THE STRUCTURE OF GUM TRAGACANTH

By

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INTRODUCTION

BILLSTON

EXTRA STRONG
The polysaccharide constituents of land plants have been divided into four main groups; 1) Cellulose and Starch, 2) Hemicellulose, 3) Mucilages and 4) Plant Gums. Such a classification is largely biological and certain polysaccharides (e.g. Gum Tragacanth) cannot be conveniently placed in any one group. A further defect in such a division is that many polysaccharides which have similar structures have a different biological function within the plant.

Cellulose and starch, the former as a structural material and the latter as a food reserve, are the most abundant plant polysaccharides. Hemicelluloses have been defined as those plant cell wall polysaccharides which occur in close association with cellulose. Mucilages exist either as secondary membrane thickening material or as intracellular substances while the term "plant gums" is generally applied to plant exudates which swell and/or dissolve in water. Gums and mucilages have been reviewed together (1-8), while the hemicellulose group is generally considered separately (1,2,5,9-11) although in a recent review of hemicelluloses, Aspinall (12) has included some of the mucilage group on the basis of similarity in chemical structure.

A large number of plants, particularly fruit trees and the thorny shrubs which are found in hot dry climates, are capable of producing gum exudates. Over one hundred species of genus Acacia produce gums known commercially under/
under the name of Gum arabic (13). This source together with gums from many species of genus Astragulus, under the name of Gum tragacanth, accounts for a large part of the world market. The marketing of gums under collective names leads to confusion in structural work and precise conclusions cannot be drawn unless the botanical origin of a particular sample is known.

A number of theories have been advanced as to the origin of the gum exudates. Many authors subscribe to the view that the gums arise as products of natural metabolism while others suggest that they arise from pathological conditions. The gums are produced in greatest quantity by injured trees growing under unfavourable conditions. It is also noted that healthy Acacia trees produce no gum. Gum tragacanth, however, is produced by seemingly healthy trees immediately after injury. Infection of the plant by micro-organisms or fungi has been considered with the supposition that the plant produces gum to seal off the infected area. A further possibility is that enzymes liberated by the pathogen may synthesise polysaccharides although, if this were the case, one might expect different pathogens to synthesise different polysaccharides on the same tree. Certain fungi contain enzymes which hydrolyse gums to their component sugars (14).

The most likely function of the gum in the plant is to seal off wounded parts to limit or prevent infection and/
and/or to conserve moisture.

The source of structural material within the plant poses a further problem. A variety of sugar residues are found in the gums and it is suggested that either starch (15, 16), cellulose or hemicellulose (17) may be the source. Traces of pentose oligosaccharides found in certain gums are taken as an indication of their function as intermediates (18). There is however insufficient evidence to settle the problem.

The mucilages are polysaccharides which form colloidal solutions in water and may often be precipitated by the addition of ionic salts. Membrane mucilages occur throughout the plant. Intracellular mucilages include the seaweed polysaccharides; mucilage cells also occur in several bulbs (Scilla and Orchis). The mucilage sometimes acts as a food reserve and it has been reported that certain mucilaginous seed coatings disappear on germination of the seed. In plants living in dry climates the mucilage may also function as a water reservoir.

The origin of the mucilage in the plant is again uncertain. It has been proposed (17) that the mucilage arises in specialised cells capable of converting cell wall material through hemicellulose into mucilage. Again others (19) have considered that the contents of many cells in the growing regions are converted into mucilage and that the cell walls are not involved. The origin of the mucilage in
the seeds of *Linum usitatissimum* L has been examined in detail by Jaretsky et al. (15,16) and it was concluded that starch is converted to an intermediate without loss of granular structure and that this material was later converted to mucilage.

Hemicelluloses are found in the cell wall closely associated with cellulose but, unlike cellulose, soluble in alkali or water. The function of the hemicelluloses is uncertain and it is suggested that they act as a plasticiser rendering flexibility to the tissues. The relationship between cellulose, hemicellulose and lignin, a third non-carbohydrate cell wall constituent, is not clearly defined and it is still debatable whether there is a chemical linkage between any two or all three substances or whether physical forces predominate.

**The Structural Investigation of Plant Gums, Mucilages & Hemicelluloses.**

**Isolation and Purification.**

Polysaccharide gums are isolated from the tree as hard glassy masses, yellow or brown in colour, in the form of flakes and/or nodules and may be contaminated by various materials such as terpenoid resins, bark, dust and insects. Purification is usually effected by solution in water or dilute alkali (under oxygen free conditions) followed by filtration/
filtration and precipitation with organic solvents. The seed mucilages, which occur as hard vitreous layers covering the seed, may be removed by milling processes or by extraction of the seeds with water or alkali. Mucilages occurring in roots and tubers may generally be extracted from the dried ground root flour with hot water. Considerable difficulty is often experienced in the isolation of the hemicellulose polysaccharides because of their close association with cellulose and lignin. Alkaline extraction (20) has proved a useful method but the necessary removal of lignin together with the possibility of alkaline degradation, may bring about changes in the structure of the polysaccharide. In a few cases, hemicellulose (arabinogalactans) may be extracted with hot water directly from the wood meal. A promising extractant in this field is dimethyl sulphoxide (21) which has the advantage that it does not saponify acyl groups.

Purification of the polysaccharide is generally effected by repeated precipitation from aqueous solution with ethanol. Polysaccharides containing uronic acid may be obtained in the free acid form by using acidified ethanol (22) or glacial acetic acid (23) as precipitant. The precipitated polysaccharide may be dried either by solvent exchange or by freeze drying

**Fractionation.**

There are often indications that the polysaccharide is heterogeneous, particularly in the case of hemicelluloses, and/
and the preparation of homogeneous polymers for structural investigation is most important. The term heterogeneous is applied to those polysaccharides built up from different constituent sugars or which, although they contain the same sugars, are markedly different in structure. The methods of investigation have reached a degree of accuracy which can lead to erroneous conclusions even if the starting material contains small amounts of contaminating polysaccharide. The problem is further complicated in that great care must be taken to avoid degradation or modification of the complex molecules during fractionation. A number of techniques have been evolved but no general method has yet been found. The difficulty in separation lies in close similarities in structure or in association by hydrogen bonding.

**Fractional Precipitation.**

Certain polysaccharides, e.g. those from agar (24), the lichenins (25) and aqueous extracts of oat flour (26), have been purified by precipitation from aqueous solutions on cooling.

Fractional precipitation may be considered under three headings.

A. Precipitation by gradual addition of non-solvent (e.g. ethanol, glacial acetic acid) to aqueous solutions has been the principal method of purifying the polysaccharides from gums and mucilages. There is, however, a marked tendency for/
for co-precipitation and, unless there is a marked difference in solubility, only a rough separation is possible.

B. Addition of ionic salts (e.g. ammonium sulphate) to aqueous solutions of some polysaccharides gives a fractionation in a manner analogous to the fractionation of proteins (27). 

C. Complex formation between some polysaccharides and various copper compounds as Fehling's solution (28), cupric chloride (29), cupric sulphate (30), cupric acetate (31), and cupriethylene diamine (p8) have been of considerable use for fractionation in the field of mucilages and hemicelluloses. Complex formation with borate in alkali has been applied to fractional extraction of hemicelluloses (32). Acidic polysaccharides can be separated from neutral polysaccharides as the former complex with quaternary ammonium salts such as cetyl trimethyl ammonium bromide (Cetavlon) (33). Cetavlon has also been used to fractionate polysaccharide borate complexes of neutral hemicelluloses (34).

Dimethyl sulphoxide has been used as a solvent for preferential extraction (21).

The preparation of polysaccharide derivatives such as acetates, methyl ethers and esters has in some cases, led to fractionation (35,36) after the application of the above techniques.

The above methods of fractionation are all applicable on a preparative scale. There are however a large number of methods outlined below which may be applied as tests of homogeneity/
homogeneity and in a few cases to the separation of small amounts of polysaccharides.

**Electrophoresis and Ionophoresis.**

Electrophoresis in a Tiselius apparatus (37) has been applied to various natural and synthetic polysaccharide mixtures with varying degrees of success. Gum arabic a has been separated from *Acacia oyanophylla* gum (38) and from Plum gum (39). Gum arabic itself appeared to be homogeneous although immunological tests suggested heterogeneity. The best results on a preparative scale have been obtained using an inert supporting medium.

Ionophoresis using paper as a support and with borate buffers has been used (40). A disadvantage of this method is that detection of the polysaccharides is rendered difficult by the reaction of cellulose with spray reagents. This difficulty is conveniently overcome by using glass fibre sheets (41). Using 2N alkali as electrolyte Smith et al. (42) have shown that a number of previously purified polysaccharides appear to be heterogeneous. The use of thick glass fibre sheets or powdered glass columns might make it possible to carry out preparative separations. The method might also be extended to examine various copper and other complexes formed by polysaccharides.
Chromatographic Separation.

Various methods for the chromatographic separation of polysaccharides are being introduced. Displacement chromatography using charcoal columns has been used to purify yeast mannan (43). There are some indications that methylated gums may be separated on alumina (44) in a similar manner. Gardell (45) has applied gradient elution from a cellulose column to the separation of mucopolysaccharides. The mixture is precipitated at the top of the column and the various polysaccharides brought selectively into solution using decreasing concentrations of aqueous ethanol with barium acetate carrier. More recently it has been shown that cellulose ion exchangers can be used for the chromatography of polysaccharide mixtures. Deuel et al. (46) have used diethylaminoethyl-cellulose to separate acidic and neutral polysaccharides using varying conditions of pH and electrolyte concentration.

Immunological Fractionation.

The fact that specific anti-pneumococcus sera give specific precipitation reactions with polysaccharides which are similar in structure to the original pneumococcus polysaccharide is utilised in immunological fractionation. Using this technique gum arabic has given a precipitate with Type II anti-pneumococcus serum and contains only one third to one fifth the amount of L-rhamnose in the original polysaccharide (47).

It is becoming increasingly apparent that heterogeneity in/
in plant polysaccharides may be the rule rather than the exception. Gum tragacanth (35), the gums from Olibanum (48) and Khaya senegalensis (49) have all been fractionated directly to give more than one polysaccharide. There are indications from immunological and electrophoretic studies that gum arabic, gum ghatti and the gum from Acacia pycnantha are also heterogeneous. Fractionation studies in the glucomannans indicate the presence of groups of polysaccharides of closely similar structure but differing in chain length and molecular weight. Whether a definite structure can be assigned to any gum characteristic of the species is not certain. It has been shown that random nodules of gum from Fagora xanthonxvoideze (50) and Brachychiton diversifolium (51) show no significant variation, however, in the case of the gum from Combretum leonense (52), there were significant differences, in particular, a wide variation in uronic acid content.

It thus seems likely that the majority of plant gums, mucilages and hemicelluloses consist of groups of polysaccharides which conform to a general pattern but which may differ in chain length slightly and molecular weight.

Preliminary Investigations.

The purified homogeneous polysaccharide is characterised by the determination of optical rotation, ash content, methoxyl and acetyl content. The equivalent weight may be determined directly by titration with dilute alkali and uronic/
Uronic anhydride content is best estimated by decarboxylation and estimation of the liberated carbon dioxide (53).

**Hydrolysis Studies.**

**Complete hydrolysis:** Hydrolysis or complete breakdown of the polysaccharide to give the component sugars may be effected by heating with dilute mineral acid. Hydrolysis with N-hydrochloric acid or sulphuric acid at 100° for 5-6 hours generally liberates neutral monosaccharides together with acidic disaccharides (aldobiouronic acids). The glycosidic link of a uronic acid is relatively stable to hydrolysis and the breakdown of this link requires more drastic conditions which may result in decomposition of sugars. Because of this difficulty it is not possible to obtain an accurate quantitative measure of the various sugars present in a polysaccharide containing a high proportion of glycuronosyl linkages.

Before the advent of partition chromatography (54,55,56) the sugars liberated from hydrolysis of a polysaccharide were separated and identified by the use of specific precipitants. Using modern chromatographic techniques, which have been well reviewed (4,57), a preliminary indication of the component sugars may be rapidly ascertained by examining the hydrolysate in basic, neutral and acidic solvents and comparing the rates of movement with those of standard sugars. Larger amounts of sugars may be separated on thick paper sheets or cellulose columns/
columns (58). The final identification is made by preparing crystalline derivatives. Flood, Hirst and Jones (59) have used chromatography for the quantitative separation of sugars which may then be estimated with any suitable micro method such as that of Somogyi (60).

**Partial hydrolysis:** By varying the conditions of hydrolysis, polysaccharides may afford disaccharides and higher oligosaccharides of structural importance as well as monosaccharides. If the polysaccharide contains uronic acid residues autohydrolysis may occur on heating in aqueous solution. The glycosidic links of those sugars in the furanose form are particularly acid labile and e.g. L-arabinose, which is frequently found in this form, may be completely removed by autohydrolysis leaving a more resistant degraded polysaccharide. The use of weak mineral acids (0.01-0.1N) in aqueous solution will also remove furanose sugars and may give reasonable yields of di- and higher oligosaccharides. Aldobiouronic acids may be isolated after hydrolysis under more vigorous conditions with 0.5-2.0N acid.

The isolation of a degraded polysaccharide is often possible after treatment with acid. Such a degraded polymer is useful in structural studies as the degraded molecules are less complex and so more amenable to constitutional studies.

Partial acetolysis of the polysaccharide followed by de-acetylation of the oligosaccharide acetates produced is/
is a procedure which has given high yields of oligosaccharides (61). The polysaccharide is treated with acetic anhydride, sulphuric acid and sometimes acetic acid in the cold for a few days to give oligosaccharides as the acetates. These are readily de-acetylated with e.g. barium methoxide in methanol and may then be separated and characterised. The method has found particular use so far with glucomannan mucilages (62,63) and hemicelluloses (64).

Enzymes have been isolated which break specific glycosidic bonds. This technique has been used extensively in studies on starch and glycogen (65,66). Structurally significant oligosaccharides have also been obtained in the field of seaweed mucilages (67). Enzymes have also been applied to glucosamannans (68) and glucomannans (69) with excellent results. It is apparent that as an increasing number of selective enzymes become available this will become a more important degradative technique.

It is known that oligosaccharides may be synthesised from monosaccharides in dilute acid solution (70) and also by the action of enzymes on monosaccharides under certain conditions. Therefore oligosaccharides which are obtained in small yield from any of the above methods may be of doubtful structural significance. Synthetic or reversion products will reach an equilibrium value while authentic hydrolysis products will break down on prolonged hydrolysis (71).
Acidic oligosaccharides may be separated by precipitation of the barium salt with ethanol, by adsorption on anion exchange resin (72) followed by elution with formic acid, or by chromatography on cellulose columns using acid eluants. The elution of neutral oligosaccharides from charcoal:celite columns with aqueous ethanol (42) generally gives a rough fractionation into di-, tri- and tetra-saccharides etc., and complete separations are usually afforded by chromatography on cellulose (73). Ionophoresis in a number of electrolytes may also be used as a criterion of homogeneity.

Hydrolysis of neutral oligosaccharides enables the identity of the component sugars to be found. A prior reduction of the oligosaccharide with potassium borohydride in aqueous solution to give the corresponding polyalcohol followed by hydrolysis yields the sugar at the reducing end as its corresponding alcohol. Methylation of the oligosaccharide (cf p.15) and subsequent hydrolysis indicates the mode of linkage of the component sugars. Further information and confirmation of the structure may be obtained using the technique of periodate oxidation (cf p.20).

Hydrolysis of aldobiouronic acids is not satisfactory and these are generally converted to the methyl ester methyl glycoside by treatment with methanolic hydrochloric acid and then reduced with lithium aluminium hydride (74) or sodium borohydride (75). The neutral disaccharide so obtained may then be examined as outlined above.
Methylation.

The replacement of all free hydroxyl groups in the polysaccharide with methoxyl groups followed by hydrolysis and identification of the partially methylated polysaccharides gives important information on the linkages between the component sugars.

One of the earliest and still one of the most important methods of methylation is that proposed by Haworth (76) in which the polysaccharide is treated with dimethyl sulphate and 30-40% aqueous sodium hydroxide. The reaction is usually repeated until there is no further increase in the methoxyl content of the polymer. An important modification of this method is to treat the polysaccharide acetate in an organic solvent such as tetrahydrofuran with dimethyl sulphate and solid sodium hydroxide (77, 78). Fear and Menzies (79) introduced thallous hydroxide and methyl iodide as reagents for the methylation of low molecular weight compounds and this was adapted by Hirst and Jones (80) to the methylation of polysaccharides in cases where the Haworth method was not successful.

The Haworth method generally gives only a partially methylated product and other methods must be applied to obtain a fully methylated polysaccharide. The most widely used of these is that of Purdie and Irvine (81) where the partially methylated material is treated with silver oxide and boiling methyl iodide. This reaction must usually be/
be applied many times and Kuhn et al. (82) have improved the reaction by using N,N,N-dimethylformamide as a solvent. Silver oxide which may cause oxidative degradation has also been replaced by barium oxide (83).

Freudenburg et al. (84) introduced sodium in liquid ammonia and methyl iodide for the methylation of starch and cellulose. Peat (85) has used this method for complete methylation of other polysaccharides and Isbell et al. (86) have adapted it for use on a micro scale.

The fully methylated polysaccharide may be purified by fractional precipitation or extraction with chloroform:petrol mixtures. The methylated polysaccharide is insoluble in hot aqueous mineral acid solutions and so cannot be hydrolysed directly. Partial hydrolysis in the cold followed by heating at 100°C to complete hydrolysis with aqueous mineral acid is usually effective. Alternative methods are methanolysis followed by acid hydrolysis of the methyl glycosides and formolysis followed by hydrolysis of the formyl esters. Care must be taken to ensure that demethylation is kept to a minimum.

The separation of the mixture of methylated monosaccharides obtained after hydrolysis is achieved by column chromatography. Partition chromatography on cellulose columns (75) has been used extensively but the use of adsorption chromatography on charcoal:celite (87) and celite (88) columns is reported. Where there is incomplete separation/
separation on the column, electrophoresis in borate buffer (89) and the use of a variety of chromatographic solvents on paper (57) is usually successful. The pure methylated monosaccharides so obtained are generally characterised by the preparation of crystalline derivatives. Recently gas liquid partition chromatography has been introduced for the separation and identification of methyl glycosides of the methylated sugars (90).

The position of the free hydroxyl groups on the partially methylated monosaccharides indicates the point of linkage to other sugar residues. In some cases ambiguity arises e.g. 2,3 di-O-methyl-L-arabinose may be derived either from arabinofuranose linked through carbon 5 or arabinopyranose linked through carbon 4. The methylation technique gives no information as to the order of linkage of the sugar residues. It is desirable if possible to methylate a degraded polysaccharide which, being less complex, gives results more easily interpreted and which may give useful information when compared with the methylated whole polysaccharide.

**Periodate Oxidation.**

The oxidation and cleavage of adjacent hydroxyl groups by the periodate ion is an extremely important tool in the elucidation of polysaccharide structure. The technique has been well reviewed (7,91,92). The cleavage may be illustrated as follows:-
The consumption of periodate, formic acid and formaldehyde release, and the unattached sugar residues may all be estimated (93) to give an indication of the mode of linkage and extent of branching.

Important variations on the method include the stepwise degradation of the polysaccharide as applied by Barry (94). Treatment of the periodate oxidised polysaccharide with phenylhydrazine and glacial acetic acid at 100° removes all fragments of the oxidised monosaccharides in the form of osazones and leaves a simpler degraded polysaccharide whose structure may be more readily determined. (See Fig. I).
Fig. I

Barry Degradation

Periodate oxidised Polysaccharide (hypothetical)

GLACIAL ACETIC ACID

PHENYLHYDRAZONE

Fig II

Smith Degradation

Above oxidised polysaccharide
In some cases this degraded polymer may again be attacked by periodate and the procedure repeated. A polysaccharide has been obtained from gum arabic after three such degradations which was resistant to further oxidation and which contained only galactose, indicating a (1-3)-linked galactan chain (95).

Smith et al. (96) have developed a similar method which avoids the use of phenylhydrazine. In its original form the periodate oxidised polysaccharide was reduced catalytically with Haney nickel and the fragments identified after hydrolysis. More recently (97,98) the polyaldehyde formed after oxidation, which decomposes on acid hydrolysis, has been reduced with borohydride to the corresponding polyalcohol. Treatment with dilute mineral acid in the cold hydrolysés the acetal linkages of those monosaccharide residues which were cleaved by the periodate. (See Fig. II). Estimation of the amounts of glycerol and erythritol provides further information on the type of residue oxidised.

Reduction and Oxidation.

Many polysaccharides contain uronic acid residues which often confer resistance to hydrolysis, methylation and other reactions. It is therefore useful to reduce these residues to the corresponding primary alcohols which are generally more readily handled. Suitable reducing agents require/
require that the acid group be either esterified and/or the polysaccharide soluble in an organic solvent. Ethylene oxide (99) has been used to prepare the glycol ester in aqueous solution which may then be reduced by borohydride. Complete esterification is difficult and a number of reactions must be carried out. Alternatively if the polysaccharide has been modified (methylated, acetylated) so that it is soluble in dry organic solvents lithium aluminium hydride may be used. Diborane has recently been used with some success on the polysaccharide acetate (100). Reduction of methylated derivatives of aldobiouronic acids and uronic acids obtained during methylation studies and of the methylated polysaccharide before hydrolysis also makes the identification of residues simpler.

The oxidation of neutral sugars to the corresponding uronic acids has been described by Aspinall (101). Using platinum catalyst with oxygen the primary alcoholic groups in the polysaccharide were converted to carboxyl groups and after hydrolysis it was possible to isolate aldobiouronic acids. L-Arabinofuranose residues, normally readily hydrolysed, in larch arabinogalactan were oxidised to uronic acid, and, on hydrolysis an arabinofuranuronosylgalactose was isolated which showed that L-arabinose residues were linked through carbon 6 of the galactose units to the galactose frame.
The Structure of Gum Arabic. (Acacia senegal).

It is useful at this stage to consider how the above methods of structural investigation have been applied to determine the structure of this polysaccharide: so far the most fully investigated gum.

Analysis of the gum shows it to contain $\text{L}$-arabinose 30%, $\text{L}$-rhamnose 11%, $\text{D}$-galactose 37%, and $\text{D}$-glucuronic acid 14%. Autohydrolysis of the gum produces a degraded polysaccharide and liberates $\text{L}$-arabinose, $\text{L}$-rhamnose and 3-0- $\text{D}$-galactopyranosyl-$\text{L}$-arabinose (102). The structure of the disaccharide was proved by methylation and hydrolysis which gave 2,3,4,6-tetra-0-methyl-$\text{D}$-galactose and 2,4-di-0-methyl and 2,5-di-0-methyl-$\text{L}$-arabinose. Although the methylation results do not distinguish between furanose or pyranose arabinose in the disaccharide, the ease of hydrolysis and the fact that only 2,6-di-0-methyl-$\text{L}$-arabinose is found in the hydrolysis products of the methylated gum indicates that it is present in the furanose form.

Hydrolysis of the gum or degraded gum with acid has led to the isolation of 3-0- $\text{D}$-galactopyranosyl-$\text{D}$-galactose (103) and an aldobiouronic acid, 6-0- ($\text{D}$-glucopyranosyluronic acid)-$\text{D}$-galactose (104,105). The structures of both disaccharides were proved by methylation.

Both the whole and degraded gum have been methylated, hydrolysed and the cleavage fragments identified.
Degraded Gum. (103).

2,3,4-tri-O-methyl-D-glucuronic acid (3 moles)
2,3,4,6-tetra-O-methyl-D-galactose (1 mole)
2,3,4-tri-O-methyl-D-galactose (5 moles)
2,4-di-O-methyl-D-galactose (3 moles)

Whole Gum. (103).

2,3,4,6-tetra-O-methyl-D-galactose
2,3,4-tri-O-methyl-L-rhamnose
2,3,5-tri-O-methyl-L-arabinose
2,5-di-O-methyl-L-arabinose
2,4-di-O-methyl-D-galactose
2,5-di-O-methyl-D-glucuronic acid
2,3,4-tri-O-methyl-D-glucuronic acid.

The methylation study on the degraded gum shows that all units are pyranose and are linked either by (1-3) or (1-6) linkages. The number of structures which can be postulated from these methylation results is limited by the isolation of the methylated aldobiuronic acid 6-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-2,3,4-tri-O-methyl-D-galactose (103). This indicates side chains containing D-glucuronic acid linked through at least one galactose residue to the main galactose chain. The methylation does not distinguish between (1-6) and/or (1-3) linkages in the galactan chain.

At/
At this stage possible structures for the main repeating unit in degraded arabic acid include:-

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The application of the Smith degradation technique (94) to the degraded gum yields a degraded galactan. Methylation and hydrolysis of this degraded galactan indicates the presence of only (1--3) galactose linkages. Such a galactan can only arise from structure IV above. The Barry degradation (94) has been applied to the whole gum and after three degradations a degraded polymer similar to that obtained from the Smith degradation of the degraded gum was isolated.
Comparing the methylated degraded gum with the methylated whole gum it is noted that only 2,4-di-O-methyl-D-galactose is isolated from the whole gum whereas 2,3,4-tri-O-methyl-D-galactose was also present in the degraded gum. 2,3,4-tri-O-methyl-D-glucuronic acid is also largely replaced by 2,3-di-O-methyl-D-glucuronic acid in the whole gum indicating the presence of labile sugars linked to position C4. Non-terminal arabinose residues are indicated by the isolation of 2,5-di-O-methyl-L-arabinose and this is confirmed by the isolation of 3-O-D-galactopyranosyl-L-arabinose. A possible structure from this evidence has been postulated below:

\[
\begin{align*}
& \text{GA} \longrightarrow R \\
& \quad \vline \mathbf{6} \\
& \quad \mathbf{Gal} \rightarrow R \\
& \quad \vline \mathbf{1} \\
& \quad \vline \mathbf{6} \\
& \quad -3 \mathbf{Gal} \longrightarrow 3 \mathbf{Gal} \longrightarrow 3 \mathbf{Gal} \longrightarrow 3 \mathbf{Gal} \\
& \quad \vline \mathbf{6} \\
& \quad \vline \mathbf{1} \\
& \quad \mathbf{R} \longrightarrow 3 \mathbf{Gal} \\
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& \quad \mathbf{R} \longrightarrow 3 \mathbf{Gal} \\
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& \quad \vline \mathbf{1} \\
& \quad \mathbf{R} \longrightarrow 3 \mathbf{Gal} \\
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& \quad \vline \mathbf{1} \\
& \quad \mathbf{GA} \\
\end{align*}
\]

R = (a) Gal 1--

R = (b) Gal 1--3 Araf 1--3 Araf 1--

R = (c) Rha 1--

R = (d) Araf 1--
The Molecular Structure of Plant Gums.

A large number of plant gums have received preliminary investigation and are characterised in that all contain uronic acid. Relatively few have been fully investigated but the results so far obtained indicate that the gums fall into two main groups: I. Plant gums with a galactan framework, II. Plant gums containing a high proportion of uronic acid. A number of gums do not, however, fall conveniently into either of the above categories and will be considered in a third section.

Gums of group I include those from the genera Acacia and Prunus and in nearly all cases the uronic acid is D-glucuronic acid and/or its 4-O-methyl derivative. Group II is smaller and the acidity of the gums is due to D-galacturonic acid and/or D-glucuronic acid and its 4-O-methyl derivative. Many are partially acetylated and resemble closely the pectins in physical properties (See p.50)

Group I: Plant gums with a galactan framework. (Table I, p.32)

Investigation of a number of plant gums by methylation and partial hydrolysis techniques has indicated that they have a central framework of D-galactose residues to which is attached a more or less complex periphery. The uronic acid content of these polysaccharides is generally less than 20% but is such that autohydrolysis takes place on heating an/
an aqueous solution of the gum. During the autohydrolysis the periphery of the molecule is removed leaving a degraded polysaccharide of simpler structure. A frequent method of investigation involves the comparison of the methylated whole and degraded gums. Table I shows the available structural evidence for a number of these gums.

The structure of Acacia *senegal* gum has already been discussed in detail (see p. 22). Other gums of this genus are similar in many respects to Acacia *senegal* gum. They all contain the same component sugars although in different proportions; the aldobiouronic acid \(6-O-\beta-D\text{-glucopyranosyluronic acid\)}-\(D\text{-galactose is common to all. Detailed methylation and partial hydrolysis studies have been made on Acacia *pycnantha* gum (106,107,108) and indicate a galactan framework of \((1\rightarrow3)\) linked galactose residues with side chains of galactose from position 6 as in Acacia *senegal*. Acacia *pycnantha* gum is slightly less complex than Acacia *senegal* gum in that all the glucuronic acid residues in the former are terminal and arabinopyranose units are absent. The unit Araf \(1\rightarrow3\) Ara 1- is found in the periphery of Acacia *pycnantha* gum. \(4-O-(\alpha-D\text{-glucopyranosyluronic acid\)}-\(D\text{-galactose has been isolated only from Acacia *karrooo* gum (109) and it is possible that this gum is heterogeneous.}

Greater diversity is observed in gums from the genus *Prunus*. A comparison of methylation studies on whole and degraded/
degraded polysaccharides from Prunus damson (90), Prunus cherry (73,110), and Prunus egg plum (114) gums indicates a framework of (1--3) and (1--6) linked D-galactose units similar to that of Acacia gums. Variation in the glucuronic acid linkage is observed and in Prunus damson, and Prunus cherry gums both terminal and (1--4) linked uronic acid is found while only non-terminal (1--4) linked uronic acid is present in Prunus egg plum gum. Mannose units occur in Prunus damson and Prunus cherry gums and in both cases the aldobiouronic acid isolated is 2-O-(\(\beta\)-D-glucopyranosyluronic acid)-D-mannose. Methylated mannose units have not been identified in the methylated gums. Where mannose is absent the aldobiouronic acid 6-O-(\(\beta\)-D-glucopyranosyluronic acid)-D-galactose is found. An examination of the periphery of Prunus polysaccharides indicates that terminal units of arabinose are common. The unit Arap 1--3 Ara 1-- is has been isolated from Prunus cherry gum. Terminal xylose units have been found in the methylation study of Prunus egg plum gum.

Mesquite gum from Prosopis juliflora (117-120), which belongs to the same family as Acacia, has been shown, by methylation studies, to resemble the Acacia and Prunus gums in that (1--3) and (1--6) linked galactose residues are present; arabinofuranose units occur as end groups and in non-terminal positions linked (1--3).

A recent/
A recent examination of gum Asafoetida (121) by methylation and partial hydrolysis techniques has shown that it also has a framework of galactose linked (1→3) and (1→6) and that terminal units are arabinofuranose, galactose and 4-0-methyl-D-glucuronic acid. Two aldobiouronic acids have been isolated and characterised as 6-0-(β-D-glucopyranosyluronic acid)-D-galactose, and the corresponding monomethyl derivative 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Variations in the linkage of acid residues are found in Citrus lemon (122,123,124) and Citrus grapefruit gums (124) although otherwise they follow the general pattern outlined above. 4-0-(4-0-methyl-α-D-glucopyranosyluronic acid)-L-arabinose and 4-0-(4-0-methyl-α-D-glucopyranosyluronic acid)-D-galactose have both been characterised from Citrus lemon gum. The closely related Commiphora myrrha (125,126) and Boswellia carterii (127) gums contain the aldobiouronic acid 6-0-(4-0-methyl-β-D-glucopyranosyluronic acid)-D-galactose and in the former 4-0-(4-0-methyl-D-glucopyranosyluronic acid)-D-galactose is also present. Although no methylation study of Spondias cythera (72,128) (Golden Apple) gum has yet been made, partial hydrolysis results indicate a general similarity to the above structures.

Three members of the family Combretaceae have been investigated. Partial hydrolysis studies of Anogeissus latifolia (129,130), Anogeissus schimperi (131,132) and Combretum/
Combretum leonense (52,133) gums suggest that there is a preponderence of (1→6) linked galactose units in the main chain with occasional arabinose residues linked (1→3). The unit -Gal 1→3 Gal 1- has also been isolated from Anogeissus latifolia and Anogeissus schimperi gums but is present to a lesser extent than in Acacia and Prunus gums. In other respects the structure follows the general pattern of the Acacia and Prunus polysaccharides with side chains of aldobiouronic acid and acid labile pentose end groups. Evidence that there is more than one contiguous arabinose unit in the side chains arises from the methylation of Anogeissus latifolia gum and from the isolation of arabinobiose from Anogeissus schimperi and Combretum leonense gums. The presence of the aldobiouronic acids 2-0-(β-D-glucopyranosyluronic acid)-D-mannose and 6-0-(β-D-glucopyranosyluronic acid)-D-galactose is indicated in both Anogeissus latifolia and Anogeissus schimperi gums while hydrolysis of Combretum leonense yields 6-0-(D-glucopyranosyluronic acid)-D-galactose and 2-0-(α-D-galactopyranosyluronic acid)-L-rhamnose. This latter aldobiouronic acid is frequently found in gums of group II.

The only uronic acid isolated from the exudate of Opuntia fulgida (114,109) (Cholla gum) is D-galacturonic acid. The gum undergoes autohydrolysis to liberate arabinose, xylose and 4/5-0-β-D-xylopyranosyl-L-arabinose and leaves a degraded/
a degraded gum of galactose and galacturonic acid residues. No aldobiouronic acid has been reported. Methylation studies indicate that the galactose is linked (1--3) and (1--6) as in Acacia and Prunus gums and that the galacturonic acid occurs as straight chain unit linked through O3 and O4. Xylose and arabinose both occur as terminal units and the latter also linked (1--5). That the periphery of cholla gum is more complex than that of other gums in this group is shown by the presence of free L-arabinose in the methylation products indicating a branching unit as

\[
\begin{array}{c}
5 \\
--3 \text{ Araf} \\
2
\end{array}
\]
# TABLE I. MAIN STRUCTURAL FEATURES OF SOME PLANT GUMS WITH A GALACTAN FRAMEWORK.

<table>
<thead>
<tr>
<th>Source</th>
<th>Acid labile groups and periphery.</th>
<th>Framework.</th>
<th>Acidic Oligosaccharides</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia senegal</em></td>
<td>Arap 1(^\beta)-3 Ara 1- Gal 1(^\alpha)-3 Ara 1- Araf 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>GA 1(^\beta)-6 Gal</td>
<td>47,71,95, 96,102-105.</td>
</tr>
<tr>
<td><em>Acacia pycnantha</em></td>
<td>Araf 1(^\beta)-3 Ara 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>GA 1(^\beta)-6 Gal</td>
<td>106,107, 108.</td>
</tr>
<tr>
<td><em>Prunus damson</em></td>
<td>Araf 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>GA 1(^\beta)-2 Man</td>
<td>22, 80.</td>
</tr>
<tr>
<td><em>Prunus cherry</em></td>
<td>Araf 1(^\beta)-3 Ara 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>GA 1(^\beta)-2 Man</td>
<td>73,109-113.</td>
</tr>
<tr>
<td><em>Prunus egg plum</em></td>
<td>Araf 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>GA 1(^\beta)-6 Gal</td>
<td>114-116.</td>
</tr>
<tr>
<td><em>Prospelia juliflora</em></td>
<td>Araf 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>4(\text{Me} )GA 1(^\beta)-6 Gal</td>
<td>117-120.</td>
</tr>
<tr>
<td><em>Citrus lemon</em></td>
<td>Araf 1(^\beta)-3 Ara 1- 4(\text{Me} )GA 1(^\alpha)-4 Ara 1-</td>
<td>-</td>
<td>4(\text{Me} )GA 1(^\alpha)-4 Gal</td>
<td>122-124.</td>
</tr>
<tr>
<td><em>Asafoetida</em></td>
<td>Araf 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>4(\text{Me} )GA 1(^\beta)-6 Gal</td>
<td>121.</td>
</tr>
<tr>
<td><em>Spondias cythera</em></td>
<td>Arap 1(^\beta)-3 Ara 1- Xyl 1(^\alpha)-3 Ara 1- 4(\text{Me} )GA 1(^\alpha)-3 Ara 1-</td>
<td>-Gal 1(^\beta)-3 Gal 1- (M)</td>
<td>4(\text{Me} )GA 1(^\beta)-6 Gal</td>
<td>72,128.</td>
</tr>
</tbody>
</table>
TABLE I continued.

<table>
<thead>
<tr>
<th>Source</th>
<th>Acid labile groups and periphery.</th>
<th>Framework.</th>
<th>Acidic Oligosaccharides.</th>
<th>References.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-Gal 1-6 Gal 1-</td>
<td>GA 1-6 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Gal 1-6 Gal 1-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anogeissus schimperi.</td>
<td>Arap 1-3 Ara 1-</td>
<td>-Gal 1-3 Gal 1-</td>
<td>GA 1-2 Man</td>
<td>131,132.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Gal 1-3 Ara 1-</td>
<td>GA 1-6 Gal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Gal 1-6 Gal 1-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combretum leonense.</td>
<td>Arap 1-3 Ara 1-</td>
<td>-Gal 1-7 Gal 1-</td>
<td>GA 1-6 Gal</td>
<td>52,133.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Gal 1-7 Ara 1-</td>
<td>GalA 1-2 Rha</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[Gal1-GalA-2Rha]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xyl 1-4/5 Ara 1-</td>
<td>-Gal 1-6 Gal 1-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY: - GA = D-glucuronic acid; 4MeGA = 4-0-methyl-D-glucuronic acid; GalA = D-galacturonic acid; Gal = D-galactose; Man = D-mannose; Rha = L-rhamnose; Fuc = L-fucose; Ara = L-arabinose; Xyl = D-xylose.
<table>
<thead>
<tr>
<th>Source</th>
<th>Branch point.</th>
<th>Terminal units.</th>
<th>Chain units.</th>
<th>Aldobiouronic acid.</th>
<th>References.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterculia gal1—2Gla1—3Gal1—4 Rha</td>
<td>Rha(1,2,5,4)</td>
<td>Gal1—2 Rha</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>135,136</td>
</tr>
<tr>
<td>Cochleocerasum</td>
<td>GalA1—2 Gal1—3Gal1—4 Rha</td>
<td>Rha(1,2,4)</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>134,132</td>
</tr>
<tr>
<td>Erythrina</td>
<td>Gal1—2 Gal1—3Gal1—4 Rha</td>
<td>Rha(1,2,4)</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>134,132</td>
</tr>
<tr>
<td>Khaya</td>
<td>Gal1—2 Gal1—3Gal1—4 Rha</td>
<td>Rha(1,2,4)</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>134,132</td>
</tr>
<tr>
<td>X. senegalensis</td>
<td>Gal1—2 Gal1—3Gal1—4 Rha</td>
<td>Rha(1,2,4)</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>134,132</td>
</tr>
<tr>
<td>Brechotia</td>
<td>Gal1—2 Gal1—3Gal1—4 Rha</td>
<td>Rha(1,2,4)</td>
<td>1—2 Rha</td>
<td>GalA1—2 Gal1—4 Gal1—4 Gal1—2 Rha</td>
<td>134,132</td>
</tr>
</tbody>
</table>
Group II: Plant gums containing a high proportion of uronic acid.

This group of gums resembles the mucilages and pectins rather than the plant gums previously discussed. The group is characterised by having a high uronic acid content, usually of the order 35-50%. Galactose residues are (1--4) linked rather than (1--3) and (1--6) linked as in gums of group I. A further difference is that gums of group II have a lower proportion of acid labile groups so that autohydrolysis to give a degraded polysaccharide is generally not possible. Hydrolysis is rendered difficult because of the high proportion of uronic acid and the isolation of oligosaccharides, other than aldobiouronic acids, by mild hydrolysis has not so far been found possible. Structural investigations are therefore limited to methylation studies and the isolation of aldobiouronic acids after hydrolysis. The structural features of a number of these gums is shown in Table II.

Two gums from the genus Khaya have been examined and are typical of the high acid gums. Khaya senegalensis (49,134) gum has been fractionated to give a major fraction A and minor fraction B, the former being a high acid polysaccharide. Khaya senegalensis A and Khaya grandifolia (132,134) gum have been examined by methylation and partial hydrolysis techniques. Both gums contain D-galacturonic acid, 4-O-methyl-D-glucuronic acid, D-galactose, L-rhamnose and in the latter a trace of L-arabinose. On hydrolysis each/
each gum has yielded \(2-O-(\beta\text{-galactopyranosyluronic acid})-\beta\text{-rhamnose}\) and \(4-O-(4-O\text{-methyl-}\beta\text{-glucopyranosyluronic acid})-\beta\text{-galactose}\). From methylation studies it is apparent that the terminal groups of both polysaccharides are galactose and \(4-O\text{-methyl-}\beta\text{-glucuronic acid}\). Galactose and galacturonic acid are present as straight chain units linked through positions \(\text{C}1\) and \(\text{C}4\) while rhamnose is found only as branch point linked \(\text{C}1\), \(\text{C}2\) and \(\text{C}4\). A number of other methylation hydrolysis products have been identified in small amount in *Khaya senegalensis* methylated gum but it is possible that some of these arise from the contaminating fraction B.

Consideration of both hydrolysis and methylation results, therefore, indicates that both polysaccharides are very similar in structure. One possible simple structure consistent with the experimental results is postulated below.

\[
\begin{align*}
-4\text{GalA} & \longrightarrow 2\text{Rha} \longrightarrow 4\text{Gal} \longrightarrow 4\text{GalA} \longrightarrow 2\text{Rha} \longrightarrow 4\text{GalA} \longrightarrow 4\text{GalA} \longrightarrow 4 \\
| & \quad | \\
1 & \quad 1 \\
\text{Gal} & \quad \text{Gal 4--1 GA4Me}
\end{align*}
\]

The uronic acid in the exudates from *Sterculia setigera* (135,136) and *Gossypium gossypium* (30) is \(\beta\text{-galacturonic acid}\) and both gums, on hydrolysis, have yielded the aldobiouronic acid \(2-O-(\beta\text{-galactopyranosyluronic acid})-\beta\text{-rhamnose}\), found in *Khaya* gums, while the presence of \(4-O-(\beta\text{-galactopyranosyluronic acid})-\beta/\)
acid)-D-galactose is also indicated. Methylation studies also show that there are strong similarities between the exudates from Sterculia setigera and Cochlospermum gossypium. In both gums galactose is present as terminal and (1--4) linked straight chain units, while rhamnose occurs as (1--2) linked chain unit. Rhamnose is also found as a terminal residue in Cochlospermum gossypium. Although D-tagatose has been identified as a hydrolysis product in Sterculia setigera gum it has not been located in the methylation products, but, in view of the fact that the proportion of end group is lower than that of branch point unit it may be that D-tagatose is present as a terminal residue. Galacturonic acid and rhamnose both occur as branch point units. It is evident, then, that although there are structural similarities between Sterculia setigera, Cochlospermum gossypium and the Khaya gums, the former pair are more complex and further investigation is required before any structure can be postulated.

Prachyochiton diversifolium (formerly Sterculia caudata) gum (49) contains 50% uronic acid identified as D-glucuronic acid. A resemblance to the other gums of this group is found in the results of a methylation study which shows that rhamnose occurs as branch point linked C1, C2 and O4 and as chain unit linked (1--2), while galactose is found as terminal residue and as chain unit linked (1--4). Partial hydrolysis/
hydrolysis suggests that the D-glucuronic acid, which occurs mainly as terminal unit, is linked to rhamnose at C2.
It may be noted that although no galacturonic acid is found in the gum hydrolysate, an increase in the proportion of galactose occurs after reduction.

The major fraction of gum tragacanth has a high uronic acid content and so may be included in this group. Gum tragacanth will be more fully discussed later (See p. 51).

Group III: Other plant gums.

A few of the plant exudates so far examined do not fall conveniently into either of the above two groups. The exudate from Achiiras sapota (137, 138) contains L-arabinose, D-xylose and D-glucuronic acid while D-galactose is absent. Methylation studies on the gum have indicated the presence of an aldobiouronic acid containing glucuronic acid and xylose but the linkage is uncertain. Other methylation products of Achiiras sapota gum show that arabinofuranose and xylose are present as terminal units. Xylose occurs as branch point linked through C1, C2 and C4 while the glucuronic acid residues are both terminal and linked (1-2). Further information is required before any structure can be postulated.

Chagual gum (Puya chilensis) (139) on graded hydrolysis liberates the aldobiouronic acid 2-0-(D-glucopyranosyluronic acid)-D-xylose (27%) together with L-arabinose (7%), D-xylose (31%) and/
and D-galactose (38%). It is noted that *Achras sapota* gum also contains an aldobiouronic acid composed of xylose and glucuronic acid; but whether there are further structural similarities to Chagual gum, or whether Chagual gum is related to the galactan framework gums of group I, must await a methylation study of this gum.

The exudate from *Phormium tenax* (140) is unusual in that only xylose and glucuronic acid are liberated on hydrolysis of the polysaccharide.
The Molecular Structure of Mucilages, Hemicelluloses and Pectin.

On the basis of chemical structure and physical properties it is convenient to consider these groups of polysaccharides together. The group may be divided into five classes:

1) Mannans - galacto- and glucomannans.
2) Xylans.
3) Arabinogalactans.
4) Mucilages containing uronic acid.
5) Pectic substances.

Mannans.

True mannans have been isolated from Ivory nuts and Coffee beans (141). Ivory nut mannan (142, 143, 144) has been fractionated into two polymers which, after methylation and partial hydrolysis studies, were shown to consist mainly of \((1\rightarrow4)\beta\) linked chains of mannopyranose with possibly some \((1\rightarrow6)\) linkages. There is evidence that the fractions differ in molecular weight (145).

Polysaccharides containing D-galactose and D-mannose have been isolated from seeds of Leguminosae. A number of these galactomannans from e.g. Guar (68, 146, 147) and Fenugreek (148, 149, 150), have been examined by partial hydrolysis or acetolysis, methylation and periodate oxidation techniques. All are similar to Ivory nut mannan in that they have a \((1\rightarrow4)\beta\) linked mannopyranose chain. Galactose occurs as non-reducing terminal single unit side chain from/
from position 6 of the mannan chain. (Fig. III).

Variations occur in the proportion and order of the galactose units along the chain and in molecular weight.

Fig. III.  
Gal 1 1  
| |  
6 6  
\-4 Man 1--4 Man 1--4 Man 1--4 Man 1--4 Man 1--

An unusual galactomannan from Coconut kernel (151) contains galactose units in the main chain and also as branch point residues.

Glucomannans are widely occurring and in coniferous woods e.g. Jack pine (152,153), Norway spruce (69,154,155), Western hemlock (156,157) amount to almost half the hemicellulose content although the proportion is lower in hard woods e.g. Red maple (158). The ratio of glucose to mannose is higher in coniferous woods than hard woods, and indeed this appears to be the only major difference between the two. Glucomannan mucilages have been isolated from *Amorphophalus* (Konjak (32), Iles mannan (65,159,160), Iris (161) and Lily (162). The basal structure in glucomannans is also a (1--4) linked chain, but glucose, as well as mannose is found in the chain and variations occur in the order of units along the chain. (Fig. IV)

Fig. IV.  
\-4 G 1--4 Man 1--4 Man 1--4 Man 1--4 G 1--4 G 1--4 Man 1--

Glucomannan/
Glucomannan fractions from the same source show only minor structural variation and little difference in the degree of polymerisation (154,155). An examination of oligosaccharides isolated from many glucomannans shows that there are rarely more than two contiguous glucose units in the chain. Exceptions arise in the glucomannans from Loblolly pine (163,164) and White spruce (165) where partial hydrolysis indicates that few if any glucose units are contiguous. Evidence of slight branching has been found in e.g. Norway spruce (72).

D-Galactose has also been found in small amounts and it is uncertain whether this is an integral part of the glucomannan or whether it arises from contaminating galactomannan. Methylation, e.g. of Jack pine (153) has shown that the galactose occurs only as terminal non-reducing units so that it is unlikely to arise from a galactan. Meier (72) has isolated, from Norway spruce glucomannan, the oligosaccharides 6-O-\text{D}-galactopyranosyl-\text{D}-mannose and 0-\text{D}-galactopyranosyl-(1-6)-0-\text{D}-mannopyranosyl-(1-4)-0-\text{D}-mannose which are similar to those isolated from galactomannans.

Xylans.

This group of polysaccharides comprises hemicellulose extracts from cereals and grasses as well as woods. L-Arabinose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid are found in association with D-xylose. Methylation and/
and the isolation of xylobiose and its homologues, together
with, in some cases, periodate oxidation studies on these
polysaccharides indicate the general occurrence of \((1\rightarrow 4)\)β
linked xylose chains throughout this group. (Fig. V A.)

Tamarind seeds (166) and Esparto grass (167) both yield
pure xylans. Cereal and grass xylans contain varying
amounts of L-arabinose as non-reducing single unit side
chain on position C3 of the xylose chain. Non-terminal
arabinose occurs in xylans of Barley husks (168) and Maize
extracts (169,170,171,172). Glucuronic acid and/or
4-0-methyl-D-glucuronic acid, when found, generally occur
as terminal units linked to C2 on the xylose chain. Uronic
acid linked to the xylan chain at C3 is found in Wheat straw
xylan (173) and at C4 in Corn cob xylan (174) (Fig. V B.)

Fig. V A. \[ -4 \text{Xyl} \rightarrow -4 \text{Xyl} \rightarrow -4 \text{Xyl} \rightarrow -4 \text{Xyl} \]

Fig. V B. \[
\begin{array}{c}
\text{(Me)GA} \\
\text{1} \\
\text{2} \\
\text{3}
\end{array} \quad \begin{array}{c}
\text{Araf} \\
\text{1} \\
\text{3}
\end{array} \\
\begin{array}{c}
-4 \text{Xyl} \\
\text{1} \\
\text{4}
\end{array} \begin{array}{c}
-4 \text{Xyl} \\
\text{1} \\
\text{4}
\end{array} \begin{array}{c}
-4 \text{Xyl} \\
\text{1} \\
\text{4}
\end{array} \begin{array}{c}
-4 \text{Xyl} \\
\text{1} \\
\text{4}
\end{array}
\]

Xylans containing glucuronic acid and/or 4-0-methyl-
D-glucuronic acid are also common in wood hemicelluloses
e.g. Loblolly pine (163), White spruce (175) and Yellow
birch (176). The uronic acid is again found attached to C2
of/
of the xylan chain. Branching has been reported in the case of the xylan from Loblolly pine (185). L-Arabinofuranose is also found with uronic acid residues in the xylans from e.g. Scots pine (177) and Sitka spruce (178). As in the cereal and grass xylans the arabinose is generally located on C5 of the xylan chain as end group (Fig. V B.) Xylan fractions (167) from a single source have frequently been found to contain the same sugars but in widely different proportions.

Arabinogalactans.

Arabinogalactans are present in greatest proportions in Larch woods and also occur in many coniferous woods. They are generally highly branched polymers. (1-6) and (1-3) linked D-Galactose units are common and L-arabinose occurs mainly as terminal non-reducing unit although in some cases it is found in non-terminal positions. Arabinogalactan extracts have shown heterogeneity in a number of cases.

European larch arabinogalactan was reported to be heterogeneous in an early report (179) but later work has shown this to be unlikely (180,181). On the basis of methylation and periodate studies a partial structure for the galactan framework has been postulated as in Fig. VI.

The presence of 3-0-L-arabinopyranosyl-L-arabinose (181) is indicated in the periphery. After catalytic oxidation (101) of the polysaccharide followed by hydrolysis, it was shown that/
that arabinose is linked to C6 of galactose. The isolation of a degraded polymer, after Smith degradation, which contained both arabinose and galactose showed that the arabinose was linked directly to the (1→3) linked galactose backbone rather than the (1→6) linked side chains.

Fig. VI.  

\[
\begin{array}{c|c}
\text{Gal} & \text{Gal} \\
\hline
1 & 1 \\
6 & 6
\end{array}
\]

Western larch arabinogalactan has also been extensively investigated. Mild hydrolysis of this arabinogalactan releases 6-\(\alpha\)-D-galactopyranosyl-\(\beta\)-galactose and 3-\(\alpha\)-L-arabinopyranosyl-L-arabinose (182), the latter in disagreement with the results of an earlier methylation study by White (183). The ease of hydrolysis of both disaccharides suggests that the reducing units are furanose. Extraction and fractionation of Western larch heartwood arabinogalactan (184) has yielded two fractions (A and B) which both contain a galactan framework similar to that of European larch. After mild hydrolysis, A has been fractionated to give two fractions (A1 and A2) (185) which were shown to contain the same type of linkage although in different proportions. It is possible that A1 and A2 arise from different polysaccharides or from two parts of the same polysaccharide joined by a weak linkage.

Arabinogalactans/
Arabinogalactans have been isolated from White spruce (186), Tamarack (187) and a number of pines (188, 189, 190). The main structural features are similar although there is variation in the ratio of arabinose to galactose and in the amount of terminal arabinose. Recently an arabinogalactan has been isolated from Maple sap (191) containing 5% L-rhamnose and with a higher proportion of (1→3) galactose linkages.

Mucilages containing uronic acid.

This group of mucilages is diverse and further examination is required throughout. Two major groups are distinguished: one based on D-xylose and the other on D-galacturonic acid, L-rhamnose and D-galactose.

Basal framework of Xylose.

A number of mucilages from Plantago species have been examined. Plantago ovata (191, 195) yields two polysaccharide fractions on extraction (A and B). Methylation or acetylation (191) of A yields two parts; A1 - a neutral polysaccharide, and A2 - an acidic polysaccharide containing 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose. Methylation of A1 and B indicates that both are based on a highly branched xylan framework and are structurally different. Acetylation of Plantago lanceolata mucilage (194) gives two fractions which have been methylated and appear to differ only in molecular weight. The general structure is similar to the mucilage of Plantago ovata. Plantago areneria mucilage (192) also yields two fractions/
### TABLE III. MUCILAGES CONTAINING URONIC ACID.

**Group I. Basal structure of xylose.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Xyl</th>
<th>Ara</th>
<th>Rha</th>
<th>Gal</th>
<th>Uronic acid</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantago areneria.</td>
<td>65</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>GalA - 7</td>
<td>191,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>192.</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>72</td>
<td>-</td>
<td>11</td>
<td>-</td>
<td>GalA - 15</td>
<td>193,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>194.</td>
</tr>
<tr>
<td>Plantago ovata A.</td>
<td>46</td>
<td>7</td>
<td>20</td>
<td>-</td>
<td>GalA - 20</td>
<td>191,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>195.</td>
</tr>
<tr>
<td>Plantago ovata B.</td>
<td>80</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>GalA - 2</td>
<td></td>
</tr>
<tr>
<td>Mimosa pudica.</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>GA - 21</td>
<td>196.</td>
</tr>
</tbody>
</table>

**Group II. Basal structure Galacturonic acid, Rhamnose and Galactose.**

<table>
<thead>
<tr>
<th>Source</th>
<th>GalA</th>
<th>Gal</th>
<th>Ara</th>
<th>Xyl</th>
<th>Rha</th>
<th>Other Sugars</th>
<th>Oligosaccharides</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okra</td>
<td>6</td>
<td>80</td>
<td>3</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>GalA 1--2 Rha</td>
<td>197-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GalA 1--Rha 1--Gal</td>
<td>199.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gal 1--Gal 1--Rha</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gal 1--4 Gal</td>
<td></td>
</tr>
<tr>
<td>Slippery elm.</td>
<td>33</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>33</td>
<td>3 MeGal</td>
<td>GalA 1--2 Rha</td>
<td>200-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>202.</td>
</tr>
<tr>
<td>Corchorus olitorius</td>
<td>3</td>
<td>60</td>
<td>12</td>
<td>6</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>198.</td>
</tr>
<tr>
<td>Flax seed.</td>
<td>+</td>
<td>-</td>
<td>12</td>
<td>27</td>
<td>29</td>
<td>L-Gal</td>
<td>GalA 1--2 Rha</td>
<td>203-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>205.</td>
</tr>
<tr>
<td>White mustard.</td>
<td>+</td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>GA</td>
<td>-</td>
<td>206.</td>
</tr>
<tr>
<td>Cress seed.</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>GalA 1-- Rha</td>
<td>206.</td>
</tr>
</tbody>
</table>
fractions, one of which contains no uronic acid. Treatment of Plantago areneria mucilage with 2N alkali also reduces the uronic acid content. Thus it seems that the Plantago mucilages contain an alkali sensitive link or closely associated polyuronide containing 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose. The alkali stable nucleus consists of D-xylose chains linked through C1 and C4 and/or C1 and C5. Side chains are composed of xylose and galactose, and in Plantago ovata A and Plantago areneria, of terminal arabinose, Plantago areneria and Plantago ovata B also contain (1-3) linked arabinofuranose.

Basal framework of uronic acid, rhamnose and galactose.

Okra mucilage and Slippery elm mucilage have been investigated in some detail and it is evident that they are closely related to the Plant gums of high uronic acid content in that the framework consists largely of galacturonic acid, rhamnose and galactose.

An investigation of Okra mucilage (188) by partial hydrolysis has led to the identification of 2-O-(D-galactopyranosyluronic acid)-L-rhamnose, O-D-galacturonic acid-O-L-rhamnose-O-D-galactose and O-D-galactose-O-D-galacturonic acid-O-L-rhamnose which suggests the presence in the molecule of the unit:

--- Gal 1-- GaLa 1--2 Rha 1-- Gal 1--
The neutral disaccharide 4-α-D-galactopyranosyl-D-galactose was also isolated. Methylation of Okra mucilage (192) has shown that the galactose units are terminal, linked (1→4) and in smaller amount linked (1→3) while rhamnose is present as branch point.

Interpretation of methylation results (202) from Slippery elm mucilage is rendered difficult as in this case the original mucilage contains large amounts of 3-O-methyl-D-galactose (201). Rhamnose is again present as branch point and has also been isolated as the free sugar from the methylated products. In view of the yield of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose on hydrolysis, it is likely that all the galacturonic acid is linked to rhamnose at C2. Methylation of Corchorus olitorius mucilage (198) indicated a similar type of structure.

A preliminary investigation has been made of the mucilages extracted from Flax (203), Mustard (206), Cress (206) and Quince seeds (195). All contain an associated polysaccharide similar in all respects to cellulose other than solubility and which may be precipitated by heating the aqueous solution for a few hours or for a shorter period with dilute acid. An unusual sugar, L-galactose, has been isolated from Flax seed mucilage (203). Partial hydrolysis of this mucilage (204) and Cress seed mucilage has shown the presence of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose although the linkage is not defined in the latter case. There is also/
also evidence for the presence of an aldotriuronic acid containing \( L \)-galactose, \( D \)-galacturonic acid and \( L \)-rhamnose in Flax seed mucilage (205). It is likely that Flax, Cress and White mustard seed mucilages are heterogeneous.

The composition of a number of other mucilages containing uronic acid have been reported but further structural investigation is required.

**Pectic substances.**

This group of polysaccharides is found in the cell walls and intercellular layers of all plant tissues. Fruits and roots have large pectic contents while amounts as low as 0.5% are found in certain soft woods. The pectic triad consists of a polygalacturonic acid (pectic acid), an araban and a galactan. The polysaccharides of the triad are closely associated and have such similar solubility characteristics that the usual extractive procedures remove all three polysaccharides together. Whether in fact they constitute three separate entities closely associated physically or are chemically linked is still uncertain although recent evidence indicates the former view (207).

Pectic acid is difficult to obtain free from araban and galactan. The pectic acid is found mainly as the methyl ester in the plant. Treatment with e.g. dry methanolic hydrochloric acid preferentially degrades the araban and galactan leaving a partially degraded pectic acid. Methylation (208)/
(208) and periodate oxidation studies (209) of this degraded material indicate a (1→4) linked polymer of D-galacturonic acid residues. Recently (207) it has been shown that pectic acids probably have associated neutral monosaccharides e.g. rhamnose and arabinose.

Hirst and Jones (210) have isolated an araban from peanut. Methylation revealed a highly branched structure with equal amounts of branch point, chain and terminal arabinofuranose units. Arabans from apple and citrus pectins have similar methylated products to that of peanut araban.

Galactans have only been isolated from the seeds of Lupinus albus (211) and Strychnos nux-vomica (212). A linear (1→4) linked structure of D-galactopyranose units is indicated from methylation results.

Gum tragacanth and the present investigation.

Gum tragacanth is obtained from plants of the genus Astragalus of which there are some 1600 species, not all of which, however, produce gum. The shrubs are found in dry locations in regions of Asia Minor and Iran. The gum is exuded as ribbons or flakes immediately after injury of the plant and it is possible that it is present as preformed metabolic product.

Gum tragacanth is valued commercially as an emulsifying and stabilising agent, particularly in the pharmaceutical, textile and paper industries.
Early work on the gum showed (213) that it was complex and consisted of at least two parts, one water soluble and one water insoluble. It was shown that samples of the gum contained xylose, arabinose, galactose, fucose and uronic acid. The major structural work in recent years is that of James and Smith (55) who, although they were unable to fractionate the gum directly, obtained three fractions after methylation; A) an acidic methylated polysaccharide, B) a neutral methylated polysaccharide and C) a fraction which may be a steroidal glycoside. More recently (214) a resolution of the water soluble gum into two fractions has been reported. The major fraction contained \( \beta \)-galacturonic acid, \( \beta \)-xylose and \( \beta \)-fucose with smaller amounts of arabinose, galactose and an unidentified sugar. This fraction may be equated with the acidic methylated polysaccharide of James and Smith and will be referred to as tragacanthic acid. The minor fraction contained \( \alpha \)-arabinose and \( \beta \)-galactose in the ratio 13:2 and small amounts of xylose and galacturonic acid; it may be equated with the neutral methylated polysaccharide isolated by James and Smith and will be referred to as the 'Arabinogalactan' fraction.

An examination of the methanalysis products of methylated tragacanthic acid and the methylated arabinogalactan shows the following structural features:-
<table>
<thead>
<tr>
<th>Fraction</th>
<th>End group</th>
<th>Straight chain unit</th>
<th>Branch unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tragacanthic acid</td>
<td>Xyl 1---</td>
<td>--2 Xyl 1---</td>
<td>-- GaLA 1---</td>
</tr>
<tr>
<td></td>
<td>Fuc 1---</td>
<td>--4 GaLA 1---</td>
<td></td>
</tr>
<tr>
<td>Arabinogalactan.</td>
<td>AraF 1---</td>
<td>--4/5 Ara 1---</td>
<td>-- Hexose 1---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4/5 Ara 1---</td>
</tr>
</tbody>
</table>

**Tragacanthic acid.**

As there is a high proportion of uronic acid in tragacanthic acid it may be placed with plant gums of group II. An examination of the methanolyis products of the methylated polysaccharide, however, shows that there is little further resemblance to these gums. Galacturonic acid linked (1--4) and as branch point has been found in the exudated from *Sterculia setigera* and *Cochlospermum gossypium*, but, in those gums the uronic acid is associated in chains with rhamnose, itself present both as branch point and chain unit. In tragacanthic acid the galacturonic acid is also (1--4) linked and in this case provides the only branch point. There is, then, a greater resemblance to the (1--4) linked galacturonic acid structure of pectic acid. That xylose is present in chains of galacturonic acid cannot be excluded as a possibility in the structure of tragacanthic acid on the basis of methylation results alone.
Arabinogalactan.

An examination of the methanolyis products from this fraction of the methylated gum indicates that it is highly branched. The only other polysaccharide so far investigated where free arabinose has been encountered in the methylation products is methylated Cholla gum. Arabinose end unit and straight chain unit are also found in the periphery of plant gums of group I but in this case the arabinose chain unit is linked (1--3) whereas in the arabinogalactan from gum tragacanth it is linked (1--4/5). A further difference lies in the uronic acid content of group I gums. Arabinogalactans free from uronic acid have been isolated from the hemicellulose components of a number of woods. Investigations, however, of e.g. European larch 6-galactan and the arabinogalactan from Western larch indicate that the periphery contains (1--3) rather than (1--4/5) linked arabinose units as well as arabinose end group. As in the case of plant gums of group I, the wood arabinogalactans have a framework of (1--6) and (1--3) linked galactose units. Although hexose branch point has been shown in the methanolyis products of the arabinogalactan from gum tragacanth the type of linkage has not been specified and so it is still uncertain whether this polysaccharide contains a similar galactan framework.

Later work by the British Cotton Industry Research Association has shown the gum to be alkali labile.

In view of the high commercial value of the gum and the possible/
possible relationship of its constituent polysaccharides to other plant polysaccharides, it is of considerable interest to investigate the structure of gum tragacanth using modern techniques. It may also be noted that fractions of the gum are at present being investigated for possible anti-cancer activity.

In the present investigation experiments have been carried out to fractionate the gum directly into homogeneous polysaccharides. Two fractions have been investigated.

Methylated tragacanthic acid has been hydrolysed and the methylated products examined. Oligosaccharides have also been obtained from this fraction by acid and enzyme hydrolysis.

Methylated arabinogalactan has been hydrolysed and the methylated products examined. Degraded polysaccharides obtained after periodate oxidation have been investigated.

The significance of these results is discussed.
The Molecular Structure of Gum Tragacanth, Discussion.

Part I. Purification and Fractionation of Gum Tragacanth.

It has long been known that gum tragacanth is heterogeneous and contains at least two polysaccharides, which have been isolated after methylation of the gum (37). An acidic polysaccharide - tragacanthic acid - containing D-galacturonic acid, D-xylose and L-fucose can be deduced from the examination of the methanolysis products of the acidic methylated polysaccharide and a neutral polysaccharide - arabinogalactan - containing L-arabinose and D-galactose can be deduced from the methanolysis products of the methylated neutral polysaccharide.

In the present investigation gum tragacanth has been subjected to a number of fractionation techniques, summarized in table VI, in an attempt to isolate tragacanthic acid and the arabinogalactan fraction directly. The reagents chosen for extraction were least likely to modify the polysaccharide so that the materials examined would be as nearly as possible representative of polysaccharides present in the original gum.

A polysaccharide similar to the tragacanthic acid above has been isolated and shown to contain small amounts of arabinose and galactose in addition to galacturonic acid, D-xylose and L-fucose. A second polysaccharide similar to the arabinogalactan has also been obtained and shown to contain small amounts of galacturonic acid in addition to D-galactose and/
## Table VI. Summary of Fractionation of Gum Tragacanth

### Part I.

| Procedure                                      | Water soluble A | Alkali soluble B | Alkali insoluble B1 | Ethanol insoluble B2 | De-ionised B3 | Neutralised with 50% Acetic acid B4 | Fractional precipitation with ethanol B5 | (70% Esterification with Ethylene oxide B6 | DEAE cellulose column A7 |
|------------------------------------------------|-----------------|------------------|---------------------|---------------------|--------------|-------------------------------------|------------------------------------------|------------------------------------------|

### Part II.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Ethanol extract</th>
<th>Crude Gum extract</th>
<th>Insoluble residue A8</th>
<th>Esterification with Ethylene oxide A9</th>
<th>DEAE cellulose column A10</th>
</tr>
</thead>
</table>

- **Water soluble A**: Fractional dissolution with Cetavlon.
- **Alkali soluble B**: Fractional precipitation with ethanol.
- **Alkali insoluble B1**: De-ionised.
- **Ethanol insoluble B2**: Alkaline precipitation with Cetavlon.
- **De-ionised B3**: Neutralised with 50% Acetic acid.
- **Insoluble residue A8**: Esterification with Ethylene oxide.
- **DEAE cellulose column A10**: Extracted with ethanol.
and L-arabinose.

A preliminary fractionation of the gum into water soluble and insoluble fractions has been made. The water insoluble fraction has been further resolved into fractions soluble and insoluble in alkali.

Fractional precipitation of the water soluble gum from aqueous solution with ethanol gave four fractions - A4, A3, which showed a gradation between a tragacanthic acid rich fraction A1 and an arabinogalactan rich fraction A4. Fractional precipitation of the alkali soluble fraction in the same way gave only two polysaccharides, B4 which was similar to A1 but with a higher equivalent weight, and B4 similar to A4 but with a lower equivalent weight.

Complex formation between acidic polysaccharides and cetavlon (cetyl trimethylammonium bromide) has been used in many cases to resolve mixtures of polysaccharides (33). The cetavlon-polysaccharide complex precipitates from aqueous solution and treatment of the precipitate with acetic acid or ionic salt solutions regenerates the polysaccharide. Addition of cetavlon to an aqueous solution of the water soluble gum gave a complex from which polysaccharide was regenerated by addition of sodium chloride solution. This polysaccharide was fractionated by addition of ethanol to the solution to give two fractions - A5, a tragacanthic acid rich fraction similar to A4, and A6 an impure arabinogalactan fraction. Addition of ethanol to the mother liquors from the original complex precipitation gave a polysaccharide-A7—an arabinogalactan rich fraction/
fraction which contained slightly less contaminating xylose and a lower proportion of uronic acid than A4. Treatment of the alkali soluble gum with cetavlon also gave an insoluble polysaccharide complex which was regenerated to give fraction B6 - an impure tragacanthic acid fraction. Treatment of the supernatant, after precipitation of the complex, with ethanol gave fraction B6 - an impure arabinogalactan.

A comparison of the tragacanthic acid fractions (A1, A5, B5, B6) shows that they contain the same sugars in approximately the same proportions, that the rotations are of the same order and that the equivalent weights are almost content. The arabinogalactan fractions (A4, A7, B4, B6) are also similar. It would then seem likely that the water soluble and alkali soluble fractions of gum tragacanth are closely related and may contain the same polysaccharides, the difference being largely one of solubility. This concept is further supported since in a previous investigation James and Smith (35) isolated only two methylated polysaccharides after methylation of the whole gum.

An improved resolution of tragacanthic acid and arabinogalactan was obtained by first converting the water soluble gum into its glycol ester by treatment with ethylene oxide. A small amount of insoluble material which formed during the reaction was isolated - A8. The esters were then fractionally precipitated with acetone to give a tragacanthic acid rich fraction - A9, which contained smaller amounts of galactose and arabinose than previous fractions, and an arabinogalactan - A10/
AIO, with slightly less xylose than so far obtained. This tragacanthic acid ester – A9 – was prepared in larger amounts and was used for further investigations into the structure of tragacanthic acid.

Attempts to fractionate the water soluble gum via its copper complex and after acetylation were unsuccessful.

Although relatively large amounts of tragacanthic acid in a reasonably pure state, could be isolated by the above procedures, the isolation of pure arabinogalactan in quantity sufficient for structural investigation was extremely laborious since it is the minor fraction. The arabinogalactan was readily isolated in a highly pure state by direct extraction of the crude gum.

The ground gum was extracted with boiling ethanol on a Soxhlet extractor and then with ethanol:water (7:3). Addition of ethanol to the 70% ethanol extract gave a good yield of a polysaccharide rich in arabinogalactan. After reprecipitation from aqueous solution this polysaccharide A14, had a higher negative rotation and higher equivalent weight than any previous fraction and also contained no xylose. Further amounts of this material were isolated for structural investigations. An examination of the sugar composition gave – arabinose 75%, galactose 12%, galacturonic acid 3%, and a trace of unidentified sugar.

An examination of tragacanthic acid ester, A9, and arabinogalactan A14 was made using analytical methods. Ionophoresis/
Ionophoresis of these fractions and the crude gum was carried out in potassium hydroxide solution on glass fibre sheets. The crude gum was resolved into three fractions, the two major fractions corresponding to tragacanthic acid and arabinogalactan, and the third being only present in trace amounts. A larger scale ionophoresis of tragacanthic acid gave a major fraction, a trace corresponding to arabinogalactan and a further very faint trace. Isolation of the major fraction and hydrolysis followed by chromatography indicated a polysaccharide containing galacturonic acid, xylose, fucose (4:4:1) and traces of galactose and arabinose. A similar examination of the arabinogalactan A14 gave a trace of material corresponding to tragacanthic acid and a major fraction which contained arabinose, galactose (6:1) and a trace of galacturonic acid, and unidentified sugar.

Diethylaminoethyl cellulose ion exchanger has been used to fractionate a number of polysaccharides (46). Tragacanthic acid - A9 - was placed on a column of DEAE cellulose in the phosphate form and eluted with increasing concentrations of phosphate buffer followed by a gradient of sodium hydroxide solution. Polysaccharide eluted by the phosphate buffer was estimated with anthrone, and polysaccharide eluted by the sodium hydroxide was estimated with carbazole. Only small amounts of polysaccharide were detected in the phosphate eluate but a major polysaccharide was found in the sodium hydroxide solution. This/
This polysaccharide was recovered and re-eluted from a DEAE column when a single symmetrical peak was obtained similar to the original peak. The polysaccharide was again recovered. Examination of this hydrolysed polysaccharide by chromatography gave galacturonic acid, xylose 40%, fucose 10%, galactose 4% and a trace of arabinose. The polysaccharide had uronic acid anhydride content of 48%.

Arabinogalactan - A14 - was fractioned on a DEAE cellulose column in a similar way. Elution with increasing concentrations of phosphate buffer gave small amounts of polysaccharide in the early stages and later a major polysaccharide fraction. Further elution with sodium hydroxide solution gave only traces of polysaccharides. The major polysaccharide was recovered and re-eluted from a DEAE cellulose column when a single symmetrical peak was obtained corresponding to that of the original polysaccharide. This polysaccharide, after precipitation with acetone and hydrolysis was shown to contain arabinose, galactose (6:1) and traces of galacturonic acid (4-5% by carbazole estimation), and unidentified sugar.

The alkali insoluble polysaccharide was only partially hydrolysed under normal hydrolysis conditions for polysaccharides but after treatment with concentrated sulphuric acid was found to hydrolyse almost completely when a large increase in the proportion of glucose was found in the hydrolysate. It would then appear likely that this fraction contains a high percentage of cellulose. It has also been noticed that in certain of the tragacanthic acid fractions, particularly those/
those obtained by fractionation of the alkaline soluble polysaccharide - II, a small amount of material precipitated on heating an aqueous solution either alone or with acid. A similar fraction was separated from the esterification reaction on the water soluble gum. This material was only completely hydrolysed after treatment with concentrated sulphuric acid and gave a chromatographic pattern similar to the hydrolysate of the alkali insoluble polysaccharide. The amount of this material was found to vary in any one fractionation and depended on how often the water soluble or alkali soluble gum was centrifuged in the preliminary fractionation.

Gum tragacanth was found to be resistant to methylation. Methylation of the gum, water soluble gum or arabinogalactan fraction using Haworth reagents gave a satisfactory yield and methoxyl content for the partially methylated arabinogalactan but not of partially methylated tragacanthic acid. Fractionation of the partially methylated gum or partially methylated water soluble gum was carried out by extraction of the almost neutral methylation reaction mixture with chloroform. Concentration of the chloroform extracts and precipitation by addition of light petroleum gave partially methylated arabinogalactan (OMe 33%). The remaining aqueous reaction mixture was then dialysed against tapwater. A small insoluble fraction corresponding to the glycoside fraction of James and Smith (37) was filtered off and partially methylated tragacanthic acid isolated by concentration of the aqueous filtrate.

Methylation of the gum has been reported by James and Smith/
Smith and although the conditions and reagent quantities reported by these workers were repeated on the water soluble and whole gums a partially methylated tragacanthic acid was not obtained in reasonable yield or methoxyl content. An attempt to methylate tragacanthic acid using sodium in liquid ammonia and methyl iodide was also unsuccessful. A satisfactory partially methylated tragacanthic acid was finally obtained using thallous hydroxide and methyl iodide, reagents which have previously been used to methylate polysaccharides resistant to methylation with Haworth's reagents.

The low yields of methylated tragacanthic acid may be due partly to alkaline degradation as it has been reported (214) that gum tragacanth is alkali labile. In the present investigation it has also been noted that degraded tragacanthic acids underwent degradation with alkali during equivalent weight determinations. It has also been shown that the yield of partially methylated tragacanthic acid improved slightly on carrying out the methylation under oxygen free conditions.

Partially methylated arabinogalactan (OMe 34%) was fully methylated using 'Purdie' reagents.

The partially methylated tragacanthic acid (OMe 31%) was also found to be resistant to complete methylation using 'Purdie' reagents in the presence of methanol, which was required as solvent. An attempt to methylate this polysaccharide fully using barium oxide and methyl iodide as described by Kuhn et al. (83) was also unsuccessful. A fully methylated polysaccharide was/
was finally obtained using 'Purdie' reagents with dry tetrahydrofuran and acetone as solvents.

Hydrolysis of the methylated polysaccharides, which will be discussed in later sections, gave, for tragacanthic acid, methylated derivatives of galacturonic acid, xylose, fucose, in smaller amount galactose, and in traces arabinose: and for the arabinogalactan methylated derivatives of galactose, arabinose and in smaller amount of galacturonic acid, and rhamnose.

The isolation of tragacanthic acid as a symmetrical peak from chromatography on a DEAE cellulose and as a single fraction on ionophoresis would indicate that it is probably homogeneous. From an examination of these fractions tragacanthic acid contains galacturonic acid 43%, xylose 40%, fucose 10%, galactose 4% and a trace of arabinose. This composition is verified by identification of hydrolysis products from methylated tragacanthic acid, which will be discussed in detail in a later section.

The arabinogalactan fraction, on the basis of the above techniques, has also been isolated as a homogeneous polysaccharide and contains arabinose 75%, galactose 12%, galacturonic acid 3% and an unidentified sugar. This composition is again verified by a study of the methylated arabinogalactan.

It has previously been reported (228) that gum tragacanth contained in addition to galacturonic acid, galactose, arabinose, xylose and fucose a further sugar, which was probably a 6-deoxy-hexose. In the present investigation a similar sugar has/
has been found only in the arabinogalactan and not in tragacanthic acid after fractionation on DEAE cellulose. This sugar was isolated in small yield from partial hydrolysis of an impure tragacanthic acid fraction when it was shown to be a 6-deoxy-hexose by the isolation of acetaldehyde after periodate oxidation. Larger amounts were isolated by hydrolysis of the crude gum and purified by fractionation on cellulose columns. This sugar was chromatographically and ionophoretically identical to L-rhamnose and to a small sample obtained by hydrolysis and fractionation of the hydrolysate from the arabinogalactan. The sugar also had a similar rotation to that of L-rhamnose. The melting points of the p-toluene-sulphonylhydrazone derivatives of the sugar and L-rhamnose were similar and undepressed in mixed melting point, indicating that the sugar was L-rhamnose.

A third glycoside fraction has been isolated from the methylation study of James and Smith. This fraction gave a red colour with concentrated sulphuric acid. Concentration of the mother liquors, from the fractional precipitation of the water soluble gum with ethanol, followed by extraction with butanol and evaporation of the butanol extracts gave a yellow powder. This powder, on hydrolysis, contained xylose, glucose and arabinose (2:2:1) and an oily liquid. The powder also gave a red colour with concentrated sulphuric acid. Larger amounts of this material have been isolated by evaporation of the ethanol extract of the crude gum. This material has been fractionally/
fractionally precipitated from ethanol solution with ethyl acetate to give a white powder; a second fraction was obtained on evaporation of the mother liquors. Both fractions give glucose, arabinose and xylose with oily liquid on hydrolysis and give a red colour with concentrated sulphuric acid. The small amount of water insoluble material isolated from methylation reaction mixtures also gave a red colour with concentrated sulphuric acid.

There would then appear to be four major fractions in gum tragacanth.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>GalA</th>
<th>Gal</th>
<th>G</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tragacanthic acid</td>
<td>43%</td>
<td>4%</td>
<td>-</td>
<td>tr</td>
<td>40%</td>
<td>10%</td>
<td>-</td>
</tr>
<tr>
<td>Arabinogalactan</td>
<td>3-4%</td>
<td>12%</td>
<td>75%</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alkali insoluble polysaccharide</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Part II. Tragacanthic Acid.

Partial Hydrolysis.

Graded hydrolysis of tragacanthic acid in two stages has given two degraded polysaccharides.

Hydrolysis of tragacanthic acid with sulphuric acid (0.05N) for 20 hours led to the isolation of degraded tragacanthic acid I. Chromatographic examination of the hydrolysate showed that fucose and a small amount of xylose had been liberated. Hydrolysis of the degraded polysaccharide and chromatography showed that it contained only xylose and galacturonic acid in almost equal amounts and that all the fucose had been liberated. Degraded tragacanthic acid I also showed an increase in optical rotation from $[\alpha]_D = +105^\circ$ to $+170^\circ$; an increase in uronic acid anhydride content from 43% to 48% and a corresponding decrease in equivalent weight.

The fully degraded tragacanthic acid II was isolated after hydrolysis of tragacanthic acid for 6 hours with sulphuric acid (0.5N). Examination of this polysaccharide and comparison with degraded tragacanthic acid I showed that although a large amount of xylose had been released, only relatively small amounts of galacturonic acid were liberated during hydrolysis. A further increase in optical rotation to $[\alpha]_D = +228^\circ$ was observed, together with an increase in uronic acid anhydride content to 69%. Direct determinations of equivalent weight with alkali gave results varying from 202 - 189 and it was noted/
noted that alkaline solutions of degraded tragacanthic acid II rapidly turned a yellow colour. It would therefore appear that this polysaccharide is partially alkali labile.

The increase in positive optical rotation during the preparation of degraded tragacanthic acids indicated that \( \alpha-L \) and \( \beta-D \) glycosidic linkages were being removed and also that, since the degraded tragacanthic acid contains a high proportion of \( D \)-galacturonic acid, \( \alpha-D \) linkages were predominant. The high positive optical rotation of degraded tragacanthic acid II and its high galacturonic acid content indicate a strong similarity to pectic acids.

Although small amounts of oligosaccharides had been observed in the hydrolysis products during the preparation of degraded tragacanthic acid I, an attempt to isolate and characterise them was unsuccessful. Hydrolysis of the whole fraction, however, showed the presence of xylose, galactose and fucose and as there was a much higher proportion of xylose than fucose and galactose, it is probable that xylobiose was present in the oligosaccharide mixture.

Partial acetolysis of tragacanthic acid, followed by de-acetylation of the oligosaccharide acetates with barium methoxide, gave a syrupy mixture of sugars which was fractionated on a charcoal:celite column using aqueous ethanol as eluant. In addition to monosaccharides, two disaccharides were eluted from the column as a mixture and were finally isolated in a pure state by thick paper chromatography.
The faster moving disaccharide I was obtained crystalline. On hydrolysis, this disaccharide gave equal amounts of fucose and xylose. After reduction with borohydride, followed by hydrolysis disaccharide I gave fucose and xylitol indicating that xylose was present as reducing group. A negative reaction to triphenyltetrazolium salt showed that position C2 of the xylose reducing unit was blocked so that the sugar was probably 2-O-L-fucosyl-D-xylose.

Methylation of disaccharide I with dimethyl sulphate and sodium hydroxide, followed by hydrolysis of the fully methylated sugar gave 2,3,4-tri-O-methyl-L-fucose and 3,4-di-O-methyl- D-xylose, identified by the formation of crystalline derivatives, together with traces of partially methylated monosaccharides probably arising from demethylation during hydrolysis.

From the optical rotation of disaccharide I it is probable that it contains an α-L-glycoside linkage.

\[
\begin{align*}
\text{Disaccharide I} & : [\alpha]_D^{20} = -60^\circ \\
\alpha-L\text{-methyl-L-Fucose} & : [\alpha]_D^{20} = -197^\circ \\
D\text{-Xylose} & : [\alpha]_D^{20} = +18^\circ
\end{align*}
\]

On this evidence disaccharide I is probably 2-O-α-L-fucopyranosyl-D-xylose.

The slower moving disaccharide II, from partial acetolysis, gave, on hydrolysis, galactose and xylose in equal amount. Reduction of the sugar with borohydride followed by hydrolysis gave galactose and xylitol indicating that xylose was present as a reducing unit. A negative reaction to triphenyltetrazolium salt again showed that position C2 of the xylose was blocked, so/
so that the disaccharide was probably 2-0-β-D-galactosyl-D-xylose.

Methylation of disaccharide II followed by hydrolysis of the fully methylated sugar gave 2,3,4,6-tetra-0-methyl-D-galactose and 3,4-di-0-methyl-D-xylose, identified by the formation of crystalline derivatives.

From the optical rotation of the disaccharide it is likely that it contains a β-D glycosidic linkage.

Disaccharide II  \([\alpha]_D = -40^\circ\)

β -methyl-D-Galactoside  \([\alpha]_D = 0.0^\circ\)

D-Xylose  \([\alpha]_D = +18^\circ\)

It is probable therefore that disaccharide II is 2-0-β-D-galactopyranosyl-D-xylose.

The enzymes Hemicellulase and Pectinase were found to have little action on tragacanthic acid. Enzymic degradation of degraded tragacanthic acid I with hemicellulase enzyme gave a disaccharide III in good yield and smaller amounts of a disaccharide/
disaccharide IV together with traces of other oligosaccharides.

Hydrolysis of disaccharide III gave xylose and galacturonic acid and hydrolysis after reduction with borohydride gave xylose and galactonic acid indicating that the disaccharide was a pseudo-aldobiouronic acid with galacturonic acid as the reducing end unit. It was also noted that disaccharide III was readily hydrolysed by H₂SO₄ sulphuric acid in contrast to the more common aldobiouronic acids which are resistant to acid hydrolysis. A positive reaction to triphenyltetrazolium salt and liberation of approximately 1 mole of formaldehyde from periodate oxidation of 1 mole of the borohydride reduced disaccharide, showed that the xylose unit is probably linked to position C3 or C4 of the galacturonic acid.

Methylation of the disaccharide followed by reduction of the methyl ester methyl glycoside with lithium aluminium hydride and hydrolysing gave 2,3,4-tri-O-methyl-D-xylose and 2,4-di-O-methyl-D-galactose, identified by the preparation of crystalline derivatives.

From the optical rotation of disaccharide III it probably contains a β-D-glycosidic linkage.

Disaccharide III  \([\alpha]_D = 0.0^\circ\)
β-methyl-D-Xyloside  \([\alpha]_D = -65.5^\circ\)
D-Galacturonic acid  \([\alpha]_D = +80^\circ\)

It is then likely that disaccharide III is 3-O-β-D-xylopyranosyl-D-galacturonic acid.

Enzymic/
Enzymic degradation of fully degraded tragacanthic acid II with hemicellulase gave disaccharide IV in good yield with smaller amounts of disaccharide III and slower moving oligosaccharides V and VI.

Hydrolysis of disaccharide IV gave only galacturonic acid. The syrup was converted to the calcium salt which was identical in optical rotation, infra-red spectrum and on chromatography to the calcium salt of 4-O-α-D-galactopyranosyluronic acid)-D-galacturonic acid (digalacturonic acid).

Oligosaccharide V gave on hydrolysis xylose galacturonic acid, and therefore probably is a trisaccharide containing one xylose unit and two galacturonic acid units. It has not been ascertained whether this trisaccharide is

(a) Xyl - GalA - GalA  (b) GalA - GalA

or (c) GalA - Xyl - GalA  Xyl

The latter possibility (c) is unlikely as hydrolysis of such a trisaccharide would give an aldobiouronic acid GalA1—Xyl resistant to acid hydrolysis. Such an aldobiouronic acid was not in fact detected on chromatograms.

Oligosaccharide VI gave only galacturonic acid on hydrolysis. The calcium salt was similar to the calcium salt of (1 - 4)-α-D trigalacturonic acid in rotation, infra-red spectrum and on chromatography. It was noted that the infra-red spectra of di- and trigalacturonic acid calcium salts were identical.
Acid hydrolysis of tragacanthic acid using the conditions normally employed for the isolation of aldobiouronic acids gave a syrup which was fractionated on IR 45-OH resin. Elution of the acidic sugars from the column with formic acid, and chromatographic examination showed the absence of any aldobiouronic acid.

The partial hydrolysis studies, therefore, show the presence of the following structural features in tragacanthic acid.

\[ L\text{-Pucp} 1^\alpha - 2 \text{D-Xyl} 1^- \]
\[ D\text{-Galp} 1^\beta - 2 \text{D-Xyl} 1^- \]
\[ D\text{-Xylp} 1^\beta - 3 \text{D-Gala} 1^- \]
\[ D\text{-GalAp} 1^\alpha - 4 \text{D-Gala} 1^- \]
\[ D\text{-GalAp} 1^\alpha - 4 \text{D-Gala} 1^\alpha - 4 \text{D-Gala} 1^- \]

\{Xyl -- Gala -- Gala or Gala -- Gala\}
\{GalA -- GalA\}
\{Xyl\}
Methylation results.

The preparation of methylated tragacanthic acid has already been discussed (see Part I). The bulk of this product was reduced with lithium aluminium hydride and the mixture of methylated sugars obtained on hydrolysis was fractionated on cellulose. The results are summarised below.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Moles</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-tri-O-methyl-D-xylose</td>
<td>2.34</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-L-fucose</td>
<td>0.35</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
<td>0.20</td>
</tr>
<tr>
<td>3,4-di-O-methyl-D-xylose</td>
<td>1.01</td>
</tr>
<tr>
<td>2,4-di-O-methyl-D-xylose</td>
<td>Minor</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-xylose</td>
<td>Minor</td>
</tr>
<tr>
<td>5-di-O-methyl-L-fucose</td>
<td>0.35</td>
</tr>
<tr>
<td>3,5-di-O-methyl-L-arabinose</td>
<td>0.11</td>
</tr>
<tr>
<td>2,4,6-tri-O-methyl-D-galactose</td>
<td>Minor</td>
</tr>
<tr>
<td>2,3,4-tri-O-methyl-D-galactose</td>
<td>0.11</td>
</tr>
<tr>
<td>4-mono-O-methyl-D-xylose</td>
<td>0.46</td>
</tr>
<tr>
<td>3-mono-O-methyl-D-xylose</td>
<td>Minor</td>
</tr>
<tr>
<td>2-mono-O-methyl-D-xylose</td>
<td>Minor</td>
</tr>
<tr>
<td>7-mono-O-methyl-L-fucose</td>
<td>Minor</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-galactose</td>
<td>0.59</td>
</tr>
<tr>
<td>2,4-di-O-methyl-D-galactose</td>
<td>0.11</td>
</tr>
<tr>
<td>2-mono-O-methyl-D-galactose</td>
<td>1.55</td>
</tr>
<tr>
<td>D-xylose</td>
<td>0.60</td>
</tr>
<tr>
<td>D-galactose</td>
<td>Minor</td>
</tr>
</tbody>
</table>
It has been shown that galactose is a component sugar of tragacanthic acid and so it is necessary to distinguish between methylated derivatives arising from this galactose and from the galactose formed by the reduction of galacturonic acid residues. $2,3,4,6$-tetra-0-methyl-$D$-galactose and $2,4,6$-tri-0-methyl-$D$-galactose must arise from the original galactose in the polysaccharide. The source of the remaining methylated galactose derivatives was determined by a comparison of the hydrolysis products from methylated tragacanthic acid and reduced methylated tragacanthic acid by paper and gas phase chromatography. Hydrolysed methylated tragacanthic acid was also fractionated on thick paper and those fractions containing methylated uronic acids were reduced with lithium aluminium hydride. The reduced methylated acids were then examined by paper and gas phase chromatography. The results of this investigation are tabulated below.

**Methylated derivatives of $D$-galactose.**

- $2,3,4,6$-tetra-0-methyl-$D$-galactose
- $2,4,6$-tri-0-methyl-$D$-galactose

**Methylated derivatives of $D$-galacturonic acid.**

- $2,3,4$-tri-0-methyl-$D$-galactose
- $2,3$-di-0-methyl-$D$-galactose
- $2,4$-di-0-methyl-$D$-galactose
- $0$-mono-0-methyl-$D$-galactose

It may then be deduced that the main structural units in tragacanthic acid are:

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyl 1-</td>
<td>19</td>
<td>-2 Xyl 1-</td>
<td>9</td>
<td>-3 Xyl 1-</td>
<td>4</td>
</tr>
<tr>
<td>Fuc 1-</td>
<td>3</td>
<td>- Fuc 1-</td>
<td>3</td>
<td>-4 GaLA 1-</td>
<td>13</td>
</tr>
<tr>
<td>GaL 1-</td>
<td>2</td>
<td>-2 Ara 1-</td>
<td>1</td>
<td>-4 GaLA 1-</td>
<td>1</td>
</tr>
<tr>
<td>GaLa 1-</td>
<td>1</td>
<td>-4 GaLa 1-</td>
<td>3</td>
<td>-4 GaLa 1-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3 GaLa 1-</td>
<td>1</td>
<td>-4 Xyl 1-</td>
<td>5</td>
</tr>
</tbody>
</table>

All sugars are pyranose.

It is evident that some galacturonic acid residues have been lost during methylation since in tragacanthic acid the molar ratio of galacturonic acid : xylose is 1 : 1.3 whereas in methylated tragacanthic acid it is 1 : 2. As a result of this loss it is not possible to give any quantitative structure for tragacanthic acid although a number of important features may be deduced.

From an examination of degraded tragacanthic acids and from partial hydrolysis studies it is probable that tragacanthic acid has a backbone of (1-4) linked $\alpha$-$\beta$-galacturonic acid residues. The failure to isolate any aldohiouronic acids during partial hydrolysis studies indicates that it is unlikely that xylose units form an integral part of this chain.

The presence of galacturonic acid residues linked through C1 and C4 has been found in the methylation study and is in agreement with a (1-4) linked galacturonic acid chain structure. Approximately 2 in every 3 residues of galacturonic acid are linked.
linked through positions 01, 03 and 04. It is probable that these residues form part of the galacturonic acid chain linked through 01 and 04 and therefore that the linkage through 03 is the main branch point. That this is the case has been confirmed by the isolation of 3-0-β-D-xylopyranosyl-D-galacturonic acid in good yield from fully degraded tragacanthic acid.

The presence of small amounts of galacturonic acid residues linked through 01 and 03 and also through 01, 02, 03 and 04 has also been indicated in the methylation study. The structural significance of these units is uncertain. In addition small amounts of terminal galacturonic acid units have been detected but, even assuming a linear chain structure, no estimate as to chain length can be made because of the degradation of acidic residues during methylation.

A possible partial structure for the central framework of tragacanthic acid incorporating the main features outlined above is:

\[-4\text{DGalAp}1\rightarrow4\text{DGalAp}1\rightarrow4\text{DGalAp}1\rightarrow4\text{DGalAp}1\rightarrow4\text{DGalAp}1\rightarrow4\text{DGalAp}1\rightarrow3\]

The ratio of terminal to non-terminal xylose in tragacanthic acid is approximately 1:1. The isolation of 3-0-β-D-xylopyranosyl-D-galacturonic acid and the high proportion of galacturonic acid residues with linkages through 03 indicates that this is the main mode of linkage between the xylose units and the galacturonic acid framework.

Almost/
Almost 50\% of the terminal xylose units are linked through positions C1 and C2. The remaining 50\% of the non-terminal xylose is present as branch point linked through C1, C2 and C3 and also C1, C2, C3 and C4. In view of the fact that these branched xylose units represent a relatively high percentage (25\%) of the xylose units it is improbable that they all arise from under-methylation, or demethylation during hydrolysis of the methylated polysaccharide and they are therefore of structural significance. Although the disaccharides 2-\(\beta\)-\(\alpha\)-D-fucopyranosyl-D-xylose and 2-\(\beta\)-\(\alpha\)-galactopyranosyl-D-xylose have been isolated by partial hydrolysis, xylobiose has not yet been characterised; it was noted, however, that on hydrolysis the unresolved mixture of oligosaccharides obtained by mild acid hydrolysis of tragacanthic acid, a considerably higher proportion of xylose than fucose and galactose was given, so that it is probable that xylobiose is present in the mixture. The presence of xylobiose would indicate that the polysaccharide has side chains containing at least two xylose units. In addition to terminal fucose almost 50\% of the fucose residues are non-terminal but the significance of these units is still uncertain.

The possible side chain units of tragacanthic acid will therefore include structures as:-
Tragacanthic acid, with its high content of galacturonic acid, may be classed with gums of group II. However, tragacanthic acid differs from these gums in sugar composition in that it contains, in addition to galacturonic acid, a high proportion of xylose and also some fucose. In physical properties e.g. viscosity, tragacanthic acid resembles the mucilages rather than the plant gums. It has also been noted that tragacanthic acid is associated with colloidal cellulose, which may be rendered insoluble by heating aqueous or acid solutions. A similar colloidal cellulose has been found in mucilages extracted from Flax, Cress, Quince and Mustard seeds.

The \((1\rightarrow4)\alpha-D-galacturonic\) acid framework of tragacanthic acid is very similar to that of pectic acid, but, although pectic acid has recently been shown to contain some neutral sugars, these do not correspond to the neutral sugars of tragacanthic acid and are present to a much lesser extent. Although the backbone structures of some plant gums from group II e.g. Khaya grandifolia and Khaya senegalensis (major fraction), contain \((1\rightarrow4)\) linked \(D\)-galacturonic acid residues these structures differ in/
in that rhamnose and galactose are also present as integral parts of the chain and that branch points are found only on the rhamnose units. The 6-deoxy hexose (fucose) present in tragacanthic acid is linked directly to xylose rather than galacturonic acid.

The xylose periphery of tragacanthic acid is unique and similar structures have not yet been found in other plant polysaccharides.
Part III. Arabinogalactan.

Hydrolysis Studies.

The arabinogalactan fraction from gum tragacanth has an arabino:galactose ratio of 6:1 and therefore differs from many hemicellulose arabinogalactans which have an arabino:galactose ratio of 1:6 or less. In addition to arabinose and galactose smaller amounts of galacturonic acid and rhamnose are also present.

Graded hydrolysis of the arabinogalactan with 0.05N sulphuric acid has given a degraded polysaccharide containing galactose and only small amounts of arabinose and rhamnose. The ready removal of arabinose indicates that it is predominantly in the furanose form.

Hydrolysis of the arabinogalactan with more concentrated acid followed by chromatography has indicated $\beta-\text{galactosyl-galactose}$, $\alpha-\text{galactosyl-galactose}$ in almost equal amounts, and also traces of $\alpha-\text{galactosyl-(1\rightarrow6)\text{-galactosyl-(1\rightarrow6)-galactose}}$ together with unidentified oligosaccharides.

Methylation Results.

The preparation of a fully methylated arabinogalactan has already been discussed. An attempt to fractionate the mixture of sugars obtained on hydrolysis of the methylated polysaccharide, by chromatography on charcoal:celite with aqueous butan-2-one as eluant was unsuccessful in that a large number of/
of fractions were mixtures of methylated sugars. Charcoal:celite columns have been successfully used for fractionation of methylated sugars from the methylated arabinogalactan of Western Larch by Bouveng et al., but this arabinogalactan is less complex than the arabinogalactan from gum tragacanth.

A second sample of methylated arabinogalactan was hydrolysed and the methylated sugars were fractionated on a cellulose column after removal of methylated uronic acids on IR 45-OH resin. The uronic acids were subsequently recovered from the resin by elution with formic acid and were finally reduced with lithium aluminium hydride and fractionated on thick paper.

The following sugars were identified.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Moles</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-tri-O-methyl-L-arabinose</td>
<td>2.06</td>
</tr>
<tr>
<td>2,5-di-O-methyl-L-arabinose</td>
<td>Minor</td>
</tr>
<tr>
<td>3,5-di-O-methyl-L-arabinose</td>
<td>0.10</td>
</tr>
<tr>
<td>2,3-di-O-methyl-L-arabinose</td>
<td>0.22</td>
</tr>
<tr>
<td>3,4-di-O-methyl-L-rhamnose</td>
<td>Minor</td>
</tr>
<tr>
<td>2,4,6-tri-O-methyl-D-galactose</td>
<td>0.27</td>
</tr>
<tr>
<td>2-mono-O-methyl-L-arabinose</td>
<td>0.22</td>
</tr>
<tr>
<td>3-mono-O-methyl-L-arabinose</td>
<td>0.70</td>
</tr>
<tr>
<td>2,3-di-O-methyl-D-galactose</td>
<td>Minor</td>
</tr>
<tr>
<td>2,4-di-O-methyl-D-galactose</td>
<td>0.35</td>
</tr>
<tr>
<td>4-mono-O-methyl-L-rhamnose</td>
<td>Minor</td>
</tr>
<tr>
<td>2-mono-O-methyl-D-galactose</td>
<td>0.46</td>
</tr>
<tr>
<td>L-arabinose/</td>
<td></td>
</tr>
</tbody>
</table>
Sugar                          Moles
L-arabinose                   0.75
D-galactose                   Minor
2,3,4-tri-O-methyl-D-galacturonic acid  0.10
2,3-di-O-methyl-D-galacturonic acid  Minor

It is possible that a number of the minor fractions detected in the methylation study arise from undermethylation of the polysaccharide or from demethylation during hydrolysis. A further source of error arises from the volatility of 2,3,5-tri-O-methyl-L-arabinose, some of which may have been lost during concentration of solutions to a syrup.

The main structural units of the arabinogalactan may then be deduced to be:

Araf 1- 20  -5 Ara 1-  2  -5 Ara 1-  7
GalAp 1-  1  -2 Ara 1-  1  -5 Ara 1-  2
  -3 Gal 1-  5  -6 Gal 1-  4
  -5 Ara 1-  7
  32
  -6 Gal 1-  5
  4 3

Partial hydrolysis studies have indicated that the galactan framework consists of (1-3) and (1-6) linked galactose units/
units. This is in agreement with methylation results which show that for every 12 galactose units, three are linked through C1 and C5, four are linked through C1, C3 and C6 and five are linked through C1, C3, C6 and C4.

It is apparent that the arabinose periphery of the arabinogalactan is very complex. In addition to a high proportion of terminal arabinose, arabinose is also linked through C1 and C5. However, single, double and triple branched arabinose units are also common with arabinose residues linked through C1, C2 and C5, through C1, C3 and C5, also through C1, C2, C3 and C5 being present.

The presence of small amount of galacturonic acid and rhamnose has been shown in the arabinogalactan. It is evident that the bulk of the galacturonic acid residues are present as end group although a small amount of galacturonic linked through C1 and C4 has been detected. The presence of rhamnose as non-terminal unit and to a lesser extent as branch point has also been found.

The technique of Smith degradation following periodate oxidation has been applied to e.g. the arabinogalactan from European larch, when it was shown that the galactan framework consisted of a (1→3) linked galactose chain with (1→6) linked side chains of galactose and arabinose.

The arabinogalactan from gum tragacanth was oxidised with sodium metaperiodate and, after destroying excess periodate with ethylene glycol, removal of iodate as barium iodate and de-ionisation/
de-ionisation, the oxidised polysaccharide was reduced with borohydride. The solution was then de-ionised and hydrolysed with acid under controlled conditions so that only hemi-acetal links and no glycosidic links were broken. After neutralisation the degraded arabinogalactan I was precipitated by addition of ethanol to the solution and the low molecular weight fraction I was recovered by concentration of the solution. Degraded arabinogalactan I was similarly treated a further twice to give degraded arabinogalactans II and III and low molecular weight fractions II and III.

Degraded arabinogalactan III on hydrolysis gave only galactose and arabinose in the approximate ratio of 1:1. Partial hydrolysis of degraded arabinogalactans I, II and III under the same conditions was followed by chromatography when the presence of almost equal amounts of 6-O-galactosyl-galactose, 3-O-galactosyl-galactose and in polysaccharide I and II traces of 0-galactosyl-(1→6)-O-galactosyl-(1→6)-galactose was shown confirming a preponderance of (1→3) and (1→6) linkages in the galactose units of the arabinogalactan.

Arabinogalactans I and II were methylated in the usual way. The methylated arabinogalactan and methylated degraded arabinogalactans I and II were then compared, after hydrolysis and methanolysis, by paper and gas phase chromatography. From these results it was apparent that the galactan framework had been largely untouched and that, although the proportion of terminal arabinose units was greatly decreased and was to a small/
small extent replaced by terminal galactose units, the arabinose periphery was still highly branched.

Chromatographic examination of the low molecular weight fractions released during the degradations showed large amounts of glycerol, which would arise from oxidation and degradation of terminal arabinofuranose units or galactose units. Small amounts of erythritol were found in the low molecular weight fractions from the second and third degradations and this would arise from (1→4) linked galactose units. In addition to these substances decreasing amounts of arabinosyl-glycerol were detected and small amounts of galactosyl-glycerol were also found after the third degradation. Arabinosyl-glycerol would arise from oxidation and degradation of units such as:

\[
\text{Araf} 1\rightarrow 5 \text{Ara} 1\rightarrow 5 \text{Ara} 1\rightarrow 2 \\
\begin{array}{c}
\text{Araf} \\
\end{array}
\]

\[
\text{Araf} 1\rightarrow 2 \text{Araf}
\]

It is evident that further degradations are required before definite information as to the nature of the galactan framework is obtained.

A comparison of the galactan frameworks of the arabinogalactan from gum tragacanth and that from the hemicellulose arabinogalactans shows that both are similar in that galactose residues are mainly linked through positions C1, C3 and C6 and to a smaller extent through C4. The galactan framework of group I plant gums is also similar in that galactose units are linked through C1, C3 and C6, but in those gums evidence indicates that/
that galactose units linked through C4 are absent.

The arabinose units in the peripheries of the hemicellulose arabinogalactans are normally either terminal or linked through C1 and C3. It has been shown, however, that Jeffrey pine arabinogalactan has in addition to terminal and (1→3) linked arabinose units a doubly linked arabinose unit. In the present case the arabinose periphery of the arabinogalactan of gum tragacanth is considerably more complex. The only other instances, so far investigated, where similar highly branched arabinose units are present, is in Cholla gum and the araban associated with pectic acid. In the case of Cholla gum the polysaccharide has a galactan framework linked in positions C1, C3 and C6 as well as a periphery of arabinose units which are terminal, linked through C1 and C5 and also as in gum tragacanth arabinogalactan linked through C1, C2, C3 and C5.

Although the arabinogalactan from gum tragacanth has been fractionated on diethylaminoethyl cellulose to give a single symmetrical peak a further possibility is that the arabinogalactan fraction is heterogeneous and consists of a highly branched araban similar to the araban associated with pectic acid and a simpler arabinogalactan with an arabinose periphery similar to the hemicellulose arabinogalactans.
EXPERIMENTAL.

General Methods.

Paper chromatography was carried out on Whatman No.1 filter paper using the following solvent systems, v./v.:—

C) Benzene:ethanol:water (169:47:15, Upper layer).
D) Butan-2-one half saturated with water.
H) Ethyl acetate:acetic acid:water (9:2:2).

Detection of sugars on chromatograms.

Reducing sugars were detected by spraying the dried chromatograms with saturated aqueous aniline oxalate and heating at 100°.

Sugar alcohols were detected by spraying with silver nitrate in acetone and then with sodium hydroxide in ethanol; after development of spots (1 - 3 minutes) the papers were treated with aqueous sodium thiosulphate and then washed with water.

Treatment of chromatograms with triphenyltetrazolium chloride in chloroform followed by sodium hydroxide in ethanol was used to detect reducing sugars with position C2 at the reducing/
reducing end free.

The rates of movement of the sugars were compared either to the solvent front ($R_f$ value) or to the rate of some convenient standard sugar e.g. galactose - $R_{Gal}$. Methylated sugars were compared with the rate of movement of 2,3,4,6-tetra-O-methyl-D-glucose in solvent A ($R_g$ value) and in other solvents with standard sugars.

Thick paper separations were carried out on Whatman No. 3MM paper which had previously been extracted with methanol. The position of sugars was determined by cutting narrow vertical strips from the paper and developing with an appropriate spray reagent. The sugars were eluted from the paper with water.

Ionophoresis (216) was carried out on Whatman No.1 filter paper in borate buffer solution at pH 10, and with a potential of 750 volts at 0.03 amp. The dried papers were sprayed with aqueous aniline oxalate acidified with glacial acetic acid. The rate of movement of sugars was compared with standard sugars.

Ionophoresis of polysaccharides was carried out on glass fibre sheets in the following systems:
A) 2N potassium hydroxide at a potential of 220 volts and 0.025 amp.
B) Borate buffer solutions at pH 10 at a potential of 1200 volts and 0.030 amp.

The dried papers were sprayed with alkaline potassium permanganate.

Column chromatography.

Cellulose columns were packed dry, washed with water and then/
then with the required solvent.

Charcoal columns were packed as a slurry of charcoal:celite (1:1) which had been previously washed with hydrochloric acid and then with water. The columns were eluted with increasing concentrations of aqueous ethanol. Slight acidity in the fractions was removed by treatment with Amberlite IR 4B-0H or IR 45-0H ion exchange resins, or where acidic oligosaccharides were present, with barium carbonate.

Fractions from columns were collected on an automatic fractionator and every 5th tube was concentrated and examined chromatographically. Fractions were bulked accordingly and were concentrated.

Solvents were purified as follows:

- Butan-1-ol was refluxed with sodium hydroxide (10g./litre) for one hour and then distilled.
- Light petroleum (b.p. 100/120°) was repeatedly shaken with concentrated sulphuric acid (100ml./litre) until the acid layer was almost colourless, and then with water until free of acid, and distilled.
- Butan-2-one was distilled.

**Ion-exchange resins.**

- Cations were removed with Amberlite IR 120-H resin and anions were removed with Amberlite IR 4B-0H or IR 45-0H resin.
- Evaporations were carried out under reduced pressure below 40°.
- Small scale hydrolysis on 5-25mg. of material were carried/
carried out at 100° in sealed glass tubes with dilute mineral acid (1 N unless otherwise stated). Where sulphuric acid was used, the solution was neutralised with barium carbonate, filtered, treated with 1R 120-N resin, filtered and evaporated to dryness. Hydrochloric acid was neutralised with silver carbonate, the solution was filtered, silver ions were removed with hydrogen sulphide, filtered, and evaporated to dryness. The organic material was extracted with hot acetone and the extracts taken to dryness.

Methyl glycosides and methyl ester methyl glycosides of sugars (1 - 100 mg.) were prepared by heating the sugar in a sealed tube at 100° with dry methanolic hydrochloric acid (4%; 1-10 ml.). The solution was neutralised with silver carbonate and concentrated.

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

Methoxyl contents were estimated by the semi-micro Zeissel method (216).

Acetyl contents were estimated by the method of Weissenberger (217).

Uronic acid anhydride contents were determined by decarboxylation (63).

Nitrogen estimations were carried out by the micro-Kjeldhal method.

Sugar estimations: Sugars were separated chromatographically (59) and after elution from the paper were estimated with periodate (59A).
Demethylation.

Methylated sugar (5-10 mg.) was treated with hydriodic acid (218) (1 ml.) in a sealed tube at 100° for 7-8 minutes. After dilution with water the solution was neutralised with silver carbonate and concentrated. Alternatively the methylated sugar in dry dichloroethane was treated with boron trichloride (219) at 70° for 30 minutes. The solution was allowed to evaporate at room temperature under anhydrous conditions, methanol was added and evaporated off. The syrup was then examined chromatographically in solvents E and F.

Borohydride reductions were effected by addition of excess potassium borohydride to an aqueous solution of the sugar and allowing to stand overnight. Excess borohydride was destroyed and potassium ions removed by addition of 1R 120-H resin. The solution was filtered, evaporated, and borate was removed by repeated addition and evaporation of methanol.

Periodate oxidation of methylated sugars was carried out by the method of Lemieux and Bauer (220). The results obtained were as follows:-

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Rp</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-O-methyl-hexose</td>
<td>0.15</td>
<td>Yellow</td>
</tr>
<tr>
<td>3-O-methyl-hexose</td>
<td>0.4-0.5</td>
<td>Pink</td>
</tr>
<tr>
<td>2-O-methyl-pentose</td>
<td>0.15</td>
<td>Yellow</td>
</tr>
<tr>
<td>3-O-methyl-L-rhamnose</td>
<td>0.20</td>
<td>Yellow (yellow U.V.)</td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td>Brown (yellow U.V.)</td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td>Brown (Pink U.V.)</td>
</tr>
<tr>
<td>2:5-di-O-methyl-D-galactose</td>
<td>0.66</td>
<td>Grey</td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>Grey</td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-galactose</td>
<td>0.15</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
Aniline derivatives of methylated sugars were prepared by dissolving equimolar amounts of sugar and freshly distilled aniline in dry ethanol and refluxing for 30 minutes under nitrogen in the dark. The solvent was evaporated and the derivative was recrystallised from ethanol.

Aldonolactones of methylated sugars were prepared by the addition of excess bromine to an aqueous solution of the sugar and allowing to stand in the dark for 2-3 days. Excess bromine was removed by aeration and the solution was neutralised with silver carbonate and evaporated to dryness. The syrup was extracted with dry acetone and crystallised.

Amides of aldonic acids were prepared by treating the lactones with dry methanolic ammonia at 0°C for 24 hours in the dark. The solution was evaporated to dryness when the derivative crystallised.

Polysaccharide precipitates unless otherwise stated were washed with ethanol and dried by solvent exchange with ethanol and then ether.

Gas-liquid-partition chromatography was carried out on a 'Pye Argon Chromatograph' instrument. Columns packed with

A) Apiezon M grease (20%) on celite at 150°C
B) Butane-diol-succinate polyester (15%) on celite at 150°C
C) Polyphenol (10%) on celite at 200°C

were used as the liquid phase. The gas flow rate (Argon) was maintained at 80-90ml. per minute. Relative retention times ($R_T$) of the methyl glycosides were compared to β-methyl-2,3,4,6-tetra-α-methyl-β-glucoside and are tabulated as follows.
<table>
<thead>
<tr>
<th>Methyl Glycoside</th>
<th>System A</th>
<th>System B</th>
<th>System C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L-Arabinose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-Me$_3^-$</td>
<td>0.58</td>
<td>1.04-1.05</td>
<td>0.83-0.84</td>
</tr>
<tr>
<td>2,3,5-Me$_3^-$</td>
<td>0.38-0.40</td>
<td>0.61 (s)</td>
<td>0.41 (s)</td>
</tr>
<tr>
<td>2,5-Me$_2^-$</td>
<td>0.41 (s)</td>
<td>1.59 (s)</td>
<td>0.63 (s)</td>
</tr>
<tr>
<td>2,4-Me$_2^-$</td>
<td>0.54 (m)</td>
<td>1.50 (w)</td>
<td>0.62 (m)</td>
</tr>
<tr>
<td></td>
<td>0.60 (a)</td>
<td>1.99 (m)</td>
<td>0.94 (m)</td>
</tr>
<tr>
<td>2,4-Me$_2^-$</td>
<td>0.71 (shoulder leading edge)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-Me$_2^-$</td>
<td>0.64 (s)</td>
<td>2.26</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.86-0.87 (w)</td>
<td>-</td>
<td>1.52-1.53</td>
</tr>
<tr>
<td>2,5-Me$_2^-$</td>
<td>0.46-0.47</td>
<td>1.99</td>
<td>0.67-0.63</td>
</tr>
<tr>
<td>3,5-Me$_2^-$</td>
<td>0.41-0.42 (w)</td>
<td>1.06</td>
<td>0.59-0.60</td>
</tr>
<tr>
<td></td>
<td>0.61 (s)</td>
<td>2.76</td>
<td>0.84</td>
</tr>
<tr>
<td>2-Me$_-$</td>
<td>0.64-0.65 (s)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.83 (w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Me$_-$</td>
<td>0.51 (w)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.70-0.71 (s)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.92 (w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D-Xylose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4-Me$_3^-$</td>
<td></td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>2,3-Me$_2^-$</td>
<td>0.45</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.51</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Me$_2^-$</td>
<td>0.51</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.63</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>3,4-Me$_2^-$</td>
<td></td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>2-Me$_-$</td>
<td>0.52</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.64</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.86?</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Methyl Glycoside</td>
<td>System A</td>
<td>System B</td>
<td>System C</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>D-xylose continued</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Me-</td>
<td>0.58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-Me-</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Galactose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-Me₄-</td>
<td>-</td>
<td>1.86 (shoulder)</td>
<td>1.53 (shoulder)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.99</td>
<td>1.62</td>
</tr>
<tr>
<td>2,3,4-Me₃-</td>
<td>1.64</td>
<td>-</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td></td>
<td>2.94</td>
</tr>
<tr>
<td>2,4,6-Me₃-</td>
<td>1.48</td>
<td>-</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>1.76</td>
<td></td>
<td>2.40</td>
</tr>
<tr>
<td>2,3,6-Me₃-</td>
<td>1.23 (s)</td>
<td>-</td>
<td>1.62 (s)</td>
</tr>
<tr>
<td></td>
<td>1.57 (w)</td>
<td></td>
<td>2.52 (s)</td>
</tr>
<tr>
<td></td>
<td>1.79 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Me₂-</td>
<td>2.10 (m)</td>
<td>-</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>2.44 (s)</td>
<td></td>
<td>4.40</td>
</tr>
<tr>
<td>2,3-Me₂-</td>
<td>1.69 (s)</td>
<td>-</td>
<td>2.45 (s)</td>
</tr>
<tr>
<td></td>
<td>2.33</td>
<td></td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td>2.62</td>
<td></td>
<td>4.19</td>
</tr>
<tr>
<td>2,6-Me₂-</td>
<td>1.57</td>
<td>-</td>
<td>2.51 (s)</td>
</tr>
<tr>
<td></td>
<td>1.98</td>
<td></td>
<td>3.21 (m)</td>
</tr>
<tr>
<td></td>
<td>2.32</td>
<td></td>
<td>3.77 (s)</td>
</tr>
</tbody>
</table>

s = strong.
m = medium.
w = weak peak
Part I. Purification and Fractionation of Gum Tragacanth.

The crude gum was obtained as white horny flakes contaminated by small pieces of bark etc.

Preliminary fractionation into water-soluble and insoluble fractions:

The crude gum (10g.) was stirred in water (1000ml.) for 24 hours to give a highly viscous solution. The solution was diluted with water (500ml.), stirred for 12 hours and then allowed to stand for 24 hours at 0°. Small particles of dirt were removed by filtration of the solution through muslin. Fraction A, the water-soluble gum, was separated from fraction B, the water-insoluble gum, by repeated centrifugation through a 'Sharples Super Centrifuge'. It was found that small amounts of B could still be removed even after six passages of the solution through the centrifuge. Fraction A (4.09g.) was obtained by concentrating the aqueous solution and then precipitating the polysaccharide by addition of ethanol containing 2% hydrochloric acid (7 vol.). Fraction B (5.85g.) was washed with water, then with ethanol and dried.

Fractional precipitation:

Fraction A (4.09g.) was dissolved in water (500ml.) and gradually increasing amounts of ethanol added with vigorous stirring. Polysaccharide precipitates A1, A2, A3, and A4 were removed at the centrifuge at 50%, 60%, 70%, and 80% concentration of ethanol. (See Table IVi). Fraction A4 was twice re-precipitated from aqueous solution with ethanol but/
TABLE IV. EXAMINATION OF FRACTIONS FROM GUM TRAGACANTH.

(i) Fractional precipitation with ethanol.

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Fr.</th>
<th>[α]_D</th>
<th>E.Wt.</th>
<th>Yield (g.)</th>
<th>Gal</th>
<th>A</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>+101</td>
<td>405</td>
<td></td>
<td>2.81</td>
<td>+++</td>
<td>tr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>+5</td>
<td>-</td>
<td>0.43</td>
<td>++</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>-35</td>
<td>-</td>
<td>0.32</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude gum</td>
<td>-50</td>
<td>1640</td>
<td>0.18</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10g.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>-65</td>
<td>575</td>
<td>2.85</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>-20</td>
<td>1100</td>
<td>0.50</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

(ii) Fractional dissolution with Cetyl trimethylammonium bromide.

<table>
<thead>
<tr>
<th>Fraction A</th>
<th>Fr.</th>
<th>[α]_D</th>
<th>E.Wt.</th>
<th>Yield (g.)</th>
<th>Gal</th>
<th>A</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5</td>
<td>+120</td>
<td>405</td>
<td>6.75</td>
<td>++</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>-40</td>
<td>1425</td>
<td>1.38</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>-35</td>
<td>1650</td>
<td>1.07</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction B</td>
<td>Fr.</td>
<td>[α]_D</td>
<td>E.Wt.</td>
<td>Yield (g.)</td>
<td>Gal</td>
<td>A</td>
<td>Ara</td>
<td>Xyl</td>
<td>Fuc</td>
<td>Rha</td>
<td>G</td>
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<tr>
<td>B5</td>
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<td>727</td>
<td>7.60</td>
<td>++</td>
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<td>+</td>
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</tr>
<tr>
<td>B6</td>
<td>-20</td>
<td>1050</td>
<td>1.53</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
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</table>

(iii) Esterification with ethylene oxide and fractionation of esters.

<table>
<thead>
<tr>
<th>Fraction A</th>
<th>Fr.</th>
<th>[α]_D</th>
<th>E.Wt.</th>
<th>Yield (g.)</th>
<th>Gal</th>
<th>A</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A8</td>
<td>-</td>
<td>-</td>
<td>1.78</td>
<td>+</td>
<td>tr</td>
<td>tr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>+104</td>
<td>383</td>
<td>7.18</td>
<td>+++</td>
<td>tr</td>
<td>tr</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>-7</td>
<td>1790</td>
<td>0.87</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(iv) Extraction of crude gum.

<table>
<thead>
<tr>
<th>Crude gum</th>
<th>Fr.</th>
<th>[α]_D</th>
<th>E.Wt.</th>
<th>Yield (g.)</th>
<th>Gal</th>
<th>A</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A14</td>
<td>-78</td>
<td>5500</td>
<td></td>
<td>3% 12% 76%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

(v) Fractionation on Diethylaminoethyl cellulose.

<table>
<thead>
<tr>
<th>Fraction A</th>
<th>Fr.</th>
<th>[α]_D</th>
<th>E.Wt.</th>
<th>Yield (g.)</th>
<th>Gal</th>
<th>A</th>
<th>Ara</th>
<th>Xyl</th>
<th>Fuc</th>
<th>Rha</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A13</td>
<td>+105</td>
<td>255mg.</td>
<td>45%</td>
<td>40%</td>
<td>tr</td>
<td>40%</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction A</td>
<td>Fr.</td>
<td>[α]_D</td>
<td>E.Wt.</td>
<td>Yield (g.)</td>
<td>Gal</td>
<td>A</td>
<td>Ara</td>
<td>Xyl</td>
<td>Fuc</td>
<td>Rha</td>
<td>G</td>
</tr>
<tr>
<td>A16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>+</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A17</td>
<td>-75</td>
<td>265mg.</td>
<td>+</td>
<td>+++</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Incomplete hydrolysis with 2N sulphuric acid, 18 hours, 100°. PI 72% sulphuric acid 3 days then 2N 12 hours 100° - large increase in G.

Chromatographic estimation: 

- ++ major
- ++ intermediate
- + minor
- tr trace

Sugar % are determined by periodate estimation after chromatographic separation.
but no decrease in the proportion of xylose or galacturonic acid was observed. The mother liquors from precipitation of A4 were evaporated to dryness leaving a brown residue. This material was dissolved in water and extracted by shaking with butan-1-ol. The pale yellow organic layer was separated and concentrated when a small amount of white amorphous material was separated - fraction C1. Hydrolysis of C1 gave glucose, arabinose and xylose. The formation of an oily precipitate during hydrolysis was also noted. When treated with concentrated sulphuric acid C1 gave a red colour.

Fraction B (5.85g.) was stirred with aqueous sodium hydroxide (1 N; 600 ml.) under nitrogen for 15 hours. Centrifugation gave an insoluble residue B1. The alkali solution was neutralised with glacial acetic acid and ethanol was added gradually with stirring to give two polysaccharide precipitates B3 and B4 at 50% and 70% concentration of ethanol respectively. (See Table IVi).

Fraction B1 was incompletely hydrolysed with sulphuric acid (2N) at 100°C for 18 hours. Treatment with 72% sulphuric acid at room temperature for 3 days and then with 2N acid for 12 hours at 100°C served to hydrolyse B1 completely when a large increase in the proportion of glucose was found.

Fractional Dissolution.

Cetyl trimethylammonium bromide (Cetavlon) is known to combine with acidic polysaccharides to give a water-insoluble polysaccharide-cetavlon complex (33). Fraction A (10g.) was dissolved/
dissolved in water to give a 1% solution. A saturated aqueous solution of cetavlon was added with stirring in slight excess. The precipitate of polysaccharide-cetavlon complex was centrifuged off and washed with water. The complex was decomposed by addition of excess aqueous sodium chloride (10%) to an aqueous suspension. Gradual addition of ethanol gave polysaccharide precipitates B5 and B6 at 40% and 70% concentration of ethanol respectively. The solution and washings from the complex were concentrated and ethanol was added to give a polysaccharide precipitate B4. (See Table IVa).

Fraction B2 (10g.) dissolved in alkali, was de-ionised with 1R 130-H resin (no polysaccharide was precipitated on neutralisation). The solution was treated with cetavlon as above and the polysaccharide-cetavlon complex removed at the centrifuge and washed with water. The complex was decomposed as above and addition of ethanol gave a polysaccharide precipitate B5. Concentration of the solution and washings followed by addition of ethanol gave a polysaccharide precipitate B6. (See Table IVb).

Fractionation of the gum as the glycol ester.

The glycol ester was prepared by the method of Deuel (221). Fraction A (10g.) in water (1000ml.) was de-ionised with 1R 130-H and 1R 4B-OH resins to give a solution of pH 2.4. Ethylene oxide (125ml.) was added slowly to the solution with shaking. After the final addition the mixture was placed on a shaker for 12 days when the solution was found to be neutral/
neutral. A precipitate A8, which formed during the reaction, was filtered off and washed with water. Addition of acetone to the solution and washings gave two polysaccharide precipitates A9 and A10 at 20% and 50% concentration of acetone respectively. (See Table IVii). During subsequent fractionations by this method it was observed that the yield of A8 depended largely on the number of times the original fraction A had been centrifuged in the preliminary fractionation. After six passages of A through the 'Sharples centrifuge' fraction A8 reduced to about 5% as compared with 15% after only one passage. Fractions A8 and B1 are similar in that both are incompletely hydrolysed with 2N sulphuric acid. In addition to the data given in Table IVii fraction A9 gave the following data.

\[ \text{OMe} \% < 1; \text{Glycol ester} 5.4\% \text{ (calculated 8\%); U.A.A. (decarboxylation) 41.3\% (corrected to free polysaccharide 43.5\%).} \]

**Extraction of the crude gum.**

The crude powdered gum (100g.) was extracted with ethanol in a Soxhlet extractor for 24 hours. The dark yellow alcohol solution was evaporated to dryness to give a yellow solid - fraction C. The residual crude gum, after the ethanol extraction, was further extracted with ethanol:water (7:3) twice for 30 hours on the shaker. The mixture was centrifuged and a polysaccharide fraction precipitated by addition of ethanol to the centrifugate. This fraction was dissolved in water and re-precipitated by gradual addition of ethanol to give a/
a major fraction of constant rotation - A14. (See Table IViv).

\[ \text{A14 : U.A.A.} \% 5.4; \text{ Ash} \ 1.0\% \text{ OMe} \ 0.5\% \]

The yield of fraction C was 5 - 4\% per 100\% of crude gum. Extraction of C with benzene, petrol or ether gave only traces of soluble material. Chromatographic examination of C in solvents A, E and F showed traces of free glucose and arabinose while the bulk of the material travelled at the solvent front. The free monosaccharides were removed by elution of the crude fraction (2g.) from a cellulose column (20 x 3cm.) with butan-l-ol saturated with water, as eluant. Hydrolysis of this purified fraction C' gave glucose, arabinose and a smaller amount of xylose. Addition of ethyl acetate to an ethanol solution of C' (2g.) gave a precipitate C2 (0.55g.) at 66% concentration of ethyl acetate. Fraction C3 (1.61g.) was recovered by evaporation of the mother liquors. C2 and C3 both gave a positive Molisch test and a red colour with concentrated sulphuric acid.

The Unidentified Sugar in the Gum.

The crude gum (10g.) was hydrolysed by heating at 100\° with sulphuric acid (1N: 1000ml.) for 12 hours. The acid solution was filtered and neutralised with barium hydroxide. After removal of barium sulphate at the centrifuge the neutral solution was de-ionised with IR 45-OH resin and IR 120-E resin and concentrated to a syrup. The syrup (7.2g.) was eluted from a cellulose column (68 x 3.8cm.) with butan-l-ol: ethanol:water (3:1:3: upper layer). The following fractions were/
were obtained. 1) Non-carbohydrate material. 2) Unidentified sugar, fucox, xylose, and arabinose. 3) Unidentified sugar (trace), fucox, xylose and arabinose. The remaining sugars on the column were eluted with water and discarded.

Examination of fraction 2. 840mg. 

Chromatography of the syrup in solvents A, E and F showed spots corresponding to arabinose (Rg 0.12 pink), xylose (Rg 0.15 pink), fucox (Rg 0.21 brown-yellow), and unidentified sugar (Rg 0.30 brown-yellow). The syrup was fractionated on a small cellulose column (57 x 2.8cm.), using solvent A as eluant, to give two fractions: a faster moving fraction 2A containing the unidentified sugar, and a slower moving fraction 2B containing fucox, xylose and arabinose.

Subfraction 2A. 95mg. Rg 0.30 [α]D = + 7° (c = 1.9)

Paper chromatography of the syrup in solvents A, E and F gave a single major spot corresponding to rhamnose and traces of faster moving material. Papers sprayed with vanillin/perchloric acid spray for deoxy sugars gave a single brown spot similar to that of rhamnose. Ionophoresis in borate buffer also gave a major spot running at the same rate as rhamnose.

The sugar was characterised by conversion into the p-toluene sulphonylhydrazone, m.p. 240-241° and mixed m.p. 241-242° with L-rhamnose p-toluene sulphonylhydrazone (m.p. 242-243°) prepared from authentic L-rhamnose.
Fractionation on Diethylaminoethyl cellulose columns (46).

A thin aqueous slurry of Whatman diethylaminoethyl cellulose (DEAE) (30 g.) was run into a column in small batches, air pressure being applied after addition of each batch, to give a column (32 x 30 cm.) of the cellulose derivative. The DEAE was generated in the phosphate form by elution with 0.5M sodium dihydrogen phosphate buffer (pH 6; 800 ml.). The column was then equilibrated with 0.005M sodium dihydrogen phosphate buffer (pH 6; 1000 ml.). After addition of the polysaccharide fraction the column was eluted with a) 0.025M (500 ml.), b) 0.05M (500 ml.), c) 0.10M (500 ml.), d) 0.25M (500 ml.) sodium dihydrogen phosphate buffer at pH 6. Elution was then continued with a linear gradient of sodium hydroxide (0.0-0.5N; 2000 ml.). A constant flow rate was maintained by the use of a water electrolysis cell at 20 ml./hour. Fractions (20 ml.) were collected and each fraction analysed for polysaccharide content. The polysaccharide in those fractions eluted with phosphate buffer was determined by the anthrone method (222) using a calibration curve based on L-arabinose as reference sugar. The polysaccharide in fractions eluted with alkali was estimated with carbazole using a calibration curve based on D-galacturonic acid (225).

Fraction A14 (335 mg.) was dissolved in 0.005M sodium dihydrogen phosphate buffer (pH 6; 6 ml.) and run on to the column which was allowed to stand for 4 hours. Elution was carried out as above. A plot of polysaccharide concentration per 20 ml. fraction against volume of eluate (Graph I) gave two/
two minor peaks A15 and A16 and one major peak A17. The fractions corresponding to each peak were bulked, dialysed against tap water overnight and de-ionised with ion-exchange resins. A15 and A16 were concentrated to a small volume and hydrolysed directly; A17 was treated with acetone to yield a polysaccharide precipitate. (See Table IVv). Fraction A17 was rechromatographed on a DEAE cellulose column when a single peak corresponding to the original peak was obtained,

\[
\text{A17 Uronic acid anhydride} = 5\% \text{ (carbazole method)} \\
= 3.2\% \text{ (decarboxylation)}
\]

Fraction A9 (355mg.) was de-esterified in sodium hydroxide (0.5N) for 3 hours at room temperature. The polysaccharide was then precipitated with ethanol, freeze dried, dissolved in 0.005M sodium dihydrogen phosphate buffer (pH 6; 5ml.) and run onto the column which was allowed to stand for 4 hours. The column was eluted as above. A plot of polysaccharide concentration per 20ml. fraction against volume of eluate (Graph II) showed two minor peaks A11 and A12 and a major peak A13. A11 and A12 were isolated in a similar manner to A15 and A16 above and hydrolysed; A13 was treated with acetone after removal of ions to give a polysaccharide precipitate. See Table IVv. Fraction A13 was rechromatographed on a DEAE cellulose column and gave a single symmetrical peak corresponding to the original peak.

\[
\text{A13 Uronic acid anhydride} = 40\% \text{ (carbazole method)} \\
= 43.2\% \text{ (decarboxylation)}
\]
Electrophoretic examination of the gum.

Samples of crude gum, fraction A9 (de-esterified) and fraction A14 were examined by electrophoresis on glass fibre sheets in borate buffer and potassium hydroxide (2N).

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Distance moved from starting line (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude gum</td>
<td></td>
</tr>
<tr>
<td>Borate buffer (3 hours)</td>
<td>Streaked.</td>
</tr>
<tr>
<td>Potassium hydroxide (7 hours)</td>
<td>0·0 Major</td>
</tr>
</tbody>
</table>

A larger scale separation of fractions A9 (de-esterified) and A14 (50mg.) was made on glass fibre sheets (12cm. wide) in potassium hydroxide. The position of spots was determined by spraying a narrow strip cut from the centre of the sheets. The major polysaccharide strips from A9 and A14 were then cut out and hydrolysed directly with sulphuric acid (2N) for 8 hours at 100°. Chromatographic examination of the hydrolysis products showed:

GalA Gal Ara Xyl Fuc Rha

A9 (major fraction) +++ tr tr +++ + -

A14 (minor fraction) + ++ +++ - - tr

Nomenclature.

A comparison of the results of fractionation (Table IV) indicates that there is a major acidic polysaccharide and a minor polysaccharide in gum tragacanth. For the remainder of this thesis the major acidic fraction will be referred to as/
as 'tragacanthic acid', and where any specific tragacanthic acid 'fraction' is mentioned the notation will be such as 'tragacanthic acid - A13'. The minor polysaccharide fraction will be referred to as the 'arabinogalactan' fraction and as with tragacanthic acid specific fractions will be referred to as 'arabinogalactan - A14'.

Methylation and Fractionation of the Methylated Polysaccharide.

Methylation A: Fraction A (10g.) in water (100ml.) was treated with dimethyl sulphate (150ml.) and aqueous sodium hydroxide (50%; 400ml.) added dropwise, simultaneously, over five hours with brisk stirring below 30°. Four further additions of Haworth reagents were made over successive days. The solution was then brought to pH 10 with dilute sulphuric acid and heated an a boiling water bath for 1 hour. The solution was then dialysed against tap water for five days and concentrated to 200ml. A small amount of material which separated during dialysis was filtered off and dried -MA1. The neutral solution was extracted with chloroform (5 times) and the dried (anhydrous sodium sulphate) chloroform extracts were evaporated to dryness to give a brown glassy solid - MA2. The aqueous solution was de-ionised with resins, neutralised with barium hydroxide, and the solution evaporated to dryness - MA3. (See Table V).

Methylation B: Fraction A (10g.) was acetylated by the method of Carson and Maclay (224). The acetate, in acetone (500ml.),
(500ml.), was methylated by seven additions of Haworth reagents as in methylation A. The corresponding fractions MB1, MB2 and MB3 were obtained. (See Table V).

Methylation C: Fraction A (10g.) was methylated as in methylation A except that the methylation reaction was conducted under nitrogen. Corresponding fractions MC1, MC2 and MC3 were obtained. (See Table V).

Methylation D: Powdered crude gum (10g.) was suspended in sodium hydroxide (50%: 350ml.) with brisk stirring under nitrogen for 24 hours. Dimethyl sulphate (150ml.) was then added dropwise over 6 hours. Six further additions of Haworth reagents were made as above. The corresponding fractions MD1, MD2 and MD3 were obtained. (See Table V).

Table V.

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Fraction</th>
<th>OMe %</th>
<th>$[\alpha]_D$</th>
<th>Yield (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction A - 10g.</td>
<td>MA1</td>
<td>20.0</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>MA2</td>
<td>35.4</td>
<td>-100(A)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>MA3</td>
<td>15.8</td>
<td>+70(W)</td>
<td>2.3</td>
</tr>
<tr>
<td>Fraction A - 10g.</td>
<td>MB1</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>(acetylated)</td>
<td>MB2</td>
<td>34.0</td>
<td>-95(A)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>MB3</td>
<td>14.8</td>
<td>+75(W)</td>
<td>2.0</td>
</tr>
<tr>
<td>Fraction A - 10g.</td>
<td>MC1</td>
<td>21.2</td>
<td>-105(A)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>MC2</td>
<td>33.5</td>
<td>-92(A)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>MC3</td>
<td>16.5</td>
<td>+80(W)</td>
<td>6.0</td>
</tr>
<tr>
<td>Crude gum - 10g.</td>
<td>MD1</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>MD2</td>
<td>33.0</td>
<td>-94(A)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>MD3</td>
<td>23.0</td>
<td>+91(W)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

C = chloroform, A = acetone, W = water.
An attempt was made to methylate tragacanthic acid glycol ester A9 (100g.) with sodium in liquid ammonia and methyl iodide according to the semi-micro method of Isbell et al. (86). The product isolated after three methylation reactions was hydrolysed directly and examined chromatographically in solvent A. The presence of substantial amounts of free galacturonic acid, xylose and fucose was demonstrated and the method was abandoned.

Fractions MA1, MB1, MC1 and MD1 were combined to give a partially methylated glycoside fraction - ML. Fractions MA2, MB2, etc., were combined to give partially methylated arabino-galactan fraction M2; while combination of MA3, MB3, etc. gave partially methylated tragacanthic acid M3.

**Methylation of Partially Methylated Tragacanthic Acid (M3)**

**with Silver Oxide/Methyl Iodide.**

Partially methylated tragacanthic acid (10g.) was dissolved in water, de-ionised with IR 120-H resin and converted to the silver salt by addition of silver oxide. The solution was concentrated and freeze dried. The solid was suspended in methyl iodide (200ml.) and refluxed for one hour under anhydrous conditions in the dark. A large amount of material remained insoluble and was dissolved by addition of dry methanol (50ml.). Silver oxide (15g.) was then added portionwise over six hours and refluxing was continued for a further four hours. The solution was then filtered and the silver residues were extracted with/
with chloroform on a Soxhlet extractor. The filtrate and chloroform extract were combined and evaporated to dryness. Yield 4.8 g. OMe - 26.7%. Three further treatments with Purdie reagents failed to raise the methoxyl content above 30%. Yield 3.0 g.: OMe 30%: Ash 0.01%.

Methylation of Partially Methylated Tragacanthic Acid (M3) with Barium Oxide and Methyl Iodide.

Partially methylated tragacanthic acid (M3) (1.75 g.) in dimethyl formamide (80 ml.) was treated with methyl iodide (20 ml.) and barium oxide (18 g.) and stirred vigorously overnight under anhydrous conditions. The mixture was filtered and the residues were exhaustively extracted with chloroform. The combined extracts (800 ml.) were extracted with water (3 x 100 ml.) and the water washings with chloroform (5 x 50 ml.). The combined dried chloroform extracts were then evaporated to dryness. Yield 0.4 g. OMe 28%.

Methylation of Tragacanthic Acid A9 with Thallous Hydroxide and Methyl Iodide.

Tragacanthic acid glycol ester A9 (10 g.) was dissolved in water (1000 ml.) and thallous hydroxide (20 g.) added with stirring under carbon dioxide free conditions. The solution was then concentrated and freeze dried. The dry product was added to methyl iodide (200 ml.) and dry methanol (150 ml.) and the mixture was refluxed for twelve hours in the dark with the/
the exclusion of moisture and carbon dioxide. The methanol and methyl iodide were distilled off under vacuum and benzene (200ml.) was added. To the resulting slurry thallous ethoxide (10g.) was added and the mixture was shaken for one hour. The benzene was then evaporated off and after addition of methyl iodide (100ml.) and methanol (50ml.), the mixture refluxed for eighteen hours as above.

The yellow residues left after removal of the methanol and methyl iodide were extracted with boiling chloroform in a Soxhlet extractor for eighteen hours. Evaporation of the chloroform extracts gave a product: Yield 3.8g.; OMe 28% \( [\alpha]_D = +90^\circ \) (chloroform).

To this product was added partially methylated tragacanthic acid (MS) (total 9.6g.) and a further methylation was carried out as above. Yield 7.0g.; OMe 51%.

Further methylations of this product were carried out with Purdie reagents. The partially methylated material was not, however, completely soluble in methyl iodide and a small quantity of methanol was added to give a solution. After three methylations a product was obtained (Yield 3.3g.; OMe 33.1%) which was still not completely soluble in methyl iodide.

A fourth Purdie methylation using tetrahydrofuran and dry acetone instead of methanol to aid solution gave a methylated polysaccharide (Yield 3.1g.; OMe 38.5%; \( [\alpha]_D = +90^\circ \)). A fifth methylation of this product (now soluble in methyl iodide) gave a fully methylated tragacanthic acid. Yield 3.0g.; OMe 38.6%; \( [\alpha]_D = +90^\circ \) (chloroform).
Methylation of the Arabinogalactan - Al4.

The arabinogalactan Al4 (5g.) was methylated by seven additions of Haworth reagents as outlined in Methylation C. A product was isolated by extraction with chloroform (Yield 4g.; OMe 34%).

This product was combined with partially methylated arabinogalactan M2 (total 6g.) and methylated fully by two reactions with Purdie reagents as above.

Fully methylated arabinogalactan was isolated. Yield 4.2g.; OMe 39.5%; $\left[\alpha\right]_D^\circ = -970$ (chloroform).
Part II. Tragacanthic Acid.

Partial Hydrolysis.

A. The preparation of Degraded Tragacanthic Acids.

1) Tragacanthic acid glycol ester - A9 was dissolved in water (100ml.) and the solution made 0.05N with respect to sulphuric acid. The solution was heated on a boiling water bath and samples (5ml.) were removed at intervals. Each sample was estimated for reducing power with Somogyi reagent - Graph 3. Degraded polysaccharide was precipitated with ethanol (5vol.) and filtered off. The solution was then neutralised with barium carbonate and examined chromatographically, as was the fully hydrolysed degraded polysaccharide (1N sulphuric acid: 10 hours).

After 25 hours the degraded polysaccharide contained only traces of fucose, galactose and arabinose and almost equal amounts of xylose and galacturonic acid. Only traces of galacturonic acid were found in the original hydrolysate. Small amounts of oligosaccharides were observed in this hydrolysate reaching a maximum after approximately 6 hours. Further quantities of this partially degraded tragacanthic acid I were prepared by hydrolysis of tragacanthic acid for 20 hours under the same conditions.

II) Tragacanthic acid glycol ester - A9 (2.5g.) was treated under the same conditions as above with 0.5N sulphuric acid. Samples/
Samples (3ml.) were taken and examined as above - Graph 3 - until no further precipitation of degraded polysaccharide with ethanol was possible (16 hours). After two hours the degraded polysaccharide was substantially free of fucoose and after 6 hours only small amounts of xylose remained with galacturonic acid. The original hydrolysate contained only small amounts of galacturonic acid after 6 hours.

The fully degraded tragacanthic acid II was prepared by hydrolysing tragacanthic acid for 6 hours under the above conditions.

An attempt to estimate the glycol ester content of tragacanthic acid glycol ester - \( A_0 \) and degraded tragacanthic acids I and II by saponification with alkali, and then estimation of the released ethylene glycol by addition of excess standard periodate solution followed by determination of the amount of periodate consumed, led to extremely high results, particularly with degraded tragacanthic acid II. The method was therefore modified so that, rather than estimate the periodate consumed by oxidation of ethylene glycol the formaldehyde released was estimated spectrophotometrically by/
by the method of McPadyen (225). Unexpectedly high results were also encountered in the determination of equivalent weight with sodium hydroxide in both degraded tragacanthic acids.

Comparison of Tragacanthic acid A9, and Degraded Tragacanthic Acids I and II.

<table>
<thead>
<tr>
<th></th>
<th>A9</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (from 5g.A9)</td>
<td>-</td>
<td>3.0g.</td>
<td>1.3g.</td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>+105</td>
<td>+175</td>
<td>+228</td>
</tr>
<tr>
<td>Glycol ester %</td>
<td>5.4</td>
<td>4.7</td>
<td>0.5</td>
</tr>
<tr>
<td>E. Wt. (NaOH)</td>
<td>383</td>
<td>326</td>
<td>202-189</td>
</tr>
<tr>
<td>U.A.A.%(decarboxylation)</td>
<td>41.3</td>
<td>46.4</td>
<td>68.5</td>
</tr>
<tr>
<td>U.A.A.%(corr. for glycol ester.)</td>
<td>43.5</td>
<td>48.5</td>
<td>68.5</td>
</tr>
</tbody>
</table>

B. Isolation of Oligosaccharides.

Attempted Isolation of Oligosaccharides by Mild Acid Hydrolysis.

It had been noted that traces of oligosaccharides occurred on chromatograms of certain fractions of the hydrolysate from the graded hydrolysis of tragacanthic acid with 0.05N acid reaching a maximum after about 6 hours. Further trial hydrolysis were carried out to find the optimum conditions for a maximum yield of disaccharides.

Tragacanthic acid - A9 (5g.) was heated at 100° in aqueous sulphuric acid (0.02N; 100ml.). Samples were taken at intervals (every 20 minutes between 4 and 8 hours), the degraded polysaccharide/
polysaccharide was precipitated by addition of ethanol and removed at the centrifuge, and the solution was neutralised with barium carbonate and concentrated. Each solution was eluted from a charcoal:celite column (1:1: 10g.) with water and then aqueous ethanol (5% and 10%). The eluates were concentrated and examined chromatographically in solvents A, D, E and H. A second hydrolysis was carried out in a similar manner but with 0.05N acid. Sugars, which did not correspond to standard monosaccharides appeared strongest on chromatograms of the hydrolysate taken after 5 hours 20 minutes (0.02N acid) and in the 5% ethanol elute from the column. These oligosaccharides were, however, still in small amount.

A larger scale hydrolysis was carried out. Tragacanthic acid - A9 (12g.) in sulphuric acid (0.02N; 750ml.) was heated at 100° for 5 hours 20 minutes. The solution was cooled and after addition of ethanol the degraded polysaccharide was removed at the centrifuge and washed with ethanol. The centrifugate and washings were neutralised with barium carbonate, filtered, de-ionised with IR 120-H resin and concentrated to give a pale yellow syrup (8.5g.).

The syrup was eluted from a charcoal:celite column (1:1: 100g.) with a linear gradient of aqueous ethanol (0.0 - 15%; 2000ml.) and then 50% ethanol (1000ml.). The following fractions were obtained.
Fraction.  Yield.  Sugars (Chromatographic analysis)
1    1.58g.  Fucose, arabinose.
2    0.286g. Fucose, xylose small amounts of galactose, arabinose and other traces.
3    0.073g. Oligosaccharides and unidentified sugar X.
4    0.101g. Oligosaccharides.

Examination of fraction 3.  0.073g.

The syrup was fractionated on thick paper in solvent H to give two fractions.

Subfraction 3A 0.024g.  $R_{	ext{Glu}}$ 0.8 - 1.50 (solvent H).

Chromatographic examination in solvents A, D, E, and H showed the presence of at least three oligosaccharides in this fraction but in no solvent was there a satisfactory separation. Hydrolysis and chromatography of the whole fraction gave xylose : fucose : galactose approximately 4:1:1.

Subfraction 3B 0.039g.  $R_{	ext{Glu}}$ 2.88 (solvent H) $[\alpha]_D = +8^\circ$.

Chromatography of the syrup in solvents A, D, E, and H showed a single brown-yellow spot (aniline oxalate). Vanillin/perchloric acid spray (266) gave a pinkish-grey spot indicating a 6-deoxy-hexose.

Ionophoresis in borate buffer gave a single spot $R_{\text{Rha}}$ 0.95.

The syrup was oxidised with sodium metaperiodate according to the method of Reeves (227) and the acetaldehyde-dimedone derivative isolated, m.p. and mixed m.p. 140 - 141°. Acetaldehyde would arise from periodate oxidation of a 6-deoxy-hexose.
Examination of fraction 4 0.101g.

Chromatography in solvents A, D, E, F, and H showed a mixture of oligosaccharides which were not satisfactorily separated. Elution of the syrup (90mg.) from a charcoal:celite column with a linear gradient of aqueous ethanol (0.0 - 15%) also failed to resolve the mixture further.

Attempted Isolation of Aldobiouronic Acid.

Tragacanthic acid - A₅ (200mg.) was heated at 100°C for 1 hour in sulphuric acid (1.0N; 20ml.). The solution was neutralised with barium carbonate, barium ions were removed with IR 120-H resin and the solution was concentrated. Chromatography in solvents E and F gave no indication of the presence of aldobiouronic acid in the hydrolysate.

A second hydrolysis was carried out on tragacanthic acid A₅ (1g.) in sulphuric acid (1N; 100ml.) for 10 hours at 100°C. The solution was then passed through a column of IR 45-OH resin (28 x 3cm.) and the neutral sugars were eluted with water (1000ml.). The resin was eluted with formic acid (1N; 300ml.). The formic acid eluant showed a negative test for sulphate and was concentrated. The final amounts of formic acid were removed by repeated additions and evaporation of water to give a syrup (520mg.).

Chromatography of the syrup in solvents E and F showed a large amount of galacturonic acid and traces of a slower moving sugar $R_{Gala} 0.54$ (solvent F). This latter fraction
was separated on thick paper in solvent \( P \) (7mg.). This fraction was converted to the methyl ester methyl glycoside and was reduced by treatment with sodium borohydride (15mg.) in water (5ml.) overnight. After de-ionisation of the solution with resins followed by acid hydrolysis only galactose was found on chromatographic examination.

Partial Acetolysis and De-acetylation of Tragacanthic Acid.

A trial experiment was carried out by adding tragacanthic acid acetate (1g.) to acetolysis mixture (10ml.: acetic acid: acetic anhydride:sulphuric acid : 10:10:1) at 0°. The mixture was then allowed to come to room temperature, placed on the shaker until the polysaccharide had dissolved (7 hours) and allowed to stand. Samples were taken after 24, 48, 60, 72 and 96 hours. Each sample was poured into water (100ml.) and the suspension was brought to pH 4 by addition of solid sodium bicarbonate. Each mixture was extracted with chloroform the chloroform extracts dried and concentrated.

The syrup was dissolved in dry methanol (5ml.) and barium methoxide (0.5M; 15ml.) was added at 0° with shaking. A flocculent precipitate formed and the mixture was left overnight at 0°. Each sample was then poured into water (50ml.), de-ionised with IR 120-H resin, filtered, concentrated and examined chromatographically in solvent \( H \). Oligosaccharides were observed in samples taken after 48, 60, 72 and 96 hours and in maximum quantity after 72 hours.

The acetolysis was repeated on a larger scale. Tragacanthic acid/
acid - A9 (15mg.) was acetylated by the method of Carson and Maclay (224). Tragacanthic acetate (12.5g.) was added slowly to acetylation mixture (500ml.) at 0°C, allowed to come to room temperature and placed on a shaker for 18 hours. The acetylation products were allowed to stand for a further 60 hours and were then precipitated by pouring the reaction mixture into water (4000ml.) at 0°C. The aqueous suspension was brought to pH 4 by addition of sodium bicarbonate and the precipitated acetates were removed at the centrifuge and washed with water. Treatment of the acetates with refluxing chloroform left a small amount of insoluble material which was discarded. The chloroform solution was dried with anhydrous sodium sulphate and concentrated to give a syrup (3.0g.).

This syrup was dissolved in dry methanol (150ml.) containing chloroform (10ml.) and barium methoxide (0.5N: 40ml.) was added at 0°C with shaking when a precipitate formed. The mixture was left overnight and, after checking that there was excess barium methoxide (phenolphthalein), was poured into water (3000ml.) when most of the precipitate dissolved. A small insoluble residue was filtered off, the aqueous solution was concentrated (1000ml.), barium ions were removed with IR 120-H resin and the solution was finally concentrated to a syrup. (3.6g.).

The syrup was eluted from a charcoal column (1:1: 160g.) with aqueous ethanol (0%, 2%, 5%, 10%, 15% all 1000ml.). Each fraction was treated with IR 45-OH resin to remove galacturonic/
galacturonic acid and any acidity from the column and was then concentrated to a syrup.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>%EtOH</th>
<th>Yield</th>
<th>Chromatographic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>900mg</td>
<td>Fuc., Ara., Xyl., Gal.</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>501mg</td>
<td>Fuc., Xyl., tr Gal., tr Rglu 1.0 orange brown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tr Rglu 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Solvent F</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>454mg</td>
<td>Xyl., tr Gal., Rglu 1.0, 0.6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>84mg</td>
<td>Material on starting line.</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>50mg</td>
<td>Material on starting line.</td>
</tr>
</tbody>
</table>

**Examination of fraction 3 0.454g.**

It was evident that the bulk of the oligosaccharides were in this fraction. Chromatography in solvents E and F showed two major oligosaccharides, a faster moving sugar A and slower moving sugar B, together with some monosaccharides. \( R_{\text{Glu}} \) values for A and B were

<table>
<thead>
<tr>
<th>Solvent</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1.4</td>
<td>0.95</td>
</tr>
<tr>
<td>F</td>
<td>1.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

An attempted fractionation of A and B by elution from a charcoal:celite column (1:1; 30g.) with a linear gradient of aqueous ethanol (0.0% - 7%) gave three fractions.

1) monosaccharides and B,  ii) B > A,  iii) A > B.

Each of these fractions was then separated on thick paper in solvent F and those areas of the paper corresponding to A and B were eluted with water.

**Fraction A/**
Fraction A  110mg.  $[\alpha]_D = -60^\circ (c = 1:1)$

The fraction crystallised on standing and was twice recrystallised from ethanol/water.  m.p. 185 - 190°(d)

$[\alpha]_D = -61^\circ (c = 0.71)$.

Hydrolysis of A (2mg.) with dilute acid gave only fucose and xylose in equal amounts.  Reduction of A (2mg.) with sodium borohydride gave fucose and xylitol indicating that xylose was the reducing unit of the disaccharide.  A negative reaction to triphenyltetrazolium salt indicated that position C2 of the xylose residue was blocked i.e. that the disaccharide was probably 2-0-L-fucosyl-D-xylose.

Methylation  The disaccharide (80mg.) in water (1ml.) was treated with dimethyl sulphate (1ml.) and aqueous sodium hydroxide (30%; 1ml.) added dropwise with stirring over 1 hour; then dimethyl sulphate (8ml.) and sodium hydroxide (30%; 14ml.) over 4 hours.  The solution was stirred overnight and two further additions were made over two successive days.  The solution was then heated on a boiling water bath to destroy excess dimethyl sulphate, cooled and extracted with chloroform.  The dried chloroform extracts were combined and concentrated (65mg.).

A trial hydrolysis followed by chromatographic examination showed two major sugars - $R_g$ 0.92, 0.75 and traces of partially methylated material.  The remainder of the methylated disaccharide was hydrolysed and fractionated on thick paper in solvent A.

Subfraction Al  28mg.  $R_g 0.92 [\alpha]_D = -118^\circ (c = 1.4)$
The/
The sugar was chromatographically pure and corresponded to 2,3,4-tri-0-methyl-L-fucose. The sugar was crystallised from aqueous ethanol m.p. and mixed m.p. 62 - 63° with authentic 2,3,4-tri-0-methyl-L-fucose. The sugar was characterised by conversion into the aniline derivative m.p. 132 - 133°.

**Subfraction A2** 30mg.  \( R_G 0.75 \left[ \alpha \right]_D = +21° (\varphi = 1.5) \).

The sugar was chromatographically pure and corresponded to 3,4-di-0-methyl-D-xylose. The sugar was characterised by conversion into 3,4-di-0-methyl-D-xylonolactone m.p. and mixed m.p. 67 - 68° with authentic sample.

**Fraction B** 60mg. \( \left[ \alpha \right]_D = -40° (\varphi = 3.0) \).

Hydrolysis of B (2mg.) with dilute acid gave only galactose and xylose in equal amounts. Reduction of the syrup (2mg.) with sodium borohydride followed by hydrolysis gave galactose and xylitol indicating that xylose was a reducing unit. A negative reaction to triphenyltetrazolium salt indicated that position C2 of the xylose unit was blocked, so that the disaccharide was probably 2-0-D-galactosyl-D-xylose.

The disaccharide was methylated in a similar manner to disaccharide A (41mg.). A trial hydrolysis showed only two major sugars \( R_G 0.88, 0.75 \). The remainder of the methylated sugar was hydrolysed and fractionated on thick paper in solvent A.
G.P.C.  

System A  

Subfraction Bl 18mg. \( R_g 0.88 \left[ \alpha \right]_D = +118 \) (\( c = 0.91 \)).  
The sugar was chromatographically pure and corresponded to 2,3,4,6-tetra-O-methyl-D-galactose. The sugar was characterised by conversion into 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. and mixed m.p. 188 - 189°.  

Subfraction B2 17mg. \( R_g 0.74 \left[ \alpha \right]_D = +23 \) (\( c = 0.85 \)).  
The sugar was chromatographically pure and corresponded to 3,4-di-O-methyl-D-xylose. The sugar was characterised by conversion into 3,4-di-O-methyl-D-xylonolactone m.p. and mixed m.p. 67 - 68°.  

The Action of Hemicellulase and Pectinase enzymes on Tragacanthic Acid and Degraded Tragacanthic Acid.  

Tragacanthic acid (100mg.), degraded tragacanthic acid I and II (100mg.) were each dissolved in water (100ml.) and brought to pH 3 by addition of acetic acid. One set of samples was digested with hemicellulase enzyme and the other with pectinase enzyme (25mg.). Samples were taken at 8 hour intervals over 24 hours; any residual polysaccharide was precipitated by addition of ethanol, filtered off and hydrolysed. The solutions and polysaccharide hydrolysates were examined chromatographically/
chromatographically in solvent H.

Both enzyme preparations showed little action on tragacanthic acid and liberated only small amount of galactose and arabinose.

The enzymolysis products from the degraded tragacanthic acids after 8 hours are tabulated below.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Xyl</th>
<th>GaLA</th>
<th>R_GaLA</th>
<th>0.72</th>
<th>0.51</th>
<th>0.38</th>
<th>0.26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tragacanthic acid I</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td></td>
</tr>
<tr>
<td>Tragacanthic acid II</td>
<td>++</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>tr</td>
<td>+</td>
</tr>
</tbody>
</table>

Increase in the time of enzymolysis gave a larger amount of monosaccharides. An examination of the products of the residual polysaccharides after complete acid hydrolysis showed xylose and galacturonic acid in the same proportions throughout. No polysaccharide could be precipitated from the digests after 32 hours.

**Large Scale Enzymolysis of Degraded Tragacanthic Acid**

Degraded tragacanthic acid I (3g.) was dissolved in water (1000ml.) at pH 3 (acetic acid) and hemicellulase (1.2g.) was added with shaking. The solution was allowed to stand for 8 hours, an equal volume of acetone was added (at 0°C) and the resulting precipitate of enzyme and residual polysaccharide removed at the centrifuge. The precipitated material (3.2g.) was redissolved in water (800ml.) at pH 3 and allowed to stand for a further 8 hours. The enzymolysis was repeated a further twice. The combined centrifugates were concentrated (500ml.) neutralised with ammonia, evaporated to dryness (2.05g.) and/
and separated chromatographically on thick paper sheets (Whatman No. 51 extra thick) in solvent F. Sugars at $R_{\text{GalA}}$ 0.72 (i), 0.51 (ii), and 0.38 (iii) were eluted from the paper.

**Fraction i** 285mg. $R_{\text{GalA}}$ 0.72 (solvent F pink).

$$[\alpha]_D^0 = +20^\circ (c = 0.57).$$

The sugar, with a similar fraction from the enzymolysis of degraded tragacanthic acid II, was isolated as the ammonium salt and was chromatographically pure. Hydrolysis of the syrup (6mg.) with N sulphuric acid for 4 hours at 100° gave xylose and galacturonic acid only in equal amount. Reduction of the sugar (5mg.) with sodium borohydride followed by hydrolysis gave xylose and galactonic indicating that galacturonic acid is the reducing unit of the disaccharide.

The free disaccharide (10mg.) after reduction with borohydride, was dissolved in water (24ml.) and oxidised by addition of sodium metaperiodate (1ml.; 0.1M). A sample (1ml.) was analysed spectrophotometrically by the method of McFadyen (225) for formaldehyde. Approximately 1 mole of formaldehyde was released per mole of sugar, which would arise from a disaccharide with a non-reducing xylose residue attached to C5 or C4 of galacturonic acid.

**Methylation** The sugar (i) (200mg) was methylated in the same manner as disaccharide A except that nitrogen was passed into solution to minimise oxidative degradation. After three additions of Haworth reagents the solution was heated to 100° to/
to destroy excess dimethyl sulphate and, after cooling, brought to pH 3 with sulphuric acid. The slightly acid solution was extracted with chloroform exhaustively, the extracts dried and concentrated (70mg.). The dry syrup was converted to the methyl ester methyl glycoside, dissolved in tetrahydrofuran (5ml.), lithium aluminium hydride in tetrahydrofuran (150mg. in 10ml.) added dropwise and the solution was refluxed for 2 hours. Excess hydride was decomposed by addition of water, the organic layer separated and the aqueous layer taken to dryness. The residue was extracted several times with acetone and chloroform and the extracts combined and taken to dryness (58mg.).

A trial hydrolysis of this neutral disaccharide with N sulphuric acid showed small amounts on nonmethylated material and a further methylation of the partially methylated disaccharide was carried out with Haworth reagents. The fully methylated disaccharide was isolated in the usual way. Examination of the hydrolysis products showed two spots R 0.93 and 0.41. The two sugars were separated on thick paper in solvent A.

Subfraction iA 25mg. R 0.41 \([\alpha]_D = +82\) (c = 1.1).

The sugar was chromatographically pure and corresponded to 2,4-di-O-methyl-D-galactose. The syrup crystallised on standing and after recrystallisation from acetone containing 1% water gave m.p. 102° and mixed m.p. 101-102° with authentic 2,4-di-O-methyl-D-galactose monohydrate m.p. 101°. The sugar/
sugar was characterised by conversion into 2,4-di-O-methyl-\(\alpha\)-phenyl-\(\alpha\)-galactosylamine m.p. and mixed m.p. 210°.

Subfraction IB 22mg.  \(R_0\) 0.98 \([\alpha]_D = +30^\circ\) (\(c = 1.1\))

The sugar was chromatographically pure and corresponded to 2,3,4-tri-O-methyl-\(\beta\)-xylose. The syrup crystallised on standing and after recrystallisation from ethanol/water gave m.p. and mixed m.p. 2,3,4-tri-O-methyl-\(\beta\)-xylose 90 - 91°.

Large Scale Enzymolysis of Degraded Tragacanthic Acid II.

Degraded tragacanthic acid II (3g.) was digested with hemicellulose enzyme (1.8g.) in a similar manner to degraded tragacanthic acid I. After three treatments with enzyme the solutions were concentrated and after neutralisation with ammonia were evaporated to dryness (1.71g.). The syrup was fractionated on thick paper (Whatman No.31 extra thick) in solvent F. Sugars with \(R_{\text{gala}}\) 0.72 (i), 0.61 (ii), 0.38 (iii), and 0.26 (iv) were eluted from the papers. Fraction i was combined with fraction i from the enzymolysis of tragacanthic I. Fraction ii 93mg.  \(R_{\text{gala}}\) 0.72 (solvent F)

This fraction was combined with fraction ii from the enzymolysis of degraded tragacanthic acid I. The fraction was chromatographically pure and corresponded to (1→4)\(\alpha\)-\(\beta\)-linked digalacturonic acid in solvents F and G. The syrup was de-ionised with IR 120-H resin and the calcium salt formed by addition of calcium carbonate to bring the solution to pH 7. Addition of acetone to the solution gave a white precipitate of the/
the calcium salt of digalacturonic acid which was filtered off and dried. \([\alpha]_D = +182^\circ (c = 1.2 \text{ in } \text{N-hydrochloric acid})\).

A comparison of the I.R. spectra of this compound with that of the calcium salt of \((1\rightarrow4)-\alpha\text{-digalacturonic acid} \), showed they were identical.

**Fraction iii** 10mg. \(R_{\text{GalA}} 0.38\) (solvent F)

The syrup was isolated as the calcium salt. Hydrolysis gave xylose \(<\) galacturonic acid.

**Fraction iv** 12mg. \(R_{\text{GalA}} 0.26\) (solvent F)

\(\text{Ca Salt } [\alpha]_D = +161^\circ (c = 0.6 \text{ in } \text{N hydrochloric acid})\)

The fraction was converted to the calcium salt and isolated by precipitation with acetone from aqueous solution. Hydrolysis of the precipitate showed only galacturonic acid. The I.R. spectra of this compound and the calcium salt of trigalacturonic acid were identical. It was noted, however, that the I.R. spectra of both di and trigalacturonic acids were also identical.

**Attempted acetylation of Tragacanthic Acid II prior to Reduction**

The reducing end groups of tragacanthic acid II (4g.) in water (100ml.) were reduced by addition of sodium borohydride (5g.). The solution was then de-ionised with resins and freeze dried. The polysaccharide was then dissolved in formamide (50ml.) and pyridine (65ml.) added dropwise over 4 hours with stirring. After stirring for 18 hours acetic anhydride (40ml.) was added over 5 hours and the reaction mixture stirred for a further 18 hours. A trial sample poured into water, acidified/
acidified with hydrochloric acid (4%) failed to give precipitate of the acetate. A second acetylation was carried out by further addition of pyridine (45ml.) and acetic anhydride (30ml.) dropwise simultaneously over 5 hours and then allowing the solution to stir for 30 hours. The polysaccharide acetate still failed to precipitate when poured into acidified water. Addition of light petroleum (b.p. 60/80°) to the reaction mixture also failed to precipitate any acetate.

An attempt to make the polysaccharide propionate by the method of Carson and McLay (234) was also unsuccessful.

Examination of Methylated Tragacanthic Acid.

Reduction of the Methylated Acid.

The fully methylated tragacanthic acid (2·8g.; OMe 38.6%) was dissolved in dry tetrahydrofuran (150ml.) and lithium aluminium hydride in tetrahydrofuran (3·0g. in 25ml.) added dropwise. The mixture was refluxed for 2 hours and further addition of hydride (1·0g.) was made. The solution was refluxed for a further 1 hour and allowed to stand for 18 hours. Excess hydride was destroyed by addition of ethyl acetate and the solution was brought to pH 4 by addition of dilute sulphuric acid. The acid solution was extracted with chloroform (4x) and the dried extracts were concentrated to a small volume. Addition of light petroleum (b.p. 60/80°) to the chloroform solution gave a precipitate of reduced methylated tragacanthic acid (1·9g.).

The/
The aqueous layer was neutralised with sodium bicarbonate and evaporated to dryness. The dry powder was extracted with chloroform and addition of light petroleum (b.p. 60/80°) to the dried extracts gave a further precipitate of reduced polysaccharide (0.25g.).

Total Yield 2.15g. OMe 31.8% \( [\alpha]_D = +78° \) (c = 1.2 chloroform)

A sample of reduced methylated tragacanthic acid was hydrolysed and examined chromatographically in solvents A, B and E. After spraying chromatograms with aniline oxalate, indicator sprays and potassium iodate/potassium iodide/starch spray, no traces of methylated uronic acid were found. A carbazole determination gave uronic anhydride < 2%.

Hydrolysis of the Reduced Methylated Tragacanthic Acid.

Methylated reduced tragacanthic acid (1.9g.) was dissolved in cold N-hydrochloric acid (125ml.) and allowed to stand at room temperature for 2 days. The solution was gradually warmed to 100°, avoiding precipitation of partially hydrolysed material, over 4 days. Hydrolysis was completed by heating the solution at 100° for 10 hours (constant rotation). The hydrolysate was neutralised with silver carbonate in the usual way and concentrated to a syrup.

Examination of the Hydrolysis Products.

The methylated sugars (1.7g.) from the hydrolysis of the methylated reduced tragacanthic acid were separated by chromatography on a cellulose column (74 x 4cm.). The sugars were/
were eluted with the following systems:

Light petroleum (b.p. 100/120°):butan-1-ol (80:20) saturated with water (until trimethyl pentoses eluted).

Light petroleum (b.p. 100/120°):butan-1-ol (70:30) saturated with water (until trimethyl hexoses eluted).

Light petroleum (b.p. 100/120°):butan-1-ol (50:50) saturated with water (until dimethyl hexoses eluted).

Butan-1-ol half saturated with water (until monomethyl hexoses eluted) and then with water.

The following fractions were obtained and examined chromatographically in solvent A. The results are tabulated as follows.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (g)</th>
<th>Colour</th>
<th>$R_g$</th>
<th>Sugars Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.397</td>
<td>red, grey-yellow</td>
<td>0.93</td>
<td>2,3,4-tri-O-methyl-D-xylose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.88</td>
<td>2,3,4-tri-O-methyl-L-fucose tr.</td>
</tr>
<tr>
<td>2</td>
<td>0.131</td>
<td>red, grey-yellow</td>
<td>0.93</td>
<td>2,3,4-tri-O-methyl-D-xylose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.88</td>
<td>2,3,4-tri-O-methyl-L-fucose</td>
</tr>
<tr>
<td>3</td>
<td>0.148</td>
<td>red-brown, grey-yellow</td>
<td>0.88</td>
<td>2,3,4,6-tetra-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
<td>di-O-methyl-L-fucose ?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
<td>3,4-di-O-methyl-D-xylose</td>
</tr>
<tr>
<td>4</td>
<td>0.172</td>
<td>red</td>
<td>0.76</td>
<td>3,4-di-O-methyl-D-xylose</td>
</tr>
<tr>
<td>5</td>
<td>0.032</td>
<td>red, pink</td>
<td>0.74</td>
<td>3,4-di-O-methyl-D-xylose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.68</td>
<td>2,4,6-tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td>6</td>
<td>0.019</td>
<td>red-brown</td>
<td>0.65</td>
<td>2,3,4-tri-O-methyl-D-galactose trace monomethyl fucose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>monomethyl fucose ?</td>
</tr>
<tr>
<td>7</td>
<td>0.020</td>
<td>red-brown, brown</td>
<td>0.68</td>
<td>2,3,6/2,3,4-tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
<td>monomethyl fucose ?</td>
</tr>
<tr>
<td>8</td>
<td>0.021</td>
<td>grey-brown</td>
<td>0.52</td>
<td>3-O-methyl-L-fucose ?</td>
</tr>
<tr>
<td>9</td>
<td>0.022</td>
<td>brown, red</td>
<td>0.55</td>
<td>dimethyl galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
<td>monomethyl xylose.</td>
</tr>
<tr>
<td>10</td>
<td>0.155</td>
<td>brown, red</td>
<td>0.43</td>
<td>2,3-di-O-methyl-D-galactose</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>0.39</td>
<td>4-O-methyl-D-xylose</td>
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<tr>
<td>11</td>
<td>0.040</td>
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<td>0.40</td>
<td>2,4-di-O-methyl-D-galactose</td>
</tr>
<tr>
<td>12</td>
<td>0.022</td>
<td>pink, brown</td>
<td>0.29</td>
<td>5-O-methyl-D-xylose</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
<td>?</td>
</tr>
<tr>
<td>13</td>
<td>0.030</td>
<td>pink, brown</td>
<td>0.29</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
<td>2-O-methyl-D-galactose</td>
</tr>
<tr>
<td>14</td>
<td>0.320</td>
<td>brown</td>
<td>0.23</td>
<td>2-O-methyl-D-galactose</td>
</tr>
<tr>
<td>15</td>
<td>0.150</td>
<td>brown, pink, brown</td>
<td>0.23</td>
<td>2-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
<td>D-Xylose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
<td>D-galactose</td>
</tr>
</tbody>
</table>
Examination of Fractions.

**Fraction 1. 0.397 g.** $R_g 0.93$, trace 0.88. $[\alpha]_D = +16^\circ (c = 1.5)$

Chromatography of the syrup in solvents A, C, D and E showed a major component ($R_g 0.93$, red) corresponding to 2,3,4-tri-$O$-methyl-$D$-xylose and a trace ($R_g 0.88$, yellow grey) corresponding to 2,3,4-tri-$O$-methyl-$L$-fucose.

**G.P.C. System A.**

$R_f 0.38, 0.45$. methyl-2,3,4-tri-$O$-methyl-$D$-xyloside.

Demethylation gave xylose.

The fraction crystallised on standing and the crystals, after drying on porous tile gave m.p. and mixed m.p. $90-91^\circ$ with authentic 2,3,4-tri-$O$-methyl-$D$-xylose,

$[\alpha]_D = +60^\circ \rightarrow +17^\circ (c = 1.2)$

The sugar was finally characterised by conversion into 2,3,4-tri-$O$-methyl-$D$-xylonolactone, m.p. and mixed m.p. $55-56^\circ$.

**Fraction 2. 0.131 g.** $R_g 0.93, 0.87 [\alpha]_D = -80^\circ (c = 1.4)$

Chromatography of the syrup in solvents A, C, D and E gave spots corresponding to 2,3,4-tri-$O$-methyl-$D$-xylose and 2,3,4-tri-$O$-methyl-$L$-fucose.

Demethylation gave xylose and fucose.

The syrup was fractionated on thick paper in solvent E to give two fractions.

**Subfraction 2A. 0.040 g.** $R_g 0.93 [\alpha]_D = +20^\circ (c = 0.8)$

Chromatography of the syrup in solvents A, C, D, and E showed a spot corresponding to 2,3,4-tri-$O$-methyl-$D$-xylose and a trace of 2,3,4-tri-$O$-methyl-$L$-fucose. The syrup partly crystallised.
crystallised on standing and after drying a few crystals on porous tile these gave m.p. and mixed m.p. 90-91° with authentic 2,3,4-tri-O-methyl-D-xylose.

**Subfraction 2B.** 0.061g.  
\[ \alpha \] D = -118° (c = 0.75)  
Chromatography of the syrup in solvents A, C, D and E showed spots corresponding to 2,3,4-tri-O-methyl-L-fucose and a trace of 2,3,4-tri-O-methyl-D-xylose.

Demethylation gave fucose and a trace of xylose.

The syrup crystallised on standing and was recrystallised as the monohydrate from ethanol containing 1% water m.p. 63-64° and mixed m.p. with authentic 2,3,4-tri-O-methyl-L-fucose monohydrate.

The sugar was characterised by conversion into the crystalline aniline derivative m.p. 132-133°.

**Fraction 3.** 0.148g.  
\[ \alpha \] D = -12° (c = 1.3)  
Chromatography of the syrup in solvents A, C, D and E gave at least three components corresponding to 2,3,4,6-tetra-O-methyl-D-galactose (\( R_g \) 0.88 red brown), dimethyl fucose (grey yellow \( R_g \) 0.80) and 3,4-di-O-methyl-D-xylose (\( R_g \) 0.76 red) with traces of trimethyl fucose and trimethyl xylose.

Demethylation gave galactose, fucose and xylose.

The syrup was fractionated on thick paper in solvent C to give two fractions.

**Subfraction 3A.** 0.044g.  
\[ \alpha \] D = +116° (c = 0.9)  
Chromatography/
Chromatography of the syrup in solvents A, C, D and E showed that the main component corresponded to 2,3,4,6-tetra-α-methyl-D-galactose together with traces of trimethyl fucose, trimethyl xylose and 3,4-di-α-methyl-D-xylose.

Ionophoresis in borate buffer gave a non mobile red brown spot and a mobile pink spot corresponding to 3,4-di-α-methyl-D-xylose.

The major sugar was characterised by conversion into 2,3,4,6-tetra-α-methyl-D-phenyl-D-galactosylamine m.p. and mixed m.p. 189-190°C.

Subfraction 3R. 0.096 g. Rf 0.80, 0.76, trace 0.88

[α]D = -40° (c = 0.98)

Chromatography of the syrup in solvents A, C, D and E showed two major components corresponding to dimethyl fucose and 3,4-di-α-methyl-D-xylose with a trace of tetra-α-methyl-D-galactose.

Demethylation gave fucose, xylose and trace of galactose.

Ionophoresis gave a non mobile brown spot and a mobile pink spot corresponding to 3,4-di-α-methyl-D-xylose.

The syrup was unaltered after treatment with sodium metaperiodate.

The syrup was fractionated on thick paper by ionophoresis in borate buffer to give three fractions.

Subfraction 3R1. 0.015 g. Rf 0.80, trace 0.76.

Chromatography and ionophoresis of the syrup showed a major component as a possible dimethyl fucose and minor components of tetramethyl galactose and 3,4-di-α-methyl-D-xylose.

Demethylation/
Demethylation gave fucose, and traces of galactose and xylose.

**G.P.C. System A.**

- \( R_T \) 0.47, 1.11 methyl dimethyl-fucoside.
- 0.61, 0.68 methyl 3,4-di-O-methyl-D-xyloside.
- 1.44 methyl 2,3,4,6-tetra-O-methyl-D-galactose.
- 1.68 methyl 3,4-di-O-methyl-D-xyloside.

**System B.**

- 1.08, 1.52 methyl-dimethyl-fucoside.
- 1.53, 1.61 methyl 3,4-di-O-methyl-D-xyloside.
- 2.0 methyl 2,3,4,6-tetra-O-methyl-D-galactoside.

**Subfraction 3B.** 0.010g. \( R_g \) 0.75 \[ \alpha \] = +22° (c = 1.0).

Chromatography and ionophoresis of the syrup gave a single spot corresponding to 3,4-di-O-methyl-D-xylose, which was characterised by conversion into 3,4-di-O-methyl-D-xyloolactone, m.p. and mixed m.p. 67-68°.

**Subfraction 3B.** 0.006g. \( R_g \) 0.82

The sugar corresponded in mobility and colour to 3,5-di-O-methyl-L-arabinose, or 2,5-di-O-methyl-L-arabinose in solvents A and E, and to 3,5-di-O-methyl-L-arabinose on ionophoresis.

Demethylation gave arabinose.

**G.P.C. System A.**

- \( R_T \) 0.42, 0.43, 0.54 methyl 3,5-di-O-methyl-L-arabinoside.

**System B.**

- \( R_T \) 1.06, 2.75 methyl 3,5-di-O-methyl-L-arabinoside.

**Fraction 4**
Fraction 4. 0·172g.  $R_G$ 0·75 trace 0·80, 0·87, 0·93
$[\alpha]_D = +14^\circ$ (c = 1·2).

Chromatography of the syrup in solvents A, C, D and E gave a spot corresponding to 3,4-di-$O$-methyl-$D$-xylose and also traces of trimethyl fucose trimethyl xylose and dimethyl fucose.

Demethylation gave xylose and a trace of fucose.

Ionophoresis in borate buffer gave a major component travelling at the same rate as 3,4-di-$O$-methyl-$D$-xylose and other traces on the starting line.

G.P.C.  System B.

$R_T$ 0·41, 0·55 trace methyl 2,3,4-tri-$O$-methyl-$D$-xyloside.

$R_T$ 0·71 trace methyl 2,3,4-tri-$O$-methyl-$L$-fucoside.

1·09 ? dimethyl fucoside.

1·33, 1·65 methyl 3,4-di-$O$-methyl-$D$-xyloside (major).

1·55, 2·13 ? methyl 2,4-di-$O$-methyl-$D$-xyloside.

1·50, 1·89 ? methyl 2,3-dimethyl-$D$-xyloside.

The syrup was fractionated on thick paper by ionophoresis in borate buffer to give two fractions.

**Subfraction 4A.**  0·020g.  $R_G$ 0·80, 0·73 and traces 0·68, 0·87, 0·93.

Chromatography in solvent A gave spots corresponding to the suspected dimethyl fucose ($R_G$ 0·80 grey yellow), 2,3-dimethyl-$D$-xylose ($R_G$ 0·73 pink), 2,4-dimethyl-$D$-xylose ($R_G$ 0·69 pink trace) and traces of trimethyl fucose and trimethyl xylose.

Demethylation gave fucose and xylose.

**Subfraction 4B.**
Subfraction 4B. 0.062 g. Rg 0.76 [α]D = +31° (c = 1.5).

Chromatography and ionophoresis of the syrup gave a single spot corresponding to 3,4-di-O-methyl-D-xylose. The sugar was characterised by conversion into 3,4-di-O-methyl-D-xylonolactone, m.p. and mixed m.p. 67 - 68°C.

Fraction 5. 0.032 g. Rg 0.68 [α]D = +43° (c = 1.6).

Chromatography of the syrup in solvents A, C, D and E gave a single spot corresponding to trimethyl-galactose and/or dimethyl-xylose.

Demethylation gave galactose and xylose.

Ionophoresis in borate buffer gave a major non mobile red spot and a trace of mobile pink spot corresponding to 3,4-di-O-methyl-D-xylose.

G.P.C. System A.

Rf 0.41 methyl, 3,4-di-O-methyl-D-xyloside (major).
0.63, 0.60, 0.70, 0.78 minor peaks possibly include methyl 2,4-di-O-methyl-D-xyloside.
1.50, 1.78 methyl 2,4,6-tri-O-methyl-D-galactoside, (major).

System B.

Rf 0.42, 0.55 minor, methyl 2,3,4-tri-O-methyl-D-xyloside.
1.37, 1.63 major, methyl 2,4-di-O-methyl-D-xyloside.
1.89, 2.07 major, methyl 2,4,6-tri-O-methyl-D-galactoside.

Fraction 6. 0.019 g. Rg 0.63 [α]D = +65° (c = 0.9).

Chromatography of the syrup in solvents A and D gave a single red spot corresponding to trimethyl-galactose.

Chromatography/
Chromatography in solvent C, however, resolved the fraction into a major red spot corresponding to a trimethyl galactose and a minor brown spot (possibly a monomethyl fucose).

Demethylation gave a galactose and a trace of xylose and fucose.

Ionophoresis gave a non mobile spot (red) and a minor mobile brown spot.

G.P.C. System A.

R_T 0.41, 0.67 minor peaks ?
0.77 ? monomethyl fucoside
1.65, 1.79 methyl 2,3,4-tri-O-methyl-D-galactoside.

The major sugar was characterised by conversion into 2,3,4-tri-O-methyl-N-phenyl-D-galactosylamine m.p. and mixed m.p. 164 - 165°.

Fraction 7 0.020g. R_g 0.68, 0.56 [α]_D = +18° (c = 1.0).

Chromatography of the fraction in solvents A, C and D gave spots corresponding to dimethyl xylose and/or trimethyl galactose.

Demethylation gave galactose and xylose with a trace of fucose.

G.P.C. System A.

R_T 0.51, 0.62 methyl 3,4/2,4-di-O-methyl-D-xyloside.
0.69, 0.95 ?
1.24, 1.66, 1.80 methyl 2,3,6/2,3,4-tri-O-methyl-D-galactoside
2.23 ?

The/
The syrup was fractionated on thick paper in solvent A to give two fractions.

**Subfraction 7A** 0.007g. \( R_f \) 0.68

Chromatography of the syrup in solvent A gave a single brown-red spot which was non mobile on ionophoresis in borate buffer.

Demethylation gave galactose and a smaller amount of xylose. This fraction possibly contains 2,3,6/2,3,4-tri\(-\)-methyl-\( D \)-galactose and 2,4-di\(-\)-methyl-\( D \)-xylose.

**Subfraction 7B** 0.008g. \( R_f \) 0.56, trace 0.70.

Chromatography in solvent A gave a major brown spot (\( R_f \) 0.56) and a minor pink spot corresponding to 3,4-di\(-\)-methyl-\( D \)-xylose.

Ionophoresis in borate buffer gave a non-mobile brown spot and trace of mobile red spot corresponding to 3,4-di\(-\)-methyl-\( D \)-xylose.

**G.P.C.**  System A.

\( R_f \) 1.35, 1.65 methyl 3,4-di\(-\)-methyl-\( D \)-xyloside.

Demethylation gave xylose and trace fucose.

**Fraction 8** 0.021g. \( R_f \) 0.50 \( [\alpha_0]_D = +32^\circ \) (c = 1.0)

Chromatography of the fraction in solvents A, C and D gave a spot moving at the same rate as 2,3/2,3,6-di\(-\)-methyl-\( D \)-galactose but differing slightly in colour (grey-brown) and minor traces. The sugar was purified by fractionation on thick paper in solvent A.

Ionophoresis in borate buffer gave a mobile grey brown spot/
spot $M_G$ 0.38.

Periodate oxidation gave the following pattern,
$R_p$ 0.10 (yellow; yellow U.V.), 0.38 (pale pink; yellow U.V.),
0.52 (grey faint? unoxidised starting material), 0.68
(brown-yellow; pink U.V.). A similar pattern might be
expected from oxidation of 3-O-methyl-D-fucose.

Demethylation gave fucose and trace of galactose.

G.P.C. System A.

$R_T$ 0.65 major $\delta$
0.94 trace $\delta$

Fraction 9 0.022g. $R_G$ 0.55, 0.47 $[\alpha]_D = +66^\circ (c = 1.1)$

Chromatography of the syrup in solvents A, C and D and
ionophoresis in borate showed that there were at least four
sugars in this fraction.

Demethylation gave galactose $\rightarrow$ fucose $\rightarrow$ xylose.

G.P.C. System A.

$R_T$ 0.38, 0.46, 0.55 $\delta$
0.57 $\delta$ methyl 3-O-methyl-D-xyloside
0.71 $\delta$ methyl monomethyl fucoside
1.91, 2.58 $\delta$ methyl di-O-methyl-D-galactoside.

In view of the complexity of this relatively small
fraction it was not investigated further.

Fraction 10 0.155g. $R_G$ 0.48, 0.39 $[\alpha]_D = +70^\circ (c = 2.0)$

Chromatography of the fraction in solvents A and C gave
two spots corresponding to 2,3-di-O-methyl-D-galactose
(brown $R_G$ 0.48) and 4-O-methyl-D-xylose (pink $R_G$ 0.38).

Demethylation/
Demethylation gave galactose and xylose.

Ionophoresis in borate buffer gave a major spot corresponding to a mixture of 2,3-di-O-methyl-D-galactose and 4-O-methyl-D-xylose. Traces of pink spots corresponding to 3-O- and 2-O-methyl-D-xylose were also observed.

Periodate oxidation gave a pattern with spots at $R_f$ 0.85, 0.73, and 0.67 corresponding to oxidised 2,3-di-O-methyl-D-galactose and at $R_f$ 0.57 (brown), a possible oxidation product of 4-O-methyl-D-xylose, and traces at $R_f$ 0.21 (yellow) from 2/3-O-methyl-D-xylose.

G.P.O. System A.

$R_f$ 0.42 minor

0.52, 0.64 minor, methyl 2-O-methyl-D-xyloside.

0.69 minor, methyl 3-O-methyl-D-xyloside.

0.68 major, methyl 4-O-methyl-D-xyloside.

1.09 minor, 1.65, 2.2-3.5, 2.62 major, 2,3-di-O-methyl-D-galactoside.

An attempt to fractionate the syrup on a charcoal:celite column using a gradient of aqueous ethanol as eluant (0 - 20%) was unsuccessful. The fraction was partially resolved on thick paper in solvent F to give three fractions.

Subfraction 10A 0.040g. $R_g$ 0.38 $[\alpha]_D = +11^\circ$ ($c = 2.0$),

The fraction was chromatographically pure and corresponded to 4-O-methyl-D-xylose on chromatography on solvents A, E and F and on ionophoresis in borate buffer. The sugar crystallised on seeding with authentic 4-O-methyl-D-xylose and gave m.p. and mixed/
mixed m.p. 102 - 104°. An X-ray powder photograph of the crystalline sugar was identical to that of 4-O-methyl-D-xylose.

**Subfraction 10B 0.045g.** $R_g 0.38, 0.46$

Chromatography of the syrup in solvents A, C, D, E and F showed it to be a mixture of 2,3-di-O-methyl-D-galactose and 4-O-methyl-D-xylose.

**Subfraction 10C 0.050g.** $R_g 0.44 \left[ \alpha \right]_D = +77^\circ (\alpha = 0.75)$.

The fraction was chromatographically pure and corresponded to 2,3-di-O-methyl-D-galactose. The sugar was characterised by conversion to 2,3-di-O-methyl-$N$-phenyl-D-galactosylamine m.p. 153 - 154°, mixed m.p. 152 - 154° with authentic sample m.p. 152 - 153°. An X-ray powder photograph of the aniline derivative was identical to that of an authentic sample.

**Fraction 11 0.040g.** $R_g 0.40 \left[ \alpha \right]_D = +66^\circ (\alpha = 2.0)$.

Chromatography of the fraction in solvents E and F gave a single spot corresponding to 2,4-di-O-methyl-D-galactose.

Demethylation gave galactose and traces of xylose.

Ionophoresis in borate buffer showed a non-mobile spot and traces corresponding to 2/3-O-methyl-D-xylose.

Periodate oxidation gave unchanged 2,4-di-O-methyl-D-galactose.

The sugar partly crystallised on standing and a few crystals after drying on porous tile gave m.p. and mixed m.p. 97 - 98° with authentic 2,4-di-O-methyl-D-galactose monohydrate. The sugar was finally characterised by conversion into 2,4-di-O-methyl-$N$-phenyl-D-galactosylamine m.p. and mixed m.p. 209 - 210°.

**Fraction 12** 0.022g.  \( R_g \) 0.29 \( [\alpha]_D = +11^\circ \) \((c = 1.1)\).

Chromatography of the syrup on solvent A gave a single pink brown spot. Examination of the fraction in solvents E and F, however, resolved the syrup into two sugars; a pink spot corresponding to 3-0-methyl-D-xylose and a brown-yellow spot.

Ionophoresis in borate buffer gave a mobile pink spot corresponding to 3-0-methyl-D-xylose and a mobile brown spot \( R_g 0.32 \).

Demethylation gave xylose and trace of galactose.

**G.P.C. System A.**

\( R_T \) 0.54 ? methyl 3-0-methyl-D-xylose  

0.77 ?

**Fraction 15** 0.050g.  \( R_g 0.28, 0.24 \) \( [\alpha]_D = +70^\circ \) \((c = 1.5)\).

Chromatography of the fraction in solvent A gave spots corresponding to 2-0-methyl-D-galactose and 3-0-methyl-D-xylose (pink \( R_g 0.28 \)). However, further examination in solvents E and F showed that this second pink spot failed to correspond to 3-0-methyl-D-xylose. The syrup was fractionated on thick paper in solvent F to give two fractions.

**Subfraction 15A** 0.009g.  \( R_g 0.23 \) \( [\alpha]_D = +85^\circ \) \((c = 0.9)\)

Chromatography and ionophoresis of the fraction gave a single spot corresponding to 2-0-methyl-D-galactose.

**Subfraction 15B** 0.018g.  \( R_g 0.28 \) \( [\alpha]_D = +54^\circ \) \((c = 0.9)\)

Chromatography and ionophoresis of the syrup gave a major pink spot which did not correspond to any of the monomethyl derivatives.
derivatives of arabinose or xylose.

Demethylation gave galactose and arabinose.

**Fraction 14** 0.320g. $R_g$ 0.23 $[\alpha]_D = +84^\circ$ ($c = 1.5$)

The sugar was chromatographically pure and corresponded to 2-0-methyl-D-galactose.

Demethylation gave galactose.

The syrup crystallised on standing and was partly recrystallised from ethanol/water to give m.p. 146 - 148° undepressed in mixed m.p. with authentic 2-0-methyl-D-galactose:

$[\alpha]_D = +52^\circ \rightarrow +84^\circ$

**Fraction 15** 0.150g. $R_g$ 0.23, 0.17, 0.09 $[\alpha]_D = +45^\circ$ ($c = 1.4$).

Chromatography of the syrup on solvents A, E and F showed spots corresponding to galactose (minor), xylose (major) and 2-0-methyl-D-galactose (minor). The partially crystalline syrup was fractionated on thick paper in solvent E to give three fractions.

**Subfraction 15A** 0.081g. $R_g$ 0.24 $[\alpha]_D = +85^\circ$ ($c = 1.0$)

The syrup was chromatographically identical to 2-0-methyl-D-galactose and crystallised on standing to give m.p. and mixed m.p. 147°.

**Subfraction 15B** 0.060g. $R_g$ 0.17 $[\alpha]_D = +20^\circ$ ($c = 4.0$).

The sugar was chromatographically identical to D-xylose and crystallised on standing to give m.p. 144 - 145° undepressed in mixed m.p. with authentic D-xylose:

$[\alpha]_D = +80^\circ \rightarrow +19^\circ$

The/
The sugar was finally characterised by conversion into dibenzylidene, diacetal D-xylose m.p. and mixed m.p. 210°.

Subfraction 15C 0·029g. R₆ 0·10 [α]D = +80° (c = 1·3)

The sugar was chromatographically pure and identical to D-galactose. The syrup crystallised on seeding with D-galactose and gave m.p. and mixed m.p. 166°. The sugar was characterised by conversion into mucic acid m.p. 210°.

Comparison of Methylated and Methylated Reduced Tragacanthic Acid

The methanolyis products of methylated and reduced methylated tragacanthic acid (100mg.) were examined by gas phase chromatography in an attempt to show which of the methylated galactose residues identified in the reduced methylated acid arose from reduction of galacturonic acid residues, and in particular if this was the source of 2,3,4-tri-O-methyl-galactose.

Examination of the methanolysis products directly in systems A, B and C was inconclusive. The methanolysis products of each polysaccharide were then hydrolysed to the free sugars and fractionated by paper chromatography in solvent E into four fractions.

I. Trimethyl hexoses, tetramethyl hexose, dimethyl and trimethyl pentoses.

II. Dimethyl hexoses and monomethyl pentoses.

III. Free monosaccharides and any fast moving methylated uronic acid.

IV. Methylated uronic acids.

Each/
Each fraction was examined by paper chromatography in solvents A, C and D, and after methanolysis by gas phase chromatography in system G. The identification of 2,3,4-tri-O-methyl-D-galactose in fraction I was still uncertain.

Examination of fraction III and IV from the methylated acid gave the following peaks on gas phase chromatography in system G.

<table>
<thead>
<tr>
<th>III</th>
<th>( R_T )</th>
<th>Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.43, 0.49, 0.56 minor.</td>
<td>0.68 major.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.13 major.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.10 major.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV</th>
<th>( R_T )</th>
<th>Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.68 minor.</td>
<td>2.14 major.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.15 trace.</td>
</tr>
</tbody>
</table>

Fraction IV was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride in the usual way and examined chromatographically in solvents A and E. The presence of 2-O-methyl-D-galactose, 2,3-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose in the approximate ratio 6:1:trace was ascertained by comparison with standard sugars.

The methyl glycosides of fraction IV after reduction were also examined by gas phase chromatography on system C when the following peaks were obtained.

\[
R_T = \begin{align*}
2.62, 2.92 & - \text{methyl 2,3,4-tri-O-methyl-D-galactoside.} \\
2.48, 3.69, 4.20 & - \text{methyl 2,3-di-O-methyl-D-galactoside.} \\
3.69, 4.65 & - \text{methyl 2,4-di-O-methyl-D-galactoside.}
\end{align*}
\]
Part III. Arabinogalactan from Gum Tragacanth.

Partial acid hydrolysis.

A) Arabinogalactan (0.25g.) was dissolved in hydrochloric acid (0.1N; 25ml.) and the solution was heated at 100°. Samples (1ml.) were removed at intervals and the rotation observed. (Graph 4). The degraded polysaccharide was precipitated with ethanol, centrifuged off, hydrolysed completely and examined chromatographically. The solution was neutralised with IR 48-OH resin, concentrated and examined chromatographically.

Graph 4

\[ [\alpha]_D \]

Increasing amounts of arabinose appeared immediately in the hydrolysate. After three hours small quantities of galactose were observed but these did not become appreciable. Rhamnose was present in small quantities throughout the hydrolysis.

Examination of the hydrolysis products of the degraded polysaccharides showed a steady decrease in the arabinose content. After 22 hours the rate of hydrolysis slowed down and at this stage a degraded galactan containing only small amounts of arabinose.
arabinose and rhamnose could be isolated.

B) Arabinogalactan (0.1g.) was dissolved in sulphuric acid (0.5N; 10ml.) and heated at 100° for 1 hour. The solution was neutralised with barium carbonate and concentrated. The syrup was eluted from a charcoal:celite column (1:1; 10g.) with water (500ml.) and then aqueous ethanol (10%; 250ml.). Chromatographic examination of the latter eluant showed sugars corresponding to (1→3) galactobiose and (1→6) galactobiose, the latter in slightly greater quantity, together with other oligosaccharides.

Examination of Methylated Arabinogalactan.

Hydrolysis of methylated Arabinogalactan.

A) The fully methylated arabinogalactan (2.2g.; 0.9% 39.5%; $[\alpha]_D = -97^\circ$) was heated for 1½ hours with formic acid (90%; 120ml.) at 100°. The solution was concentrated almost to dryness, hydrochloric acid (0.5N; 200ml.) was added and the solution was heated at 100° for 11 hours. The solution was filtered and neutralised by passage through a column of IR 45-OH resin.

Separation of the methylated sugars on a charcoal:celite column.

The eluate and water washings from the resin column were concentrated (1.25g.) and placed on a charcoal:celite (1:1; 50 x 4.5cm) column and eluted with a linear gradient of water/butan-2-one (0 - 5%; 8000ml.)

A preliminary examination of fractions from the column in solvent/
solvent A showed that there was a considerable overlap of methylated sugars. The probable content of each fraction, based largely on $R_f$ values and comparison with standard methylated sugars in at least two of the solvents A, B, C, D, E and F, is tabulated below.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (mg.)</th>
<th>$R_f$</th>
<th>Colour</th>
<th>Probable sugars present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78.1</td>
<td>0.24</td>
<td>pink</td>
<td>Arabinose</td>
</tr>
<tr>
<td>2</td>
<td>7.9</td>
<td>0.24</td>
<td>pink</td>
<td>arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.32</td>
<td>pink</td>
<td>monomethyl arabinose</td>
</tr>
<tr>
<td>3</td>
<td>87.2</td>
<td>0.34</td>
<td>pink</td>
<td>monomethyl arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.42</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10.1</td>
<td>0.30</td>
<td>brown</td>
<td>2-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>0.30</td>
<td>brown</td>
<td>2-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.41</td>
<td>yellow</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.4</td>
<td>0.53</td>
<td>brown</td>
<td>di-methyl-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.63</td>
<td>pink</td>
<td>2,3-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>7</td>
<td>22.0</td>
<td>0.49</td>
<td>brown</td>
<td>2,4-di-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.67</td>
<td>dark grey</td>
<td>2,3-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>0.62</td>
<td>brown</td>
<td>2,5/2,6-di-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.71</td>
<td>dark grey</td>
<td>di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>9</td>
<td>8.5</td>
<td>0.68</td>
<td>red grey</td>
<td>2,3-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>red brown</td>
<td>tri-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.83</td>
<td>grey brown</td>
<td>3,5/2,5-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>10</td>
<td>7.0</td>
<td>0.73</td>
<td>red brown</td>
<td>tri-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
<td>grey brown</td>
<td>3,5/2,5-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>11</td>
<td>13.1</td>
<td>0.75</td>
<td>red brown</td>
<td>tri-O-methyl-β-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.80</td>
<td>grey brown</td>
<td>3,5/2,5-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>grey</td>
<td>2,5,5-tri-O-methyl-L-arabinose</td>
</tr>
</tbody>
</table>

In view of the difficulties involved in isolation of pure methylated sugars from many of the above fractions they were not/
not investigated further.

The resin column, used to neutralise the hydrolysate, was eluted with $N$ formic acid to strip off any methylated uronic acid residues. This elute was concentrated (0.15 g.), examined chromatographically and found to contain a substantial amount of 2,3,5-tri-$O$-methyl-$L$-arabinose together with small amounts of methylated uronic acids.

Hydrolysis of the methylated Arabinogalactan.

B) Fully methylated arabinogalactan (3.1 g.) was suspended in hydrochloric acid ($2N$; 100 ml.) at room temperature for two days when almost all had dissolved. Methanol (100 ml.) was added to aid solution of the small residue insoluble in the aqueous acid and the solution was heated gradually to 100°C. care being taken that no partially methylated material precipitated, and the methanol was allowed to evaporate off. After dilution with water so that the solution was $N$ with respect to acid, hydrolysis was continued for a further 16 hours (constant rotation). The solution was neutralised with silver carbonate in the usual way, concentrated to 100 ml., a small amount of IR 45-OH resin was added to remove uronic acids and filtered. The resin was repeatedly washed with water ($10 \times 50$ ml.) and these washings and the neutral solution concentrated to dryness.

Separation of the methylated sugars on a cellulose column.

The neutral syrup (2.96 g.) was separated on a cellulose column (74 x 4.0 cm.). The sugars were eluted with the following solvents. Light petroleum (b.p. 100/110): butan-1-ol (70:30) saturated/
saturated with water. (until trimethyl hexoses eluted).
Light petroleum (b.p. 100/120): butan-1-ol (50:50) saturated
with water. (until dimethyl hexoses eluted).
Butan-1-ol half saturated with water, (until monomethyl
hexoses eluted) and then with water.

The following fractions were obtained and examined
chromatographically in solvent A. The results are tabulated
below.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (mg)</th>
<th>Colour</th>
<th>$R_g$</th>
<th>Sugars present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>961</td>
<td>grey</td>
<td>0.95</td>
<td>2,5,5-tr-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>2</td>
<td>181</td>
<td>grey</td>
<td>0.95</td>
<td>2,5,5-tri-O-methyl-L-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>grey</td>
<td>0.89</td>
<td>3,4-di-O-methyl-L-rhamnose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>grey</td>
<td>0.85</td>
<td>2,5/3,5-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>3</td>
<td>282</td>
<td>brown-red</td>
<td>0.73</td>
<td>2,4,6-tri-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown-red</td>
<td>0.67</td>
<td>2,3-di-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>brown</td>
<td>0.57</td>
<td>4-mono-O-methyl-L-rhamnose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>0.47</td>
<td>2,4-di-O-methyl-D-galactose tr.</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>brown</td>
<td>0.46</td>
<td>2,3-di-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>0.42</td>
<td>2,4-di-O-methyl-D-galactose tr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td>0.38</td>
<td>2-mono-O-methyl-D-galactose</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>pink</td>
<td>0.38</td>
<td>2-mono-O-methyl-L-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>0.42</td>
<td>2,4-di-O-methyl-D-galactose tr.</td>
</tr>
<tr>
<td>7</td>
<td>81</td>
<td>brown</td>
<td>0.42</td>
<td>2,4-di-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td>0.38</td>
<td>2-mono-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>8</td>
<td>121</td>
<td>brown</td>
<td>0.42</td>
<td>2,4-di-O-methyl-D-galactose</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>brown</td>
<td>0.42</td>
<td>2,4-di-O-methyl-D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td>0.34</td>
<td>5-mono-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>10</td>
<td>228</td>
<td>pink</td>
<td>0.34</td>
<td>5-mono-O-methyl-L-arabinose</td>
</tr>
<tr>
<td>11</td>
<td>256</td>
<td>brown</td>
<td>0.29</td>
<td>2-mono-O-methyl-D-galactose</td>
</tr>
<tr>
<td>12</td>
<td>490</td>
<td>pink</td>
<td>0.31</td>
<td>methylated uronic acid tr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td>0.21</td>
<td>L-arabinose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>0.14</td>
<td>D-galactose tr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink</td>
<td>0.07</td>
<td>methylated uronic acid.</td>
</tr>
</tbody>
</table>
Examination of Fractions.

Fraction 1. 0.961g. $R_g 0.95 \left[\alpha\right]_D = -40^\circ (c = 1.2$).

This fraction was obtained as a mobile syrup corresponding in solvents A, C and D in colour (grey; pink U.V.) and mobility to 2,3,5-tri-O-methyl-L-arabinose.

Demethylation gave arabinose.

The sugar was characterised by conversion into 2,3,5-tri-O-methyl-L-arabonamide m.p. 135 - 136° and mixed m.p. 134 - 136° with authentic 2,3,5-tri-O-methyl-L-arabonamide m.p. 134 - 136°.

Fraction 2. 0.181g. $R_g 0.95, 0.89, 0.85, 0.71$ \[ \left[\alpha\right]_D = -20^\circ (c = 0.51) \].

Chromatography of the syrup in solvents A, C and D showed spots corresponding to 2,3,5-tri-O-methyl-L-arabinose, tetramethyl galactose or dimethyl rhamnose (brown-yellow), 2,5/3,5-di-O-methyl-L-arabinose (grey), and 2,3-di-O-methyl-L-arabinose/2,4,6-tri-O-methyl-D-galactose (pink).

Demethylation gave arabinose, galactose and rhamnose.

The syrup (170m.g.) was fractionated on a cellulose column (45 x 2cm.) using light petroleum (b.p. 100/120):butan-1-ol (80:20) saturated with water as eluant. Four fractions were collected.

Subfraction 2A 0.028g. $R_g 0.95 \left[\alpha\right]_D = -39^\circ (c = 1.4$).

Chromatography of the syrup in solvents A, C, D and E showed a single spot corresponding to authentic 2,3,5-tri-O-methyl-L-arabinose.

Subfraction 2B/
Subfraction 2B 0.018g.  \( R_G 0.39, 0.85 [\alpha]_D = +34^\circ (\rho = 0.9) \)

Chromatography of the syrup in solvents A, C and D showed a major component \( (R_G 0.39 \text{ brown-yellow}) \) and a minor component \( (\text{grey}) \) corresponding to 2,5/3,5-di-O-methyl-L-arabinose. Papers sprayed with trisphenyltetrazolium chloride gave only a slight positive reaction at \( R_G 0.85 \).

Ionophoresis in borate buffer showed the major component to be highly mobile and gave traces corresponding to 2,5- and 3,5-di-O-methyl-L-arabinose.

Demethylation gave arabinose and rhamnose.

\[ \text{OMe} = 30.1\% \text{ (Calculated for dimethyl rhamnose 32.2\%).} \]

G.P.C.  System B.

\( R_T 0.97 \text{ major, methyl 3,4-di-O-methyl-L-rhamnoside.} \)

\( 2.02 \text{ minor, methyl 2,5-di-O-methyl-L-arabinoside.} \)

These results indicate that the major component of this fraction is probably 3,4-di-O-methyl-L-rhamnose.

Subfraction 2C 0.085g.  \( R_G 0.85, 0.71 [\alpha]_D = -18^\circ (\rho = 0.85) \).

Chromatography of the syrup in solvents A, C and D showed a major sugar corresponding to 2,5/3,5-di-O-methyl-L-arabinose and a minor component corresponding to 2,3-di-O-methyl-L-arabinose and/or 2,4,6-tri-O-methyl-D-galactose.

The syrup was fractionated on thick paper by ionophoresis in borate buffer to give two major fractions.

Subfraction 2C1 0.045g.  \( R_G 0.85 \)

Chromatography and ionophoresis showed a single spot corresponding to 2,5-di-O-methyl-L-arabinose. The sugar was characterised/
characterised by conversion into 2,5-di-O-methyl-L-arabonolactone m.p. 59 - 60°, after recrystallisation from ethyl acetate, in good agreement with the literature values. The m.p. was depressed in mixed m.p. with 3,5-di-O-methyl-L-arabonolactone.

**Subfraction 2C2** 0.050 g. Rg 0.85

Chromatographic and ionophoretic examination showed a single spot corresponding to 3,5-di-O-methyl-L-arabinose. The sugar was characterised by conversion into 3,5-di-O-methyl-L-arabonolactone, after recrystallisation from ethyl acetate, m.p. and mixed m.p. 76 - 77°.

**Subfraction 2D** 0.024 g. Rg 0.71 $\left[\alpha\right]_D = +94° \ (c = 1.2)$.

Chromatography of the syrup in solvents A, C and D showed a partially resolved spot corresponding to 2,3-di-O-methyl-L-arabinose and/or 2,4,6-tri-O-methyl-D-galactose.

Demethylation showed galactose and arabinose.

**G.P.C. System A.**

Rf 0.42, 0.54, 0.69 methyl 2,3-di-O-methyl-L-arabinoside 1.25

1.52, 1.80 methyl 2,4,6-tri-O-methyl-D-galactoside.

**Fraction 3** 0.282 g. Rg 0.69, 0.65 $\left[\alpha\right]_D = +92° \ (c = 1.0)$.

Chromatography of the syrup in solvents A, C and D showed two partially resolved spots corresponding to 2,3-di-O-methyl-L-arabinose and/or 2,4,6-tri-O-methyl-D-galactose.

Demethylation gave arabinose and galactose.

**G.P.C. System A.**

Rf 0.41, 0.53, 0.59 methyl 2,3-di-O-methyl-L-arabinoside 1.26 trace ?
1.52, 1.80 methyl 2,4,6-tri-O-methyl-D-galactoside.

The syrup (260mg.) was fractionated on a charcoal:celite column (30 x 1.9cm.) using a linear gradient of aqueous ethanol (5 - 25%) as eluant. Three fractions were collected.

Subfraction 3A 0.098g. $R_g 0.64 \ [\alpha]_D^0 = +101^\circ (c = 0.5)$. The syrup was chromatographically pure and corresponded to 2,3-di-O-methyl-L-arabinose. The sugar was characterised by conversion into 2,3-di-O-methyl-L-arabinonamide, recrystallised from ethanol, m.p. and mixed m.p. 160 - 161°.

Subfraction 3B 0.024g. $R_g 0.69, 0.64$.

The syrup was shown by chromatographic examination to be 2,3-di-O-methyl-L-arabinose and/or 2,4,6-tri-O-methyl-D-galactose. Subfraction 3C 0.121g. $R_g 0.70 \ [\alpha]_D^0 = +90^\circ (c = 1.2)$. This fraction was chromatographically pure and corresponded to 2,4,6-tri-O-methyl-D-galactose. The sugar was characterised by conversion into 2,4,6-tri-O-methyl-N-phenyl-D-galactosylamine, after recrystallisation from ethyl acetate/acetone as needles, m.p. and mixed m.p. 172 - 173°.

Fraction 4 0.064g. $R_g 0.57, 0.54, 0.42 \ [\alpha]_D^0 = +77^\circ (c = 0.8)$. Chromatography of the syrup in solvents A, C and D showed three sugars, one corresponding to 2,4-di-O-methyl-D-galactose ($R_g 0.42$, brown), the others at $R_g 0.54$ as a brown spot, and at $R_g 0.57$ as a brown-yellow spot.

The fraction (0.060g.) was fractionated on thick paper in solvent D.

Subfraction 4A/
Subfraction 4A 0.010g.  \( R_G \) 0.44, 0.38 (trace).

The syrup corresponded to 2,4-di-O-methyl-D-galactose on chromatography in solvents A, C and D and also contained a trace of 2-O-methyl-L-arabinose.

Subfraction 4B 0.007g.  \( R_G \) 0.40

The sugar corresponded to 2-O-methyl-L-arabinose in solvents A, C and D.

Subfraction 4C 0.040g.  \( R_G \) 0.54 - 0.57

\[ [\alpha]_D = +20^\circ \ (c = 0.8) \]

Chromatography of the syrup in solvents A, C and D partially resolved two spots at \( R_G \) 0.54 (brown) and at \( R_G \) 0.57 (brown-yellow; yellow U.V.).

Periodate oxidation of the syrup gave the following pattern: \( R_f \) 0.60 (grey), 0.76 (grey-brown), 0.82 (grey-brown).

Hydrolysis of the syrup with sulphuric acid gave no change in the chromatographic pattern.

Ionophoresis in borate buffer for 6 hours gave 2 spots with mobilities of +2.0cm (brown) and +7.0cm (grey-yellow). An ionophoretogram sprayed with triphenyltetrazolium chloride gave a positive reaction for both sugars indicating that in both cases position C2 has a free hydroxyl group.

Demethylation gave galactose and rhamnose.

A thick paper separation was made by ionophoresis in borate buffer.

Subfraction 4C1 0.019g.  \( R_G \) 0.54 \[ [\alpha]_D = +14^\circ \ (c = 0.4) \]

The/
The sugar crystallised on standing and was recrystallised from acetone/water and had m.p. 130 - 133°.

Demethylation gave galactose.

Periodate oxidation gave spots at $R_g$ 0.75 (brown), 0.85 (brown) $\text{OMe} = 26\%$ (Calculated for dimethyl galactose 29.8%).

Subfraction 4C2 0.015g. $R_g 0.57 \ [\alpha]_D = +10^\circ (\alpha = 0.5)$.  
The sugar crystallised on standing and was recrystallised from acetone/water and had m.p. 115 - 115°.

Demethylation gave rhamnose.  
$\text{OMe} = 16\%$ (Calculated for monomethyl 6-deoxy hexose 17.2%)  
The sugar is unlikely to be 2-0-methyl-L-rhamnose as it gives a positive reaction with triphenyltetrazolium salt and no methoxymalondialdehyde was found on periodate oxidation of fraction 4C. The crystalline sugar was compared in mixed m.p. with 3- and 4-0-methyl-L-rhamnose.

\- 3-0-methyl-L-rhamnose m.p. 114 - 115°  
\- 4-0-methyl-L-rhamnose m.p. 121 - 122°  
\- 3-0-methyl-L-rhamnose + 4C2 m.p. 102 - 104°  
\- 4-0-methyl-L-rhamnose + 4C2 m.p. 115 - 113°  

It is therefore probable that the sugar is 4-0-methyl-L-rhamnose.

Fraction 5 0.066g. $R_g 0.46, 0.42$(trace), 0.38(trace).  
$\ [\alpha]_D = +105^\circ (\alpha = 1.4)$.  

Chromatography of the syrup in solvents A, C and D showed a major fraction corresponding to 2,3/2,6-di-0-methyl-D-galactose/
D-galactose \( (R_g \ 0.46, \ \text{brown}) \) and minor fractions corresponding to 2,4-di-O-methyl-D-galactose \( (R_g \ 0.46, \ \text{brown}) \) and 2-O-methyl-L-arabinose \( (R_g \ 0.38, \ \text{pink}) \).

A fractionation of the syrup on thick paper in solvent D gave two fractions.

**Subfraction 5A** 0.009 g.  \( R_g \ 0.41, \ 0.38 \).

Chromatography in solvents A, C and D gave two spots corresponding to 2,4-di-O-methyl-D-galactose and 2-O-methyl-L-arabinose respectively.

**Subfraction 5B** 0.040 g.  \( R_g \ 0.47 \ \left[ \alpha \right]_D^0 = +91^\circ \ (c = 0.8) \).

Chromatography in solvents A, C and D showed that the syrup contained only 2,3- and/or 2,6-di-O-methyl-D-galactose.

Periodate oxidation gave the characteristic pattern for 2,3-di-O-methyl-D-galactose \( (R_P \ 0.63, \ 0.75, \ 0.88) \) and a trace of methoxymalondialdehyde \( (R_P \ 0.18, \ \text{yellow}) \) which possibly arises from a trace of 2,6-di-O-methyl-D-galactose or 2-O-methyl-L-arabinose.

The sugar was characterised by conversion into 2,3-di-O-methyl-N-phenyl-D-galactosylamine m.p. 137 - 139°. An X-ray powder photograph of the crystals was identical to that of an authentic sample.

**Fraction 6** 0.047 g.  \( R_g \ 0.42, \ 0.38 \ \left[ \alpha \right]_D^0 = +104^\circ \ (c = 1.1) \).

The fraction, on chromatography in solvents A, C, D and F, gave two spots; the major spot corresponded to 2-O-methyl-L-arabinose \( (R_g \ 0.38, \ \text{pink}) \) and the minor to 2,4-di-O-methyl-D-galactose \( (R_g \ 0.42, \ \text{brown}) \).

**Demethylation/**
Demethylation gave arabinose and a trace of galactose. Periodate oxidation gave spots $R_p 0.20$ (yellow), 0.54 and 0.67 (grey-pink) corresponding to the oxidation products of a 2 methyl pentose and also a spot $R_p 0.42$ (brown) corresponding to unchanged 2,4-di-O-methyl-\(\beta\)-galactose.

The syrup (0.060g.) was fractionated on thick paper in solvent C.

Subfraction 6A 0.054g. $R_G 0.38$.

The syrup gave a single spot on chromatography in solvents A, C, D and E corresponding to 2-O-methyl-\(L\)-arabinose. The sugar was characterised by conversion to the p-toluene-sulphonylhydrazone derivative m.p. 144 - 146\(^\circ\) and in mixed m.p. 145 - 148\(^\circ\) with authentic 2-O-methyl-\(L\)-arabinose-p-toluene-sulphonylhydrazone, m.p. 147 - 148\(^\circ\).

Subfraction 6B 0.004g. $R_G 0.42$.

Chromatography of the syrup on solvents A and F gave a single spot corresponding to 2,4-di-O-methyl-\(\beta\)-galactose.

Fractions 7 0.081g. $R_G 0.42, 0.58 \left[\alpha\right]_D = +91\(^\circ\) (c = 1.8).

Chromatography in solvents A, C, D and F gave spots corresponding to 2,4-di-O-methyl-\(\beta\)-galactose and 2-O-methyl-\(L\)-arabinose in approximately equal amounts.

Demethylation gave arabinose and galactose.

The syrup partly crystallised on standing and after drying a few crystals on porous tile these gave m.p. and mixed m.p. with authentic 2,4-di-O-methyl-\(\beta\)-galactose monohydrate 98 - 99\(^\circ\).
Periodate oxidation gave a pattern corresponding to the oxidation of a 2 methyl pentose, and unchanged 2,4-di-O-methyl-D-galactose.

**Fraction 8** 0.121g. $R_g 0.41, 0.58 \ \left[\alpha\right]_D^0 = +84^\circ (\sigma = 1.2)$.

The syrup crystallised immediately and was recrystallised from acetone containing 1% water and gave m.p. and mixed m.p. with authentic 2,4-di-O-methyl-D-galactose monohydrate 101 - 102$^\circ$.

$\left[\alpha\right]_D^0 = +120 \rightarrow +85^\circ (\sigma = 0.7)$

Chromatography of the sugar in solvents A, C, and D gave a spot corresponding to 2,4-di-O-methyl-D-galactose and a trace corresponding to 2-O-methyl-D-arabinose. The sugar was characterised by conversion into 2,4-di-O-methyl-N-phenyl-D-galactosylamine m.p. and mixed m.p. 210$^\circ$.

**Fraction 9** 0.040g. $R_g 0.41, 0.52 \ \left[\alpha\right]_D^0 = +85^\circ (\sigma = 0.8)$

Chromatography of the syrup in solvents A, C and D gave spots corresponding to 2,4-di-O-methyl-D-galactose (brown $R_g 0.41$) and 3-O-methyl-D-arabinose (pink, $R_g 0.52$). Periodate oxidation gave spots $R_p 0.24$ (weak yellow), 0.72 (strong grey) corresponding to the oxidation products of a 3-O-methyl-pentose, and $R_p 0.42$ corresponding to unchanged 2,4-di-O-methyl-D-galactose.

The syrup partly crystallised on standing.

Demethylation gave galactose and arabinose.

**Fraction 10** 0.288g. $R_g 0.32 \ \left[\alpha\right]_D^0 = +113^\circ (\sigma = 2.2)$

The syrup gave a single spot in solvents A, D and F and corresponded to 3-O-methyl-D-arabinose.

Periodate oxidation gave spots $R_p 0.18$ (weak yellow) and 0.72 (strong grey) corresponding to the pattern for a 3-O-methyl-pentose.
Demethylation gave arabinose.

The sugar was characterised by conversion to the lactone of the aldonic acid m.p. 58 - 59° on crystallisation from water and drying over phosphorous pentoxide. The lactone was purified by sublimation under vacuum and gave crystals m.p. 76 - 77° and mixed m.p. 75 - 76° with authentic 3-O-methyl-L-arabonolactone m.p. 75 - 77°.

**Fraction 11** 0.256g. $R_G 0.29$, trace 0.21. $\left[\alpha\right]_D = +62^\circ $ ($c = 2.0$).

Chromatography of the syrup in solvents A, D and F showed a major spot corresponding to 2-O-methyl-D-galactose and also a trace of arabinose. The syrup crystallised almost completely on standing and part was recrystallised from ethanol and gave m.p. 145 - 146° undepressed in mixed m.p. with 2-O-methyl-D-galactose.

$$\left[\alpha\right]_D = +55 \rightarrow +84^\circ .$$

**Fraction 12** 0.506g. $R_G 0.31$, 0.21, 0.14, 0.07.

$$\left[\alpha\right]_D = +82^\circ $ ($c = 2.1$).

Chromatography of the syrup in solvents A, B, F and H showed spots corresponding to galactose ($R_G 0.14$), arabinose ($R_G 0.21$), and methylated acids ($R_G 0.31$ and 0.07).

The syrup was fractionated on thick paper (Whatman No. 17 extra thick) in solvent F to give four fractions.

**Subfraction 12A** 0.025g. $4R_G 0.14 \left[\alpha\right]_D = +80^\circ $ ($c = 1.2$).

The syrup crystallised on standing and was recrystallised from ethanol, m.p. 166 - 167° undepressed in mixed m.p. with D-galactose.

$$\left[\alpha\right]_D = +140 \rightarrow +80^\circ $$
The sugar was further characterised by conversion into mucic acid m.p. and mixed m.p. 210 - 211°.

Subfraction 12B 0.240g. \( R_G 0.21 \) \( [\alpha]_D = +104° \) (c = 1.4).

The syrup crystallised on standing and gave m.p. 158 - 159° undepressed in mixed m.p. with L-arabinose.

\( [\alpha]_D = +160 \rightarrow +104° \)

The sugar was further characterised by conversion to L-arabinose-p-toluenesulphonylhydrazone m.p. and mixed m.p. 154 - 155°.

Subfraction 12C 0.052g. \( R_G 0.07 \)

This fraction was combined with fraction 13A.

Subfraction 12D 0.017g. \( R_G 0.31 \)

This fraction was combined with fraction 13B.

Fraction 13 The IR 45-OH resin, used to remove uronic acids from the hydrolysate of methylated arabinogalactan, was eluted with formic acid (1N: 250ml.). The eluate was concentrated and the final traces of formic acid removed by frequent evaporations with water. The syrup (101mg.) was examined chromatographically in solvent B and contained a considerable proportion of neutral methylated sugars in addition to methylated uronic acids. The syrup was de-ionised with IR 120-H resin, neutralised with barium carbonate and evaporated to dryness. The barium salts were then extracted with dry acetone. The residual barium uronates were dissolved in water, de-ionised with IR 120-H resin and fractionated on thick paper (Whatman No. 17 extra thick) in solvent F to give two fractions.

Subfraction 13A/
Subfraction 13A  0.028g. Rg 0.07

The syrup was chromatographically pure and was combined with fraction 12C. The combined syrup (80mg.) was converted to the methyl ester methyl glycoside, dissolved in dry tetrahydrofuran (10ml.), lithium aluminium hydride (120mg.) in tetrahydrofuran (10ml.) was added dropwise and the solution was refluxed for 4 hours and allowed to stand for a further 18 hours. The excess hydride was destroyed by addition of water and the mixture evaporated to dryness before being exhaustively extracted with chloroform. The chloroform extracts were concentrated to give a syrup (41mg.). This syrup (Rg 0.49, brown [α]D = +85°) was chromatographically identical to 2,3-di-O-methyl-D-galactose. The sugar was characterised by conversion into 2,3-di-O-methyl-N-phenyl-D-galactosylamine m.p. 133 - 135°. An X-ray powder photograph of this derivative was identical to that of an authentic sample.

Subfraction 13B  0.013g. Rg 0.51

The syrup was chromatographically pure and was combined with fraction 12D. The combined syrup was reduced in the same manner as fraction 13A to give a syrup (10mg.). This syrup (Rg 0.72, red-brown, [α]D = +115°) crystallised and was recrystallised from ethanol m.p. 80 - 81° undepressed in mixed m.p. with 2,3,4-tri-O-methyl-D-galactose.

[α]D = +147 → +120°
Periodate Oxidation and Smith Degradation of the Arabinogalactan.

A trial periodate oxidation was carried out on the arabinogalactan - Al4 (0.5g.), dissolved in water (48ml.), by addition of sodium metaperiodate (0.3M: 24 ml.). Samples were withdrawn at intervals and the periodate consumed was determined volumetrically by the method of Fleury and Lange (229).

Results: Graph V.

Time in hours: 0 1.0 2.25 5.66 18.5 28.5 42.6 72
Moles 10^-6 consumed/0 0.22 0.41 0.52 0.58 0.60 0.61 0.62
mole sugar.

Normal oxidative glycol cleavage was considered to be complete after about 30 hours. Extrapolation of the straight line part of Graph V to correct for overoxidation gives a consumption of 0.58 moles of periodate per sugar residue (M.Wt. 137).

Arabinogalactan - Al4 (17g.), dissolved in water (1630ml.) was therefore oxidised for 30 hours with sodium metaperiodate (0.3M: 815ml.) in the dark. Ethylene glycol (17g.) was added to destroy excess periodate. After 10 minutes a sample was taken and the formic acid which was released during the oxidation of the polysaccharides was estimated by titration with standard sodium hydroxide to a methyl red end point, when it was found that 0.04 moles of formic acid were released per sugar residue (M.Wt. 137). The remaining solution was de-ionised with IR 120-H resin, iodate ions were removed at the centrifuge after precipitation as barium iodate by addition of/
of barium hydroxide until the solution was neutral, and the solution was treated with potassium borohydride (14 g.) for 24 hours. Excess of borohydride was destroyed and the solution was de-ionised with IR 120-H resin. After concentration to a smaller volume the solution was made with respect to sulphuric acid and allowed to stand at room temperature for three hours. The sulphuric acid was neutralised with barium hydroxide, and after removal of barium sulphate at the centrifuge, degraded arabinogalactan \( \text{I} \) (7 g.) was recovered by addition of ethanol to the solution. Low molecular weight material \( \text{I} \) was recovered by concentration of the solution and removal of borate with methanol.

A trial periodate oxidation was carried out as above on degraded arabinogalactan \( \text{I} \) (0.5 g.) with the following results:

Graph VI

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>18</th>
<th>27</th>
<th>78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles 10(^{-4}) consumed</td>
<td>0</td>
<td>0.15</td>
<td>0.32</td>
<td>0.41</td>
<td>0.47</td>
<td>0.52</td>
<td>0.55</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Normal oxidative cleavage was considered to be complete after about 20 hours and extrapolation of Graph VI to correct for overoxidation gave a consumption of 0.52 moles of periodate per mole of sugar residue (M.Wt. 144).

A larger scale oxidation of arabinogalactan \( \text{I} \) (3.5 g.) was carried out with periodate under the same conditions as above for 20 hours. The excess periodate was destroyed with ethylene glycol and estimation of formic acid gave a release of 0.06 moles/
moles per sugar residue (M.Wt. 144). The oxidised polysaccharide was subjected to Smith degradation as outlined above when a degraded arabinogalactan II (1.5g.) was recovered. The low molecular weight material II was also recovered from solution.

A third periodate oxidation and Smith degradation was carried out on degraded arabinogalactan II (0.75g.). The periodate consumption was followed spectrophotometrically (230).

Graph VII

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0 1 2 4 8 20 48 72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles 10^-4 consumed</td>
<td>0 0.24 0.41 0.55 0.60 0.62 0.64 0.64</td>
</tr>
</tbody>
</table>

Extrapolation of Graph VII gives a periodate consumption of 0.61 moles per sugar residue (M.Wt. 147). This periodate oxidised polysaccharide was subjected to Smith degradation as before when degraded arabinogalactan III (0.35g.) was isolated and low molecular weight material III was recovered.

Comparison of Arabinogalactan and Degraded Arabinogalactans.

Hydrolysis.

Each arabinogalactan was examined chromatographically after complete and partial hydrolysis (0.5N sulphuric acid; 1 hour).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Complete hydrolysis</th>
<th>Partial hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinogalactan</td>
<td>-72</td>
<td>1</td>
</tr>
<tr>
<td>Arabinogalactan I</td>
<td>-21</td>
<td>2</td>
</tr>
<tr>
<td>Arabinogalactan II</td>
<td>+15</td>
<td>2</td>
</tr>
<tr>
<td>Arabinogalactan III</td>
<td>+48</td>
<td>1</td>
</tr>
</tbody>
</table>

**Methylation.**

Fully methylated arabinogalactan I (OMe 40.3%) and II (OMe 41%) were prepared by treatment of arabinogalactans I and II with 6 additions of dimethyl sulphate and sodium hydroxide and then 2 additions of methyl iodide and silver oxide in the usual way. The methylated polysaccharides and methylated arabinogalactan were examined by chromatography after hydrolysis and by gas phase chromatography after methanolysis.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>System A</th>
<th>System B</th>
<th>System C</th>
<th>Paper Chr. A, C, E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5 Me₃ Ara</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2,5 Me₃ Ara</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2,5 Me₂ Ara</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ tr</td>
</tr>
<tr>
<td>3,5 Me₂ Ara</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ tr</td>
</tr>
<tr>
<td>2 Me Ara</td>
<td>++</td>
<td>+ tr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Me Ara</td>
<td>+++</td>
<td>+ tr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ara</td>
<td>+++</td>
<td>+ tr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6 Me₄ Gal</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>tr?</td>
</tr>
<tr>
<td>2,4,6 Me₃ Gal</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2,3,4 Me₃ Gal</td>
<td>?</td>
<td>tr?</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2,4 Me₂ Gal</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2 Me Gal</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Gal</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td></td>
</tr>
</tbody>
</table>

Sugars bracketed were not distinguished by paper chromatography.
Low Molecular Weight Material.

This material was examined chromatographically in solvent F. Papers sprayed with aniline oxalate for reducing sugars gave a negative reaction. As there was no free monosaccharide in the material the syrups were hydrolysed directly with acid and the hydrolysates examined chromatographically, when in addition to glycerol and erythritol arabinose and galactose were detected. It is probable that these latter two sugars arise from hydrolysis of arabinosylglycerol and galactosyl-glycerol, but, as the sugars travelling at $R_{\text{Ara}}$ 1.10 and 0.95 were not isolated and hydrolysed separately, such a conclusion is only tentative.

<table>
<thead>
<tr>
<th>Before Hydrolysis</th>
<th>Glycerol</th>
<th>Erythritol</th>
<th>$R_{\text{Ara}}$ 1.10</th>
<th>0.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinogalactan I</td>
<td>strong</td>
<td>-</td>
<td>++</td>
<td>tr</td>
</tr>
<tr>
<td>Arabinogalactan II</td>
<td>strong</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>Arabinogalactan III</td>
<td>strong</td>
<td>tr</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After Hydrolysis</th>
<th>Glycerol</th>
<th>Erythritol</th>
<th>Ara</th>
<th>Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinogalactan I</td>
<td>strong</td>
<td>-</td>
<td>++</td>
<td>tr</td>
</tr>
<tr>
<td>Arabinogalactan II</td>
<td>strong</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>Arabinogalactan III</td>
<td>strong</td>
<td>tr</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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