arabinose derivatives isolated are of structural significance and are not demethylation products. It is clear that 80% of the arabinose residues occur in furanose form as terminal groups, the majority of which constitute single unit side chains. In some cases, however, doubly linked arabinose residues may be interposed between end groups and other sugar residues and some, at least, of these must also be in furanose form. The isolation of 2:4-di-O-methyl-L-arabinose, however, indicates the presence of 3-linked L-arabopyranose residues in the gum, while 2:3-di-O-methyl-L-arabinose may arise from either a pyranose or furanose residue. It is known that not all the arabinose is readily removable by mild hydrolysis and it appears that some arabinose residues are present in the more resistant part of the polymer.

The most important glucuronic acid derivatives isolated were 2:3:4-tri-O-methyl-D-glucuronic acid from the methylated degraded gum and 2:3-di-O-methyl-D-glucuronic acid from the methylated gum. This indicates that much of the acid occupies a penultimate position in side chains, with an arabinose residue as the terminal group. Some tri-O-methyl-D-glucuronic acid was also isolated from the methylated gum and a little di-O-methyl-D-glucuronic acid from the methylated degraded gum. Neutral sugar residues linked /
linked to acids in the methylated gum are those of 2,4-di-O-methyl-D-galactose, 4-O-methyl-D-mannose and 2-O-methyl-D-galactose, while in the methylated degraded gum the corresponding residues are those of 2,3,4-tri-O-methyl-D-galactose, 3,4,6-tri-O-methyl-D-mannose and di-O-methyl-D-galactose (probably the 2,3-dimethyl ether). Arabinose residues must be attached to these sugars through position 3 of galactose and through positions 3 and 6 of mannose.

From the information so far available, it is evident that the following structural features are present in the gum (II).

<table>
<thead>
<tr>
<th>R</th>
<th>R</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

... 1 Gal 6 ... 1 Gal 6 ... 1 Gal 6 ... 1 Gal 6 ....

| 4 |

... 1 Gal 6 - 1 GA 4 - R

... 1 Ma 2 - 1 GA 4 - R

| 3 | 6 |

| R | R |

II.

R represents mainly terminal L-arabofuranose residues, but in some cases may represent L-rhamnopyranose residues or side chains composed of two or more arabinose units.
A small proportion of xylose is present in gum ghatti, but hydrolysis of the methylated gum gave only a trace of 2:3:4-tri-O-methyl-D-xylose, and no other xylose derivative was detected. It seems probable that xylose may not be a true component of the gum, but may arise from a contaminating polysaccharide which was lost during the various stages of methylation. Smith (25) has recently suggested that gum ghatti is heterogeneous and it is possible that a xylan may be present as well as the gum acid.

In contrast to the loss of xylose during methylation of the gum, rhamnose persisted through all the operations involved and was eventually identified as 2:3:4-tri-O-methyl-L-rhamnose. It seems likely, therefore, that terminal rhamnose residues form an integral part of the gum and do not arise from a contaminating polysaccharide. Rhamnose had not previously been identified in gum ghatti, although Aspinall, Hirst and Wickström reported its probable presence (101).

Results so far described give no information regarding the length of side chains; the aldobiouronic acid units may be linked directly to the main /
main backbone of galactose residues (III; n = 0), or one or more galactose residues may be interposed between the aldobiouronic acid and the main chain (III; n ≥ 1).

\[ \text{Gal} \overset{6-1}{\text{Gal}} \overset{6-1}{\text{Gal}} \overset{4}{\text{Gal}} = \left( \text{Gal} \overset{6}{\text{Gal}} \right)_n = \text{Gal} \overset{6}{\text{Gal}} \overset{1}{\text{GA}} \]

III.

In the latter case, it might be expected that both a 1:6- and a 1:4-linked galactobiose would be obtained by partial hydrolysis, whereas in the former case only 1:6-linked galactose polymers would be isolated.

The position of mannose in the gum presents another unresolved problem. In the hydrolysate of the methylated gum, mannose occurred almost exclusively as the 4-methyl ether, indicating one main mode of linkage of mannose residues. It has been shown (101) that glucuronic acid is linked to both mannose and galactose residues, but that one part of mannose and one part of glucuronic acid are present in one equivalent of gum, implying that some /
some of the mannose is linked to neutral sugar only. If the latter deduc-
tion is correct, it should be possible to isolate neutral mannose-
containing oligosaccharides by partial hydrolysis.

With a view to providing information on these problems and other points of fine structure, a partial hydrolysis study of gum ghatti was carried out.

Trial hydrolyses showed that a maximum yield of oligosaccharides was obtained by boiling the gum with 0.1 N sulphuric acid for 90 minutes, whereas more prolonged heating destroyed the oligosaccharides.

A large scale partial hydrolysis of the gum with 0.1 N sulphuric acid gave a mixture of polysaccharide, mono- and oligosaccharides. The polysaccharide material was precipitated with ethanol and subjected to a further mild hydrolysis, again yielding polysaccharide, mono- and oligo-
saccharides. The mono- and oligosaccharides from both hydrolyses were combined and fractionated by chromatography on charcoal:Celite (69). Most of the fractions obtained required further separation by chromatography on small cellulose columns and eventually nine pure oligosaccharides were obtained.

A series of galactose polymers (IV) was shown to be present by the isolation of the first three members \((n = 0, 1 \text { and } 2)\)
Fraction 5 was shown to contain 6-O-β-D-galactopyranosyl-D-galactose (V), since hydrolysis of the methylated derivative gave equal parts of 2:3:4-tri-O-methyl-D-galactose and 2:3:4:6-tetra-O-methyl-D-galactose. A β-linked disaccharide was indicated by the optical rotation which was the same as that obtained for 6-O-β-D-galactopyranosyl-D-galactose recently isolated from golden apple gum (68).
Fractions 11 and 15 were also found to contain galactose polymers which gave galactose and 6-O-galactosylgalactose on partial hydrolysis. Fraction 11, after purification, had an optical rotation indicative of \( \beta \)-linkages and the structure of the oligosaccharide was established (IV; \( n = 1 \)) since methylation and hydrolysis afforded one part of 2:3:4:6-tetra-0-methyl-D-galactose and two parts of 2:3:4-tri-0-methyl-D-galactose.

Fraction 15 was shown to be the third member of the series (IV; \( n = 2 \)) by the linear relationship existing between the number of hexose units and \( \log \frac{R_F}{1 - R_F} \) [Graph 1] (166). The optical rotation of the tetraose indicated \( \beta \)-linkages.

Fractions 8, 13, 17 and 19 were found to contain the first four members of a second homologous series (VI).

\[
\text{Gal } p 1 ( \beta 6 \text{ Gal } p 1)_n \beta 3 \text{ Ar}
\]

VI.

Hydrolysis of fraction 8 gave galactose and arabinose, while reduction and hydrolysis gave galactose only, indicating a galactosylarabinose. The structure of the disaccharide was proved to be 3-O-\( \beta \)-D-galactopyranosyl-L-arabinose, since hydrolysis of the methylated derivative yielded equal /
equal parts of 2:3:4:6-tetra-O-methyl-D-galactose and 2:4-di-O-methyl-L-arabinose, with a trace of 2:5-di-O-methyl-L-arabinose. The arabinose residues were present mainly in pyranose form, giving rise to 2:4-di-O-methyl-L-arabinose, while a trace of the 2:5-dimethyl ether arose from a small proportion of arabinofuranose residues. In view of the L-arabopyranose residues present, the optical rotation of the disaccharide (+62°) is consistent only with a β-linkage. 3-O-β-D-galactosyl-L-arabinose (VII) has not previously been isolated from a plant gum, but residues of 3-O-β-D-galactosyl-L-arabinose (VIII) have been shown to be present in gum arabic (33) and Acacia cyanophylla gum (80). In the case of the former gum, the disaccharide was isolated as the fully methylated derivative, but the unmethylated disaccharide was obtained from Acacia cyanophylla gum and had \([\alpha]_D + 152^\circ\), indicative of an α-linkage.

VII

[Chemical structure image]

VIII
Hydrolysis of fraction 13 gave galactose and arabinose, while reduction and hydrolysis gave galactose only. After partial hydrolysis of the oligosaccharide, chromatography indicated galactose, arabinose, 3-O-galactosylarabinose and 6-O-galactosylgalactose. Hydrolysis of the methylated derivative afforded equal parts of 2:3:4:6-tetra-O-methyl-D-galactose, 2:3:4-tri-O-methyl-D-galactose and 2:4-di-O-methyl-L-arabinose with a trace of 2:5-di-O-methyl-L-arabinose, proving the structure to be \( \text{O-\(\beta\)-D-galactopyranosyl-(1 \rightarrow 6)-O-\(\beta\)-D-galactopyranosyl-(1 \rightarrow 3)-L-arabinose} \). The optical rotation of the trisaccharide indicated \( \beta \)-linkages.

Fractions 17 and 19 were also found to yield galactose and arabinose on hydrolysis. Partial hydrolysis of both oligosaccharides gave galactose /
galactose, arabinose, 6-O-galactosylgalactose and 3-O-galactosylarabinose, while reduction and partial hydrolysis gave only galactose and 6-O-galactosylgalactose.

Quantitative estimation of the component sugars showed that fraction 17 contained one part of arabinose and 2.8 parts of galactose, while fraction 19 contained one part of arabinose and 3.7 parts of galactose. These results indicate that the oligosaccharides are the third and fourth members of the series (VI; n = 2 and 3) and their identity was confirmed by the linear relationship obtained on plotting \( \log \frac{R_F}{1 - R_F} \) against the number of sugar residues in the oligosaccharide [Graph 2] (166).

Small quantities of two further hexose-containing disaccharides were isolated. Fraction 7 was found to contain a galactosylglucose, although glucose residues had not previously been identified in gum ghatti. The significance of this disaccharide cannot at present be explained.

Fraction 10 contained a small amount of a galactose polymer which was chromatographically identical with 5-O-2-D-galactosyl-D-galactose (IX). Lead tetraacetate oxidation (165) of the latter and of the galactobiose from fraction 10 gave the same mixture of products, including lyxose, whereas the 1:4-linked disaccharide, cellobiose, gave a completely different mixture of oxidation products which did not include lyxose. The galactobiose from fraction /
fraction 10 had a similar melting point and optical rotation to those recorded for 3-O-β-D-galactopyranosyl-D-galactose, isolated from Acacia pycantha gum (83) and the X-ray powder photograph of the galactobiose from fraction 10 was identical with that of 3-O-β-D-galactopyranosyl-D-galactose obtained by partial hydrolysis of larch E-galactan (169).

IX.

In addition to the pure oligosaccharides isolated, chromatographic evidence indicated mixtures of pentose-containing disaccharides, mainly in fractions 3 and 4. These mixtures contained two major components, one of which /
which corresponded with 3-O-β-L-arabopyranosyl-L-arabinose. Traces of a number of other (presumably) pentose-containing disaccharides occurred in admixture with other sugars, but the total yield of pentose-containing substances was small and it is probable that some, at least, may be reversion products. 3-O-β-L-Arabopyranosyl-L-arabinose is known to occur as a reversion product of L-arabinose in acid solution (66). It is accompanied, under these conditions, by two other arabinose-containing disaccharides, 4-O-β-L-arabopyranosyl-L-arabinose and O-β-L-arabopyranosyl β-L-arabopyranoside and it is not yet known if the latter occur among the hydrolysis products of gum ghatti. It is possible that the hydrolytic conditions employed were not sufficiently mild to permit the isolation of pentose-containing disaccharides in quantity, but a careful examination of the autohydrolysis products of the gum failed to reveal any such disaccharides. It is likely that gum ghatti contains a comparatively low proportion of arabinosylarabinose units.

It has been shown (170) that two disaccharides are synthesised from D-galactose in 37% hydrochloric acid solution, the major product being 6-O-α-D-galactosyl-D-galactose. In view of the absence of this disaccharide from the partial hydrolysis products of gum ghatti, it is very improbable that any of the galactose polymers isolated were reversion products; the homologous series (IV and VI) almost certainly represent true hydrolysis products. Both 6-O-galactosylgalactose and 3-O-galactosylarabinose were detected during trial /
trial hydrolyses of the gum and it was observed that the concentration of both reached a maximum after heating the acid solution for 90 minutes, while more prolonged heating destroyed the disaccharides. This behaviour is characteristic of oligosaccharides liberated by hydrolysis, but not of reversion products, which tend to an equilibrium concentration.

Methylation studies have indicated a main chain of 1:6-linked galactose residues in gum ghatti and this evidence is confirmed by the isolation, in quantity, of the series of 1:6-linked galactose polymers (IV). β-Linkages shown to exist in this series indicate a β-linked galactose chain in the gum.

5-0-β-D-Galactosyl-D-galactose was isolated in small quantity only and is of minor structural importance compared with 6-0-β-D-galactosyl-D-galactose, but the 1:3-linked galactobiose may arise from branched galactose residues. It has been shown that methylation and hydrolysis of the degraded gum gave, among other sugars, 2:3-di-0-methyl-D-galactose with a much smaller amount of 2:4-di-0-methyl-D-galactose, indicating that branching occurs mainly through position 4 of galactose but also, to a lesser degree, through position 3. It was suggested, therefore, that acid-bearing side chains were attached mainly through position 4 of galactose residues, but some side chains, other than those composed only of pentose units, are also attached through position 3. If these latter side chains contain aldobiouronic acid units, the isolation of /
of 3-O-β-D-galactosyl-D-galactose suggests that at least one galactose residue must be interposed between the main chain and the aldobioseuronic acid residue. It is, however, possible that the degraded gum contains two types of side chains, one consisting of aldobioseuronic acid units and the other of galactose residues only.

Attempts to detect a 1:4-linked galactobiose were unsuccessful, although fractions containing disaccharides were carefully examined. Definite conclusions cannot be based on negative evidence alone, but it seems likely that aldobioseuronic acid units are directly attached to the main chain through position 4 of galactose residues (X).

There was no trace of any mannose-containing polymer among the partial hydrolysis products. This, again, provides negative evidence only, but it suggests that mannose residues may be confined to aldobioseuronic acid units and do not occur in combination with neutral sugar residues alone. Methylation studies have indicated that mannose residues occur almost entirely in one type of combination (XI); a unique mode of linkage might be
be expected if mannose residues were always joined to the same sugar units.

\[ \text{Ar} \ 1 - 3 \text{ Ma} \ 6 - 1 \text{ Ar} \]

It is known that some, at least, of the mannose residues are linked to D-glucuronic acid units but quantitative estimations suggest that only about half of the mannose is so combined. Since no evidence has been obtained regarding the linking of mannose residues to neutral sugars only, it seems probable that the quantitative estimation of mannose in the gum was incorrect. The only other mannose-containing gums which have been extensively investigated are those of damson (87) and cherry (89), and in these the whole mannose content appears to be present in aldobiouronic acid units.

The presence of the homologous series (VI) is an interesting feature of gum ghatti. The maximum value of n is not known, only the first four members of the series having been isolated, but it is likely that these oligosaccharides are derived from the main chain of the gum and that long chains of 1:6-linked galactose units are joined together by 1:3-linked arabinose residues. Methylated oligosaccharides of the galactosylarabinose series (VI) yielded mainly pyranose derivatives of arabinose, but this does not imply that the arabinose residues were present in pyranose form in the gum. In fact it is /
is more likely that they occurred as L-arabofuranose residues and that the furanoside linkage was cleaved by mild hydrolysis, after which the arabinose unit reverted to pyranose form.

It has been shown that not all the arabinose is liberated from gum ghatti during autohydrolysis. Chromatography of the hydrolysed degraded gum showed an easily detectable proportion of arabinose, but no derivatives of arabinose were isolated from the hydrolysis products of the methylated degraded gum; a trace of 2:3:5-tri-O-methylarabinose was indicated by chromatographic evidence only. On the other hand, methylated gum ghatti yielded the anticipated proportion of arabinose derivatives. It therefore appears that arabinose was lost during methylation of the degraded gum, but not during methylation of the whole gum. A possible explanation is that arabinose residues in the degraded gum are susceptible to alkaline degradation, whereas those in the whole gum are protected from attack, and this would be the case if gum ghatti contains chains of galactose units joined together by 1:3-linked arabofuranose residues. The furanoside linkage would be split by autohydrolysis, giving shorter chains with reducing arabinose residues in the terminal position (XII).
3-Linked arabinose residues with free reducing groups readily undergo alkaline degradation (171) and it might be expected that they would be lost during methylation in sodium hydroxide solution.

Scission of the main galactose chain in the manner suggested would also give rise to non-reducing terminal galactose residues. These additional terminal groups might account for the appreciable proportion of 2:3:4:6-tetra-O-methyl-D-galactose isolated from the hydrolysis products of the methylated degraded gum.

It is not yet possible to put forward a suggested structure for gum ghatti, and it is doubtful if a discrete repeating unit exists. The evidence so far available suggests that the polymer contains the structural features shown.
shown (XIII), although it is still not certain that aldobiouronic acid units are directly attached to the main chain and the nature of the sugar residues linked to mannose requires further investigation. Useful information might be obtained by a study of the partial hydrolysis products of the periodate oxidised gum.

R
\[ \begin{array}{c}
3 \\
4 \\
1 \\
6 \\
R
\end{array} \]
\[ \begin{array}{c}
1 \text{ Gal 6} \\
\end{array} \]
\[ \begin{array}{c}
R - 3 \text{ Ma 2} - 1 \text{ GA 4} - R \\
6 \\
R
\end{array} \]

\[ \begin{array}{c}
3 \\
4 \\
1 \\
3 \\
R
\end{array} \]
\[ \begin{array}{c}
1 \text{ Gal 6} \\
\end{array} \]
\[ \begin{array}{c}
3 \\
4 \\
3 \\
1 \text{ Gal 6} \\
\end{array} \]

XIII.

R represents mainly terminal L-arabofuranose residues, but in some cases may represent L-rhamnopyranose residues or side chains composed of two or more arabinose units.

Gum /
Gum ghatti exhibits the general structure common to most glucuronic acid-containing gums; the backbone of galactose residues bears side chains containing aldobiouronic acid units, and to this framework are attached acid-labile pentose residues. The detailed structure of the gum, however, shows some less usual features.

A comparison may be made with the Prunus gums [Table I, p. 24] which contain residues of D-glucuronic acid, D-galactose, L-arabinose, D-xylose and, in the case of damson and cherry gums, D-mannose. Only one aldobiouronic acid, either 6-O-β-D-glucuronosyl-D-galactose or 2-O-β-D-glucuronosyl-D-mannose has been isolated from each Prunus gum examined, whereas both these aldobiouronic acids have been obtained from gum ghatti.

Other gums which bear some resemblance to gum ghatti are the Acacia gums and the gum of Hakea acicularis [Table I, p. 24]. The latter contains the same sugar residues as gum ghatti and yields one aldobiouronic acid, 2-O-β-D-glucuronosyl-D-mannose. 6-O-β-D-Glucuronosyl-D-galactose has been isolated from all the Acacia gums so far examined, but these differ from gum ghatti in containing an appreciable proportion of L-rhamnose and no xylose or mannose.

Methylation studies of gum arabic [p. 14] and Acacia pyxantha (85), damson, cherry and egg plum gums [Table III, p. 28] have indicated mixtures of 1:6- and 1:3-linked galactose residues, of which the latter are often the more
more important. Gum arabic is now known to consist of a backbone of 1:3-linked galactose residues with 1:6-linked galactose side chains. Gum ghatti, in contrast, contains mainly 1:6-linked galactose residues with acid-bearing side chains attached through position 4.

Several of the Prunus and Acacia gums have been found to yield pentose-containing disaccharides [Table II, p. 27] and, in a few cases, aldobioaromic acid (62, 80) under very mild hydrolytic conditions. No oligosaccharides have been obtained by autohydrolysis of gum ghatti; this is explained by the fact that most of the arabinose is present as single unit side chains and the aldobioaromic acid forms part of the resistant nucleus of the molecule.

3-O-α-D-Galactosyl-L-arabinose has been obtained by autohydrolysis of gum arabic (33) and Acacia cyanophylla gum (80) and must come from the outer structure of the polymer, since it is liberated under very mild hydrolytic conditions. The significance of this disaccharide may be contrasted with that of 3-O-3-D-galactosyl-L-arabinose, which is liberated from gum ghatti under stronger hydrolytic conditions and has been shown to be the first member of a homologous series (VI). The main galactose chains in gum ghatti are thus shown to be joined together by 1:3-linked arabinose residues, a feature which has not so far been detected in any other plant gum. It seems probable, however, that similarly linked arabinose residues may be present in the /
the gum of *Anogeissus schimperi*.

Gum ghatti is one of the most complicated of the plant gums and it is to be expected that the gum of *Anogeissus schimperi*, of the same plant genus, will prove structurally similar. It has already been shown (102, 103) that *A. schimperi* gum contains residues of D-glucuronic acid, D-galactose, D-mannose, L-arabinose, D-xylose and a trace of L-rhamnose and that it yields, on partial hydrolysis, at least one aldobiouronic acid, probably 2-O-β-D-glucuronyl-D-mannose. So far, the only gums from which the disaccharide 6-O-β-D-galactosyl-D-galactose has been isolated are gum ghatti and golden apple gum (68), and 3-O-β-D-galactosyl-L-arabinose has been obtained only from gum ghatti. However, work now in progress on *A. schimperi* gum has indicated the presence of both 3-O-β-D-galactosyl-L-arabinose and 6-O-β-D-galactosyl-D-galactose (140). In view of the structural similarities which have been shown to exist between gums from plants of the same genus, it is probable that the gum of *A. schimperi* will be found to contain 1:6-linked galactose chains joined together through arabinose residues. No other gum of the genus *Anogeissus* has been investigated and it would therefore be of interest to make a detailed comparison of gum ghatti and the gum of *A. schimperi*. 
BIBLIOGRAPHY


(2) J.K.N. Jones and F. Smith, ibid., 1949, 4, 243.


(4) E.L. Hirst, Endeavour, 1951, 10, 106.


(6) E.L. Hirst and J.K.N. Jones, Research, 1951, 4, 411.


(8) E.L. Hirst, J., 1942, 70.


(12) S.P. James and F. Smith, J., 1945, 739.

(13) H. El-Khadem and M.M. Megahed, J., 1956, 3953.


(19) /


(21) J.K.N. Jones and J.B. Pridham, unpublished work; quoted by
     E.L. Hirst and J.K.N. Jones, Modern Methods of


(23) E.J. Bourne, A.B. Foster and P.M. Grant, J., 1956, 4311.


(26) G.S. Berenson, S. Rosenman and A. Dorfman, Biochim. Biophys. Acta,
     1955, 17, 75.


(28) W.J. Whelan, Modern Methods of Plant Analysis, Springer-Verlag,


(31) S.A. Barker, E.J. Bourne, M. Stacey and D.H. Whiffen,
     Chem. & Ind., 1953, 196.


(33) F. Smith, J., 1959, 744.


(36) /
(38) S.M. Partridge, Nature, 1946, 158, 270.
(43) D.J. Bell, Modern Methods of Plant Analysis, Springer-Verlag, Berlin, 1955, 2, 30.
(47) W.N. Haworth, J., 1915, 107, 8.
(48) T. Purdie and J.C. Irvine, J., 1905, 85, 1021.
(49) E.L. Hirst and J.K.N. Jones, J., 1938, 496.
(54) W.J. Whelan and K. Morgan, Chem. & Ind., 1954, 73.
(55) /
(60) A.B. Foster, *Chem. & Ind.*, 1952, 1050.
(72) J. Jackson and F. Smith, *J.*, 1940, 79.
(75) /
(75) F. Smith, J., 1939, 1724.

(76) J. Jackson and F. Smith, J., 1940, 74.

(77) F. Smith, J., 1940, 1035.


(81) A.M. Stephen, J., 1951, 646.


(85) G.O. Aspinall and A. Nicholson, unpublished work.


(87) E.L. Hirst and J.K.N. Jones, J., 1938, 1174.


(89) J.K.N. Jones, J., 1939, 558.


(94) Idem, J., 1949, 3141.

(95) /
(95) E.L. Hirst and J.K.N. Jones, J., 1946, 506.
(96) Idem, J., 1939, 1482.
(98) E.L. Hirst and J.K.N. Jones, J., 1948, 120.
(99) A.M. Stephen, J., 1956, 4437.
(100) E. Anderson and L. Harris, J. Amer. Pharm. Assoc., 1952, 44, 529.
(102) R.J. McIlroy, J., 1952, 1918.
(103) G.O. Aspinall, B.J. Auret and H. Wilkie, unpublished work.
(104) R.J. McIlroy, J., 1957, 4447.
(110) Idem, ibid., 1953, 75, 4692.
(111) Idem, ibid., 1954, 76, 4906.
(112) R.J. McIlroy, J., 1951, 1372.
(114) Idem, ibid., 1954, 13E, 452.
(128) Idem, ibid., 1956, 32, 125.
(130) Idem, ibid., 1947, 69, 622.
(131) Idem, ibid., 1947, 69, 2264.
(132) Idem, ibid., 1948, 70, 367.
(133) J.J. Gurneen and P. Smith, J. 1948, 1141.
(135) F. Smith, J., 1951, 2646.
(136) /
(138) L. Beaufraune, Compt. rend., 1946, 222, 1056.
(139) E.L. Hirst and S. Dunstan, J., 1953, 2332.
(140) G.O. Aspinall and T.B. Christensen, unpublished work.
(144) R.S. Tipson, C.C. Christman and P.A. Levene, J. Biol. Chem., 1939,
128, 609.
(149) F. Brown, E.L. Hirst, L. Hough, J.K.N. Jones and W.H. Wadman,
(150) S.P. Jones and F. Smith, J., 1945, 746.
(151) Idem, J., 1945, 749.
(152) F. Pregl, Quantitative Organic Microanalysis, Churchill, London,
1945, 146.
(154) A.E. Heron, R.H. Reed, H.E. Stagg and H. Watson, Analyst, 1954, 79, 571.
(156) /
(160) J.C. Irvine and J.P. Scott, J., 1913, 575.
(163) M. Abdel-Akher and F. Smith, ibid., 1951, 73, 5859.
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B.J.A.
47. Gum Ghatti (Indian Gum). Part II.* The Hydrolysis Products obtained from the Methylated Degraded Gum and the Methylated Gum.

By G. O. Aspinall, (Mrs.) Barbara J. Auret, and E. L. Hirst.


It was shown in Part I that gum ghatti (Indian gum) from Anogeissus latifolia, Wall, is composed of the following sugar residues, L-arabinose (5 parts), D-galactose (3 parts), D-mannose (1 part), and D-glucuronic acid (1 part), together with the small amounts of xylose and a 6-deoxyhexose (probably rhamnose). Most of the arabinose residues are located in the outer parts of the molecular structure since autohydrolysis of the gum acid leads to the removal of these residues and the formation of a degraded gum, in which only a small proportion of arabinose residues are attached to the more resistant backbone of galactose, mannose, and glucuronic acid residues. Further hydrolysis of the gum under more drastic conditions gives D-galactose and a mixture of two aldobiouronic acids, characterised as 6-O-3-D-glucuronosyl-D-galactose and 2-O-3-D-glucuronosyl-D-mannose. An examination of the methylated sugars formed on hydrolysis of the methylated gum and the methylated degraded gum is now reported, and partial structures for the gum are discussed.

A sample of degraded gum ghatti (substantially arabinose-free), prepared by autohydrolysis of the gum acid, was converted into its fully methylated derivative. The methylated polysaccharide was hydrolysed, and the hydrolysate (containing acidic components as barium salts) was separated into an ether-soluble fraction and an ether-insoluble residue. The neutral methylated sugars present in the ether-soluble fraction were fractionated chromatographically on cellulose, giving 2:3:4:6-tetra-O-methyl-, 2:3:4-tri-O-methyl-, 2:3- and 2:4-di-O-methyl-D-galactose, all of which were characterised as crystalline derivatives. Although no methyl ethers of D-mannose could be isolated in pure form and identified as crystalline derivatives, the presence of 2:3:5-tri-O-methylarabinose was established by chromatography, paper ionophoresis, the detection of 2:3:5-tri-O-methylarabinose when a fraction containing the sugar was oxidised with periodate, and the observation that a fraction containing the sugar in admixture with tetra- and 2:3:4-tri-O-methylgalactose was converted into the corresponding mixture of aldonamides, which gave a positive Weerman test. This sugar was the most important mannose derivative present, although traces of a di-O-methyl- (probably 3:4-) and 4-O-methyl-mannose were also observed. In addition, traces of 2:3:5-tri-O-methylarabinose and 2:3:4-tri-O-methyl-xylose and rhamnose were detected chromatographically, but in insufficient quantity to be of structural significance. The ether-insoluble residue afforded further quantities of 2:3:4-tri- and di-O-methylgalactose, which were separated from the barium salts on filter sheets. The acidic fraction, consisting mainly of methylated aldobiouronic acids, was submitted to further acid hydrolysis under vigorous conditions, and again the acidic were separated from the neutral sugars.

* The paper by Aspinall, Hirst, and Wickström (J., 1955, 1160) is to be regarded as Part I.
The neutral fraction contained 2:3:4-tri-O-methyl-D-galactose, identified as the aniline derivative, and 3:4:6-tri-O-methylmannose and 2:3-di-O-methylgalactose were identified chromatographically. The main component of the acidic fraction was identified chromatographically as 2:3:4-tri-O-methylglucuronic acid, and, after reduction of the methyl ester methyl glycoside and hydrolysis, as 2:3:4-tri-O-methylglucose. The minor component of the acidic fraction was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride, and hydrolysed to give 2:3-di-O-methylglucose together with small amounts of tri-O-methylglucose and tri- and di-O-methylgalactose. Some of the glucuronic acid residues in the degraded gum, therefore, were present in non-terminal positions linked through C(4) and C(4).

It is clear from these results that the gum contains a backbone of 1:6-linked D-galactopyranose units (I: R = H). Although both 2:3: and 2:4-di-O-methyl-D-galactose were characterised, evidence from ionophoresis and from chromatographic examination of the periodate oxidation products of the di-O-methylgalactose fractions showed the 2:3-dimethyl ether to be the main component of the mixture. (When mixtures of these two sugars are present, the 2:4-dimethyl ether is more readily characterised as the relatively insoluble aniline derivative.) It follows that the main branching point in the degraded gum is through position 4 of galactose, although some small proportion of branching may also occur through position 3. Since the main products of hydrolysis of the methylated aldobiouronic acids were 2:3:4-tri-O-methylglucuronic acid, 2:3:4-tri-O-methylgalactose, and 3:4:6-tri-O-methylmannose, the aldobiouronic acid groups are present as terminal groups (II and III; R = H). These groups are, therefore, attached as side-chains probably through position 4 of galactose residues in the backbone. At present, there is no indication whether these units are attached directly to the backbone or whether 1:6-linked galactose residues are interposed. The structural significance of the non-reducing D-galactopyranose end groups in the degraded gum is not yet clear. Although these end groups may terminate another type of side-chain, it is also possible that they are present at the non-reducing end of the backbone and arise from scission of the main chain during the autohydrolysis.

Fully methylated gum ghatti was hydrolysed, and the hydrolysate (containing acidic components as barium salts) was separated into an ether-soluble fraction (A) and an ether-insoluble residue (B). The residue (B) was then separated by chromatography on cellulose to give neutral sugars (C), a pure sample of 2-O-methyl-D-galactose, and an acidic fraction. The combined neutral sugars (A) + (C) were chromatographed on cellulose, giving pure samples of most of the major components and mixtures containing the minor components, which were refractionated. The following methylated sugars were characterised as crystalline derivatives: 2:3:5-tri-, 2:3-, 2:4-, 2:5-, and 3:5-di-O-methyl-L-arabinose, 2:3:4:6-tetra-, 2:3:4-tri-, 2:4-di-, and 2:mono-O-methyl-D-galactose, 4-O-methyl-D-mannose, and 2:3:4-tri-O-methyl-L-rhamnose. Traces of some other sugars were detected chromatographically, but these were present in insufficient amount to be of structural significance. The acidic fraction, which was contaminated by a small amount of 2-O-methylgalactose, was converted into the corresponding mixture of
methyl ester methyl glycosides, which was reduced with lithium aluminium hydride and hydrolysed to give a mixture of neutral sugars. The methylated sugars were fractionated on cellulose, and the following sugars were identified as crystalline derivatives: 2:3:4-tri- and 2:3-di-O-methyl-D-glucose, 2:4-di- and 2-mono-O-methyl-D-galactose, and 4-O-methyl-D-mannose. The isolation of 2:3:4-tri- and 2:3-di-O-methyl-D-glucose indicates the presence in the methylated gum of residues of 2:3:4-tri- and 2:3-di-O-methyl-D-glucuronic acid. 2:4-Di-O-methyl-D-galactose and 4-O-methyl-D-mannose are the main neutral fragments arising from the aldobiouronic acid groupings. It is probable that the 2-O-methyl-D-galactose arose entirely from the contaminating neutral sugar and not from hydrolysis of a partially methylated aldobiouronic acid. In addition, a trace of 2:3:4-tri-O-methylgalactose was detected chromatographically.

In view of the several stages involved in the separation and identification of the many methylated sugars formed on hydrolysis of the methylated gum, it is not possible to give more than an approximate estimate of the proportions of some of the constituent sugars arising from the neutral part of the gum. Since even more operations were involved in the identification of the acidic residues and of the neutral sugar residues attached thereto, and since the hydrolysates of acidic polysaccharides are usually accompanied by some decomposition, it is again only possible to estimate their relative proportions approximately. The significance of these results, taken together with previous results, may be assessed most conveniently by considering, in turn, the L-arabinose residues removed during the autohydrolysis, the backbone of 1:6-linked D-galactopyranose residues, and the aldobiouronic acid units. The approximate composition of the gum determined in Part I and expressed as parts per equivalent weight of gum acid provides a useful working model.

The quantity of 2:3:5-tri-O-methyl-L-arabinose isolated from the methylated gum accounts for approximately four of the five parts of L-arabinose present per equivalent of gum. Although relatively small amounts of 2:3:4-tri-O-methyl-L-rhamnose, 2:3:4:6-tetra-O-methyl-D-galactose, and 2:3:4-tri-O-methyl-D-glucuronic acid were also present as units of the methylated gum, terminal L-arabofuranose residues account for most of the non-reducing end groups in the gum. The fifth part of L-arabinose is accounted for by approximately equal amounts of 2:3-, 2:4-, 2:5-, and 3:5-di-O-methyl-L-arabinose. Three of these four sugars represent units not susceptible to attack by periodate. Since it was shown in Part I that about 20% of the arabinose residues in the gum are not attacked by periodate, it follows that most, if not all, of these sugars are of structural significance and do not arise from incomplete methylation of the gum or from demethylation during hydrolysis. These non-terminal arabinose residues must also occur in the outer parts of the molecule since they are removed as free arabinose during the autohydrolysis of the gum acid. It is clear, therefore, that the majority of L-arabinose residues in the gum occur in the furanose form as single-unit side-chains attached to the more resistant part of the structure. In a few cases, however, non-terminal L-arabinose residues must be interposed between the end groups and the other sugar residues. The small proportion of L-arabopyranose residues in the gum, as shown by the isolation of 2:4-di-O-methyl-L-arabinose, is of particular interest as until recently 3 L-arabinose had been found in combination only in the furanose form.

The D-galactose residues present in the gum occur in three main types of combination as shown by the isolation of 2:3:4-tri- (<1 part), 2:4-di- (>1 part), and 2-mono- (>1 part) O-methyl-D-galactose from the methylated gum. Since the corresponding residues in the methylated degraded gum afford 2:3:4-tri- and 2:3-di-O-methyl-D-galactose, it follows that the preferred mode of attachment of arabinose is to position 3 of galactose, although it is possible that some arabinose residues may also be linked to position 4. It is probable, however, that most of the 2-O-methyl-D-galactose represents a double branching point, to arabinose through position 3, and to aldobiouronic acid through position 4. These results are in reasonable agreement with the results of periodate
oxidation of the gum (Part I) where it was shown that about a third of the galactose residues in the gum were attacked by periodate. There is no evidence at present as to the role of the very small proportion of D-galactopyranose end groups.

The sugar residues present in the aldobiouronic acid groupings in the methylated gum are those of 2:3:4-tri-O-methyl-D-glucuronic acid, 2:3-di-O-methyl-D-glucuronic acid (main acid component), 2:4-di-O-methyl-D-galactose, and 4-O-methyl-D-mannose, whereas the corresponding residues in the methylated degraded gum are those of 2:3:4:tri-O-methyl-D-glucuronic acid (main acid component), 2:3-di-O-methyl-D-glucuronic acid, 2:3:4-tri-O-methyl-D-galactose, and 3:4:6-tri-O-methyl-D-mannose. It follows that arabinose residues are attached to these sugars through position 3 of galactose, positions 3 and 6 of mannose, and through position 4 of some glucuronic acid residues. Apart from chromatographic and ionophoretic evidence for traces of 3:4:6-tri-O-methylmannose, the only derivative of D-mannose found in the hydrolysate from the methylated gum was the 4-methyl ether, indicating one main mode of linkage of mannose residues in the gum. The evidence adduced in Part I indicated, on the one hand, the presence of one part of mannose and one part of glucuronic acid per equivalent of gum, and, on the other hand, the linking of glucuronic acid to both galactose and mannose, and suggested that some mannose residues in the gum may be linked to neutral sugar residues only. Experiments to provide further evidence on this point are in progress.

In Part I it was shown that hydrolysis of gum ghatti affords small amounts of xylose and a 6-deoxyhexose (probably rhamnose) in addition to the main constituent sugars. The isolation of 2:3:4-tri-O-methyl-L-rhamnose from the methylated gum confirms the presence of L-rhamnose residues in the gum. No other methyl ethers of rhamnose were detected. Since this sugar has persisted throughout the various operations it seems probable that it is an integral part of the gum structure and does not arise from a contaminating polysaccharide. On the other hand, only traces of xylose derivatives (as the 2:3:4-trimethyl ether) could be detected on hydrolysis of the methylated gum. It is unlikely, therefore, that xylose is a constituent of the gum itself.

Our present knowledge of the molecular structure of gum ghatti may be summarised in terms of the partial structures (I, II, and III), with the substituent groups R representing mainly single L-arabofuranose residues, but in a few cases more complex arabinose-containing side-chains terminated again by L-arabofuranose residues. Experiments to determine the mode of attachment of the aldobiouronic acid side-chains (II and III) to the backbone of galactose residues (I) will be reported later. It is already clear that gum ghatti resembles several other plant gums, notably damson, cherry, and egg plum gums, in containing a high proportion of L-arabofuranose residues in the outer parts of the molecular structure. It differs, however, from these gums in containing galactose residues mutually linked mainly through C_4 and C_6, and not through C_3 also. Gum ghatti differs also in this respect from gum arabic, which is now known to contain a backbone of 1:3-linked D-galactopyranose units to which are attached side-chains of 1:6-linked galactose units.

**Experimental**

Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5:1:3:3, upper layer); (B) ethyl acetate–acetic acid–water (3:1:3, upper layer); (C) butan-1-ol–acetic acid–water (4:1:5, upper layer); (D) butan-1-ol–ethanol–water (4:1:5, upper layer); (E) benzene–ethanol–water (169:47:15, upper layer); (F) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (G) butan-2-one, half saturated with water containing 1% of ammonia. Unless otherwise stated, chromatography of methylated sugars was carried out in solvent D and R_6 values refer to rate of movement relative to 2:3:4:6-tetra-O-methyl-D-glucose in that solvent. Demethylations of methylated sugars were carried out by the procedure of Hough, Jones, and Wadman. Paper ionophoresis was carried out in borate buffer at pH 10 at a
potential of 500 v. Aniline derivatives of methylated sugars were prepared by refluxing the sugar in ethanolic aniline for 30 min.; further heating resulting in darkening of the solution. Optical rotations were observed at 18° ± 2°.

Samples of the gum acid and the degraded gum acid were prepared as described in Part I. During the preparation of the degraded gum the autohydrolysis of the gum acid caused the release of arabinose and only traces of xylose, rhamnose, and galactose. The various samples of degraded gum acid had slightly different optical rotations, $[\alpha]_D$ (as barium salt) varying from +2° to +9°. These samples were combined for subsequent experiments since the chromatographic patterns, after hydrolysis with n-sulphuric acid for 6 hr. at 100°, were similar in each case showing galactose in quantity, two aldobiouronic acids, small amounts of arabinose and mannose, and a trace of xylose.

**Preparation and Hydrolysis of Methylated Degraded Gum.** —The degraded gum (15 g.) was methyleated extensively with methyl sulphate and sodium hydroxide following the procedure of Brown, Hirst, and Jones. The product isolated as methylated degraded gum acid (8-2 g.; OMe, 36-9%) was further methylated with methyl iodide and silver oxide, giving methylated degraded gum (6-7 g.). Fractional precipitation of the methylated polysaccharide from chloroform by light petroleum gave fraction (a) (3-7 g.; OMe, 44-4%), which had $[\alpha]_D -28°$ ($c 1-0$ in CHCl$_3$), and fraction (b) (3-0 g.; OMe, 45-1%), which had $[\alpha]_D -11-5°$ ($c 1-0$ in CHCl$_3$). Chromatographic examination of the hydrolysates of the two fractions in solvent D showed similar complex mixtures of sugars.

Methylated degraded gum (fraction a; 3-2 g.) was suspended in n-hydrochloric acid (500 ml.) at 35° for 7 days, and the resulting solution was heated at 100° for 12.5 hr. (constant rotation), cooled, neutralised with silver carbonate, and filtered, and the filtrate was treated with hydrogen sulphide to remove silver ions, filtered, and concentrated. The resulting syrup was dissolved in water, and the solution was neutralised with barium carbonate, filtered, and concentrated to a dark syrup (3-0 g.). The dry syrup was repeatedly extracted with dry ether, to give an ether-soluble fraction (1-92 g.) and an ether-insoluble residue.

**Examination of the Ether-soluble Fraction.** —The syrup was separated on cellulose (60 × 3-4 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7:3; later 1:1) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eight fractions, and a further fraction was obtained by elution of the cellulose with water.

**Fraction 1.** The syrup (23 mg.) contained a mixture of sugars (R$_D$ 0.97—0.93) and a trace of tri-O-methylgalactose (R$_D$ 0.72). Chromatographic examination in solvent E showed 2: 3: 4: 4-tri-O-methylxylose and two sugars having similar mobilities and staining properties with aniline oxalate to 2: 3: 4-tri-O-methylrhamnose and 2: 3: 5-tri-O-methylarabinose.

Demethylation gave galactose and traces of xylose and arabinose. The remainder of the syrup was hydrolysed with n-hydrochloric acid at 100 for 6 hr., and chromatography showed much di-O-methylgalactose in the hydrolysate. The origin of the latter sugar is obscure.

**Fraction 2.** The syrup (97 mg.) had $[\alpha]_D +100°$ ($c 0-34$) and R$_D$ 0.89. The sugar was identified as 2: 3: 4: 6-tetra-O-methyl-d-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 180—190°.

**Fraction 3.** Chromatography of the syrup (92 mg.) showed three components corresponding to 2: 3: 4: 6-tetra-O-methylgalactose, 3: 4: 6-tri-O-methylmannose, and 2: 3: 4-tri-O-methylgalactose, R$_D$ 0.89, 0.82, and 0.72. Demethylation gave galactose and mannose. Paper ionophoresis showed a component having the same mobility as 3: 4: 6-tri-O-methyl-d-mannose.

Chromatographic examination of the products of periodate oxidation showed 2: 3: 5-tri-O-methylarabinose (R$_D$ 0.95) in addition to unchanged starting material. The syrup (50 mg.) was converted into the corresponding mixture of aldonic acids, which, after treatment with sodium hypochlorite and addition of semicarbazide, afforded hydrazodicarbonamide, m. p. 268° and mixed m. p. (with sample of m. p. 263—264°) 264°.

**Fraction 4.** The chromatographically pure syrup (0-904 g.) had $[\alpha]_D +118°$ ($c 0-42$) and R$_D$ 0.72 (Found: OMe, 41-7. Calc. for C$_9$H$_{18}$O$_6$: OMe, 41.8%). Demethylation gave only galactose. The sugar was identified as 2: 3: 4-tri-O-methyl-d-galactose by conversion into the aniline derivative, m. p. 165—167° and mixed m. p. (with sample of m. p. 150—161°) 150—162°. The sugar subsequently crystallised from acetone-ether as the monohydrate, m. p. 71° and mixed m. p. (with sample m. p. 73—76°) 72—73°.

**Fraction 5.** The syrup (112 mg.) contained two components, R$_D$ 0.72 (2: 3: 4-tri-O-methylgalactose) and 0-60. The second component showed similar chromatographic and iono-
phoretic behaviour to 3:4-di-O-methyl-D-mannose. The mixture gave galactose and mannose on demethylation. Attempts to separate the two components by chromatography on Amberlite resin IRA-400 (borate form) were unsuccessful.

**Fraction 6.** The syrup (203 mg.) contained two components, having $R_d$ 0-49 and 0-75 respectively, the coloration of the former with aniline oxide suggesting 2:3-di-O-methylgalactose. Demethylation gave only galactose. Separation of the syrup (190 mg.) on filter sheets using solvent D gave fractions 6a (70 mg.) and 6b (54 mg.). Paper ionophoresis of fraction 6a showed 2:3-di-O-methylgalactose and a trace of the 2:4-dimethyl ether. Chromatographic examination of the products of periodate oxidation confirmed the presence of 2:3-di-O-methylgalactose. An authentic sample of 2:3-di-O-methyl-D-galactose when oxidised with periodate showed three oxidation products with $R_f$ 0-66 (grey), 0-78 (brown), and 0-87 (grey) respectively, whereas 2:4-di-O-methyl-D-galactose showed only unchanged sugar ($R_f$ 0-37). The sugar had $[\alpha]_D +69^0 \rightarrow +80^0 (c 0-37)$ and the identity of the main component was proved by conversion into 2:3-di-O-methyl-N-phenyl-D-galactosylamine, m. p. 125—128° and mixed m. p. 124—127°. Fraction 6b was hydrolysed with n-hydrochloric acid for 6 hr. at 100° and chromatographic examination showed only 2:3:4-tri-O-methylgalactose; it is probable that this fraction contains a polymer of 2:3:4-tri-O-methyl-D-galactose arising from incomplete hydrolysis of the methylated polysaccharide.

**Fraction 7.** The syrup (91 mg.) contained at least two components (having $R_d$ 0-48 and 0-75 respectively). Separation of the mixture (88 mg.) on filter sheets using solvent D gave fractions 7a (53 mg.) and 7b (17 mg.). Paper ionophoresis and chromatographic examination of the products of periodate oxidation indicated the presence in fraction 7a of 2:3- and 2:4-di-O-methylgalactose. The presence in the mixture of 2:4-di-O-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 202—204°. Fraction 7b contained the same substance ($R_f$ 0-76) as fraction 6b and small amounts of di-O-methylgalactose. On hydrolysis the main component gave 2:3:4-tri-O-methylgalactose.

**Fraction 8.** The syrup (31 mg.) contained a di-O-methylgalactose ($R_G$ 0-45) and small amounts of 4-O-methylmannose and an unidentified sugar ($R_G$ 0-65).

**Fraction 9.** The syrup (48 mg.) contained a complex mixture of acidic and neutral sugars and was not examined further.

**Examination of the Ether-insoluble Fraction.**—The ether-insoluble residue was treated with Amberlite resin IR-120(H) to remove barium ions, and concentration gave a syrup (0-714 g.) (equiv. wt., 597). Chromatography showed neutral sugars in addition to acidic substances. The syrup (0-63 g.) was dissolved in water and neutralised with barium carbonate, and the resulting mixture was separated on filter sheets using solvent D, to give barium salts (A) (365 mg.), and fractions B (i) (100 mg.) and B (ii) (92 mg.), both contaminated with acidic substances. Chromatography showed fraction B (i) to contain mainly 2:3:4-tri-O-methylgalactose ($R_G$ 0-72), and fraction B (ii) to contain di-O-methylgalactose (ionophoresis and chromatography of the periodate oxidation products indicating the 2:3-isomer to be the main component). A sample (25 mg.) of barium salts (A) was treated with Amberlite resin IR-120(H) to give a mixture of acids (equiv. wt., 370, indicating mainly aldobiouronic acid) which on hydrolysis yielded acidic and neutral sugars.

Barium salts (A) (330 mg.) were converted into the corresponding acids (307 mg.) which were hydrolysed with n-hydrochloric acid at 100° for 7 hr. After neutralisation with silver carbonate, separation on filter sheets in solvent D afforded neutral sugars (fractions C (i)—C (iv)) and silver salts (D). The silver salts (D) were converted into acids (143 mg.) which were separated on filter sheets by solvent C into fractions D (i) (48 mg.) and (D) (ii) (36 mg.).

Fraction C (i) (9 mg.) contained 3:4:6-tri-O-methylmannose ($R_G$ 0-82). Fraction C (ii) (35 mg.) contained 2:3:4-tri-O-methyl-D-galactose ($R_G$ 0-72), identified as the aniline derivative, m. p. 159—162° and mixed m. p. 156—158°. Fraction C (iii) (26 mg.) contained 2:3:4-tri-O-methylgalactose ($R_G$ 0-72) and a trace of a second component ($R_G$ 0-60). Fraction C (iv) (28 mg.) contained di-O-methylgalactose ($R_G$ 0-49), shown by ionophoresis and chromatography of the periodate oxidation products to be mainly the 2:3-dimethyl ether. Fraction (i) was chromatographically pure 2:3:4-tri-O-methylglucuronic acid. Attempts to characterise the sugar by conversion into the crystalline amide of methyl 2:3:4-tri-O-methyl-α-D-glucuronoside failed. The syrupy product was treated with methanolic hydrogen chloride, and the resulting ester was reduced with lithium aluminium hydride and hydrolysed. Chromatography showed only 2:3:4-tri-O-methylglucose ($R_G$ 0-85). Fraction D (ii), which contained at least
three components, was converted into the methyl ester methyl glycosides, reduced with lithium aluminium hydride, and hydrolysed. Chromatography showed 2 : 3-di-O-methylglucose, small amounts of tri- and di-O-methylgalactose, and a trace of 2 : 4-tri-O-methylglucose.

Preparation and Hydrolysis of Methylated Gum.—The gum acid (25 g.) was methylated extensively with methyl sulphate and sodium hydroxide by the procedure of Brown, Hirst, and Jones. The product isolated as the methylated gum acid (18 g., OMe, 35-0%; ash, 5-8%) was further methylated with methyl iodide and silver oxide (three treatments) to give methylated gum (7.3 g.), \([\alpha]_D^{20} = -72^\circ (c \ 1.0\ \text{in CHCl}_3)\) (Found: OMe, 42-8%).

The methylated gum (7.3 g.) was refluxed with methanolic 2% hydrogen chloride (500 ml.) for 12 hr. (constant rotation). Methanol was removed under reduced pressure and the product was heated with 0-5% hydrochloric acid (600 ml.) on the boiling-water bath for 12 hr. (constant rotation). The cooled solution was neutralised with silver carbonate, then filtered, and hydrogen sulphide was passed through the filtrate to precipitate silver ions, and the filtrate was concentrated. Sugars were extracted from the residue with methanol, and the resulting syrup was dissolved in water, neutralised with barium carbonate, filtered, and concentrated to a syrup (7-3 g.).

The mixture of methylated sugars (7.3 g.) was repeatedly extracted with dry ether to give ether-soluble sugars (A) (4.76 g.) and an ether-insoluble residue (B) (2.10 g.). The ether-insoluble sugars (B) were separated into neutral and acid fractions by chromatography on cellulose (50 x 2.6 cm.) with butan-1-ol, 80% saturated with water, as eluant, four fractions being isolated. Fraction (C) (0.80 g.) contained a mixture of neutral sugars. Fraction (D) (0.224 g.) contained chromatographically pure 2-O-methyl-D-galactose, m. p. 167-168° (from acetone–water), \([\alpha]_D^{19} + 55^\circ (5 \text{ min.}) \rightarrow + 89^\circ (120 \text{ min., const.) (} c \ 1.91\ \text{in H}_2\text{O})\). Fraction (E) (0.14 g.) contained a mixture of 2-O-methylgalactose and acidic sugars. Fraction (F) (0.398 g.) contained acidic components and a trace of 2-O-methylgalactose.

Examination of Neutral Methylated Sugars.—The ether-soluble sugars (A) and fraction (C) were combined and separated on cellulose (76 x 3.5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water as eluants, to give eighteen fractions. A further fraction (19) (64 mg.) was obtained by elution of the cellulose with water.

Fraction 1. The syrup (119 mg.) had \([\alpha]_D^{19} + 15^\circ (c \ 0.39)\), and chromatography showed a main component having \(R_f\) 1-03 (cf. 2 : 3 : 4-tri-O-methyl-D-rhamnose) and traces of other sugars. Chromatography in solvent E showed a second component travelling faster. Hydrolysis of a sample with N-sulphuric acid, followed by neutralisation with barium carbonate and chromatography showed tri-O-methylrhamnose, 2 : 3 : 5-tri-O-methylarabinose, and the barium salt of an acid (at the starting line of the paper). Treatment of a second sample with cold barium hydroxide, followed by neutralisation with carbon dioxide and chromatography, gave a similar result. The remaining syrup (ca. 80 mg.) was treated with cold 5% barium hydroxide solution for 30 min., and the solution was neutralised with carbon dioxide, filtered and concentrated. The product was separated on a filter sheet with solvent D, to give fractions la and lb. Fraction la contained 2 : 3 : 4-tri-O-methylrhamnose \((R_f \ 1-03)\) and a trace of 2 : 3 : 5-tri-O-methylarabinose \((R_f \ 0.97)\). The main component was identified by conversion into 2 : 3 : 4-tri-O-methyl-N-phenyl-L-rhamnosylamine, m. p. and mixed m. p. 98—100°. Fraction lb (barium salt) was deionised with Amberlite resin IR-120(H), and chromatography in solvent C showed 2 : 3 : 4-tri-O-methylglucuronic acid. Conversion of the acid into the methyl ester methyl glycoside with dry methanolic hydrogen chloride, followed by reduction with lithium aluminium hydride in methanol, hydrolysis with N-hydrochloric acid, and chromatography in solvent D, showed only 2 : 3 : 4-tri-O-methylglucose. It is concluded that tri-O-methylglucuronic acid was present in fraction 1 as an ester.

Fraction 2. Chromatography of the syrup (150 mg.) showed 2 : 3 : 5-tri-O-methylarabinose and a trace of 2 : 3 : 4-tri-O-methylrhamnose. The optical rotation, \([\alpha]_D^{19} - 41^\circ (c \ 0.59)\), indicated almost pure 2 : 3 : 5-tri-O-methylrhamnose (cf. 2 : 3 : 5-tri-O-methyl-L-arabinose, \([\alpha]_D^{19} - 39.5^\circ)\).

Fraction 3. The syrup (1.835 g.), which had \([\alpha]_D^{19} - 42.5^\circ (c \ 0.68)\), was almost pure 2 : 3 : 5-tri-O-methyl-L-arabinose with a trace of 2 : 3 : 4 : 6-tetra-O-methylgalactose. Demethylation gave arabinose and a trace of galactose. The identity of the main component was confirmed by conversion into 2 : 3 : 5-tri-O-methyl-L-arabonamide, m. p. 132—133° and mixed m. p. (with sample of m. p. 129—130°) 129°.
Fraction 4. Chromatography of the syrup (34 mg.), which had $[\alpha]_D +66^\circ$ (c 0-48), showed a single component, $R_f$ 0-97. Re-examination in solvent E showed 2 : 3 : 4-tri-O-methylxyllose, 2 : 3 : 5-tri-O-methylarabinose, and an unidentified sugar. Demethylation gave xylose, arabinose, and galactose.

Fraction 5. Chromatography of the syrup (34 mg.) which had $[\alpha]_D +30^\circ$ (c 0-50) in solvent E, showed 2 : 3 : 4-6-tetra-O-methylgalactose and 2 : 3 : 5-tri- and di-O-methylarabofuranose. The presence of 2 : 3 : 4-6-tetra-O-methyl-D-galactose was shown by conversion into the aniline derivative, m. p. and mixed m. p. 179—180°.

Fraction 6. The syrup (17 mg.) had $[\alpha]_D -33^\circ$ (c 0-29) and chromatography showed a main component with $R_d$ 0-83, giving a brown stain (and yellow fluorescence in ultraviolet light) with aniline oxalate (cf. 3 : 5-di-O-methyl-L-arabinose, $R_d$ 0-83). Demethylation gave arabinose and traces of galactose and mannose. Paper ionophoresis showed that 3 : 4-6-tri-O-methylmannose ($R_d$ 0-82) and 2 : 5- ($R_d$ 0-85) and 3 : 5-di-O-methylarabinose ($R_d$ 0-83) could be readily distinguished in mixtures; fraction 6 showed mainly 3 : 5-di-O-methylarabinose with small amounts of the other two sugars.

Fraction 7. The syrup (230 mg.), which had $[\alpha]_D -24^\circ$ (c 0-42), was shown by chromatography and ionophoresis to contain approximately equal amounts of 2 : 5- and 3 : 5-di-O-methylarabinose. Attempts to separate the two components by gradient elution from charcoal containing borate buffer (pH 10) with butan-2-one were unsuccessful. The major portion (160 mg.) was fractionated by elution from Amberlite resin IRA-400 (borate form) with 0.5M-boric acid. Although much sugar was irreversibly absorbed on the resin, two ionophoretically pure fractions 7a (40 mg.) and 7b (10 mg.) were obtained. Fraction 7a was identified as 2 : 5-di-O-methyl-L-arabinose by conversion into 2 : 5-di-O-methyl-L-arabinonamide, m. p. 122° and mixed m. p. 123—124°. Fraction 7b was identified as 3 : 5-di-O-methyl-L-arabinose by conversion into 3 : 5-di-O-methyl-L-arabinolactone, m. p. 65° and mixed m. p. (with sample m. p. 69—71°) 67—69°.

Fraction 8. Chromatography and ionophoresis showed the syrup (80 mg.) to contain 2 : 5- and 3 : 5-di-O-methylarabinose, 3 : 4-6-tri-O-methylmannose, and a trace of 2 : 3 : 4-tri-O-methylgalactose. Attempts to separate the components by chromatography on charcoal containing borate buffer failed.

Fraction 9. The syrup (174 mg.), which had $[\alpha]_D +72^\circ$ $\rightarrow$ $+110^\circ$ (c 0-42), contained two components, $R_d$ 0-65 and 0-70 respectively, present in approximately equal quantities and indistinguishable from 2 : 3-di-O-methylarabinose and 2 : 3 : 4-tri-O-methylgalactose. Demethylation gave arabinose and galactose. The syrup was converted into the corresponding mixture of aldonoamides, from which 2 : 3-di-O-methyl-L-arabinonamide readily crystallised, m. p. 154° and mixed m. p. 153—154°.

Fraction 10. The chromatographically pure syrup (299 mg.) had $[\alpha]_D +109^\circ$ (c 0-51) and $R_d$ 0-70. Recrystallisation from acetone–ether afforded 2 : 3 : 4-tri-O-methyl-N-galactosylamine hydrate, m. p. 66—67°. The derived 2 : 3 : 4-tri-O-methyl-N-phenyl-L-galactosylamine had m. p. and mixed m. p. 161—162°.

Fraction 11. Chromatography of the syrup (59 mg.), which had $[\alpha]_D +88^\circ$ (c 0-33), showed 2 : 3 : 4-tri-O-methylgalactose ($R_d$ 0-70) and a trace of an unknown sugar ($R_d$ 0-52). The main component was identified by conversion into 2 : 3 : 4-tri-O-methyl-N-phenyl-D-galactosylamine, m. p. and mixed m. p. 164—165°.

Fraction 12. Chromatography of the syrup (155 mg.) showed a main component, $R_d$ 0-60, and a small amount of 2 : 3 : 4-tri-O-methylgalactose. Demethylation gave arabinose and a small amount of galactose. The optical rotation, $[\alpha]_D +118^\circ$ (c 0-38), indicated that the major component was a di-O-methyl-L-arabopyranose. Chromatographic examination in solvent F differentiated the sugar from 2 : 3- and 3 : 4-di-O-methylarabinose, and ionophoresis showed 3 : 4-di-O-methylarabinose to be absent. The syrup (60 mg.), when heated with ethanolic aniline, afforded an aniline derivative, m. p. 129—130° and mixed m. p. (with sample, 139—140°) 125—126°, whose X-ray powder photograph was identical with that of 2 : 4-di-O-methyl-N-phenyl-L-arabinosylamine.

Fraction 13. Chromatography of the syrup (280 mg.), which had $[\alpha]_D +55^\circ$ (c 0-29), showed a major component ($R_d$ 0-49), a small amount of tri-O-methylgalactose, and a trace of a sugar ($R_d$ 0-32) suspected of being 2-O-methylarabinose. Demethylation gave galactose and a trace of arabinose. Paper ionophoresis showed three components, a small component travelling at the same rate as 2 : 3-di-O-methylgalactose, an unidentified component in traces ([?]) 2-O-
methylarabinose], and the main component stationary (cf. 2 : 4-di-O-methylgalactose). Chromatography of the products of periodate oxidation showed 2 : 4-di-O-methylgalactose (Re 0.37, unchanged), and small amounts of oxidation products with Re 0.66 (grey), 0.78 (brown), and 0.15 (bright yellow). The first two oxidation products are formed from 2 : 3-di-O-methylgalactose, and the third from 2-O-methylaldoses (probably from 2-O-methylarabinose). The major component was identified as 2 : 4-di-O-methyl-D-galactose by conversion into the anilide derivative, m. p. and mixed m. p. 206—208°.

Fraction 14. The chromatographically pure sugar (393 mg.) crystallised from acetone—water and had m. p. and mixed m. p. (with 2 : 4-di-O-methyl-D-galactose monohydrate) 97—99° and \( [\alpha]_D +133° \rightarrow +89° \) (equil.) (c 0.54) (Found: OMe, 27.8. Calc. for C\(_6\)H\(_{14}\)O\(_4\): OMe, 27.4%). The derived 2 : 4-di-O-methyl-N-phenyl-D-galactosylamine had m. p. and mixed m. p. 213—214°.

Fraction 15. Chromatography of the syrup (100 mg.) showed 2 : 4-di-O-methylgalactose, a sugar with \( R_1 \) 0.36, and a trace of (\( \beta \)) 2-O-methylarabinose. Demethylation gave galactose, mannose, and a trace of arabinose. Ionophoresis showed di-O-methylgalactose (stationary), and a second sugar moving faster than 2- and 3-O-methylmannose. Separation of the syrup (100 mg.) on cellulose with solvent G gave fractions 15a and 15b. Fraction 15a contained 2 : 4-di-O-methylgalactose and (\( \beta \)) 2-O-methylarabinose (\( R_1 \) 0.32) and gave galactose and arabinose on demethylation. Chromatography of the products of periodate oxidation \( \delta \) showed unchanged di-O-methylgalactose and a component, Re 0.15 (bright yellow), formed from 2-O-methylaldoses. Fraction 15b was identified as 4-O-methyl-D-mannose by conversion into 4-O-methyl-D-mannosonolactone, m. p. and mixed m. p. 159—160°.

Fraction 16. Chromatography of the syrup (47 mg.) showed 4-O-methylmannosone (\( R_0 \) 0.36) and a second sugar in smaller amount (\( R_0 \) 0.32). The optical rotation, \( [\alpha]_D +43° \rightarrow +37° \) (c 0.33) (cf. 4-O-methyl-D-mannose, \( [\alpha]_D +32° \rightarrow +22° \)), and methoxyl content (Found: OMe, 17.3. Calc. for C\(_6\)H\(_{12}\)O\(_4\): OMe, 16.9). Calc. for C\(_6\)H\(_{14}\)O\(_4\): OMe, 18.9%). were consistent with those of a mixture of 4-O-methyl-D-mannose and a mono-O-methyl-L-arabinose. Furthermore, chromatography showed periodate oxidation products with Re 0.60 (brown) and 0.15 (yellow) identical with those from 4-O-methyl-D-mannose and 2-O-methylaldoses. Attempts to characterise the sugar by conversion into 4-O-methyl-D-glucosone failed, although an impure fraction, m. p. 135—136°, was shown by circular paper chromatography to contain the desired compound together with a second component.

Fraction 17. Chromatography showed the syrup (84 mg.) to contain 2 : 4-di-O-methylgalactose, 4-O-methylmannosone, and 2-O-methylgalactose.

Fraction 18. The chromatographically pure sugar (431 mg.; \( R_0 \) 0.25) crystallised from glacial acetic acid and had m. p. and mixed m. p. (with 2-O-methyl-D-galactose) 146—147° and \( [\alpha]_D +64° \rightarrow +91° \) (equil.) (c 0.37). After recrystallisation from acetone—water the sugar had m. p. 154°.

 Examination of Acidic Components. — Acidic fractions (F) and (19) (as barium salts), containing a trace of 2-O-methylgalactose, were combined and dissolved in water, barium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup (376 mg.). The mixture of acids was refluxed with methanolic 1-3% hydrogen chloride (50 ml.) for 6 hr. The product, after neutralisation with silver carbonate and concentration, was dissolved in formaldehyde dimethyl acetal (40 ml.), lithium aluminium hydride (0.2 g.) was added, and the solution was refluxed for 2 hr. Excess of hydride was destroyed by water, the acetal layer was separated, the aqueous layer was taken to dryness, and the residue was extracted with chloroform and acetone. The combined organic extracts were concentrated to a syrup (330 mg.) which was hydrolysed with N-hydrochloric acid (30 ml.) for 6 hr. at 100° to give, after neutralisation and concentration, a syrupy mixture of sugars (230 mg.). Separation of the methylated sugars on cellulose (50 × 2.5 cm.) with light petroleum (b. p. 100—120)—butan-1-ol (1 : 1), saturated with water, as eluant, gave nine fractions. Fraction a (21 mg., \( R_0 \) 0.95) was identified as 2 : 3 : 4-tri-O-methyl-D-glucose by conversion into the anilide derivative, m. p. and mixed m. p. 134—135°. Fraction b (19 mg.; \( R_0 \) 0.72 and 0.58) contained 2 : 3 : 4-tri-O-methylgalactose and 2 : 3-di-O-methylglucose (major component). Fraction c (47 mg.; \( R_0 \) 0.58) was identified as 2 : 3-di-O-methyl-D-glucose by chromatography of the sugar and its periodate oxidation products (Re 0.73 (bright yellow) and 0.63 (brown), ionophoresis, and by conversion into 2 : 3-di-O-methyl-D-gluconophenyldrazide, m. p. 173—174° and mixed m. p. (with sample, m. p. 168—169°) 169—171°.
Fraction \( d \) (11 mg.; \( R_f 0.58 \) and 0.54) contained 2:3-di-O-methylglucose and a trace of an unidentified sugar. Fraction \( e \) (9 mg.; \( R_f 0.49 \)) was identified as 2:4-di-O-methyl-d-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 212—214°. Fraction \( f \) (10 mg.; \( R_f 0.49 \) and 0.36) contained a mixture of 2:4-di-O-methylgalactose and 4-O-methylmannose. Fraction \( g \) (20 mg.; \( R_f 0.36 \)) was identified as 4-O-methyl-d-mannose by chromatography of the sugar and its periodate oxidation product [\( R_f 0.60 \) (brown)], ionophoresis, and by conversion into 4-O-methyl-d-mannoolactone, m. p. and mixed m. p. (with sample of m. p. 161—162°) 150—151°. Fraction \( h \) (13 mg.; \( R_f 0.32 \)) contained at least two components giving periodate oxidation products having \( R_f 0.60 \) (brown) and 0.15 (bright yellow). Fraction \( j \) (8 mg.; \( R_f 0.25 \)) was identified as 2-O-methyl-d-galactose by chromatography of the sugar and its periodate oxidation product \( R_f 0.23 \) (bright yellow), and as the crystalline sugar, m. p. and mixed m. p. 139—140°.

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Department of Chemistry, University of Edinburgh. [Received, August 26th, 1957.]

1 Hough, Jones, and Wadman, J., 1949, 2511.
3 For references see Aspinall and Schwarz, Ann. Reports, 1955, 52, 267.
4 Hirst and Jones, J., 1938, 1174; 1939, 1482; 1946, 506.
5 Jones, J., 1939, 558; 1947, 1055; 1949, 3141.
6 Hirst and Jones, J., 1947, 1064; 1948, 120; 1949, 1757.
9 Brown, Hirst, and Jones, 1949, 1761.
10 Baker and Haworth, J., 1925, 365.
12 Lock and Richards, J., 1955, 3025.

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