STRUCTURAL INVESTIGATIONS OF *ACACIA* AND OTHER POLYSACCHARIDES

by

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SUMMARY

The polysaccharide gum from Acacia podalyriaefolia is essentially an arabinagalactan, containing in addition rhamnose and glucuronic acid to the extent of about 3%.

The gum has a weight-average molecular weight of between 31,500 and 33,500, is monodisperse, and has an equivalent weight of 3585.

Graded acid hydrolysis afforded mainly arabinose together with galactose, rhamnose and two degraded polysaccharides. Trace amounts of $\beta - 1 \rightarrow 3$- and $\beta - 1 \rightarrow 6$- linked di- and tri- saccharides were also obtained.

The gum has been subjected to four sequential Smith Degradations and the polysaccharides obtained from the first three degradations have been analysed by methylation, methanolysis and GLC, and the molecular weights of the four polysaccharides have been determined.

Methylated A. podalyriaefolia gum has been hydrolysed and the methyl ethers were separated quantitatively on a cellulose column.

Possible structures for the four Smith degradation products have been proposed and in the light of the experimental results, a possible structural repeating unit in the macromolecule of A. podalyriaefolia gum has been proposed.

A series of D-galactitol methyl ethers, and a number of L-arabinitol and L-rhamnitol methyl ethers have been acetylated and their retention times and molar response factors determined by GLC on an ECNSS-M-coated column.

The hydrolysates of nine methylated polysaccharides have been converted to the acetylated methylated glycitols and analysed by GLC. Possible structural fragments from molecules of six of these polysaccharides have been proposed.

Part of the work reported in this Thesis has been published, viz.,

N.M.R. Spectroscopy of Methyl Ethers of D-Galactitol, E.B. Rathbone, G.R. Woolard and A.M. Stephen S.Afri. Medical J., 42, 973 (1968). and

Gas-Liquid Chromatography of The Acetates of D-Galactitol And Its Methyl Ethers, G.R. Woolard, A.M. Stephen, and E.B. Rathbone, S.Afri. Medical J., 42, 973-974 (1968).

A further part has been accepted for publication, viz.,

Application of Gas-Liquid Chromatography To The Structural Investigation of Polysaccharides - lV, P.I. Bekker, A.M. Stephen and G.R. Woolard, Tetrahedron, in press.

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GENERAL INTRODUCTION

The taxonomic relationships between the various botanical classes have for many years been of interest to both botanists and chemists. Classifying plants taxonomically by the types of chemical components they contain, chemists have attempted to substantiate the botanical classification of a plant into a particular order, family, genus and species.

Chemical investigations have shown that the gums exuded from *Acacia* species differ markedly from gums exuded from trees of a different genus, indicating a means of chemical classification.

The gums exuded from different *Acacia* species have been shown to differ in their chemical composition, and, more recently, inter-nodule variations have been noted in the gum exuded from the same tree (e.g. reference 1 and 2); analytical investigations of different forms of the gum from *A. senegal* [3,4] have revealed minor differences; and the composition of the gum exuded from *A. cyanophylla* is markedly different from that of *A. pycnantha* despite the close botanical connection.[5] These differences, which seldom exceed five percent, are not large enough to affect the taxonomic classification.

Despite these differences in gum exudates, it is possible to generalise to some extent as regards the chemical composition to be expected in a certain type of plant. In the case of *Acacia* gum exudates, for example, it is to be expected that:

(a) the equivalent weight will be generally in the region of 1,000 - 2,000,

(b) all will contain glucuronic acid linked normally to $C_{(6)}$, but occasionally to $C_{(4)}$ of D-galactose,

(c) the "backbone" of the molecule will consist of D-galactose units linked $1 \rightarrow 3$ and $1 \rightarrow 6$,

(d) all the exudates will contain L-rhamnose and L-arabinose in addition to D-galactose and D-glucuronic acid, and

(e) none of the polysaccharides will contain D- or L-xylose (see reference 6 for a report on the identification of trace amounts of xylose, ribose and fucose in Acacia gums).

A different pattern is noticed, however, for the species of Prunus which have been studied. The main differences are

(a) the presence of D-xylose and D-mannose and normally the absence of D- or L-rhamnose, and

(b) the occurence of D-glucuronic acid in this genus, linked in some cases to $C_{(2)}$ of D-mannose and in others (as in Acacia species) to $C_{(6)}$ of D-galactose.

Kaplan and Stephen [7] have investigated a number of methylated Acacia gum polysaccharides; GLC analysis of the methanolysates of these indicated that the polysaccharide from A. elata had the simplest structure and that from A. podalyriaefolia was the closest related structurally. Detailed chemical investigations were undertaken to demonstrate the simplicity of the structures of the polysaccharides from the gums of A. elata* and A. podalyriaefolia, and to show their chemical taxonomic relationships with other Acacia gums.

Part I of this thesis presents an elucidation of the structure of the polysaccharide from A. podalyriaefolia gum and Part II illustrates the use of GLC separation of the acetates of partially methylated glycitols, derived from methylated gums, as a technique for the elucidation of polysaccharide structures.

*Investigated by P. I. Bekker.

PART I

1 INTRODUCTION

1.1 The characterization, function and origin of gums

Gums are characterized by being insoluble in alcohol and organic solvents generally, but with water they dissolve or swell to form mucilaginous mixtures.

Polysaccharides are components of almost all living organisms, and are most abundant in the higher orders of land plants and seaweeds. They fill diverse roles in the physiology of animals, plants and micro-organisms. They serve as structural materials and as fuel reserves. Polysaccharides serve structurally in the exoskeletons of insects, as surface materials they protect tissues from desiccation, and as gums are exuded from plants to seal and protect wounds, preventing further invasion of the tissue by micro-organisms.[8]

There is no agreement as to the origin of plant gum exudates. Some favour the theory [9,10] that they are products of normal plant metabolism, while others suggest that they arise from some pathological condition. Healthy *Acacia* trees grown under favourable conditions of moisture, soil and temperature do not produce gum; when grown under adverse conditions, the secretion of gum is favoured. This evidence then favours the latter view. The gums are thought by others to be produced as a result of infection of the plant by micro-organisms.[9] Two types of bacteria have been isolated from the exudates of *A. binervata* [9] and *A. pennineris.* The chief type, *Bacterium Acaciae*, when grown on artificial media, produced a slime which contained a gum of the arabino-galactan type. Innoculation of peach trees with *Bacterium Acaciae* isolated for *A. binervata* produced gummosis.[9]

Stewart has indicated that the translocation of gums from living cells into adjacent dead cells along ray parenchyma cells may represent a means of excretion of these waste metabolic products.[10]

1.2 The chemistry of Acacia gum polysaccharides.

The exudates from *Acacia* trees are generally known as "gum arabic" or "gum acacia", but this term should be used to denote the gum from A. senegal only, as chemical investigations have shown that the gums exuded from different Acacia species differ in their composition.

Although several hundred Acacia species are known botanically, only thirteen have been studied chemically to any extent; a few species have been examined in minor detail, from some particular aspect.[11, 12, 13,14] In the ensuing discussion only those which have received detailed examination will be described.

1.2.1 Acacia senegal gum

Early investigations [15, 16, 17, 18] showed gum arabic to be a neutral salt of an organic acid, arabic acid, and that D-galactose and L-arabinose were produced on acid hydrolysis. Later the presence of L-rhamnose[19,20] and glucuronic acid[20], the latter isolated as an aldobiouronic acid composed of D-galactose and D - glucuronic acid, were noted in the hydrolysate of A. senegal gum. A study of the aldobiouronic acid showed it to be 6-O-(D-glucuronosyluronic acid)- D- galactose [21] and its synthesis [22] established it as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose.

Investigations of arabic acid by Smith and Jackson[19,23, 24, 25,26] led to the isolation of L-rhamnose, L-arabinose,
3 - O - D -galactopyranosyl - L - arabinose [27],
3 - O - D -galactopyranosyl - D - galactose,
6 - O -(D-glucopyranosyluronic acid) - D - galactose and a degraded acid. 3 - O - β - L - arabinopyranosyl - L - arabinose was shown by Andrews and Jones [28] to be a constituent unit of the gum.

The nature and mode of linking of the D-galactose and D-glucuronic acid residues in the degraded acid was ascertained by methylation [24]; hydrolysis showed 2,3,4,6-tetra-O-methyl-D-galactose (1 mol.), 2,3,4 - tri-O-methyl-D - galactose (5), 2,4-di-O-methyl-D - galactose (3) and 2,3,4-tri-O-methylglucuronic acid (3). The isolation of 6-O -(2,3,4-tri-O-methyl-β-D-glucosyluronic acid)-2,3,4-tri-O-methyl-D-galactose from methylated degraded arabic acid indicated that the side chains containing the D-glucuronic acid must be linked with the main D-galactose chain through at least one D-galactose residue, otherwise the D-galactose moiety of the methylated aldobiouronic acid would have been 2,4-di-O-methyl-D-galactose.

A possible structure for degraded arabic acid is shown by I (page 7).

The branch point D-galactopyranose unit is linked through $C_{(3)}$ and $C_{(6)}$, giving rise to 2,4-di-O-methyl-D-galactose on methylation and hydrolysis. The mode of linkage is not represented as the methylation data cannot define whether it is joined to other D-galactose units in the main galactose chain by a 1->3 linkage and to the side chain by a 1->6 linkage , whether the reverse is the case, or whether they are randomly linked.

Methylation of gum arabic followed by hydrolysis and fractionation of the mixture of methyl ethers afforded 2,3,4-tri-O-methyl-L -rhamnose, 2,3,5-tri-O-methyl-L-arabinose, 2,5-di-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose, 2,3-di-O-methyl-D-glucuronic acid and 2,3,4-tri-O-methyl-D-glucuronic acid. Identification of these fragments showed that the labile sugar residues, L-arabinose, L-rhamnose and 3-O-D-galactopyranosyl-L-arabinose were linled to the nucleus of arabic acid in the form of L-arabinofuranose, L-rhamnopyranose and 3-O-D-galactopyranosyl-L-arabinofuranose.

Methylated degraded arabic acid gave 2,3,4-tri-O--methyl-D -galactose and 2,4-di-O-methyl-D-galactose on hydrolysis, whereas the only partially methylated D-galactose derivative isolated from methylated arabic acid was 2,4-di-O-methyl-D-galactose. It follows, therefore, that the labile sugar residues were attached at $C_{(3)}$ of those galactose units in degraded arabic acid which gave rise to 2,3,4-tri-O-methyl-D-galactose on hydrolysis of the methylated degraded acid. Methylated gum arabic afforded mainly

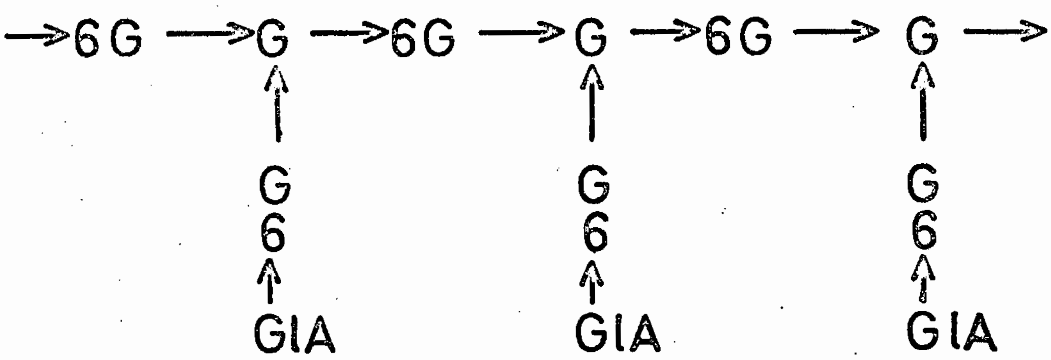
2,3-di-*O*-methyl-*D*-glucuronic acid and only a small quantity of 2,3,4-tri-*O*-methyl-*D*-glucuronic acid on hydrolysis, the latter being the only methyl derivative of *D*-glucuronic acid isolated from the hydrolysate of methylated degraded arabic acid. Some of the labile sugar residues are therefore attached through $C_{(4)}$ of the *D*-glucuronic acid residues.

A number of formulae, differing only in detail, could be assigned to gum arabic. One of these is shown by structure II (page 7).

Arabic acid was degraded[29] according to the procedures described by Barry.[30] After three oxidation-degradation procedures all arabinose was removed and the residual galactan was only slightly degraded on a subsequent Barry degradation. These results showed that the galactose residues in the main chain of arabic acid are linked by $1 \rightarrow 3$ glycosidic bonds.

Oxidation of degraded arabic acid with periodic acid, followed by reduction of the polyaldehyde to the polyalcohol and mild acid hydrolysis of the acetal linkages[31] resulted in the formation of a polysaccharide containing *D*-galactose and *D*-lyxitol (*D*-arabinitol), the latter arising from the oxidation of the reducing end-group. Further degradation followed by methylation, hydrolysis of the methylated derivative and identification of the methyl ethers confirmed the $1 \rightarrow 3$ linkage of the galactose units in the main galactan chain.

Osmotic pressure determinations showed the methylated degraded arabic acid to have a number-average molecular weight, $\bar{M}_n$,[24] of approximately 4,800, compared with 220,000 for the number - average molecular weight of gum arabic by similar methods of measurement. Extensive degradation of the gum macromolecule occurred during hydrolysis. This degradation is much greater than could have arisen from the simple removal of arabinose and rhamnose from the periphery. The postulate[9] that blocks of degraded units might have been interconnected by labile arabinofuranose residues has been rejected.[32] Moreover there is no



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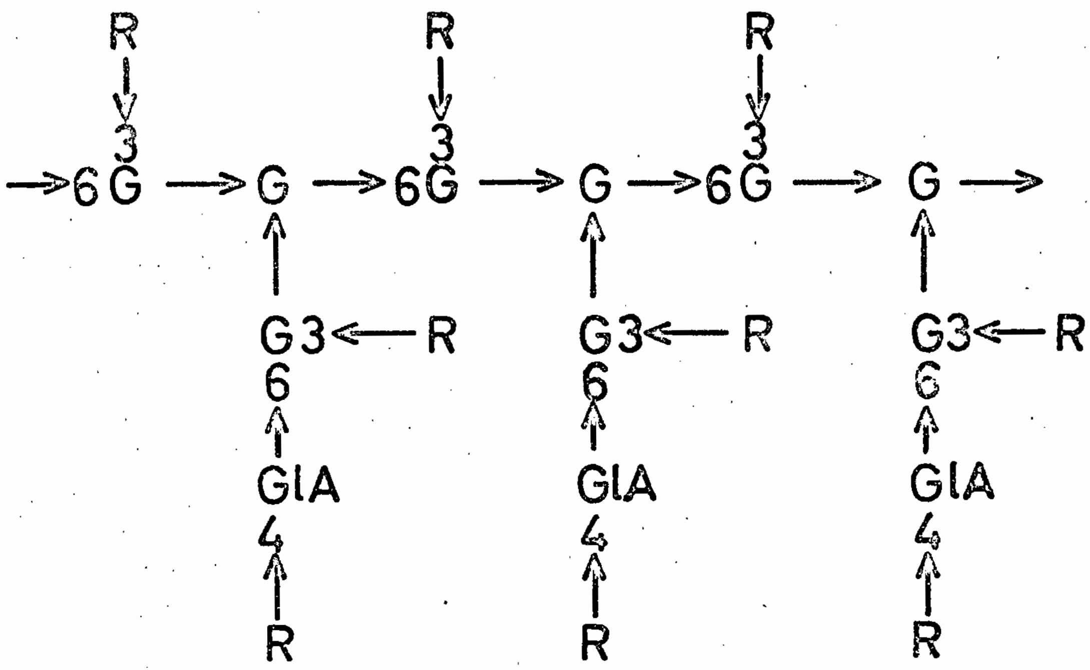


II

G = D-galactopyranose.

GlA = D-glucuronic acid.

R = L-arabinofuranose,

L-rhamnopyranose, or

3-O-D-galactopyranosyl-L-arabinose

evidence from methylation studies for the presence of any galactofuranose residues; this implies that certain galactopyranosidic bonds must be unusually reactive under the mild conditions of hydrolysis.

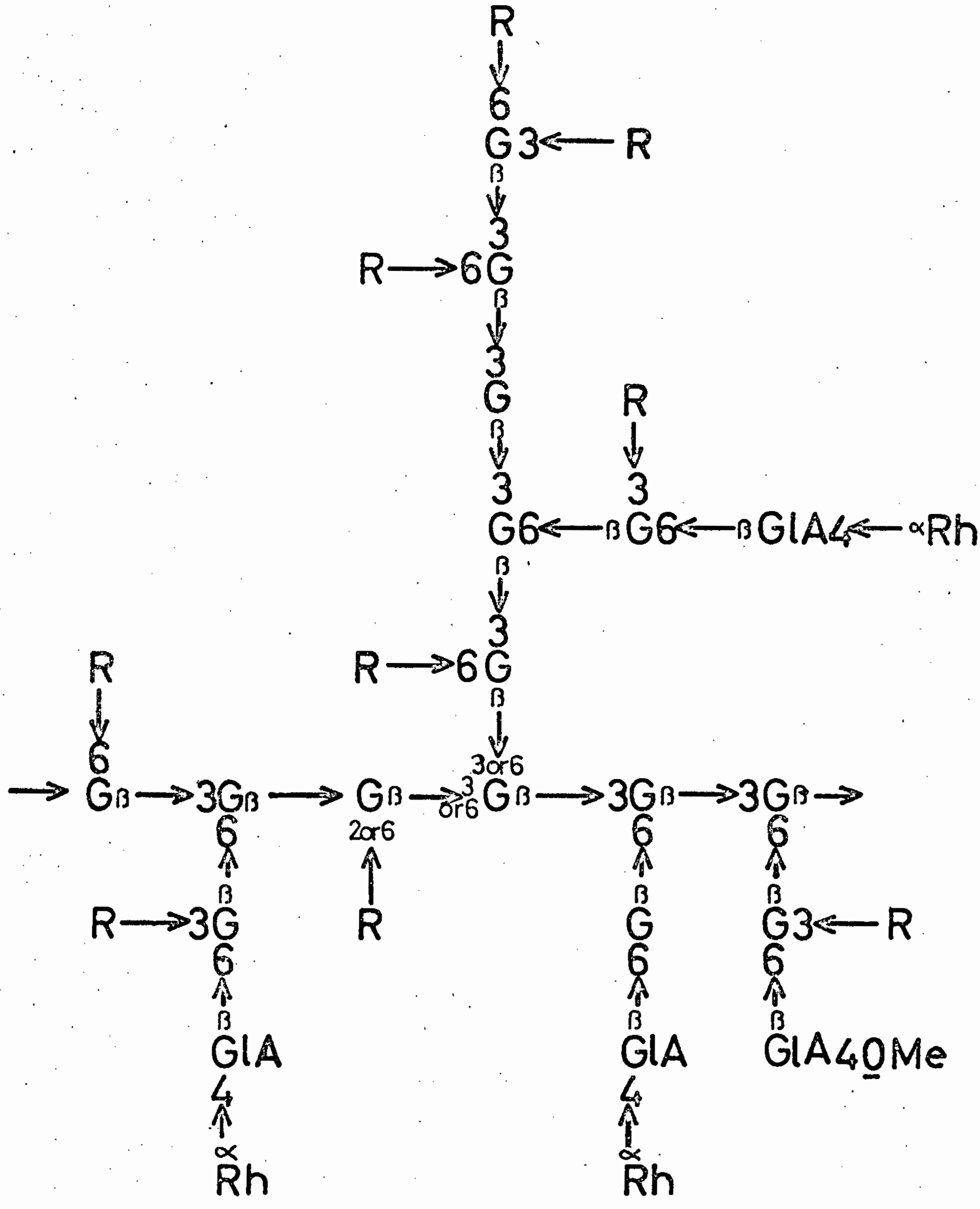
Anderson, Hirst and Stoddart[33] have subjected A. senegal gum to seven successive Smith degradations. The methylated derivatives of each polysaccharide obtained from the first five degradations were analysed, after methanolysis, by GLC. Partial acid hydrolysis of the polysaccharide obtained after five degradations afforded 6-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-β-D-galactopyranosyl-D-galactose and a β1→3 linked galactotriose. These results, together with the evidence obtained from autohydrolysis of the gum, led these workers to propose structure III as a possible repeating unit in A. senegal gum.

1.2.2 Acacia pycnantha gum

Although containing the same sugar residues as other Acacia gums, A. pycnantha gum differs from gum arabic[19,23,24,25,26,31] and from the gums of other species.[5,34,35,36,37,38,39.]

Hydrolysis of the gum under controlled conditions resulted in the release of L-arabinose, L-rhamnose, trace amounts of D-galactose, a disaccharide, identified as 3-*O*-L-arabinofuranosyl-L-arabinose and a degraded gum. Methylation of the degraded gum, followed by hydrolysis and identification of the methyl ethers afforded 2,3,4,6,-tetra-, 2,4,6-tri- and 2,4-di-*O*-methyl-D-galactose and a methylated aldobiouronic acid together with trace amounts of 2,6-di- and 2-*O*-methyl-D-galactose. The methylated aldobiouronic acid was shown to arise from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. These results suggested a highly branched, stable framework of 1→3- and 1→6- linked D-galactopyranose residues, for which structures IV and V could be proposed. The isolation of the methylated aldobiouronic acid indicated that the acidic unit (VI) must be attached as side chains.

Evidence in favour of structure IV was obtained from the application of Barry's method of degradation[30] to the polysaccharide.



G = D-galactopyranose.

GlA = D-glucopyranosyluronic acid.

GlA4OMe = 4-O-methyl-D-glucopyranosyluronic acid.

Rh = L-rhamnopyranose.

R = L-arabinofuranose.

L-arabinofuranosyl(1→3)-arabinofuranose.

L-arabinopyranosyl-β(1→3)-arabinofuranose.

L-arabinofuranosyl(1→3)-arabinofuranosyl(1→3)-arabinofuranose.

L-arabinopyranosyl-β(1→3)-arabinofuranosyl(1→3)-arabinofuranose.

D-galactopyranosyl-α(1→3)-arabinofuranose.

III

Partial acid hydrolysis of the Barry degraded polysaccharide afforded 3-O-β-D-galactopyranosyl - D- galactose, together with small amounts of a 1→3 - linked galactotriose, thus proving the 1→3 linkage of galactose residues in the basal chain.

Methylation of A.pycnantha gum and characterisation of the methyl ethers released on hydrolysis showed 2,3,4-tri-O-methyl-rhamnose, 2,3,5-tri- and 2,5-di-O-methyl-L-arabinose, 2,3,4,6,-tetra-, 2,3,4- and 2,4,6-tri- and 2,4-di-O-methyl-D-galactose, together with a methylated aldobiouronic acid 2,3,4-tri-O-methyl-6-O-(2,3,4-tri-O-methyl-β-D-glucopyranosyluronic acid) - D-galactose. Trace amounts of 2,6-di- and 2-O-methyl-D-galactose as well as two methyl ethers of arabinose were noted in the hydrolysate.

A consideration of these results led Aspinall, Hirst and Nicolson to propose structure VII for A.pycnantha, with the aldobiouronic acid units VI attached in an unknown manner.

1.2.3 Acacia cyanophylla gum

The gummy exudate from A.cyanophylla[5,37] contained the same monomeric components as A.pycnantha gum, but in different molecular proportion. Autohydrolysis of A.cyanophylla gum afforded L-rhamnose, L-arabinose, D-galactose, 3-O-α-D-galactopyranosyl-L-arabinose, 6-O-(β-D-glucopyranosyl uronic acid)-D-galactose and a degraded acidic polysaccharide. The degraded acid had an equivalent weight of 700, and was considered by Charlson to be identical with the degraded gum obtained on hydrolysis of A.pycnantha gum.

1.2.4 Acacia mearnsii gum

The gum from A.mearnsii[36] (syn. A.decurrens var. mollis, A. mollissima) afforded L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid in the molar ratios of 6:1:5:1, on acid hydrolysis. In a comparative investigation carried out on the above-mentioned sample of South African origin and a sample of Jamaican origin, Aspinall, Carlyl and Young[40] found the latter sample to be composed

$$\begin{array}{ccccccccc} \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow \\ & 6 & & & & 6 & & & \\ & \uparrow & & & & \uparrow & & & \\ & G & & & & G & & & \end{array}$$

IV

$$\begin{array}{ccccc} \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow \\ & 6 & & 6 & \\ & \uparrow & & \uparrow & \\ & G & & G & \\ & 3 & & 3 & \\ & \uparrow & & \uparrow & \\ & G & & G & \end{array}$$

V

$$GlA \rightarrow 6G \longrightarrow$$

VI

$$\begin{array}{ccccccccc} \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow & 3G & \longrightarrow \\ & 6 & & 6 & & 6 & & 6 & \\ & \uparrow & & \uparrow & & \uparrow & & \uparrow & \\ & G & & R & & G & & R & \\ & 6 & & & & & & & \\ & \uparrow & & & & & & & \\ & R & & & & & & & \end{array}$$

VII

G = D - galactopyranose.

GlA = D - glucopyranosyluronic acid.

R = L - arabinofuranose,

L - arabinofuranosyl(1→3)-arabinofuranose,

or L-rhamnopyranose.

of the same four sugars in the molar ratios of 4.9:1:5.6:1.6. Some of the glucuronic acid in the Jamaican sample was present as the 4-O-methyl ether.

Autohydrolysis of the free acid liberated the labile residues of L-arabinose, L-rhamnose, 3-O-β-L-arabinopyranosyl-L-arabinopyranose,[38] 3- and 6-O-β-D-galactopyranosyl-D-galactose,[40] affording a degraded acidic polysaccharide A, which was composed solely of residues of galactose and glucuronic acid. The highly branched nature of the degraded gum A was established by the detection of the methyl glycosides of 2,3,4-tri-O-methylglucuronic acid, and 2,3,4,6-tetra-, 2,3,4-and 2,4,6-tri-, and 2,4-di-O-methyl-D-galactose on methanolysis of the methylated degraded gum A. Smith degradation of degraded gum A afforded degraded gum B. Partial acid hydrolysis and methylation studies showed degraded gum B to be a galactan consisting mainly of 1 ⟶3- linked sugar residues.

Partial acid hydrolysis of A.mearnsii gum resulted in the release of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 6-O-(4-O-methyl-β-D-glucopyranosyl uronic acid)-D-galactose. Two acidic trisaccharides were also detected.[40]

On the basis of these results, Aspinall and co-workers have proposed structure VIII as a possible repeating unit for degraded gum A.

Samples of A.mearnsii of both South African and Jamaican origin were converted into the methylated derivatives and portions of these derivatives were reduced with lithium aluminium hydride. GLC analysis of the methanolysates of the methylated polysaccharides and of the reduced methylated polysaccharides showed that both of the methylated gums contained residues of the following sugars: 2,3,4-tri-O-methyl-rhamnose, 2,3,5-tri- and 2,3-and 2,5-di-O-methylarabinose, 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, and 2,4-di-O-methyl-galactose and 2,3,4-tri-and 2,3-di-O-methyl-glucuronic acid. No substantial differences in the relative proportions of the constituent sugar units in the two methylated polysaccharides were observed.

A comparison of the sugar constituents of methylated A. mearnsii gum with those from methylated degraded gum A indicated that the peripheral residues removed during the autohydrolysis of the gum included end-groups of L-rhamnopyranose, L-arabinofuranose and (in small proportion) L-arabinopyranose, together with 3- and 5-O- substituted L-arabinofuranose residues. The exact points of attachment of the various outer-chain units to the inner parts of the polysaccharide structure are not yet known, but these do include $C_{(4)}$ of the unmethylated D-glucuronic acid residues and $C_{(3)}$ or $C_{(6)}$ or both of D-galactose residues. Structure IX was proposed to accommodate the known structural features of A. mearnsii gum.

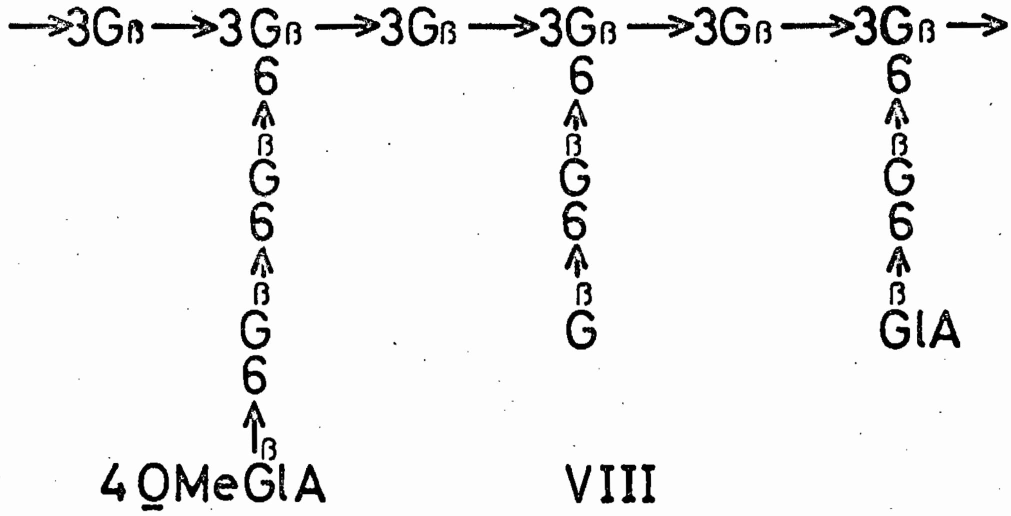
1.2.5 Acacia karroo gum

The gum from A. karroo [38,41] differs from those gums discussed previously in having a positive specific rotation. Autohydrolysis of the gum afforded L-rhamnose, L-arabinose, D-galactose, two neutral reducing disaccharides, a mixture of acidic oligo - saccharides and a degraded acidic polysaccharide. One of the neutral reducing oligosaccharides had the same chromatographic mobility as 3-O-α-D-galactopyranosyl-L-arabinose, while the other was identified as 3-O-β-L-arabinopyranosyl-L-arabinopyranose.

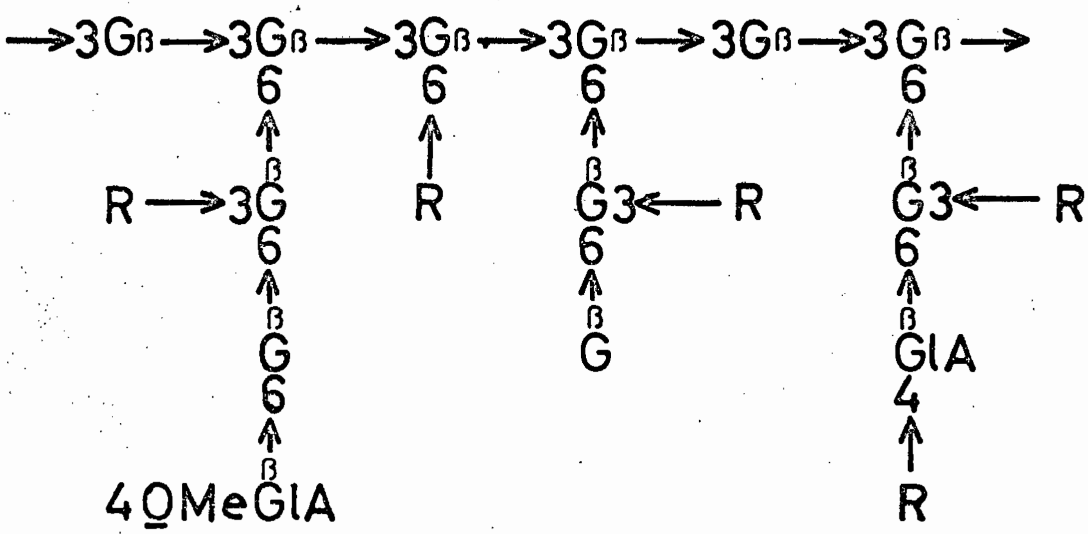
Hydrolysis of A. karroo gum with 0.5N-sulphuric acid afforded the above-mentioned monosaccharides and two aldobiouronic acids. The aldobiouronic acids were characterised as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 4- O - (α-D-glucopyranosyluronic acid)-D-galactose.

1.2.6 Acacia arabica gum

The gummy exudate from A. arabica [42] has a high positive specific rotation and contains 4-O-methyl-D-glucuronic acid in addition to L-rhamnose, L-arabinose, D-galactose and D-glucuronic acid, present in the gums from the above-mentioned Acacia species. As the rhamnose content was very low, it was assumed to be present in the



VIII



IX

G = $\underline{\underline{D}}$-galactopyranose.

GlA = $\underline{\underline{D}}$-glucopyranosyluronic acid.

4$\underline{O}$MeGlA = 4-$\underline{O}$-methyl-$\underline{\underline{D}}$-glucopyranosyluronic acid.

R = $\underline{\underline{L}}$-arabinofuranose,

$\underline{\underline{L}}$-arabinofuranosyl-(1→3)-$\underline{\underline{L}}$-arabinofuranose,

$\underline{\underline{L}}$-arabinofuranosyl-(1→5)-$\underline{\underline{L}}$-arabinofuranose,

$\underline{\underline{L}}$-arabinopyranose,

$\underline{\underline{L}}$-arabinopyranosyl-β(1→3)-$\underline{\underline{L}}$-arabinofuranose,

or $\underline{\underline{L}}$-rhamnopyranose.

L-form, because of its occurrence in this form in other Acacia gums.[9]

Partial acid hydrolysis of A. arabica gum resulted in the release of almost all the arabinose, trace amounts of galactose and 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose affording a degraded gum A. The degraded acid A was composed of D-galactose (86%) L-arabinose (2%) and D-glucuronic acid (11%)

An examination of the hydrolysate of methylated degraded gum A showed the presence of 2,3,4-tri-O-methyl-L-arabinose(tr) 2,3,4,6-tetra-(++), 2,3,6-(+/2), 2,4,6-(++) and 2,3,4-tri(+++), 2,6-(+) and 2,4-di-(+++) and 2-O-methyl-D-galactose (+), and 2,3,4-tri-O-methyl-D-glucuronic acid (++). These results indicated that the galactose residues in the basal chain are present in a highly branched framework of β1→6- and β1→3-linked D-galactose residues. A GLC examination of the methyl glycosides of the methylated reduced product, obtained after reduction of methylated degraded gum A with lithium aluminium hydride, followed by re-methylation and methanolysis, showed the presence of 2,3,4,6-tetra-O-methyl-D-glucose in addition to the above-mentioned neutral O-methyl sugars. This confirmed that 4-O-methyl -D-glucuronic acid residues were present as non-reducing end-groups in degraded gum A.

Partial methanolysis of methylated degraded gum A indicated the presence of the methylated aldobiouronic acids, identified as 4-O-(2,3,4-tri-O-methyl-α-D-glucopyranosyluronic acid)-2,3,6-tri-O-methyl-D-galactose and 6-O-(2,3,4,-tri-O-methyl-β-D-glucopyranosyluronic acid)-2,3,4-tri-O-methyl-D-galactose, together with small amounts of 6-O-(2,3,4-tri-O-methyl-β-D-glucopyranosyluronic acid)-2,4-di-O-methyl-D-galactose. The isolation of these methylated aldobiouronic acid residues indicated that X, XI and XII are possible structural fragments attached to the galactan framework of degraded gum A. In addition to these uronic acid residues, L-arabinopyranose and D-galactopyranose residues occupy terminal non-reducing positions.

Some idea of the distribution of β1 ⟶ 3- and β1 ⟶ 6-linkages within the galactan framework was obtained by partial acid hydrolysis and methylation analysis of the degraded gum B, isolated after a Smith degradation of degraded gum A. The isolation of both β1 ⟶ 3- and β1 ⟶ 6-galactobioses on partial acid hydrolysis and the identification of 2,3,4,6-tetra-(++), 2,4,6-(++++), and 2,3,4-tri-(+), 2,6-(+) and 2,4-di-(++), and 2-O-methyl-D-galactose(+), and 2,3,4-tri-O-methyl-D-glucuronic acid (trace) indicated that structure XIII is a possible structural fragment of degraded gum A. These results suggested that the structure of the gum is based on a branched framework of β1 ⟶ 3- and β1 ⟶ 6- linked D-galactose residues in which no "main chain" appears.

Two disaccharides obtained from the diffusate of the mild acid hydrolysis of the whole gum were identified as 3-O-β-L-arabinopyranosyl-L-arabinose and 3-O-β-L-arabinofuranosyl-L-arabinose. As both arabinobioses were released by relatively mild acid hydrolysis conditions, Anderson has suggested that in each case the reducing arabinose residue was initially present in the furanose form in the arabinose-containing side chain.

An examination of the methyl ethers released on acid hydrolysis of methylated *A. arabica* gum indicated the presence of 2,3,5- and 2,3,4-tri-, and 2,5-, 3,5- and 3,4-di-O-methyl-L-arabinose, 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid together with trace amounts of 2,3,4-tri-O-methyl-L-rhamnose, 4-O-methyl-L-arabinose and 2,3,4,6-tetra-, 2,3,6-, 2,4,6- and 2,3,4-tri- and 2-O-methyl-D-galactose, D-galactose and L-arabinose. The small amounts of 4-O-methyl-L-arabinose, L-arabinose, 2-O-methyl-D-galactose and D-galactose were not thought to be structurally significant, and probably arose from

$$GlA_{\alpha} \rightarrow 4G_{\beta} \rightarrow$$

or

$$4OMeGlA_{\alpha} \rightarrow 4G_{\beta} \rightarrow$$

X

$$GlA_{\beta} \rightarrow 6G_{\beta} \rightarrow$$

or

$$4OMeGlA_{\beta} \rightarrow 6G_{\beta} \rightarrow$$

XI

$$\begin{array}{ccc} GlA_{\beta} \rightarrow & 6G_{\beta} & \rightarrow \\ & 3 & \\ & \uparrow & \end{array}$$

or

$$\begin{array}{ccc} 4OMeGlA_{\beta} \rightarrow & 6G_{\beta} & \rightarrow \\ & 3 & \\ & \uparrow & \end{array}$$

XII

$$\begin{array}{ccc} \rightarrow 3G_{\beta} \rightarrow & 6G_{\beta} & \rightarrow 3G_{\beta} \rightarrow \\ & 3 & \\ & \uparrow & \\ & R & \end{array}$$

XIII

G = D-galactopyranose.

GlA = D-glucopyranosyluronic acid.

4OMeGlA = 4-O-methyl-D-glucopyranosyluronic acid.

R = L-arabinopyranose,
D-galactopyranose,
$\rightarrow$4-D-galactopyranose,
or $\rightarrow$6-D-galactopyranose.

either incomplete methylation or de-O-methylation during hydrolysis.

Methylation evidence for the whole gum and for degraded gum A suggested that the arabinose-containing side-chains are attached to the galactan framework at the $C_{(3)}$ and/or $C_{(6)}$ positions of certain D-galactose residues.

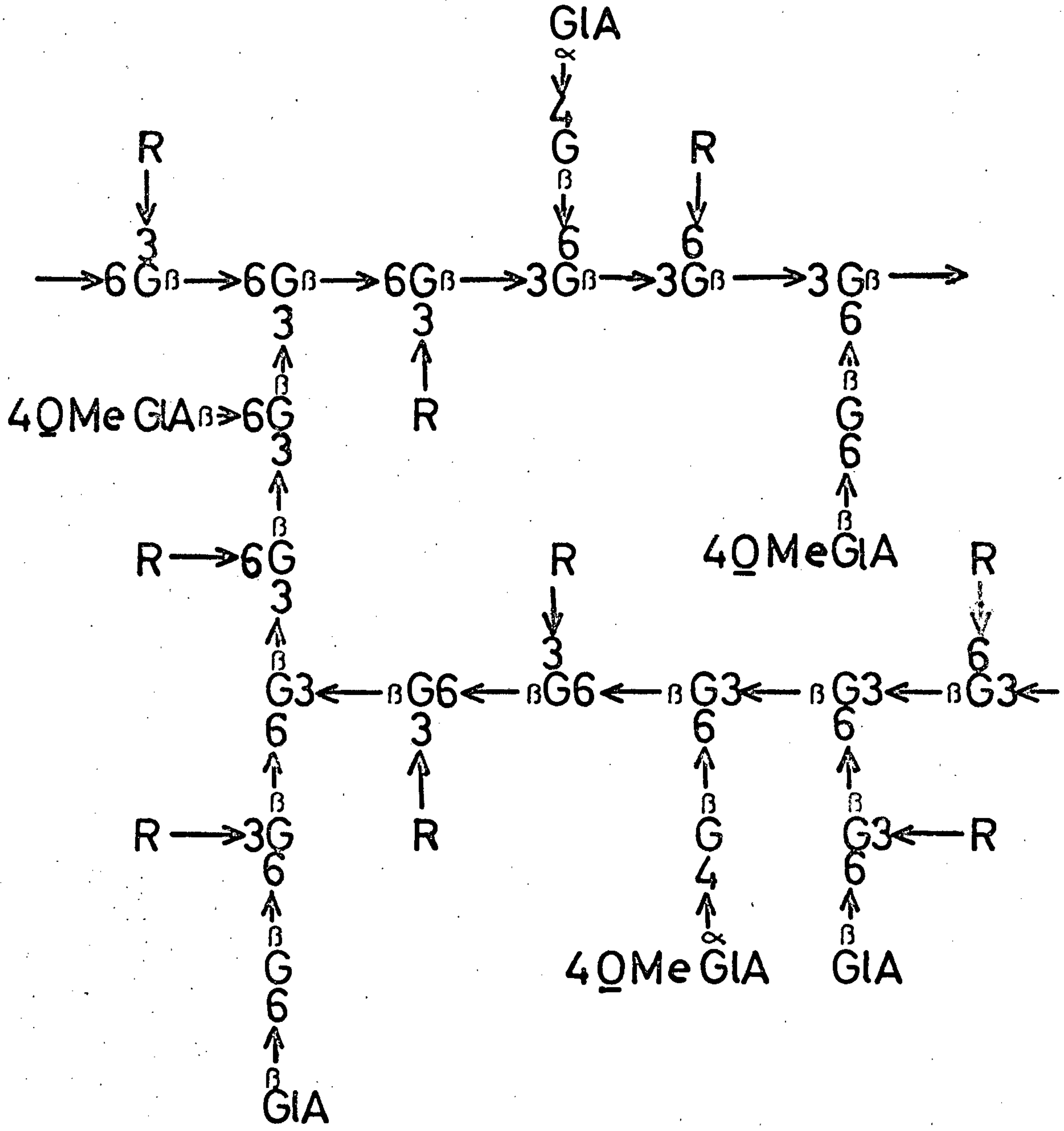
A. arabica gum was subjected to four successive Smith degradations and the complete fragmentation of the macromolecule showed that long chains or periodate-resistant β1→3- linked D-galactose residues are not such an important feature of A. arabica gum as they are of A. senegal gum.[33] In A. arabica gum, blocks of β1→3- linked D-galactose units must be interspersed by blocks of periodate-vulnerable β1→6-linked D-galactose residues.

The four polysaccharides obtained during the Smith degradation sequence were converted to the O-methyl derivatives. On methanolysis, the O-methyl derivatives gave the methyl glycosides of 2,3,5-tri-, 2,5- and 3,5-di-O-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,4-di-O-methyl-D-galactose. The identification of some 2,5- and 3,5-di-O-methyl-L-arabinose from the methylated derivative of the polysaccharide obtained after the fourth Smith degradation, indicated that some of the arabinose-containing side-chains in A. arabica gum must contain at least six 1,3- and/or 1,2-linked L-arabinose units.

A consideration of these results led Anderson, Hirst and Stoddart to propose XIV as a possible structural fragment from an A. arabica gum molecule.

1.2.7 Acacia laeta var. hashab gum.

A. laeta R.Br. ex Benth. is a natural hybrid of A. senegal (L.)Willd. and A. mellifera (Vahl.)Benth., and it occurrs in two varieties: A. laeta var. hashab (which resembles A. senegal) and A. laeta var. mellifera (which resembles A. mellifera).



G = $\underline{\underline{D}}$-galactopyranose.

GlA = glucopyranosyluronic acid.

4OMeGlA = 4-$\underline{O}$-methyl-$\underline{\underline{D}}$-glucopyranosyluronic acid.

R represents $\underline{\underline{L}}$-arabinofuranose- and $\underline{\underline{L}}$-arabinopyranose-containing side-chains. Some of these side-chains are at least six units long and contain ----3-$\underline{\underline{L}}$-arabinfuranose 1---- , ----2-$\underline{\underline{L}}$-arabinofuranose 1----, and ----2-$\underline{\underline{L}}$-arabinopyranose 1---- residues. They may be terminated by $\underline{\underline{L}}$-arabinofuranose or $\underline{\underline{L}}$-arabinopyranose residues.

XIV

The exudate from *A. laeta* var. *hashab* showed inter-nodule variations[6], the variations from the average being similar in extent to those found for *A. seyal* gum [2] and more marked than those found for *A. nilotica* gum.[1]

The polysaccharide from *A. laeta* var. *hashab* was shown to contain L-rhamnose, L-arabinose, D-galactose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid. Partial acid hydrolysis of the gum afforded two neutral disaccharides [43] and two aldobio-uronic acids.[6] The neutral disaccharides were characterised as 3-O-β-D-galactopyranosyl-D-galactose and 6-O-β-D-galactopyranosyl-D-galactose, and the aldobiouronic acids were shown to be 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Autohydrolysis of *A. laeta* gum released all of the rhamnose, nearly all of the arabinose, some galactose, three disaccharides and afforded a degraded gum A.[43] The disaccharides were characterised as 3-O-β-L-arabinofuranosyl-L-arabinose, 3-O-β-L-arabinopyranosyl-L-arabinose[28] and 3-O-α-D-galactopyranosyl-L-arabinose.[27] The arabinofuranosyl-L-arabinose disaccharide was only obtained when the whole gum was autohydrolysed in a dialysis bag immersed in distilled water at 80°.

An examination of methylated degraded gum A showed the presence of 2,3,4,6-tetra-(++), 2,4,6-(++), 2,3,4-tri-(++), 2,4-di-(+++) and 2-O-methyl-D-galactose (+), 2,3,4-tri-O-methyl-D-glucuronic acid (+++), and trace amounts of 2,6-di-O-methyl-D-galactose and 2,3,4- and 2,3,5-tri-O-methyl-L-arabinose.

Smith degradation of degraded gum A afforded a degraded gum B, which on partial acid hydrolysis gave 3-O-β-D-galactopyranosyl-D-galactose and a trace of 6-O-β-D-galactopyranosyl-D-galactose. An examination of the O-methyl derivative of degraded gum B indicated the presence of 2,3,4,6-tetra(+), 2,4,6-(++++++), 2,3,4-(trace), and 2,3,6-tri-(trace), 2,6-(+) and 2,4-di-(++), and 2-O-methyl-D-galactose (+). These results suggested that degraded gum B is a galactan, composed predominantly of β-D-(1 ⟶3)-glycosidic linkages with a few β-D-(1 ⟶6) branch-points. Structure XV was proposed as a possible structural fragment of degraded gum A.

Methylated A. laeta gum was methanolysed and the methyl glycosides of 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri- and 2,5-di-O-methyl-L-arabinose, 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,4-di-O-methyl-D-galactose, and 2,3,4-tri- and 2,3-di-O-methyl-D-glucuronic acid were identified by GLC analysis. The GLC traces were virtually identical with those obtained [33] for a methanolysate of methylated A. senegal gum, and are indicative of a strong structural similarity between the gums of A. senegal and A. laeta var. hashab.

A sample of A. laeta gum was subjected to eight Smith degradations and polysaccharides 1 - 6 and 8 were methylated, methanolysed and analysed by GLC. The number-average molecular-weights, $\bar{M}_n$, of polysaccharides 1-8 were estimated by molcular-sieve chromatography. [32,33] The results showed that the degraded polysaccharides 5 - 8 were not simple, linear β-D-(1 ⟶3)- linked galactans. Although 2,4,6-tri-O-methyl-D-galactose was the predominant O-methyl sugar obtained from methylated polysaccharides 5 - 8, the identification of 2,4-di-O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose indicated occasional branching at the $C_{(3)}$ and $C_{(6)}$ positions. The increase in the molar proportion percent of 2,4,6-tri-O-methyl-D-galactose from polysaccharides 5 to 8 indicated a progressive decrease in the degree of branching.

A. laeta and A. senegal gums are closely similar on an overall molecular basis. As in the case of A. senegal gum[33], there is no proof for a "main-chain" in A. laeta gum.

1.2.8 Acacia drepanolobium gum.

A. drepanolobium gum [44], a further example of an Acacia gum having a positive specific rotation, was shown to contain L-rhamnose, L-arabinose, D-galactose, D-glucuronic acid

$$\begin{tikzcd} {} \arrow[r] & 3G_\beta \arrow[r] & 3G_\beta \arrow[r, dash] & 6G_\beta \arrow[r] & 3G_\beta \arrow[r] & {} \\ & & & R \arrow[u, "3"'] & & \end{tikzcd}$$

G = D-galactopyranose.

R = L-arabinopyranose,

L-arabinofuranose,

D-galactopyranose,

or D-galactopyranosyl 1—6-D-galactopyranose.

XV

and 4-O-methyl-D-glucuronic acid in the molar ratios of 1:52:38:7:2, and afforded four aldobiouronic acids on hydrolysis. The aldobiouronic acids were characterised as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

A. drepanolobium gum was fractionated into a water-soluble gum, a fraction soluble in dilute sodium chloride solution and an insoluble gel. An examination of two series of extraction showed no striking differences in the composition of these fractions.

1.2.9 Acacia nilotica gum.

The gum exudate from A. nilotica, having a high, positive specific rotation and containing only trace amounts of rhamnose, showed inter-nodule variations.[1]

Partial acid hydrolysis of the gum released arabinose, galactose, glucuronic acid and four aldobiouronic acids. The aldobiouronic acids were characterised as 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid) - D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

1.2.10 Acacia nubica gum

The gum from A. nubica [45], having a high, positive specific rotation, was shown to contain L-rhamnose, L-arabinose, D-galactose, and D-glucuronic acid in the molar ratios of 1:59:33:7.

Graded acid hydrolysis of the gum afforded two aldobiouronic acids, which were characterised as 6-O-(β-D-glucopyranosyluronic acid)- D-galactose and 4-O-(α-D-glucopyranosyluronic acid)-D-galactose. A. nubica gum differs from other acacia gums which have high methoxyl contents and high, positive specific rotations [1, 42, 44]; A. nubica gum has a low methoxyl content and consequently small proportions (if any) of 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.[46]

Autohydrolysis of *A. nubica* gum afforded 3-*O*-β-L-arabinofuranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, a β-(1→3)- linked-L-arabinose tri-saccharide, and a degraded gum A of weight-average molecular-weight, $\bar{M}_w$, 5730.

Partial acid hydrolysis of degraded gum A gave 3-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-β-D-galactopyranosyl-D-galactose, the β-D-(1→3)- and β-D-(1→6)-linked galactose trisaccharides, and a fraction which was probably a mixture of tri- and tetra- saccharides. An examination of the *O*-methyl derivative of degraded gum A indicated the presence of 2,3,4-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-, 2,3,6- and 2,4,6-tri-, and 2,4-di-*O*-methyl-D-galactose, and 2,3,4-tri-*O*-methyl-D-glucuronic acid.

Smith degradation of degraded gum A afforded degraded gum B, which was subjected to partial acid hydrolysis. Paper-chromatographic examination of the neutralised hydrolysate showed galactose, traces of arabinose and glycerol and three oligosaccharides. The oligosaccharides had the same paper chromatographic mobilities as authentic 6-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-β-D-galactopyranosyl-D-galactose and the β-(1→3)-linked D-galactose trisaccharide. Methylation of degraded gum B and analysis of the methanolysate by GLC showed the methyl glycosides of 2,3,4,6-tetra-(+++), 2,4,6-(+++) and 2,3,4-tri (+) and 2,4,-di-*O*-methyl-D-galactose (+) together with a trace of 2,3,5-tri-*O*-methyl-L-arabinose.

After methylation of the whole gum, cellulose-column chromatography showed the presence of 2,3,4- and 2,3,5-tri-, 2,5,- and 3,5,-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra, 2,3,4-, 2,3,6- and 2,4,6-tri-, 2,6- and 2,4-di and 2-*O*-methyl-D-galactose, and 2,3,4-tri-O-methyl-D-glucuronic acid.

Five sequential Smith degradations were carried out on the whole gum, and the five degraded polysaccharides (S1-S5) were examined by partial acid hydrolysis and methylation.

The results suggested that A. nubica gum has a highly branched galactan framework, some branches terminated by D-glucuronic acid (0.3% of the acid present as its 4-O-methyl derivative) linked mainly β-(1$\rightarrow$6) to galactose with some α-(1$\rightarrow$4) linkages. Galactose residues were shown to be linked β-(1$\rightarrow$3) and β-(1$\rightarrow$6), the latter type predominating.

Chains of L-arabinose residues, attached mainly at $C_{(3)}$ of the D-galactose residues, were shown to be at least six units long, and terminated by L-arabinofuranose and L-arabinopyranose residues linked β-(1$\rightarrow$3) to L-arabinose. The chains contained (1$\rightarrow$2)-linked L-arabinofuranose residues, with a smaller proportion of (1$\rightarrow$3)-linkages. The results did not define whether chains occurred which were exclusively (1$\rightarrow$2)- or (1$\rightarrow$3)-linked, or whether both types of linkages occurred in one chain.

A. nubica gum resembles A. arabica gum[42] as both are dextrorotatory and these gums must have structural differences from A. senegal [33] gum which is laevorotatory.

A. nubica gum resembles other Acacia gums in giving a degraded gum consisting mainly of D-galactose residues linked β-(1$\rightarrow$3) and β-(1$\rightarrow$6). Unlike the gums from A. senegal[33] and A. pycnantha[35], β-D-(1$\rightarrow$6)-linkages predominate in A. nubica gum. As in A. senegal gum[33], the L-arabinose chains are attached to $C_{(3)}$ of D-galactose; in A. pycnantha[35] they are attached to $C_{(6)}$ of D-galactose and in A. seyal gum they are attached to both $C_{(3)}$ and $C_{(6)}$ of D. galactose.

1.2.11 Acacia seyal gum

The gum from *A. seyal* [2] was shown to contain rhamnose, arabinose, galactose and glucuronic acid. The monosaccharides obtained on hydrolysis of a representative sample showed these residues to be present in the approximate ratios of 3:46:38:12.5.

Gums from nine individual nodules were examined as crude gum, and after purification by (i) precipitation, (ii) electro-dialysis and (iii) ion-exchange. The chemical composition and physical behaviour of the gum was found to vary from nodule to nodule.

Passage through a column of diethylaminoethylcellulose showed that the purified gum from individual nodules is heterogenious, two components, A and B being present. The proportion of component A in different nodules was found to vary from 34% to 41%; components A and B contained 12.5% and 15.3%, respectively, of glucuronic acid.

Acid hydrolysis of *A. seyal* gum [46] afforded four aldobiouronic acids, characterised as 6-O-(β-D-glucopyranosyl-uronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid) - D-galactose and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

1.2.12 Acacia sundra gum

The gum from *A. sundra*[39,47] was shown to contain L-rhamnose, L-arabinose, D-galactose, D-glucuronic acid and a component in trace amounts, which had the same paper chromatographic mobility as xylose. The neutral sugars were found to be present in the molar ratio of 1:2:3.

Autohydrolysis of *A. sundra* gum released L-rhamnose, L-arabinose and trace amounts of D-galactose and xylose, affording a degraded gum A.

Hydrolysis of degraded gum A resulted in the release of galactose and arabinose (trace) and gave two aldobiouronic acids. One of the acids was characterised as

6-O-(β-D-glucopyranosyluronic acid)-D-galactose, the other was present in only trace amounts.

1.2.13 Acacia catechu gum

A. catechu gum gave L-rhamnose, L-arabinose, D-galactose and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose on hydrolysis.[48]

Autohydrolysis of A. catechu gum afforded a degraded gum which had equivalent weight 850 and contained D-galactose and D-glucuronic acid in the ratio of 4:1.[49] The O-methyl derivative of the degraded gum was dissolved in chloroform and fractionally precipitated with petrol ether (b.p. 60-80°). The methoxyl contents and the specific rotations for the four fractions were:

(a) 39.1%, - ; (b) 42.5%, 45.2°;

(c) 43.8%, 48° and (d) 43.8%, 48°.

Fractions (c) and (d) were methanolysed, neutralised (Ag_2CO_3), treated with $Ba(OH)_2$, neutralised (CO_2) and separated into neutral methyl glycosides and barium salts of the acidic sugars. Acid hydrolysis followed by chromatographic separation of the neutral methyl glycosides afforded 2,3,4,6 tetra-(+), 2,3,4-tri-(+) and 4,6-di-O-methyl-D-galactose (++). The acidic sugar was characterised as 2,3,4-tri-O-methyl-D-glucuronic acid.

2. DISCUSSION OF RESULTS.

2.1 Introduction.

Acacia podalyriaefolia, A. Cunn. (*A. Fraseri*, Hook, *A. Caley*, A.Cunn.), or Queensland Silver Wattle [50, 51] is a tall, glabrous shrub having slightly greenish-grey foliage, the "leaves" being rather broad, sickle-shaped and many-nerved, and the branches covered with a soft pubescence. It has a handsome spreading habit and produces dense heads of globular flowers about January-March, and it is for this reason an ornamental tree in South Africa.[52] The pods are long and narrow and very much curved. The species is widely spread in Australia, being found on the Queensland and New South Wales coast, particularly along the river banks, and scattered throughout Victoria. From these trees *A. podalyriaefolia* gum exudes and solidifies as a dark brown plastic solid.

The gum used in the present work was collected by Professor A.M. Stephen from trees in Bryanston, Johannesburg, following heavy snowfalls, during July, 1964.

The *Acacia* species was identified at the Bolus Herbarium, University of Cape Town, by courtesy of the Director.

2.2 Purification of *A. podalyriaefolia* gum.

Precipitation of *A. podalyriaefolia* gum from the relatively limpid, aqueous solution with ethanol afforded a white, fibrous mass. This material was re-dissolved in water and re-precipitated with ethanol. The polysaccharide, after being washed with acetone and ether, was dissolved in water and deionised with Amberlite IR-120 (H^+) resin. The solution was neutralised ($BaCO_3$), filtered to remove any insoluble barium salts, and deionised with Amberlite IR-120(H^+) resin. The solution was shell frozen and freeze-dried. Unless otherwise stated, this purified polysaccharide was used for the structural investigations described in this thesis.

The gum gave a positive Molisch test, did not reduce Fehling's solution, and an analysis, as galactose, showed the gum to be composed entirely of carbohydrate residues. The gum had a low, positive specific rotation, $[\alpha]_D$ +5.3° (c 2.83) and analysis showed C, 42.1; H, 7.0; N, <0.1; S, <0.1 and OMe, 1.4%. The methoxyl groups present in polysaccharides from a number of Acacia species[53] have been shown to occur as the 4-O-methyl derivative of D-glucuronic acid.[46]

The equivalent weight of the gum, as an acid, was determined by titration with 0.01 M - NaOH, using phenol phthalein as indicator. The mean value of three determinations was found to be 3585, indicating a uronic anhydride content of 4.9%, which is slightly higher than the uronic anhydride content of 3.5% found by Kaplan and Stephen.[7]

Analysis of an hydrolysate of A. podalyriaefolia gum showed the polysaccharide to contain residues of L-rhamnose, L-arabinose, D-galactose and D-glucuronic acid in the molar ratios of 1:15:79:5.

The sedimentation and diffusion coefficients for A. podalyriaefolia gum were found to be 3.684×10^{-13} cm sec^{-1} dyne^{-1} g^{-1} and 7.81×10^{7} cm^{2}sec^{-1} respectively, and substitution of these constants in Svedberg's equation showed a weight-average molecular weight, $\bar{M}_w$, for A. podalyriaefolia gum of 33,500. (See section 3.7 for the determination of the coefficients).

Gel filtration of A. podalyriaefolia gum on Biogel P-300 showed a weight-average molecular weight, $\bar{M}_w$, of 31,500. Re-chromatography of some of the fractions obtained, indicated an unusually narrow distribution of molecular weights ranging from 25,500 to 31,500, about half of the material having a molecular weight of 31,500 (see fig. 1).

2.3 Partial acid hydrolysis of A. podalyriaefolia gum.

Mild acid hydrolysis was carried out in order to determine which monosaccharide residues could easily be removed, to

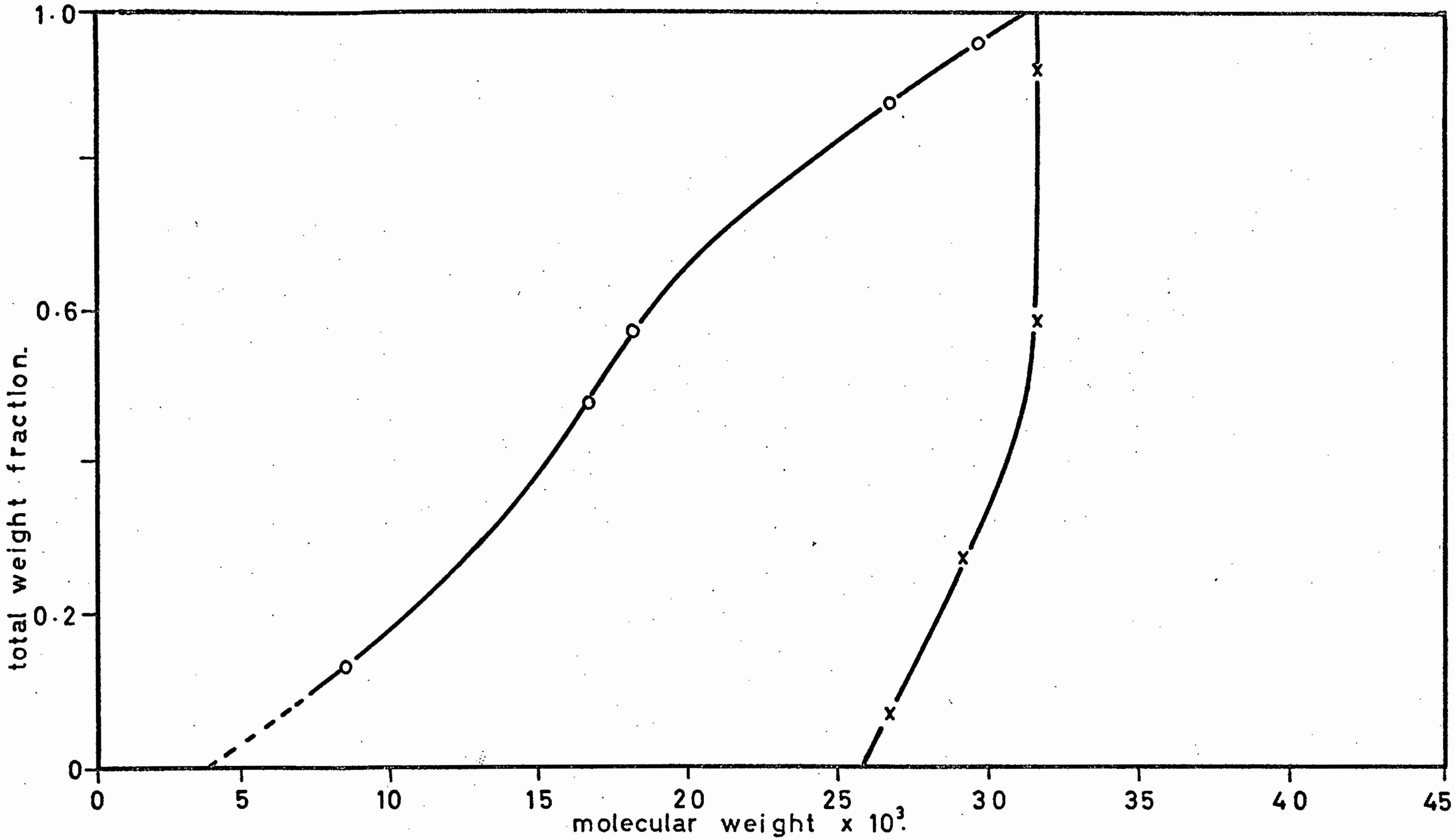


Fig.1 Integral molecular weight distribution curves of A.podalyriaefolia gum —x—x— and degraded gum B-o—o—.

investigate the possibility of obtaining oligosaccharides and also to investigate the residual degraded polysaccharide. Kaplan and Stephen[7] have reported the presence of non-reducing arabinofuranose and rhamnopyranose end-groups in A. podalyriaefolia gum; the mild condition of acid hydrolysis would remove many of these acid-labile side-chains, leaving the basal chain intact.

In an analytical experiment (not reported) a sample of the gum was hydrolysed in $0.01\underline{\underline{N}}$-$H_2SO_4$ at 96^o for 51 hours. The degraded polysaccharide was recovered by neutralisation ($BaCO_3$) and precipitation in 80% ethanol. The supernatant layer on centrifugation was concentrated to a syrup, and an examination by paper chromatography showed arabinose (+++), galactose (+), rhamnose (+) together with trace amounts of oligosaccharides.

In order to investigate the probability of the oligomers being formed and then further degraded during hydrolysis, the partial hydrolysis was repeated on a larger scale, under identical conditions, and interrupted after 15 hours by neutratisation of ($BaCO_3$), and removal of the low molecular weight material by precipitation of the degraded polysaccharide A with 80% ethanol. The recovered degraded polysaccharide A was further hydrolysed for 36 hours and the low molecular weight material separated, as previously, from degraded Polysaccharide B.

Paper chromatographic examination of the aqueous-ethanol supernatant layers showed strong similarities, the only difference being that a higher proportion of L-rhamnose was removed in the earlier stages of the hydrolysis. The combined weights of the two syrups was 430mg, approximately 25% of the weight of starting material. Paper chromatography showed the oligosaccharides to constitute approximately 5% of this weight or 1% of the weight of starting material. As the oligosaccharides constituted such a small proportion of the total gum, it was considered unnecessary to isolate and characterise these products. Paper chromatography in solvents (a), (b), (c) and (d) showed the oligosaccharides to have the same mobilities as 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose and O-β-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→3)-D-galactose.

Methanolysis and GLC analysis of the derived O-methyl derivative of degraded gum A showed the presence of 2,3,5-tri-O-methyl-L-arabinose (+), 2,3,4,6-tetra-(++++++), 2,3,6-tri-O-methyl-D-galactose (tr), 2,4,6-tri-O-methyl-D-galactose (++), 2,3,4-tri-O-methyl-D-galactose (+++), 2,4-di-O-methyl-D-galactose (++++++), 2,6-di-O-methyl-D-galactose (tr), and 2,3,4-tri-O-methyl-D-glucuronic acid(tr). Hydrolysis and paper chromatographic examination of the O-methyl derivative of degraded gum A showed also the presence of 2-O-methyl-D-galactose (tr) and 4-O-methyl-D-galactose(tr). The increase in the relative molar proportions of 2,4,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose in the O-methyl derivative of degraded gum A showed a decrease in the degree of branching in the macromolecule.

Further acid hydrolysis caused the specific rotation to change from +13° for degraded gum A to +26.4° for degraded gum B. The specific rotation of degraded gum B is identical with that obtained for the degraded gum obtained on partial acid hydrolysis of A. elata gum.[54]

GLC analysis of theglycitol acetates derived from degraded gum B and a determination of the equivalent weight showed this product to contain L-arabinose, D-galactose and D-glucuronic acid in the molar ratios of 1.5:93.5:5.

The O-methyl derivative of degraded gum B was methanolysed, and analysis by GLC showed the methyl glycosides of 2,3,5-tri-O-methyl-L-arabinose (tr), 2,3,4,6-tetra-O-methyl-D-galactose (+++), 2,3,6-tri-O-methyl-D-galactose (tr), 2,4,6-tri-O-methyl-D-galactose (++), 2,3,4-tri-O-methyl-

D-galactose (+) and 2,4-di-O-methyl-D-galactose (+++).
The absence of 2,3,6-tri-O-methyl-D-galactose in the methanolysates of degraded gum A and B showed there were no $1\rightarrow 4$-or $1\rightarrow 5$-linked galactose residues in the basal chain. The relative increase in the molar proportions percent of 2,4,6- and 2,3,4-tri-O-methyl-D-galactose as shown on GLC analysis of the methanolysates of methylated degraded gums A and B, has indicated that the labile L-arabinofuranose and L-rhamnopyranose non-reducing end-groups must be attached to the $C_{(3)}$ and $C_{(6)}$ positions of certain D-galactose residues in A. podalyriaefolia gum. These D-galactose residues, which give methyl 2,4-di-O-methyl-D-galactose in the methanolysate of methylated A. podalyriaefolia gum occur as the methyl glycosides of 2,4,6- and 2,3,4-tri-O-methyl-D-galactose in the methanolysates of methylated degraded gums A and B.

The partial acid hydrolysis investigation did not follow the theoretical consideration of Kuhn[55], who predicted that for random degradation of a uniform, linear macromolecule, the yield of the n-membered fragment is given by $n\alpha^2(1-\alpha)^{n-1}$, where α is the degree of scission. The maximum yield of the fragment is then $n[2/(n+1)]^2[(n-1)/(n+1)]^{n-1}$, and occurs when $\alpha = 2/(n+1)$. Further calculations showed that the maximum possible yield of dimer plus trimer amounts to 46%, although in practice this value seldom reached 35%.

The sedimentation and diffusion coefficients for degraded gum B were found to be respectively 2.873×10^{-13} cm sec $^{-1}$ dyne^{-1} g^{-1} and 10.94×10^{7} cm^2 sec^{-1}, indicating a weight-average molecular-weight, $\bar{M}_w$, of 18,650.

A diffusion coefficient of 10.94×10^{7}cm^{2}sec^{-1} suggested an average molecular weight of approximately 9,000 (see fig. 16) and a sedimentation coefficient of approximately 1.2×10^{-13}cm sec $^{-1}$dyne^{-1}was expected (see fig. 17).
The determined value was considerably higher than the predicted value, indicating the presence of higher molecular weight material in degraded gum B. These results showed that degraded

gum B was polydisperse, the low molecular weight material diffusing rapidly, resulting in a high diffusion coefficient, and the high molecular weight material sedimenting rapidly, resulting in a high sedimentation coefficient.

Biogel filtration of degraded gum B on Biogel P - 300 (fig. 1) confirmed the fact that degraded gum B was polydisperse.

Degraded gum B was reduced with sodium borohydride, hydrolysed in $\underline{\underline{N}}$-$H_2SO_4$ and the neutralised hydrolysate was examined by paper chromatography. Neither galactitol nor arabinitol was detected, further supporting a high average molecular weight for this degraded product. The absence of arabinitol in the hydrolysate does not, however, preclude the presence of internal acid-labile arabinofuranose residues in the basal chain of A. podalyriaefolia polysaccharide. The galactopyranosidic bonds are therefore resistant towards the mild conditions of acid hydrolysis, and A. podalyriaefolia gum is, in this respect, different from A. senegal gum[32,33] ($\bar{M}_w$ 600,000) and A. arabica gum,[42] ($\bar{M}_w$ 2.6X10^6), which are unusually sensitive to these conditions.

In order to investigate the possibility that the conditions of partial acid hydrolysis were too mild, degraded gum B was subjected to further investigation. The degraded gum was dialysed against distilled water and the non-dialysable material was hydrolysed in 0.05$\underline{\underline{N}}$-$H_2SO_4$ at 96° for 50 hours. The neutralised hydrolysate was concentrated and dialysed against distilled water. The diffusate was shown by paper chromatography to be composed entirely of D-galactose. No non-dialysable material was obtained, indicating the complete fragmentation of degraded gum B.

The diffusate from degraded gum B gave a trace of carbohydrate containing material, and was not further investigated.

Improved yields of di- and tri- saccharides are obtained if these products, once formed, are protected. Cellobiose octa acetate, for example, is partly protected by crystallisation during the acetolysis of cellulose.[56] Painter [57] has degraded polysaccharides using enzyme preparations; the reaction mixture was enclosed in a cellophane bag and the smaller degraded products were removed by dialysis. Painter has recently used a water-soluble polystyrene sulphonic acid resin [58] instead of the enzyme preparation.

In an attempt to increase the yields of di- and tri-saccharides in the degradation of A. podalyriaefolia gum, a sample of the polystyrene sulphonic acid resin was prepared and a portion of the gum was hydrolysed in a cellophane bag at 80°. Examination of the dialysate by paper chromatography showed this product to be of a similar composition to the aqueous-ethanol soluble material obtained on hydrolysis of A. podalyriaefolia gum with 0.01 N-H_2SO_4; there being no improvement in the yield of oligosaccharides. An examination of the polysaccharide residue, recovered from the polystyrene sulphonic acid resin by precipitation with ethanol, showed that very little structural degradation of A. podalyriaefolia gum had occurred.

The close structural resemblance between degraded gum B and the degraded gum obtained from A. elata, and the similarity between the aqueous-ethanol soluble components released on acid hydrolysis of the two Acacia gums, have supported the results of Kaplan and Stephen[7], who regarded A. elata as a template upon which the more complex structures are laid. Of the eight Acacia species studied, A. podalyriaefolia gum was considered to be the closest related structurally. The basal-chain was considered [7] to be composed of β-1→3-linked D-galactopyranose residues with multiple short branches at $C_{(6)}$. The identification of the methylglycosides of 2,4,6-tri-O-methyl-D-galactose and

2,3,4-tri-*O*-methyl-*D*-galactose in the methanolysates of methylated degraded gums A and B indicated the presence of both β-1→3- and β-1→6-linked *D*-galactopyranose residues in the basal chain of *A. podalyriaefolia* gum.

2.4 Methylation of *A. podalyriaefolia* gum, and isolation of the methyl ethers released on acid hydrolysis.

A sample of partially methylated *A. podalyriaefolia* gum, which had been methylated[7] according to the procedures of Haworth and Kuhn , was soluble in methyl iodide, and was, therefore, methylated using Purdie's reagents until no increase in the methoxyl content was observed. The methylated polysaccharide showed no absorption in the hydroxyl region of the infrared spectrum.

The methylated polysaccharide was dissolved in 98% formic acid and hydrolysed at 96° for 30 minutes.[41] De-*O*-methylation can, however, take place under the conditions.[59] Water was added and the product, after evaporation of the solvent, heated in *N*-H_2SO_4. After cooling, filtration and evaporation, the syrup was shown by paper chromatography to contain 2,3,5-tri-*O*-methyl-*L*-arabinose, 2,3,4,6-tetra-O-methyl-*D*-galactose, 2,3,6-tri-*O*-methyl-*D*-galactose 2,4,6-tri-*O* methyl-*D*-galactose, 2,3,4-tri-O-methyl-*D*-galactose, 2,4,-di-*O*-methyl-*D*-galactose, 2-*O*-methyl-*D*-galactose and methylated uronic acid.

The mixture of partially methylated sugars and sugar acids was separated by gradient elution from a column of cellulose and fractions were combined according to evidence obtained from paper and thin-layer chromatography.

Fractions containing sugars which could be eluted from a GLC column as the methylglycosides, namely tetra-, tri-, and di-*O*-methylgalactose, tri-, and di-*O*-methylarabinose and 2,3,4-tri-*O*-methylrhamnose,[7] were analysed quantitatively.

Difficulty was encountered in this respect as peaks appeared at T 2.6 and 6.1 in all fractions. Evaporation of a portion of the butan-1-ol, used as eluant, evaporation of water from the residue and GLC analysis showed peaks with identical retention times. Response factors were not determined for these unknown components and a factor of 1.00 was applied to their peak areas in the calculation of the weight of sugars in each fraction. It is for this reason that the total weight of the component sugars in a fraction is not equal to the weight of the fraction. In some fractions the areas of the unknown peaks were considerable, for example fractions 1 and 2, where only half the total weight was considered to be carbohydrate, whereas in others the areas were negligible, for example fraction 3.

Table I shows a comparison of the molar proportions of the component sugars obtained by (a) assay of the derived neutralglycitol acetates by GLC, and sugar acids by titration

(b) assay of the methyl glycosides by GLC, and

(c) isolation and characterisation of the partially methylated sugars obtained from the cellulose column.

Assay	Molar proportions percent			
	Uronic acids	galactose	arabinose	rhamnose
a	5.0	79	15	1
b	2.0	86	12.0	0.5
c	2.5	89.4	7.4	0.7

Table I.

The quantity of L-rhamnose recovered from the hydrolysis and separation of the monosaccharides of A. podalyriaefolia gum (see section 3.2.2) indicated as much as two molar proportions percent of rhamnose being present in the gum. Analysis of rhamnose as its 2,3,4-tri-O-methyl ether showed considerably less than 2% and it is possible that the volatile methyl ether is lost on evaporation of the solvent. All rhamnose present in A. podalyriaefolia gum exists as non-reducing L-rhamnopyranose end-groups.

The total amount of arabinose methyl ethers recovered from the column was approximately 50% of the original arabinose content in the gum, as analysed by method a, Table 1. The assumption that the peaks at T 2.6 and 6.1 have response factors of 1.00 might be incorrect. Although showing large peaks on GLC relative to the methyl glycoside peaks, the proportion by weight might be very low. This is supported by borohydride reduction of fraction 2, acetylation and analysis of the 2,3,5-tri-O-methyl-L-arabinose as 1,4-di-O-acetyl-2,3,5-tri-O-methyl-L-arabinitol (see Part II), when only one peak was obtained with a retention time identical with authentic 1,4-di-O-acetyl-2,3,5-tri-O-methyl-L-arabinitol. It is thus apparent that 2,3,5-tri-O-methyl-L-arabinose constitutes more than 50% of this fraction.

No 3,4-di-O-methyl-L-arabinose was detected and the report[7] that trace quantities were present in A. podalyriaefolia gum is therefore incorrect. The absence of both 3,4-di-O-methyl-L-arabinose and 2,3-di-O-methyl-L-arabinose is proof that all of the arabinose in the gum occurs in the furanose form.

The isolation of 2,5-di-O-methyl-L-arabinose, and a trace of 3,5-di-O-methyl-L-arabinose indicated that some of the arabinose is present as chain units. These units are resistant to periodate oxidation and account for the persistence of L-arabinose in the Smith degraded gums (see discussion below).

3,5-di-O-methyl-L-arabinose has been isolated from the hydrolysate of the methylated gums of A. pycnantha [35], A. arabica[42], A. karroo [41] and was reported present in the methanolysates [7] (as analysed by GLC) of the gums of A. mearnsii, A. cyanophylla and A. giraffae. The sugar is also present in the hydrolysate of methylated A. elata gum.[60] The isolation of 0.2 residues of 3,5-di-O-methyl-L-arabinose in every 100 sugar residues indicates that the macromolecule of A. podalyrinefolia gum should contain 500 sugar residues. This would mean a molecular weight of between 75,000 and 85,000. As the experimentally determined molecular weight is roughly half this value, either the estimation of 3,5-di-O-methyl-L-arabinose is incorrect or one out of every two or three molecules of A. podalyriaefolia gum contains a unit of arabinofuranose linked 1 → 2 in a side chain.

The structural significance of the galactopyranose residues substituted at $C_{(4)}$, shown by the isolation of 2,3,6-tri-O-methyl-D-galactose, is uncertain. Whilst this could arise from the de-O-methylation of 2,3,4,6-tetra-O-methyl-D-galactose residues during hydrolysis or methanolysis, it is known that the axial 4-hydroxyl group is resistant to methylation. If the presence of 2,3,6-tri-O-methyl-D-galactose in the methylated gum arises from either undermethylation during the methylation procedures, or de-O-methylation in the subsequent fragmentation of the polysaccharide, different quantities would have been expected from different experiments. Kaplan found a value of 5 molar proportions percent[7], identical with the value found on methanolysis and GLC analysis, as well as by cellulose column chromatography, of the sample methylated by the author.

As, already indicated, partial acid hydrolysis, even after 15 hours, reduced the quantity of 4-O-substituted galactopyranose residues, as shown by methanolysis and GLC analysis of methylated degraded gums A and B, to negligible amounts, the 1 ⟶ 4-linked galactopyranose residues must therefore occur with an acid-labile residue attached to the $C_{(4)}$ position.

The isolation of 4-O-(α-D-glucopyranosyluronic acid)-D-galactose [61], from a number of Acacia polysaccharides [38,46] shows that D-glucuronic acid is glycosidically linked to the $C_{(4)}$ position of D-galactopyranose units in these gums. Although both D-glucuronic acid and the 4-O-substitutes D-galactose residues occur to the extent of 5%, the results obtained on partial acid hydrolysis of A. podalyriaefolia gum do not support the glycosidic linkage of the glucuronic acid to the $C_{(4)}$ position of D-galactose; the degraded gums contained D-glucuronic acid, but did not contain the 4-O-substituted D-galactopyranose residues.

The adjacent hydroxyl groups at $C_{(2)}$ and $C_{(3)}$ should be cleaved by periodate oxidation during the Smith degradation sequence affording threitol on reduction. No threitol was, however, detected in the reduced-oxidised polysaccharide obtained from A. podalyriaefolia gum (see section 3.3.2 table 14).

The presence of 2,3,6-tri-O-methyl-D-galactose in the hydrolysate of methylated A. podalyriaefolia gum must therefore arise from undermethylation of the axial 4-hydroxyl group of certain D-galactopyranose residues.

The occurrence of trace quantities of 2,6-di-O-methyl-D-galactose (3,4-di-O-substituted) and 2,3-di-O-methyl-D-galactose (4,6-di-O-substituted) in the hydrolysate of methylated gums have often been regarded as being due to either incomplete methylation or to de-O-methylation [42], rather than contributing to the structure of the gums.

The structural significance of the triply O-substituted D-galactopyranose units is not clear. The quantity of 2-O-methyl-D-galactose recovered from the cellulose column constituted 10% of the gum, while the 4-O-methyl-D-galactose was present to the extent of 1.5%. On the grounds of branch-points exceeding end-groups, as analysed, there seems to be a good case for regarding these O-methyl galactoses as arising from partial failure to methylate the axial 4-hydroxyl groups of D-galactose.

D-glucuronic acid residues appear as both non-reducing end-groups and chain-units, linked through $C_{(4)}$ by some other sugar, in the gum of A. podalyriaefolia. The molar proportions of end-group 2,3,4-tri-O-methyl-L-rhamnopyranose and chain-unit 2,3-di-O-methyl-D-glucuronic acid were very similar, supporting the observation by Charlson [62] that the ratio of L-rhamnopyranose to D-glucuronic acid in the gums of A. senegal[9], A. cyanophylla[5] and A. mearnsii [36] was 1:1. This led him to suggest that L-rhamnopyranose may be the labile sugar glycosidically linked to the $C_{(4)}$ position of D-glucuronic acid. The isolation of the disaccharide, 4-O-α-L-rhamnopyranosyl-D-glucopyranose, derived from the structural unit α-L-rhamnopyranose 1→ 4-β-D-glucuronic acid 1→ , obtained from carboxyl reduced A.senegal gum [63], further supported this observation.

The methyl pentose and the sugar acid therefore occur bound in exactly the same manner as in other Acacia polysaccharides, where they represent a high proportion of the carbohydrate residues present.

Under ideal conditions the number of end-groups in the macromolecule should equal the number of branch-points. Poor agreement was found in this respect in the investigation of the methylated A. podalyriaefolia gum. The results show (a) end-groups 34, (b) chain-units 18 and (c) branch-points 61.

The relatively low proportion of end-groups is due to the handling losses of 2,3,5-tri-O-methyl-L-arabinose, which is lost on evaporation of the solvent and possibly to a low recovery of

2,3,4-tri-O-methyl-D-glucuronic acid. The former is substantiated by the fact that the total quantity of L-arabinose methyl ethers eluted from the column (7.4 molar proportions) is considerably lower than the assay for arabinose (16 molar proportions) in the hydrolysate of the whole gum. The relatively high proportion of branch points in the molecule is due to the presence of a large proportion of 2-O-methyl-D-galactose. Although the presence of these residues is authenticated by the isolation and characterisation of 2-O-methyl-D-galactose, it probably arises from the partial failure of the methylating agents to methylate the axial 4-hydroxyl group.[42]

Two unidentified carbohydrate components were eluted from the column. One of these was eluted together with 2,3,4-tri-O-methyl-D-galactose and had a chromatographic mobility in solvent (a), which was greater than that of the sugar. After acid hydrolysis 2,3,4-tri-O-methyl-D-galactose, as well as 2,3,4,6-tetra-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose were indicated by paper chromatography. Methanolysis and GLC analysis of the fraction showed the above mentioned sugars, together with a trace of 2,4,6-tri-O-methyl-D-galactose. Paper chromatography of the hydrolysate of the fraction after borohydride reduction showed 2,3,4,6-tetra-O-methyl-D-galactose and 2,4-di-O-D-methylgalactose. This component must be an oligosaccharide, and probably contains a large proportion of α linkages as the specific rotation was $+137^{\circ}$. The other unidentified component was eluted together with 4-O-methyl-D-galactose and was separated from this sugar by crystallisation of the 4-O-methyl-D-galactose. Both GLC of the methanolysate and paper chromatography of an hydrolysate showed this component to consist mainly of 2,4-di-O-methyl-D-galactose with trace amounts of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose.

Paper chromatographic examination of an hydrolysate after borohydride reduction showed all of the above-mentioned sugars. It can be concluded that this component is an oligosaccharide carrying methoxyl groups at the $C_{(2)}$ and $C_{(4)}$ positions.

2.5 Periodate oxidation studies on *A. podalyriaefolia* gum.

Analytical periodate oxidation of the polysaccharide for 114 hours indicated that 0.57 mol. periodate was consumed per 100g of carbohydrate, the theoretical amount calculated from the model being 0.57 mol. per 100g.

Periodate oxidation of a larger amount of *A. podalyriaefolia* polysaccharide was terminated after 73 hours, when 0.57 mol. periodate had been consumed per 100 g carbohydrate, by precipitation of the iodate and excess periodate ions with barium carbonate. The polyaldehyde was reduced with sodium borohydride to the polyalcohol, and excess borohydride was decomposed on addition of solid carbon dioxide. Cations were removed with Amberlite IR-120 (H^+) resin and the borate was removed on evaporation of methanol from the reduced-oxidised polysaccharide. The evaporation was carried out at 35° and prolonged heating was avoided in an attempt to minimise the loss of glycerol.

The colorimetric assays of the galactose and glycerol recovered from a preparative-scale chromatogram of an hydrolysate of reduced-oxidised *A. podalyriaefolia* gum showed these two components to be present in a molar ratio of 45:55. GLC analysis of the glycitol acetates, derived from an hydrolysate, showed the molar ratio percent to be 39:59. The GLC analysis also showed the presence of arabinose (1.8), rhamnose(0.2) and a component which had the same retention time as tetra-*O*-acetylerythritol

The reduced-oxidised polysaccharide was hydrolysed according to the Smith degradation conditions, and the product, after neutralisation, filtration and evaporation, was fractionated into

(i) a methanol-acetone(1:1) soluble fraction,

(ii) a methanol soluble fraction, and

(iii) an insoluble residue - Polysaccharide A.

(i) The methanol-acetone soluble fraction was shown by paper chromatography to contain essentially glycerol together with at least eight other minor components, one of which had $\underline{R}_{gal}$ 1.41 (solvent a) and coloured with cold ammoniacal silver nitrate spray after paper chromatographic separation. This fraction contained no free reducing sugars, and was shown by GLC separation of the glycitol acetates, derived from an hydrolysate, to contain 95 molar proportions percent of glycerol. Attempts to prepare the tris-p-nitrobenzoate of glycerol gave the derivative, but in very small yield. Assay for total sugar content (as galactose) gave a result which was in good agreement with that obtained on GLC analysis of the derived glycitol acetates.

An attempted fractionation of this fraction on Dowex-1-resin (OH^- form) afforded mainly glycerol, which was characterised by paper chromatography, GLC analysis of the derived acetate and as the tris-p-nitrobenzoate derivative. Four minor fractions were also obtained, and shown by hydrolysis and GLC analysis of the derived glycitol acetates to be composed of glycerol, rhamnose, arabinose, galactose and the unidentified component having the same retention time as tetra-O-acetyl-erythritol

The molar proportions percent of the monomeric components (see Table 16) were similar to the values found on assay of the hydrolysate of the fraction as a whole (see Table 15). The recovery from the column was 60% and the component having $\underline{R}_{gal}$ 1.41 was not eluted.

A second portion of the methanol-acetone soluble material was fractionated on a column of cellulose, using butan-2-one-water azeotrope as eluant. Glycerol and a number of minor components, including that having $\underline{R}_{gal}$ 1.41 were obtained. A portion of the last-mentioned component was reduced with borohydride, and paper chromatography of the product showed only glycerol. The component having R_{gal} 1.41 is therefore glyceraldehyde, which would not be eluted from the Dowex-1-resin. Hydrolysis of the reduced fraction, followed by paper chromatography of the neutralised solution showed no reducing sugars. The paper chromatographic mobility was not identical with freshly prepared D-glyceraldehyde,[64] and it is possible that the fraction eluted from the column had dimerised during the working-up procedures.

(ii) The intermediate methanol soluble fraction was chromatographically similar with fraction (i) above, but was shown by hydrolysis and GLC analysis of the derived glycitol acetates to contain a higher proportion of glycosidically bound sugars.

(iii) The insoluble residue, Polysaccharide A, recovered in 20% yield, had $[\alpha]_D$ +20° and a weight-average molecular weight 2985. Hydrolysis and GLC analysis of the derived glycitol acetates showed Polysaccharide A to be essentially a galactan, containing D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of = 94:2:1:3, the glycerol being linked to the reducing end-group. A molecular weight of 2985 requires glycerol, 5.6%.

Repeated recrystallisation of the glycitol acetate mixture, derived from Polysaccharide A, afforded hexa-O-acetyl-D-galactitol, identified by m.p. and mixed m.p. with an authentic sample.

Methylation of polysaccharide A, followed by methanolysis and GLC analysis, showed the following components in the molar ratios given:

2,3,5-tri-O-methyl-L-arabinose (3.5) 3,4-di-O-methyl-L-arabinose? (0.5)
2,3,4,6-tetra-O-methyl-D-galactose (8), 2,3,6-tri-O-methyl-D-galactose (1),
2,4,6-tri-O-methyl-D-galactose (61) 2,3,4-tri-O-methyl-D-galactose (8),
2,4-di-O-methyl-D-galactose (2), 2,6-di-O-methyl-D-galactose (14),
and 2,3,4-tri-O-methyl-D-glucuronic acid (1). The presence of

2-O-methyl-D-galactose (1) was estimated from a paper chromatogram of an hydrolysate of methylated polysaccharide A.

An analytical estimation of the periodate uptake by Polysaccharide A showed that 0.23 mol.periodate was consumed per 100g after 68 hours. During the preparative-scale oxidation of Polysaccharide A 0.20 mol periodate was consumed per 100 g. The "model", as found by analysis of the above methyl ethers requires 0.24 mol periodate. The polyaldehyde was recovered as previously, and the derived reduced-oxidised Polysaccharide A was shown by GLC assay of the glycitol acetates, obtained from an hydrolysate of the polyalcohol , to contain D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of 87: 3.5:0.5:8. A component having the same retention time as tetra-O-acetyl erythritol constituted 1% of the total area of the peaks on the chromatogram.

Mild acid hydrolysis (Smith degradation conditions) followed by fractionation of the product, after neutralisation, afforded :

(i) a methanol-acetone (1:1) soluble fraction, shown by GLC assay of the glycitol acetates, derived from an hydrolysate, to contain D-galactose, L-arabinose, L-rhamnose, glycerol and the unidentified component mentioned previously in the molar ratios of 38:7:1:53:(1), and

(ii) an insoluble residue, Polysaccharide B, having $[\alpha]_D$ +40^{o} and an approximate molecular weight 1,940. GLC analysis of the glycitol acetates, derived from an hydrolysate of Polysaccharide B, showed D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of 93:2.8:0.8:3.5. The model structure of Polysaccharide B requires glycerol 6.65%.

Methylation of Polysaccharide B followed by methanolysis and GLC analysis showed the following methyl glycosides with the molar proportion of each ether in parenthesis:

2,3,4-tri-O-methyl-L-arabinose (2),

3,4-di-O-methyl-L-arabinose ? (0.3),

2,3,4,6-tetra-O-methyl-D-galactose (8),

2,3,6-tri-O-methyl-D-galactose (1),

2,4,6-tri-O-methyl-D-galactose (54),

2,3,4-tri-O-methyl-D-galactose (7),

2,4,-di-O-methyl-D-galactose (5),

2,6-di-O-methyl-D-galactose (21),

2-O-methyl-D-galactose (1) (shown by paper chromatography) and 2,3,4-tri-O-methyl-D-glucuronic acid (1).

Polysaccharide B consumed 0.277 mol. periodate per 100g of carbohydrate on periodate oxidation. The "model" as found by analysis of the above methyl ethers requires: 0.33 mol. periodate per 100g. Borohydride reduction gave reduced-oxidised polysaccharide B, which was shown by GLC analysis of the glycitol acetates, derived from an hydrolysate, to contain D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of 84:4:0.5:11.

Mild acid hydrolysis (Smith degradation conditions) of reduced-oxidised Polysaccharide B, followed by fractionation of the neutralised solution afforded

(i) a methanol acetone (1:1) soluble fraction, which on hydrolysis and GLC analysis of the derived glycitol acetates showed D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of 29:7:0.4:63, and

(ii) an insoluble residue, Polysaccharide C, having $[\alpha]_D$ +28° and an approximate molecular weight 1,750. GLC assay of the glycitol acetates, derived from an hydrolysate, showed D-galactose, L-arabinose, L-rhamnose and glycerol in the molar ratios of 90:4.5:0.3:5.2.

Methylation of the Polysaccharide C, methanolysis and GLC analysis showed the methyl glycosides of the following methyl ethers, together with their molar proportion in parenthesis: 3,4-di-O-methyl-L-arabinose? (3.5), 2,3,4,6-tetra-O-methyl-D-galactose (20), 2,3,6-tri-O-methyl-D-galactose (3.5), 2,4,6-tri-O-methyl-D-galactose (61.5), 2,3,4-tri-O-methyl-D-galactose (2.5) and 2,6-di-O-methyl-D-galactose (9).

Polysaccharide C was oxidised with periodate, 0.290 mol. periodate being consumed per 100g carbohydrate. The "model" as found by analysis of the above methyl ethers, requires: 0.31 mol. periodate per 100g carbohydrate. The oxidised polysaccharide was reduced with borohydride and the product was subjected to mild acid hydrolysis (Smith degradation conditions). Fractionation, as previously, afforded:

(i) a methanol-acetone (1:1) soluble fraction, which was shown by GLC analysis of the glycitol acetates, derived from an hydrolysate, to contain D-galactose, L-arabinose and glycerol in the molar ratios of 37:10:48; an unidentified component, constituting approximately 5%, was also present in the hydrolysate, and

(ii) an insoluble residue, Polysaccharide D, having an approximate molecular weight of 800. Polysaccharide D was shown by GLC analysis of the glycitol acetates, derived from an hydrolysate, to contain D-galactose, L-arabinose and glycerol in the molar ratios of 47:1:45.

These analytical results are discussed more fully in section 2.6.

2.6 A possible repeating unit in the macromolecule of A. podalyriaefolia gum.

Polysaccharide D, obtained after four successive Smith degradations, was shown to contain D-galactose, a trace of arabinose and glycerol. The approximate molecular weight estimation has indicated that this product is a tetraose with the reducing end group glycosidically bound to glycerol. The molar proportion of glycerol required by the model is 20%.

A possible structure for this degraded product is shown by structure XV1, and the results are listed in table 2.

The molar proportion of arabinose suggests that one out of twelve molecules contains an arabinose residue.

The molecular weight estimation for Polysaccharide C indicated that approximately ten sugar residues are present in the molecule. A possible structure for this polysaccharide is shown by structure XV11 and a comparison between the analytical results and those calculated from the model structure appear in table 3.

The estimated molecular weight, obtained from XV11, is lower than that calculated from the model. The analysed quantity of periodate consumed per 100g of polysaccharide agrees with that required by the model. The molar proportion of glycerol bound to the polysaccharide, 5.2 molar proportion percent, is less than an amount of 7.2 molar proportion percent required by the model. This could result from either handling losses or the inability to measure accurately the area of the sharp glycerol peaks on the GLC traces.

The structural significance of the 3,4-di-O-methyl-L-arabinose (2-O-substituted) and the 2,3,6-tri-O-methyl-D-galactose (4-O-substituted) residues is uncertain. It is possible that the basal chain contains 1→4- linked galactopyranose residues, which were exposed during the previous Smith degradation. The assay of 1→4- and 1→6- linked galactose residues is lower than that required by the model.

Cleavage of the 1→6-linked galactopyrose residue present in the chain of the model and removal of the galactopyranose end-groups will afford two molecules of Polysaccharide D.

The model structure for Polysaccharide B, which follows from the application of the analytical results to the model of

Polysaccharide C, is shown by structure XVlll, and the analytical results and those calculated from the model are presented in table 4.

The estimated molecular weight of Polysaccharide B is lower than that calculated from the model, and the amount of periodate consumed by the polysaccharide, 0.277 mol. periodate per 100g polysaccharide, is lower than 0.337 mol. periodate required by the model structure. The analysed molar proportions percent of glycerol bound to the polysaccharide and glycerol released during the Smith degradation of Polysaccharide B are lower than the theoretical quantities calculated from the model structure.

The ratio of end-groups to branch-points, as analysed, strongly suggests under methylation of the polysaccharide. The 2,6-di-O-methyl-D-galactose, which cannot be accounted for, arises from partial failure of the methylating agents to methylate the 4-hydroxyl group. The presence of 2,3,4-tri-O-methyl-D-galactose could arise from de-O-methylation of the primary 6-O-methyl groups, this being supported by the low assay for D-galactopyranose end-groups. The presence of 2,3,4-tri-O-methyl-L-arabinose, which has not previously been detected in A. podalyriaefolia gum or its degraded products , suggests that two out of seven molecules of Polysaccharide B contains an arabinopyranose non-reducing end-group. One out of seven contains a non-reducing glucopyranosyluronic acid end-group, linked to $C_{(4)}$ of D-galactose.

Periodate oxidation of A. podalyriaefolia gum resulted in considerable degradation. This is shown by the decrease in the weight-average molecular weight from 33,500 for A. podalyriaefolia gum to 2985 for Polysaccharide A. The molecular weight determination has suggested that Polysaccharide A consists of approximately eighteen sugar residues.

Application of the analytical results to the model structure of Polysaccharide B gave a possible model structure, shown in structure lX, for Polysaccharide A. The analytical results, together with the theoretical values calculated from the model, are presented in table 5. Good agreement between the analytical and theoretical values was found. The molar proportions percent of glycerol bound to Polysaccharide A and glycerol released during the Smith degradation are, as previously, lower than the theoretical values. The presence of 2,6-di-O-methyl-D-galactose in methylated Polysaccharide A results from undermethylation.

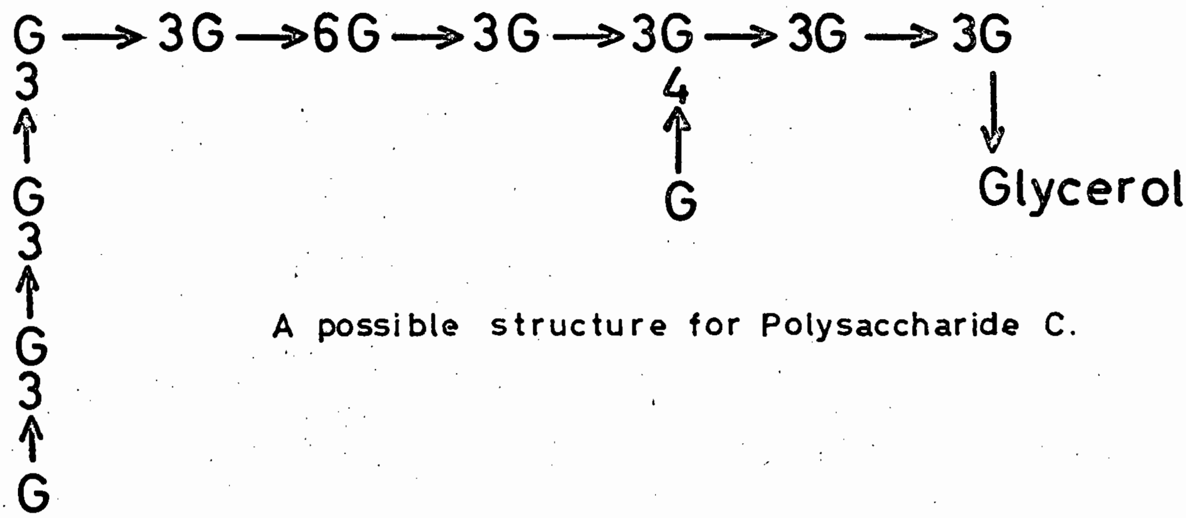
Polysaccharide A is considered to be the repeating unit in the basal chain of A. podalyriaefolia gum and structure XX, a possible fragment from A. podalyriaefolia gum molecule, shows the minor substituant side-chains attached to Polysaccharide A. The weight-average molecular weight of 33,500, determined from the diffusion and sedimentation coefficients, and a weight - average molecular weight of 31,500, determined by gel-filtration of the gum suggests that the macromolecule is composed of five or six repeating units.

The amount of periodate consumed by the gum is identical with the theoretical amount and the molar proportions of the methyl ethers present in the O-methyl derivative of the gum agree favourably with the theoretical proportions. The relatively large quantity of 2,3,4-tri-O-methyl-D-galactose could arise from de-O-methylation of the primary methoxyl groups at $C_{(6)}$. The large amount of 2-O-methyl-D-galactose cannot be accounted for, and must arise from undermethylation.

G → 3G → 3G → 3G → Glycerol

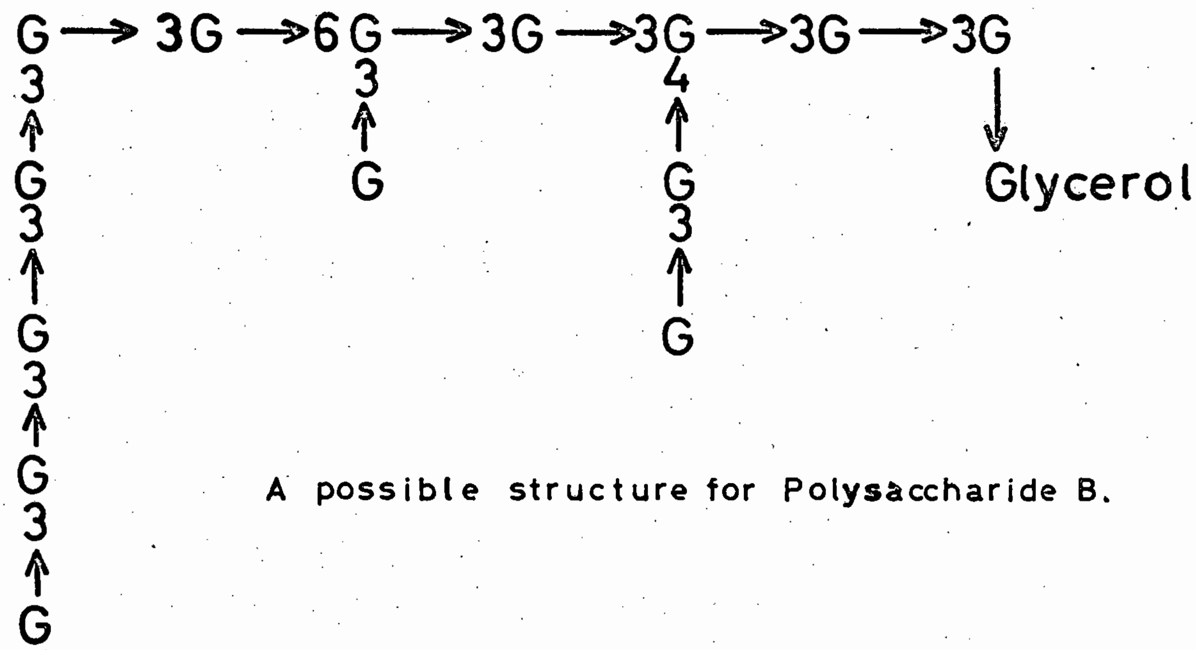
A possible structure for Polysaccharide D.

XVI.



A possible structure for Polysaccharide C.

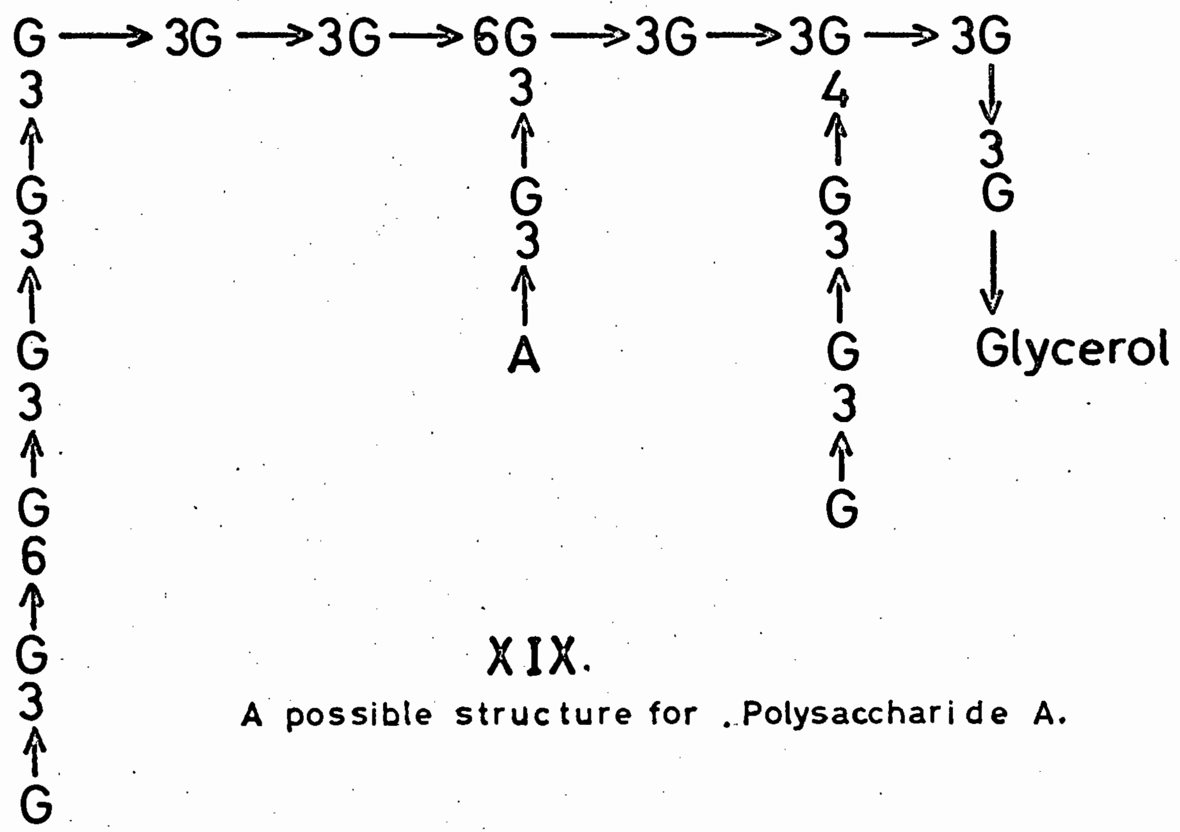
XVII.



A possible structure for Polysaccharide B.

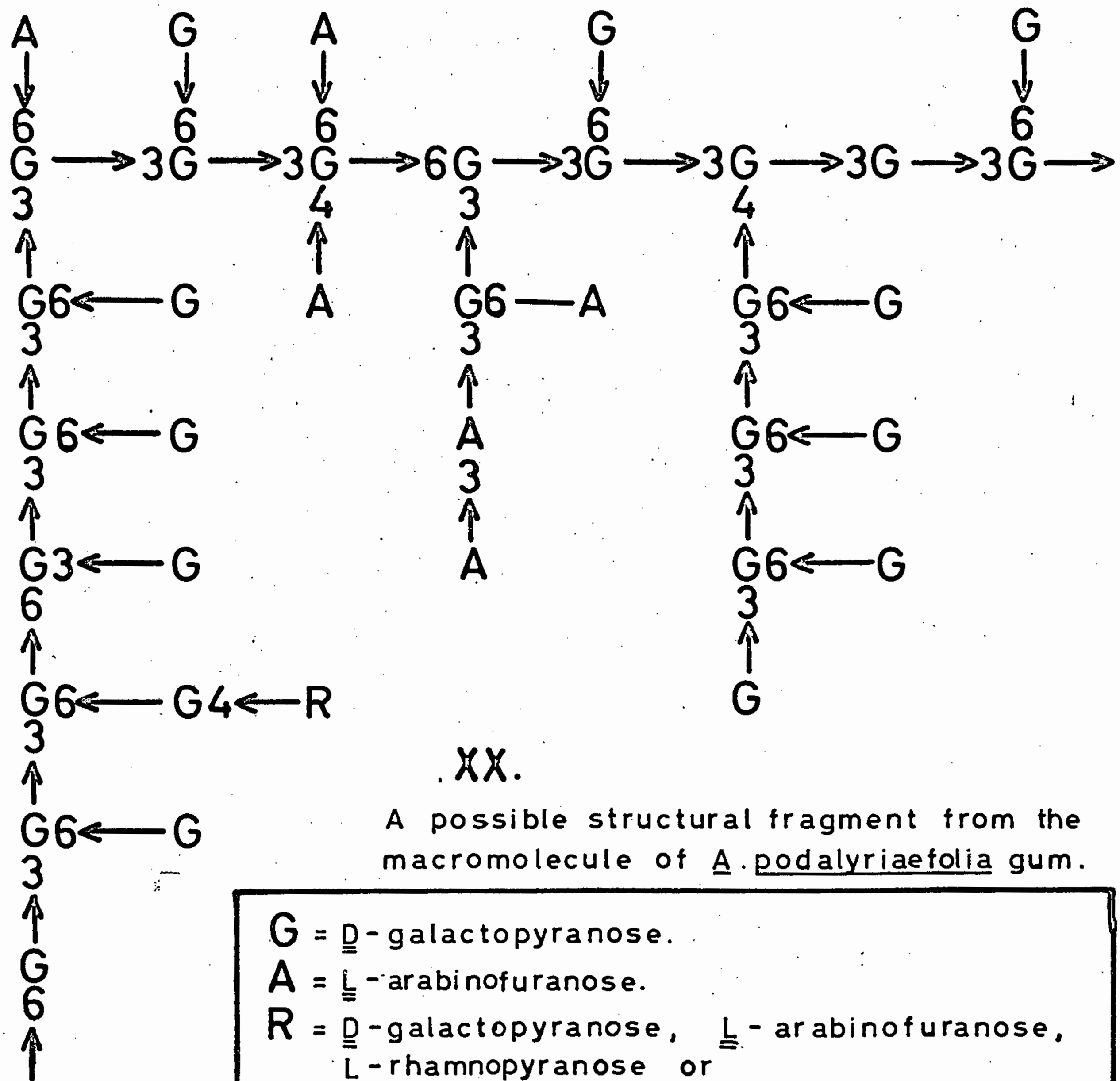
XVIII.

G = D-galactopyranose.



XIX.

A possible structure for Polysaccharide A.



XX.

A possible structural fragment from the macromolecule of A. podalyriaefolia gum.

G = D-galactopyranose.
A = L-arabinofuranose.
R = D-galactopyranose, L-arabinofuranose, L-rhamnopyranose or D-glucopyranosyluronic acid.

Assay	Found	Model
Molecular weight	$800^{\pm}$	768

Table 2

Assay	Found	Model
Molecular weight	1750	1902
mol. periodate consumed per 100g Polysaccharide C	0.29	0.31
mol. percent periodate bound to Polysaccharide C	5.2	7.2
mol. percent glycerol in reduced-oxidised Polysaccharide C	-	33
methyl ethers in methylated Polysaccharide C		
3,4-di-O-methyl-L-arabinose	3.5	-
2,3,4,6-tetra-O-methyl-D-galactose	20	18
2,3,6-tri-O-methyl-D-galactose	3.5	-
2,4,6-tri-O-methyl-D-galactose	61.5	64
2,3,4-tri-O-methyl-D-galactose	2.5	9
2,6-di-O-methyl-D-galactose	9	9

Table 3

Assay	Found	Model
Molecular weight	1,940	2,388
mol. periodate consumed per 100g Polysaccharide B	0.277	0.337
mol. percent glycerol bound to Polysaccharide B	3.5	6.65
mol.percent glycerol in reduced-oxidised Polysaccharide B	11	26.8
methyl ethers in methylated Polysaccharide B		
2,3,4-tri-O-methyl-L-arabinose	2	-
3,4-di-O-methyl-L-arabinose	0.3	-
2,3,4,6-tetra-O-methyl-D-galactose	8	21
2,3,6-tri-O-methyl-D-galactose	1	-
2,4,6-tri-O-methyl-D-galactose	54	64
2,3,4-tri-O-methyl-D-galactose	7	-
2,4-di-O-methyl-D-galactose	5	7
2,6-di-O-methyl-D-galactose	21	7
2-O-methyl-D-galactose	1	-
2,3,4-tri-O-methyl-D-glucuronic acid	0.7	-

Table 4

Assay	Found	Model
molecular weight	2,985	3,002
mol. periodate consumed per 100g Polysaccharide A	0.23	0.24
mol. percent glycerol bound to Polysaccharide A	3.0	5.5
mol. percent glycerol in reduced-oxidised Polysaccharide A	8	26.2
methyl ethers in methylated Polysaccharide A		
2,3,5-tri-*O*-methyl-*L*-arabinose	3.5	5.5
3,4-di-*O*-methyl-*L*-arabinose	0.5	-
2,3,4,6-tetra-*O*-methyl-*D*-galactose	8	11
2,3,6-tri-*O*-methyl-*D*-galactose	1	-
2,4,6-tri-*O*-methyl-*D*-galactose	61	67
2,3,4-tri-*O*-methyl-*D*-galactose	8	5.5
2,4-di-*O*-methyl-*D*-galactose	2	5.5
2,6-di-*O*-methyl-*D*-galactose	14	5.5
2-*O*-methyl-*D*-galactose	1	-
2,3,4-tri-*O*-methyl-*D*-glucuronic acid	1	-

Table 5

Assay	Found		Model
Molecular weight	33,500 31,500		-
mol. periodate consumed per 100g *A. podalyriaefolia* gum	0.57		0.57
mol. glycerol in reduced-oxidised *A. podalyriaefolia* gum	52		49
methyl ethers in methylated *A. podalyriaefolia* gum	GLC	*	
2,3,4-tri-*O*-methyl-L-rhamnose	0.5	0.7	-
2,3,5-tri-*O*-methyl-L-arabinose	11	6.2	13.5
2,5-di-*O*-methyl-L-arabinose	-	1.0	2.7
3,4-di-*O*-methyl-L-arabinose	1 ?	-	-
3,5-di-*O*-methyl-L-arabinose	-	0.2	-
2,3,4,6-tetra-*O*-methyl-D-galactose	26	25.7	29.9
2,3,6-tri-*O*-methyl-D-galactose	5	5.2	2.7
2,4,6-tri-*O*-methyl-D-galactose	3	2.8	2.7
2,3,4-tri-*O*-methyl-D-galactose	7	7.6	2.7
2,3-di-*O*-methyl-D-galactose	-	1.0	-
2,4-di-*O*-methyl-D-galactose	38	33.6	38
2,6-di-*O*-methyl-D-galactose	tr	1.2	2.7
2-*O*-methyl-D-galactose	(8)	10.3	2.7
4-*O*-methyl-D-galactose		1.5	-
D-galactose	-	0.5	-
2,3,4-tri-*O*-methyl-D-glucuronic acid	2	1.1	-
2,3-di-*O*-methyl-D-glucuronic acid	tr	1.4	- (2.7)

Table 6

* Cellulose column chromatography.

3. EXPERIMENTAL

3.1 General experimental conditions.

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

(a) butan-1-ol - ethanol-water (4:1:5, upper layer),

(b) ethylacetate-pyridine-water (10:4:3),

(c) butan -2-one - acetic acid-water (2:1:1)

(d) butan-1-ol - ethanol-water (1:1:1),

(e) butan-2-one - acetic acid - saturated boric acid (9:1:1) and

(f) butan-2-one - water azeotrope.

TLC was carried out on silica gel G with (g) chloroform - methanol mixtures in various proportions (generally 5:1, v/v), and with solvent (d). Paper ionophoresis was conducted for 2 - 4 hours at 10 V/cm in 0.1 M-borate buffer at pH 9.2.[65]

R_{gal}, R_G and M_g refer to rates of movement relative to galactose, 2,3,4,6-tetra-O-methylglucose, and glucose respectively. Infrared spectra were measured with a Unicam SP 100 instrument and ultraviolet absorptions were measured on a Beckman DB Spectrophotometer

GLC analyses for Part I of this thesis were carried out on a Beckman GC-2A instrument fitted with flame ionization detector, using helium as carrier gas. Glycitol acetate mixtures were separated on a 3 foot, 1/4 inch o.d. copper column of 3% ECNSS-M on 100-120 mesh Gas Chromosorb Q at 180°.[66] Mixtures of methyl glycosides were analysed on a 3 foot, 1/4 inch o.d. copper column of 14% ethylene glycol succinate polyester on 80 - 100 mesh Gas Chromosorb W at 155°.[7,67] Retention times (T values) were measured relative to hexa-O-acetylmannitol (actual retention time ca. 40 minutes) and methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside (actual retention time ca. 4 minutes) respectively.

GLC analyses for Part II of this thesis were carried out on a Beckman GC-4 instrument fitted with dual flame ionization detectors, using helium as carrier gas. The acetates of the partially methylated glycitols were separated on a 12 foot 1/8 inch o.d. copper column of 3% ECNSS-M on 100 - 120 mesh Gas Chromosorb Q at 175°.[68] Retention times (_T_ values) were measured relative to 1,5-di-_O_-acetyl-2,3,4,6-tetra-O-methyl-_D_-galactitol (actual retention time _ca._ 4 minutes).

Unless otherwise stated, solutions were concentrated at 35-40° and 20 mm in rotary evaporators, specific rotations were equilibrium values for aqueous solutions at _ca._ 20°, m.ps are uncorrected, and sugars were revealed as characteristically-coloured spots using the _p_-anisidine hydrochloride reagent.[69] Compounds separated by TLC were revealed by spraying with $2\underline{N}$-H_2SO_4 and heating the plates at 120°. Sugars were assayed by the phenol-sulphuric acid procedure developed by Smith and his co-workers.[70]

The methylated sugar fractions obtained in Part I, section 3.4, were weighed after being dried in _vacuo_, and identified (against standard substances) by a variety of procedures including the measurement of $[\alpha]_D$, paper chromatography in solvents (_a_) and (_b_), TLC, demethylation with hot HBr aq,[71] periodate oxidation[72] before and after reduction with borohydride[73] to the corresponding glycitols (many of them crystalline), and by N.M.R. spectroscopy (Varian A-60 spectrometer, D_2O solutions).[74] In all cases the sugar fractions were converted to their methyl glycosides by heating portions in 2% methanolic HCl for 6 hours (in sealed tubes at 96°) and examined by GLC using standard sugars for comparison.

3.2 Purification and analysis of A. podalyriaefolia gum.

3.2.1 Purification

A portion of the gum (16.50g) was dissolved in water (250 ml) and the relatively limpid, acidic solution was filtered to remove small pieces of wood and bark. Slow addition of ethanol (1.5 l) precipitated the polysaccharide as a fibrous mass.

This material was redissolved in water and precipitated in 80% ethanol. The polysaccharide was washed with ethanol, acetone and with ether and the dried product was dissolved in water and deionised with Amberlite IR - 120 (H^+) resin. The solution was neutralised ($BaCO_3$), filtered to remove any insoluble barium salts, and deionised with Amberlite IR 120 (H^+) resin. The solution was shell frozen and freeze-dried.

The white amorphous powder (11.60g) had $[\alpha]_D$ +5.3° (c, 2.83) and a weight-average molecular weight of 33,500, calculated from the sedimentation and diffusion coefficients (see section 3.6.4) and a weight-average molecular weight of 31,500, obtained on gel-filtration on Biogel P-300. Found: equivalent weight 3585; uronic anhydride, 5; C, 42.1; H, 7.0; N, <0.1; S, <0.1 and OMe[53], 1.4%.

The supernatant from the precipitations, together with the washings were evaporated to dryness, and the dark brown oil (288 mg) refluxed in acetone, affording a soluble fraction (270 mg) and an insoluble residue (18 mg). Hydrolysis of the acetone-soluble fraction (70 mg) in N-H_2SO_4 (48 hr. at 96°) gave an insoluble fraction (59 mg) and a colourless supernatant. The centrifugate was neutralised ($BaCO_3$) and shown by paper chromatography in solvents (a) and (b) to contain galactose, arabinose and rhamnose. GLC of the derived glycitol acetates indicated that the three sugars were present in the ratios 2:1:trace. The acetone insoluble fraction, and the insoluble material obtained on acid hydrolysis of the acetone-soluble fraction gave a negative result when tested for carbohydrate.

3.2.2 Assay of the sugars released on hydrolysis of Acacia podalyriaefolia gum.

Acacia podalyriaefolia gum was hydrolysed in N-H_2SO_4 (for 6 hr at 96°), neutralised ($BaCO_3$), filtered and gave sugars chromatographically identical with galactose, arabinose and rhamnose on paper chromatography in solvents (a), (b) and (f).

The hydrolysate was assayed for neutral sugar composition (1) by phenol-sulphuric acid after preparative-scale paper chromatography in solvent (a) and (2) by GLC of the derived glycitol acetates (a) on 3% SE-52 coated support, (b) on 3% ECNSS-M coated support. The molar ratios found for A. podalyriaefolia gum hydrolysate, are shown in Table 7 (uronic anhydride content calculated from equivalent weight).

Assay	Galactose	Arabinose	Rhamnose	Uronic anhydride
(1)	79	16	-	5%
(2a)	78	17	-	5%
(2b)	79	15	1	5%

Table 7.

3.2.3 Separation and characterisation of the neutral sugars of A. podalyriaefolia gum.

The gum (1.22g) was hydrolysed in N-H_2SO_4 (50 ml, for 10 hours at 96°), and the cooled solution was neutralised (Ba CO_3), filtered, treated with Amberlite IR-120 (H^+) resins and concentrated to a syrup (1.10g). The sugars were separated on Whatman No 3 MM papers in solvent (a) to give the following three monosaccharide-containing fractions.

Fraction (i) A crystalline sugar (600mg) having $[\alpha]_D$ +80° (c 1.47), m.p. 164-5°, and the same paper chromatographic mobility as D-galactose. The derived glycitol acetate had the same retention time (1.15) as authentic hexa-O-acetyl-D-galactitol.

Fraction (ii) A crystalline sugar (90 mg) having $[\alpha]_D$ + 90° (c 0.94), m.p. 154-5°, and the same paper chromatographic mobility as L-arabinose. The derived glycitol acetate had the same retention time (0.38) as authentic penta-O-acetyl-L-arabinitol.

Fraction (iii) A syrup (24 mg) having $[\alpha]_D$ +5.0° (c 1.20), and the same paper chromatographic mobility as L-rhamnose. The derived glycitol acetate had the same retention time (0.22) as authentic penta-O-acetyl-L-rhamnitol.

3.3 Partial acid hydrolysis of *A. podalyriaefolia* gum.

3.3.1 0.01 N-sulphuric acid hydrolysis

3.3.1.1 Preparation of degraded gum A.

A. podalyriaefolia gum (1.60g) was dissolved in 0.01N-H_2SO_4 and heated on a water bath at 96° for 15 hours. The cooled solution was neutralised ($BaCO_3$), filtered and concentrated (15 ml). The polysaccharide was precipitated on slow addition of ethanol, was washed with ethanol, acetone and ether and dried as degraded gum A (1.36g), having $[\alpha]_D$ +13° (*c* 2.79), (Found: equivalent weight 3,170; uronic anhydride, 5.5; galactose, 85; arabinose, 9; rhamnose, 0.5%).

The aqueous ethanol supernatant from the precipitation of degraded gum A, together with all washings, was concentrated to a syrup A (238 mg), having $[\alpha]_D$ +64° (*c* 2.16). Examination of this fraction by paper chromatography in solvents (a), (b), (c) and (d) showed it to be a mixture of at least six components, as indicated in table 8.

3.3.1.2 Methylation of degraded gum A.

Degraded gum A (108 mg) was methylated according to the procedures of Haworth and Purdie to give a product (80 mg) having $[\alpha]_D$ -12° (*c* 1.00 in chloroform), and lacking absorption due to hydroxyl groups in the infra-red spectrum. Methanolysis of a portion, followed by GLC examination of the methyl glycosides gave the results shown in table 9. Paper chromatography of an hydrolysate of the methyl glycosides in solvent (a) indicated the presence of trace amounts of 2-O- and 4-O-methyl-D-galactose in addition to those sugars found by GLC assay.

3.3.1.3 Preparation of degraded gum B.

Degraded gum A (1.00g) was further hydrolysed in 0.01N-H_2SO_4 (45 ml) for 36 hours at 96°. The cooled solution was neutralised ($BaCO_3$), filtered and fractionated as previously, affording degraded gum B (718 mg), having $[\alpha]_D$ +26.4° (*c* 2.58) and weight-average molecular weight of 18,650, as determined from the sedimentation and diffusion coefficients (see section 3.6). Gel filtration on Biogel P-300 showed this product to be polydisperse. (Found: Equivalent weight 3,542; uronic anhydride 5.0; D-galactose 93.5; L-arabinose 1.5%), and a syrup B (197 mg) having $[\alpha]_D + 64^{\circ}$ (c 2.19). The composition of this syrup is indicated in table 8.

Degraded gum B (54 mg) was dissolved in water (7 ml) and reduced with sodium borohydride (100 mg) for 48 hours. Excess of borohydride was decomposed with Amberlite IR-120 (H^+) resin and the borate removed by evaporation with methanol. Paper chromatographic examination of the hydrolysate in solvent (e) against standards showed galactose and arabinose (trace), but neither galactitol nor arabinitol, indicating a high polymer.

Degraded gum B (23mg) was hydrolysed with 0.5N - H_2SO_4 (2 ml) on a boiling water bath for 1 hour. The cooled solution was neutralised ($BaCO_3$), filtered and the syrup obtained on concentration shown by paper chromatography in solvents (a) and (b) to be composed of galactose, together with trace quantities of arabinose, 3-O-β-D-galactopyranosyl-D-galactose, 6-O-β-D-galactopyranosyl-D-galactose and O-β-D-galactopyranosyl (1⟶3)-O-β-D-galactopyranosyl-(1⟶3) D-galactose (identified by paper chromatography against standards).

3.3.1.4 Methylation of degraded gum B.

Degraded gum B (140 mg) was methylated to give a product (150 mg) having $[\alpha]_D -23^{\circ}$ (c 2.27 in chloroform), (Found: C, 52.6; H, 7.9; OMe, 38.0 and ash, 0.3%).

Methanolysis of a portion of this derivative and examination of the methyl glycosides by GLC gave the results presented in table 9. Paper chromatographic separation of an hydrolysate of the methylated material in solvent (a) confirmed the presence of the major components, and showed the absence of both 2-O and

4-O-methyl-D-galactose.

3.3.1.5 0.05 N-sulphuric acid hydrolysis of degraded gum B.

Degraded gum B (270 mg) was dissolved in water (30 ml) and dialysed against distilled water. The diffusate (10 mg) was shown by paper chromatography to be composed of D-galactose and L-arabinose. The non-dialysable material (255 mg) was dissolved in 0.05 N-H_2SO_4 and heated at 96° for 50 hours. The cooled solution was neutralised (Ba CO_3), filtered and the concentrated solution dialysed against distilled water. The diffusate was concentrated, shell frozen and freeze-dried. The white powder (238 mg) was shown by paper chromatography in solvents (a) and (b) to be composed of D-galactose and L-arabinose (trace). The non-dialysable material (7 mg) gave a positive Molisch test, did not move from the origin on paper chromatography (solvent (a)) and showed only galactose on hydrolysis and paper chromatography.

3.3.2 Attempted partial acid hydrolysis of A. podalyriaefolia gum with a water-soluble polystyrene sulphonic acid resin.

The water-soluble polystyrene sulphonic acid resin was prepared according to the method of Painter.[58] A. podalyriaefolia gum (1.22 g) was dissolved in 0.029 N-polystyrene sulphonic acid resin (100 ml) and the mixture enclosed in a cellophane bag and dialysed against water at 60° for 3 days. The dialysate was replaced by fresh water every two hours during the day. The dialysate was neutralised (Ba CO_3), filtered and evaporated, affording a dark brown syrup (325 mg). This syrup contained a large quantity of the resin, which dialysed together with the monosaccharides. Judging from the intensity of the spot at the origin on a paper chromatogram, the amount of resin present constituted more than 50% of the weight of the syrup. It is probable that the resin was degraded during the hydrolysis, as it was purified by dialysis at 70°. The paper chromatogram of this syrup showed the presence of D-galactose, L-arabinose and L-rhamnose in an approximate ratio of 1:6:trace.

The non-dialysable material (800 mg) recovered from the polystyrene sulphonic acid resin solution by precipitation with ethanol, having $[\alpha]_D$ +9.8° (*c* 2.24), was shown by GLC analysis of the glycitol acetates to contain D-galactose, L-arabinose and L-rhamnose in the molar ratios of 84:15:1. These results showed that the polysaccharide was only slightly degraded during the hydrolysis.

Examination of the sugars released on acid hydrolysis

Sugar*	R_{gal}	Syrup A	Syrup B
galβ→3 galβ →3 gal	0.14	tr	tr
galβ→6 gal	0.28	tr	tr
galβ→3 gal	0.46	tr	tr
Galactose	1.00	+	+
Arabinose	1.44	+++	+++
Rhamnose	2.40	+	+/2

Table 8

*identified by paper chromatography.

Examination of the methanolysis and hydrolysis products of methylated degraded gums A and B

O-methyl sugar	R_G (in solvent (a))	Molar proportions % gum A	gum B
2,3,5-tri-O-methyl-L-arabinose	1.00	5	0.5
3,4-di-O-methyl-L-arabinose	-	1?	1
2,3,4,6-tetra-O-methyl-D-galactose	0.92	32	34
2,3,6-tri-O-methyl-D-galactose	0.77	1	tr
2,4,6-tri-O-methyl-D-galactose	0.77	12	20
2,3,4-tri-O-methyl-D-galactose	0.74	15	11
2,4-di-O-methyl-D-galactose	0.55	31	32
2,6-di-O-methyl-D-galactose	-	2	-
2-O-methyl-D-galactose	0.37	tr	-
4-O-methyl-D-galactose	0.29	tr	-
2,3,4-tri-O-methyl-D-glucuronic acid	0.10-0.20	1	1.5

Table 9

Characteristics of the degraded polysaccharides and methylated degraded polysaccharides of *A. podalyriaefolia* gum.

	Polysaccharides		Methylated polysaccharides				
	$[\alpha]_D$ (in H_2O)	galactose:arabinose: rhamnose: uronic acid	$[\alpha]_D$ (in $CHCl_3$)	OMe	C	H	ash
Degraded gum A	$+13^{\circ}$	85:9:0.5:5.5	-12°	-	-	-	-
Degraded gum B	$+26.4^{\circ}$	93.5:1.5:5.0	-23°	38.0	52.6	7.9	0.3

Table 10

3.4 Methylation of A. podalyriaefolia gum, separation and identification of the methylated monosaccharides released on acid hydrolysis.

3.4.1 Methylation of Acacia podalyriaefolia gum.

A sample of partially methylated *A. podalyriaefolia* gum (8.27g), which had been methylated[7] according to the procedures of Haworth and Kuhn in 1964, was dissolved in methyl iodide (50 ml) and freshly precipitated silver oxide (3 g) added. The mixture was refluxed at 45° (bath temperature) for 6 days, filtered to remove silver oxide and silver iodide, and evaporated to dryness. Three further Purdié methylations were carried out, and the clear glass so obtained (7.48 g), having $[\alpha]_D$ -44° (*c* 3.31 in chloroform), (Found: C, 52.0; H, 7.8; OMe, 41.4; ash 0.5%) dried in *vacuo* to constant weight.

3.4.2 Hydrolysis of methylated A. podalyriaefolia gum.

The bulk of the methylated polysaccharide (5.82g) was heated (at 96°) in 98% formic acid (20 ml) for 30 minutes. Water was added and the product, after evaporation, heated in *N* - H_2SO_4 (120 ml at 96°) for 7 hours, neutralised($BaCO_3$) and filtered through a filter aid, Celite 535. Paper chromatographic examination of the hydrolysate in solvents (*a*) and (*b*) showed the following components, identified against standards. R_G values in parenthesis quoted for solvent (*a*).

2,3,5-Tri-*O*-methyl-*L*-arabinose	(1.02)
2,3,4,6-Tetra-*O*-methyl-*D*-galactose	(0.92)
2,3,6- and 2,4,6-Tetra-*O*-methyl-*D*-galactose	(0.78)
2,3,4-Tri-*O*-methyl-*D*-galactose	(0.72)
2,4-Di-*O*-methyl-*D*-galactose	(0.53)
2-*O*-Methyl-*D*-galactose	(0.35)
Uronic acids	(0.23)

The filtrate was evaporated to dryness and the resulting syrupy mixture of methylated sugars and sugar acids (as Ba salts) fractionated by cellulose column chromatography.

3.4.3 Chromatographic separation of methylated sugars.

The above hydrolysate was placed on a water-jacketed column of cellulose (105 X 5 cm), and eluted with mixtures of light petroleum (b.p. 100-120°) and water-saturated butan-1-ol (proportions of alcohol being increased from 1:7 to 6:1 in 9 steps; total volume 35 l.), with water-saturated butan-1-ol, and finally with ethanol and water. Fractions (on the average 40 ml each) were collected hourly in beakers, samples being screened by paper chromatography in solvents (a) and (b) and by TLC in solvent (g). In this way 20 sugar-containing fractions* were obtained. The R_G values quoted in the ensuing description of these fractions were measured in solvent (a); the T values are all for the methyl glycosides derived from the sugars.[75]

Fraction 1. A syrup (65 mg; eluted with petrol: butanol; 7:1) having $[\alpha]_D$ +5° (c 3.02), R_G 1.04, T 0.44s and 0.59w. Quantitative GLC analysis indicated this fraction to contain 2,3,4-tri-O-methylrhamnose (22 mg) and 2,3,5-tri-O-methyl-arabinose (2 mg). De-O-methylation in 48% HBr gave a series of methyl ethers of rhamnose, which were identified by paper chromatography against standards.

Fraction 2. A syrup (395 mg; eluted with petrol: butanol; 7:1) consisting of 2,3,4-tri-O-methylrhamnose (4 mg) and 2,3,5-tri-O-methylarabinose (200 mg), having $[\alpha]_D$ -24.3° (c 2.34), R_G 1.04 (trace) and 1.00, T 0.44 vw and 0.59s 0.78w. De-O-methylation showed a series of arabinose methyl ethers and arabinose, identical (paper chromatography) with those obtained similarly from authentic 2,3,5-tri-O-methyl-

*These were made up as follows, fraction numbers preceding the numbers of beakers used in the collection: 1, 1-18; 2, 19-90; 3, 91-130; 4, 131-137; 5, 138-150; 6, 151-176; 7, 177-203; 8, 204-220; 9, 221-237; 10, 238-276; 11, 277-362; 12, 363-381; 13, 382-391; 14, 392-413; 15, 414-563; 16, 564-590; 17, 591-601; 18, 602-637; 19, 638-657; 20, 658-upwards.

L-arabinose. N.M.R. spectroscopy indicated anomeric forms (α predominating) of 2,3,5-tri-O-methyl-L-arabinose (overlapping OMe signals, 9p).

Fraction 3. A syrup (1016 mg; eluted with petrol: butanol; 7:2) having $[\alpha]_D$ +90.5° (c 1.50), R_G 0.91, T 1.95, containing 2,3,4,6-tetra-O-methyl-D-galactose (1004 mg), showed a series of galactose methyl ethers and galactose on de-O-methylation. Reduction with sodium borohydride and recrystallisation (from cyclohexane) gave 2,3,4,6-tetra-O-methyl-D-galactitol, m.p. and mixed m.p. (with a sample derived from methylated Acacia elata gum [60]) 68-69°, $[\alpha]_D$ +9.1° (c 1.51 in ethanol), R_f 0.47 (TLC, solvent (f)). L it. 76, m.p. 68-70°, $[\alpha]_D$ +12.5 (c 2.25 in ethanol).

Fraction 4. A syrup (8 mg; eluted with petrol: butanol, 7:3) consisting of equal weights of 2,3,4,6-tetra-O-methylgalactose, T 1.97s, and 3,5-di-O-methylarabinose, T 1.35w 3.13s.

Fraction 5. A syrup (23 mg; eluted with petrol: butanol; 7:3) having $[\alpha]_D$ +16.7° (c 0.87), R_G 0.91 and 0.88, M_g 0 and 0.75, T 1.95w, 2.28s 4.53w and 1.35w 3.16w, containing 2,3,4,6-tetra-O-methylgalactose (2 mg), 2,5-di-O-methylarabinose (19 mg) and 3,5-di-O-methylarabinose (2 mg). De-O-methylation gave arabinose and a series of its methyl ethers.

Fraction 6. A syrup (43 mg; eluted with petrol: butanol; 7:3) having $[\alpha]_D$ +52.5° (c 1.78), R_G 0.91, 0.88 and 0.83, T 1.97w, 2.28s 4.53w and 3.77w 4.52w 5.17w 5.82w, corresponding to 2,3,4,6-tetra-O-methylgalactose (1 mg), 2,5-di-O-methylarabinose (12 mg) and 2,3,6-tri-O-methylgalactose (2 mg). De-O-methylation showed a series of methyl ethers of arabinose and galactose and the sugars themselves.

Fraction 7. A syrup (100 mg; eluted with petrol: butanol, 7:3) having $[\alpha]_D$ +85.5 (c 1.20), R_G 0.78, T 3.76s 4.75w 5.18w 5.77m, containing 2,3,6-tri-O-methylgalactose (93 mg) and 2,4,6-tri-O-methylgalactose (7 mg). De-O-methylation gave a series of methyl ethers of galactose and galactose. Recrystallisation of the borohydride-reduced derivative from ether-light petroleum (b.p. 60-80°) afforded needles, m.p. and mixed m.p. (with a sample obtained from methylated *Acacia karoo* gum)[41] 86-87°, R_f 0.32 (TLC, solvent (f)). Periodate oxidation and paper chromatography of this product (2,3,6-tri-*O*-methyl-D-galactitol) confirmed the identity of the main sugar component.

Fraction 8. A syrup (102 mg; eluted with petrol: butanol; 7:3) having $[\alpha]_D$ + 92.5° (*c* 1.28), R_G 0.78, *T* 3.73s 4.75w 5.13w 5.70m and showing two spots on TLC (solvent (f)), corresponding to 2,3,6-tri-*O*-methylgalactose (72 mg) and 2,4,6-tri-*O*-methyl-galactose (30 mg). A portion of fraction 8 was reduced with sodium borohydride and oxidised with periodate: paper chromatography then confirmed the identity of 2,3,6-tri-*O*-methylgalactose in this fraction and TLC showed the presence of unchanged 2,4,6-tri-*O*-methylgalactitol after the periodate treatment. De-*O*-methylation of the sugar mixture gave a similar series of methyl ethers to those found for fraction 7.

Fraction 9. A syrup (84 mg; eluted with petrol: butanol; 7:3) having $[\alpha]_D$ +89° (*c* 1.15), R_G 0.78 and 0.74, *T* 3.75w 4.97m 5.78s and 9.30m, corresponding to 2,3,6-tri-*O*-methylgalactose (11 mg), 2,4,6-tri-*O*-methylgalactose (43 mg) and 2,3,4-tri-*O*-methyl-galactose (27 mg).

Fraction 10. A crystalline component (258 mg; eluted with petrol: butanol; 7:3) having $[\alpha]_D$ +102° (*c* 2.25), R_G 0.73, *T* 5.41 vw and 9.30s, corresponding to 2,4,6-tri-*O*-methylgalactose (18 mg) and 2,3,4-tri-*O*-methylgalactose (216 mg). Recrystallisation (from ether containing a trace of acetone) afforded needles, m.p. and mixed m.p. (with authentic 2,3,4-tri-*O*-methyl-α-D-galactose) 61 - 63°. The derived glycitol had m.p. and mixed m.p. (with a sample derived from methylated *Virgilia oroboides* gum[77]) 122-123° (Lit.[76], m.p. 122-123°), R_f 0.29 (TLC, solvent (f)). The crystalline sugar was shown by N.M.R. spectroscopy to consist of the α form, with OMe signals corresponding to those found for an authentic specimen of 2,3,4-tri-O-methyl-D-galactose. After mutarotation $H_{(1)}\beta$ (doublet) and $C_{(2)}$ β (OMe) signals were observed.

Fraction 11. A syrup (47 mg; eluted with petrol: butanol; 7:5) having $[\alpha]_D$ +137° (c 1.99), R_G 0.70 and 0.78, T 1.92s, 5.08vw 5.66 vw, 9.43m and 22.75m 26.75s, containing 2,3,4,6-tetra-O-methylgalactose (17 mg), 2,4,6-tri-O-methylgalactose (1 mg), 2,3,4-tri-O-methylgalactose (8 mg) and 2,4-di-O-methylgalactose (19 mg). Paper chromatography of the hydrolysate of this fraction both before and after borohydride reduction showed the above-mentioned methylated sugars. De-O-methylation of the mixture gave a series of methyl ethers of galactose including 2,3,4,6-tetra-O-methylgalactose and galactose. It appears as if the constant R_G 0.78 (not present after hydrolysis) and T 1.92 and 22.75 26.75 are due to an oligosaccharide of 2,3,4,6-tetra-O-methylgalactose and 2,4-di-O-methylgalactose containing a large percentage of α linkages (see also fraction 18).

Fraction 12. A syrup (30 mg; eluted with petrol: butanol; 7:6) having $[\alpha]_D$ + 69° (c 1.25), R_G 0.61, M_g 0.30, T 13.00s 16.10w 18.50m 24.42vw, corresponding to 2,6-di-O-methyl-D-galactose (27 mg). De-O-methylation and paper chromatography gave galactose and one spot corresponding to a methyl ether (2- and/or 6-). Periodate oxidation and paper chromatography gave products characteristic of a 2,6-di-O-methylhexose.

Fraction 13. A syrup (20 mg; eluted with petrol: butanol; 7:7) having $[\alpha]_D$ + 79° (c 0.86), R_G 0.55 and 0.61, M_g 0.30, T 13.06s 15.85w 18.01m 23.70w, was judged to be a mixture of 2,3-di-O-methylgalactose (10 mg) and 2,6-di-O-methylgalactose (10 mg). De-O-methylation and paper chromatography gave galactose and monomethyl ethers thereof.

Fraction 14. A syrup (27 mg; eluted with petrol: butanol; 7:7) having $[\alpha]_D$ + 102° (c 1.12), R_G 0.55 and 0.61, M_g 0.30, T 13.77s 16.70w 18.35m 23.80m. This corresponded to 2,3-di-O-methyl-D-galactose (25 mg) containing a trace of 2,6-di-O-methylgalactose

(2 mg). Periodate oxidation and paper chromatography of the derived glycitol, R_f 0.14 (TLC, solvent (f)), gave the expected products.

Fraction 15. The crystalline sugar (1149 mg; eluted with petrol: butanol; 7:7) having $[\alpha]_D$ +86.5° (c 1.56), R_G 0.55, M_g 0.27, T 23.30m 27.00s, containing 2,4-di-O-methyl-D-galactose (1121 mg) separated as needles from chloroform-light petroleum (b.p. 60-80°), m.p. and mixed m.p. (with 2,4-di-O-methyl-α-D-galactose) 106-107°. The derived methyl 2,4-di-O-methyl-β-D-galactoside had m.p. 166-167° (lit.,[41, 78] m.p. 162°, 167°), T 24.3. Reduction of the sugar and recrystallisation of the glycitol (from ethyl acetate) yielded needles, m.p. and mixed m.p. (with a sample derived from methylated Acacia elata gum[60]) 135-136°, $[\alpha]_D$ +27.8° (c 2.28 in methanol), R_f 0.16 (TLC, solvent (f)); lit.,[76] m.p. 134-135°, $[\alpha]_D$ +23.5 (c 2.04 in methanol). Periodate oxidation of the glycitol gave the expected sugar (2,4-di-O-methyllyxose) on paper chromatography. The crystalline sugar was shown to be the α anomer by NMR spectroscopy, sharp OMe singlets corresponding to OMe at $C_{(2)}$ α and $C_{(4)}$ being observed; after mutarotation $H_{(1)}$ β (doublet) and $C_{(2)}$ β OMe signals appeared.

Fraction 16. The crystalline sugar (323 mg; eluted with petrol: butanol; 7:21) having $[\alpha]_D$ +81° (c 1.11), R_G 0.38, M_g 0.34, separated from methanol-acetone as nodules m.p. and mixed m.p. (with 2-O-methyl-β-D-galactose) 155-156°. Periodate oxidation and paper chromatography gave the expected methoxymalondialdehyde. Reduction and recrystallisation (from methanol-ethyl acetate) afforded 2-O-methyl-D-galactitol, m.p. 105°, $[\alpha]_D$ +4.8° (c 1.04 in methanol), R_f 0.06 (TLC, solvent (f)). The structure of the sugar (β-form, mutarotating slowly) was confirmed by comparison of its NMR spectrum with that of an authentic sample.

Fraction 17. The mixture (51 mg; eluted with petrol: butanol; 7:21) having $[\alpha]_D$ +93° (c 2.52), R_G 0.38, 0.29, M_g 0.34, 0.23, was judged from paper chromatography and ionophoresis to be composed of 2-*O*-methylgalactose and 4-*O*-methylgalactose in the ratio 1:2.

Fraction 18. A partly-crystalline mixture (39 mg; eluted with petrol: butanol; 7:42) having $[\alpha]_D$ +76.5° (*c* 1.94), R_G 0.30, 0.43, M_g 0, 0.24, was judged to be composed of 4-*O*-methylgalactose (1 part) and an unidentified component (2 parts). After treatment with mixed ion-exchangers and charcoal, the syrup crystallised as needles, m.p. 207° (after having been washed with acetone). Periodate oxidation and paper chromatography gave the product expected of 4-*O*-methyl-*D*-galactose. The mother liquor (R_G 0.43) was methanolysed, analysed by GLC and shown to contain the following methylated sugars (molar proportions % indicated in parenthesis):

2,3,4,6-tetra-*O*-methylgalactose	(1)
2,3,6-tri-*O*-methylgalactose	(9)
2,3,4-tri-*O*-methylgalactose	(2) and
2,4-di-*O*-methylgalactose	(88).

The appearance of a large proportion of 2,4-di-*O*-methylgalactose (confirmed by hydrolysis and paper chromatography) suggests the component R_G 0.43 is an oligosaccharide of 2,4-di-*O*-methylgalactose. The presence of 4-*O*-methyl-β-*D*-galactose in the original partly-crystalline fraction was indicated by the $H_{(1)}$ β and OMe signals in the NMR spectrum.

Fraction 19. A crystalline component (15 mg; eluted with water-saturated butanol) having R_G 0.19, chromatographically homogeneous and identical with galactose.

Fraction 20. A syrup eluted with aqueous ethanol was separated from salts by extraction with ethanol. The syrupy extract (256 mg), having R_G 0.10 - 0.20, was passed through Amberlite IR -120 (H^+) resin to remove Ba, concentrated and freeze-dried. The residue (182 mg) was heated under reflux in 2% methanolic HCl for 8 hours, neutralised (Ag_2CO_3), filtered, concentrated, then saponified in aqueous 0.15 N-Ba $(OH)_2$ for 6 hours. The solution was treated with Amberlite IR - 120 (H^+) resin and then passed through Duolite A4 (OH^-) resin (16-36 mesh, column 5 x 1 cm) and eluted with water at 4^o.

The neutral material (fraction 20a, 50 mg) thus eluted had $[\alpha]_D$ +56.5° (c 2.50). Analysis by GLC showed fraction 20a to contain the methyl glycosides of the following sugars (molar proportion indicated in parenthesis)

2,3,4,6-tetra-O-methylgalactose	(9)
2,3,6-tri-O-methylgalactose	(7)
2,4,6-tri-O-methylgalactose	(13)
2,3,4-tri-O-methylgalactose	(18)
2,6-di-O-methylgalactose	(5) and
2,4-di-O-methylgalactose	(48)

Acid hydrolysis and paper chromatography of this fraction confirmed the identity of the above-mentioned methyl ethers of galactose and indicated the presence of 2-O-methylgalactose.

Elution of the Duolite column at 4^o with N - NaOH until the eluate gave a negative Molisch response, removal of the sodium by the addition of Amberlite IR-120 (H^+) resin and cautious evaporation yielded an acid fraction (20 b, 140 mg), portions of which were hydrolysed for paper chromatography and methanolysed for GLC analysis. These analyses showed fraction 20 b to be composed of the following methyl ethers (molar proportions indicated in parenthesis):

2,3,4,6-tetra-O-methylgalactose	(1)
2,3,6-tri-O-methylgalactose	(7)
2,4,6-tri-O-methylgalactose	(4)
2,3,4-tri-O-methylgalactose	(20)
2,3,4-tri-O-methylglucuronic acid	(31) and
2,3-di-O-methylglucuronic acid	(37) .

Examination of the acid fraction. The bulk of the acid fraction 20 b (125 mg) was treated with methanolic HCl to convert the acids to their methyl ethers and the sugars to the methyl glycosides, and the neutralised solution ($Ag_2 CO_3$) reduced in THF (20 ml) with LAH (81 mg), working up the product by the addition of wet ethyl acetate, evaporation, addition of water (5 ml) and sodium citrate (107 mg) to de-complex the sugar from the aluminium; evaporation and extraction into chloroform gave a syrup, which was extracted into methanol and filtered, affording a syrup A (98.2 mg). A portion of syrup A was methanolysed and analysis by GLC showed the following sugars, together with the molar proportions (in parenthesis):

2,3,4,6-tetra-O-methylgalactose	(1)
2,3,6-tri-O-methylgalactose	(16)
2,4,6-tri-O-methylgalactose	(5)
2,3,4-tri-O-methylgalactose	(3)
2,3,4-tri-O-methylglucose	(14) and
2,3-di-O-methylglucose	(61).

Paper chromatography of the hydrolysate confirmed the presence of above-mentioned sugars.

Methanolysis and methylation of syrup A (12 mg) followed by GLC analysis showed the following sugars

2,3,4,6-tetra-O-methylglucose (T 1.00 m 1.52s; 75 mol.)

2,3,4,6-tetra-O-methylgalactose (T 2.00w; 23) and

unidentified components (2).

The remainder of syrup A was hydrolysed in N-H_2SO_4 and neutralised ($Ba CO_3$) and the hydrolysate (51.4 mg), having $[\alpha]_D$ +68.5° (c 2.56), was chromatographed on Whatman No. 3 MM paper in solvent (a) and separated into the following three fractions:

Fraction (i). A syrup (15 mg) having $[\alpha]_D$ +60° (c 0.65 in methanol), R_G 0.90, T 1.92w, 2.88m 4.24s, 3.74s 4.24s 5.28w 5.90m, 9.52w, 11.60s, 14.24m 21.95w, containing the following sugars (molar proportion in parenthesis):

2,3,4,6-tetra-O-methylgalactose (2)

2,3,6-tri-O-methylgalactose (29)

2,4,6-tri-O-methylgalactose (1)

2,3,4-tri-O-methylgalactose (3)

2,3,4-tri-O-methylglucose (23) and

2,3-di-O-methylglucose (42).

The occurrence of a large quantity of 2,3-di-O-methylglucose in this fraction, as well as in the methanolysate of the LAH reduced fraction 20b above, is entirely due to demethylation of 2,34-tri-O-methylglucose on methanolysis.

Methylation, methanolysis and GLC assay of this fraction showed:

2,3,4,6-tetra-O-methylglucose (T 1.00 m 1.52s) and

2,3,4,6-tetra-O-methylgalactose (T 1.92).

Periodate oxidation of fraction (i) after borohydride reduction gave the same sugar as was produced similarly from authentic 2,3,4-tri-O-methylglucitol.

Fraction (ii). A mixture (13.2 mg) having $[\alpha]_D$ +97° (c 0.49) and $[\alpha]_D$ +48.5° (c 0.62 in methanol), R_G 0.79, 0.68, T 2.88 vw 4.70w, 3.82s 4.70w 5.34w 5.96m, 9.56w and 11.50m 14.20m 21.30m, containing the following (molar proportions indicated in parenthesis):

2,3,6-tri-O-methylgalactose (52)

2,4,6-tri-O-methylgalactose (4)

2,3,4-tri-O-methylgalactose (6)

2,3,4-tri-O-methylglucose (1) and

2,3-di-O-methylglucose (37).

Methylation, methanolysis and GLC assay of fraction (ii) showed a mixture of

2,3,4,6-tetra-O-methylglucose (T 1.00 m 1.52s) and

2,3,46-tetra-O-methylgalactose (T 1.92s).

Periodate oxidation of the borohydride reduced fraction (ii) gave the expected products.

Fraction (iii). A syrup (19.2 mg) having $[\alpha]_D$ +49.2° (c 0.67) and $[\alpha]_D$ +59° (c 0.56 in methanol), R_G 0.68, T 3.79vw 5.85vw and 11.55s 14.20m 21.35w, containing the following sugars (molar proportions indicated in parenthesis):

2,3,6-tri-O-methylgalactose (1)

2,4,6-tri-O-methylgalactose (1) and

2,3-di-O-methylglucose (98).

Methylation, methanolysis and GLC assay of fraction (iii) showed

2,3,4,6-tetra-O-methylglucose (T 1.00m 1.52s) and

2,3,4,6-tetra-O-methylgalactose (T 1.92vw).

Periodate oxidation of the borohydride reduced fraction (iii) gave the same product as was produced similarly from authentic 2,3-di-O-methylglucitol, together with a slower moving sugar. (The latter was found to be resistant to further periodate treatment, but could be 3,4-di-O-methylxylose from partial oxidation of the glucitol). The T values found for the sugar were 11.55s 14.20m 21.35w, the shape of the three peaks differing from those found for this sugar obtained from Acacia karroo,[41] where the order was w, m, s. This fraction could well be a mixture of di-O-methylglucoses, including the 2,3-derivative.

The weights and molar proportions of the sugars eluted from the column are presented in Table 11 and diagramatically represented in a histogram (fig. 2). Included in Table 11 are the results obtained previously by methanolysis and GLC assay of the methyl glycosides.[7]

Proportions of sugar residues in hydrolysate of methylated *Acacia podalyriaefolia* gum.

Methylated sugar	wt(mg)	mol. %	mol. % GLC
2,3,4-Tri-*O*-methylrhamnose	26	0.7	0.5
2,3,5-Tri-*O*-methylarabinose	202	6.2	11
2,5-Di-*O*-methylarabinose	31	1.0	-
3,4-Di-*O*-methylarabinose	-	-	1?
3,5-Di-*O*-methylarabinose	6	0.2	-
2,3,4,6-Tetra-*O*-methylgalactose	1033	25.7	26
2,3,6-Tri-*O*-methylgalactose	196	5.2	5
2,4,6-Tri-*O*-methylgalactose	105	2.8	2
2,3,4-Tri-*O*-methylgalactose	287	7.6	7
2,3-Di-*O*-methylgalactose	35	1.0	-
2,4-Di-*O*-methylgalactose	1189	33.6	38
2,6-Di-*O*-methylgalactose	44	1.2	tr
2-*O*-Methylgalactose	340	10.3	8
4-*O*-Methylgalactose	47	1.5	
Galactose	15	0.5	-
2,3,4-Tri-*O*-methylglucuronic acid	46	1.1	2.0
2,3-Di-*O*-methylglucuronic acid	52	1.4	tr

Table 11

From these values, the following pattern is evident:

(a) end groups 34 (b) chain groups 18 (c) branch-points 61.

3.5 Periodate oxidation studies on *Acacia podalyriaefolia* gum

3.5.1 Analytical estimation of periodate uptake.

A. podalyriaefolia gum (148 mg) was dissolved in water (50 ml) and a solution of sodium meta - periodate (0.012 *M*, 50 ml) was added and the reaction mixture kept in the dark and at room temperature (*ca* 20°). At intervals aliquots (5 ml)

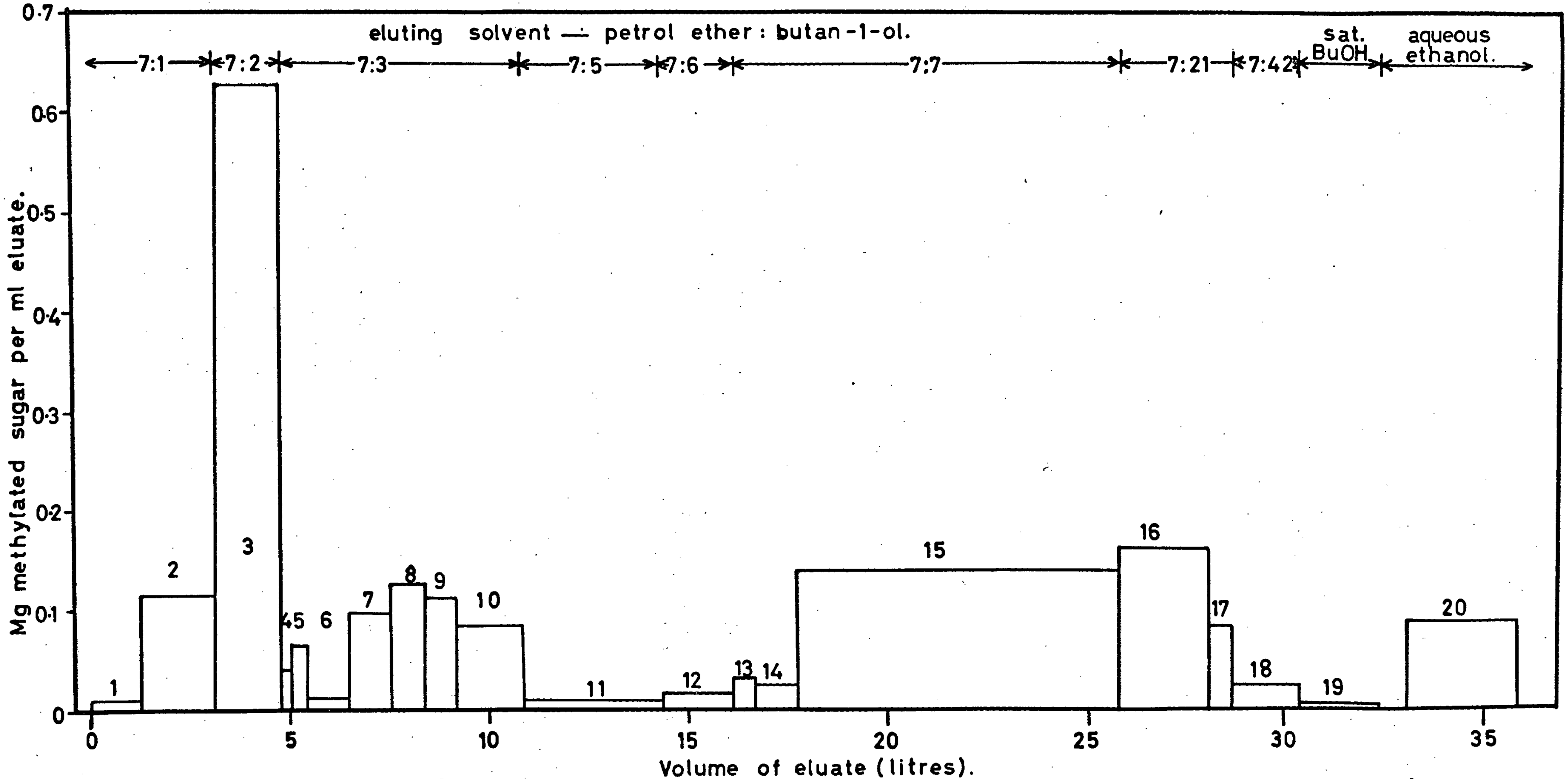


Fig. 2. Histogram of the hydrolysis products of methylated A. podalyriaefolia gum.

were removed and assayed for periodate content.[79] After 54 hours, when 0.53 mol.periodate per 100g carbohydrate had been consumed, the solution was adjusted to 0.025M with respect to sodium meta-periodate. As was expected, the rate of oxidation was not seriously altered as indicated by the change in the curve (fig. 3), which shows the rate of oxidation with time. The results are presented in table 12

Time (hours)	Mol. periodate consumed per 100g carbohydrate
0.50	0.100
2.50	0.374
4.50	0.406
6.50	0.415
11.00	0.454
28.00	0.501
54.00	0.529
70.00	0.560
114 .00	0.572

Table 12

3.5.2 Preparative-scale periodate oxidation, borohydride reduction and "Smith degradation" of Acacia podalyriaefolia gum. - Preparation of polysaccharide A.

3.5.2.1 Periodate oxidation of Acacia podalyriaefolia gum.

A. podalyriaefolia gum (5.06g) was dissolved in water (200 ml) and sodium meta-periodate (8.15g) added, and the volume adjusted to (500 ml). The reaction mixture was kept in the dark and at room temperature, aliquots being assayed at intervals for periodate content. The results are presented in table 13 and fig. 3.

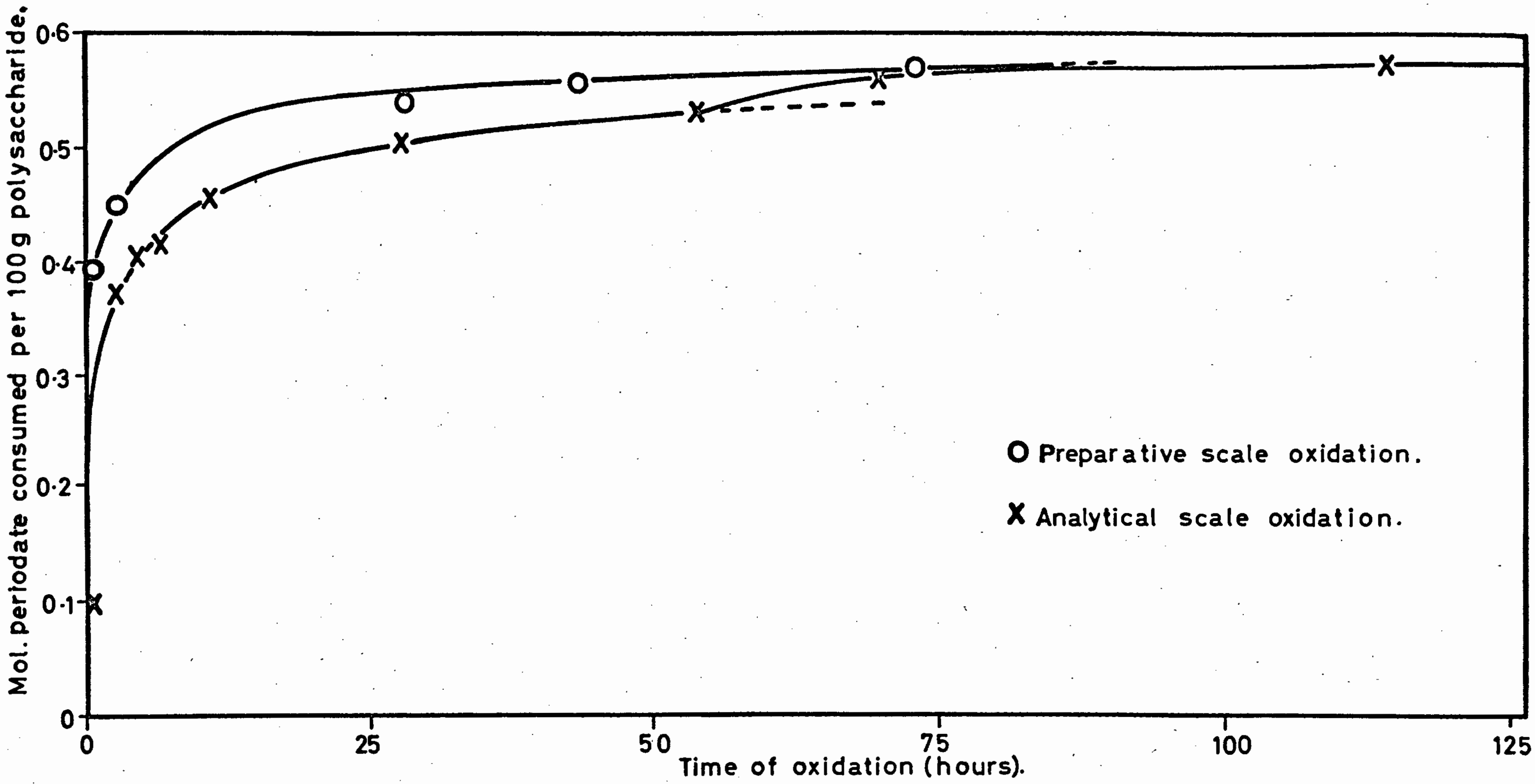


Fig. 3. Periodate oxidation of A. podalyriaefolia gum.

Time (hours)	Mol periodate consumed for 100g carbohydrate
0.50	0.391
2.50	0.452
28.00	0.537
43.00	0.556
73.00	0.569

Table 13

When the reaction had proceeded for the above period, it was terminated by the addition of barium carbonate (12g) with stirring for 24 hours. The solution was filtered through celite 535 and the volume reduced to (80 ml).

3.5.2.2 Sodium borohydride reduction of oxidised Acacia podalyriaefolia gum.

The polyaldehyde was reduced to the polyalcohol with sodium borohydride (3.5g) for 72 hours. The excess borohydride was converted to the borate by lowering the p H with solid carbon dioxide. Cations were removed with Amberlite IR - 120 (H^+) resin and the borate removed on evaporation with methanol, and the reduced-oxidised A. podalyriaefolia gum (2.99g) recovered by freeze drying.

Examination of reduced-oxidised Acacia podalyriaefolia gum.

A portion of the reduced-oxidised gum was hydrolysed in N-H_2SO_4, neutralised (Ba CO_3), and chromatographed in solvents (a), (b) and (c) and shown to contain galactose (+), arabinose (tr) and glycerol (+), the latter being the only visible glycitol. The hydrolysate was assayed for total sugar content (methods (a) and (b)) and for reducing sugar content (method c)

(a) Steam was passed through a solution of the hydrolysate (10.1 mg) to remove any glycollic aldehyde and the resulting solution was assayed with phenol-sulphuric acid[70] showing a sugar content of 3.8 mg in the portion, indicating a total sugar content of 1.113g in the derived reduced-oxidised sample of A. podalyriaefolia gum.

(b) A second portion of the hydrolysate (10.3 mg) was assayed according to the method of Bahl and co-workers.[80] The sample was dissolved in 2.5% methanolic hydrogen chloride and heated at 96° for three hours; when Fehling's test was negative. The solution was neutralised (Ag_2CO_3), filtered and evaporated to dryness. Methanol (2X30 ml) was evaporated from the residue, which was finally heated for three hours at 80° under reduced pressure to remove traces of glycollic aldehyde dimethyl acetal which might have been present. Aliquots of an aqueous solution were assayed by the phenol-sulphuric acid method for total sugar content, which was found to be 3.5 mg, indicating that the reduced-oxidised sample contained 1.020g of sugar.

(c) The third method used was that of Somogyi and co-workers.[81] A portion of the hydrolysate (10.2 mg) was dissolved in water (10 ml) and aliquots (1 ml) were treated with an alkaline copper sulphate solution (3 ml) and the acid molybdate reagent (2 ml). Colorimetric estimation showed this portion to contain 2.8 mg of sugar, indicating a total sugar content of 820 mg in the reduced-oxidised sample.

Preparative-scale chromatography of a further portion of the hydrolysate in solvent (a), elution of the galactose and glycerol and assay of the galactose by the phenol-sulphuric acid method and of the glycerol by sodium meta-periodate oxidation and estimation of the formaldehyde produced by the chronotropic acid method,[82] indicated the molar proportions of galactose and glycerol to be 45 and 55 respectively.

GLC of the derived glycitol acetates showed the components and their molar proportions listed in table 14.

Monomer	Molar proportion %
Glycerol	58.5
Erythritol?	0.5
Rhamnose	0.2
Arabinose	1.8
Galactose	39.0

Table 14

3.5.2.3 Mild acid hydrolysis of reduced-oxidised *Acacia podalyriaefolia* gum.

The reduced-oxidised polysaccharide (2.69g) was dissolved in $\underline{N}$-H_2SO_4 and hydrolysed at 20° for 2.5 hours ("Smith degradation" conditions[31]). The solution was neutralised (Ba CO_3), filtered and evaporated to dryness. The white solid was fractionated as follows:

(i) A syrup (ca. 2g, probably containing water) soluble in methanol-acetone (1:1, 100 ml) having $[\alpha]_D$ +4° (c 1.07 in methanol), and containing essentially glycerol, but also at least eight other components, one of which had R_{gal} 1.41 and coloured with cold ammoniacal silver nitrate, as shown by paper chromatography in solvent (a). No free reducing sugars were revealed on paper chromatography.

Hydrolysis of a portion and assay of the derived glycitol acetates by GLC indicated the molar proportions of the monomeric components as listed in table 15.

Monomer	Molar proportion %
Glycerol	95.0
Erythritol?	0.8
Rhamnose	1.0
Arabinose	1.6
Galactose	1.6

Table 15

A portion of fraction (i) (10 ml) was evaporated with benzene to constant weight (193 mg). The residue was dissolved in pyridine (10 ml), p-nitro-benzoylchloride (1.2g) was added and the solution heated at 96° for one hour, then left at room temperature overnight. Water was added and the solution extracted with chloroform, washed with N-HCl, 10% $NaHCO_3$ and finally with water. Evaporation and crystallisation from aqueous ethanol afforded glycerol-tris-p-nitrobenzoate (27 mg), m.p. and mixed m.p. (with an authentic sample) 194-195°.

A portion of fraction (i) (5 ml, 96 mg) was dissolved in water (50 ml) and sodium-meta-periodate (0.7g) was added and the solution kept at room temperature for 48 hours. Excess periodate was decomposed with ethylene glycol and the formic acid released was titrated with 0.02 M-NaOH solution. The amount of formic acid released was equivalent to 14 mg glycerol present in the extract.

Steam was passed through a portion of fraction (i) (1 ml) to remove any glycollic aldehyde and the residue was assayed for total sugar content (as galactose), and found to be 8.3% (by weight), which is in good agreement with a molar proportion of 4.2% found by GLC.

Attempted fractionation of fraction (i).

The syrup (20 ml; 377 mg) was charged to a column (3x21 cm) of Dowex-1-resin (OH^- form, 50-100 mesh) and eluted with carbon dioxide free water. Fractions (5 ml) were collected and screened by paper chromatography in solvent (a). In this way, the following five fractions were obtained.

Fraction (a). A syrup (133 mg) having $[\alpha]_D$ 0° (c 6.6), R_{gal} 2.55, was chromatographically homogeneous and identical with glycerol in solvent (a). The derived acetate had the same retention time as authentic triacetin. The derived p-nitrobenzoate derivative had m.p. and mixed m.p. (with authentic glycerol-tris-p-nitrobenzoate) 193-195°.

Fraction (b). A syrup (39 mg) having $[\alpha]_D$ 0^o (c 7.25), R_{gal} 1.30 (major), 1.62 and 2.55, the latter identical with glycerol. Hydrolysis and GLC assay of the derived glycitol acetates showed this fraction to be composed of glycerol (18 mg), erythritol? (2.2 mg), rhamnose (1.5 mg), arabinose (6.2 mg) and galactose (6.2 mg).

Fraction (c). A syrup (7 mg) having R_{gal} 1.30 and 1.62 (tr), $[\alpha]_D$ 0^o (c 3.5) was shown by hydrolysis and GLC assay of the derived glycitol acetates to be composed of glycerol (4 mg), erythritol? (1.2 mg) and arabinose (1.7 mg).

Fraction (d). A syrup (29 mg) having R_{gal} 0.90, 1.30, $[\alpha]_D$ 0^o (c 2.5) was shown by GLC assay of the glycitol acetates to be composed of glycerol (12.5 mg), rhamnose (1 mg) and arabinose (1 mg) and an unidentified component.

Fraction (e). A syrup (18.2 mg) having R_{gal} 0.90, 1.30 (tr) $[\alpha]_D$ 0^o (c 3.0) was shown by GLC assay of the glycitol acetates to be composed of glycerol (5.7 mg), arabinose (5.2 mg) and galactose (0.6 mg).

The results are presented in table 16.

Monomer	wt (mg)	Molar proportion %
Glycerol	173.2	91.7
Erythritol?	3.4	1.3
Rhamnose	2.5	0.7
Arabinose	14.1	4.5
Galactose	6.8	1.8

Table 16

The component having R_{gal} 1.41, and reacting with cold ammoniacal silver nitrate spray reagent was not detected and is probably aldehydic in nature, and is decomposed on this column.

An attempted fractionation of fraction (i) (500 mg) on a column of cellulose, using butan-2-one-water azeotrope as eluant, afforded glycerol and a number of minor components together with a fraction having $\underline{R}_{gal}$ 1.41, which coloured with cold silver nitrate spray reagent. The greater proportion of the "$\underline{R}_{gal}$ 1.41" fraction was eluted together with glycerol, but a small quantity (16 mg) was obtained pure.

Reduction of a portion with sodium borohydride and paper chromatography in solvent (a) showed glycerol, but lacked components at $\underline{R}_{gal}$ 1.41. Hydrolysis of the reduced fraction, neutralisation and paper chromatography showed no reducing sugars. The paper chromatographic mobility of this fraction was, however, not identical with freshly prepared D-glyceraldehyde.[64]

(ii) An intermediate fraction (0.20 g), soluble in methanol, showing similar components on paper chromatography as (i). Hydrolysis and GLC assay of the derived glycitol acetates showed the molar proportions of the monomeric components listed in table 17.

Monomer	Molar proportion %
Glycerol	57
Erythritol ?	tr
Rhamnose	tr
Arabinose	4
Galactose	39

Table 17

(iii) An insoluble residue, Polysaccharide A, (1.03g; 20% yield) having $[\alpha]_D$ +20° ($\underline{c}$ 2.75), and weight-average molecular weight 2985 (see section 3.6), was shown by hydrolysis of a portion and GLC of the derived glycitol acetates to be composed of the monomeric components listed in table 18.

Monomer	Molar proportion %
Glycerol	3
Erythritol ?	tr
Rhamnose	1
Arabinose	2
Galactose	94

Table 18

The glycitol acetate mixture was repeatedly recrystallised from ethanol and the white needles had $[\alpha]_D$ +20° (c 0.81 in chloroform) and m.p. 160-163° and mixed m.p. (with authentic hexa-O-acetylgalactitol) 159-160°.

Preparative-scale paper chromatography of the hydrolysate of Polysaccharide A, elution of the sugars and assay indicated a molar ratio of galactose to arabinose of 30:1.

Methylation of Polysaccharide A (154 mg) according to the procedures of Haworth and Purdie gave a methylated derivative (80 mg), lacking hydroxyl bands in the infrared spectrum, and having $[\alpha]_D$ +2° (c 1.53 in chloroform). Methanolysis and GLC assay of the methylated polysaccharide showed the methyl ethers, together with their molar proportions presented in table 19.

O-Methylsugar	Molar proportion %
2,3,5-Tri-O-methylarabinose	3.5
3,4-Di-O-methylarabinose	0.5 ?
2,3,4,6-Tetra-O-methylgalactose	8
2,3,6-Tri-O-methylgalactose	1
2,4,6-Tri-O-methylgalactose	61
2,3,4-Tri-O-methylgalactose	8
2,4-Di-O-methylgalactose	2
2,6-Di-O-methylgalactose	14
2-O-methylgalactose*	1
2,3,4-Tri-O-methylglucuronic acid	1

Table 19

*estimated for paper chromatogram.

Hydrolysis of the methylated derivative and paper chromatography confirmed the presence of the major components and indicated the presence of 2-O-methylgalactose in the hydrolysate.

From these results the following pattern emerges (i) end groups 12.5 (ii) chain-groups 71.5 (iii) branch-points 18 and (iv) ratio of galactose : arabinose as 95.4

3.5.3 Analytical estimation of the periodate uptake by Polysaccharide A.

Polysaccharide A (25.06 mg) was dissolved in water, sodium meta-periodate (50 mg) was added, and the volume adjusted to 25 ml. The reaction mixture was kept as previously. Aliquots were removed at intervals and the periodate content measured spectrophotometrically.[83] The results are presented in table 20 and fig.4.

Time (hours)	mol. periodate consumed per 100g carbohydrate
0.50	0.069
3.25	0.115
8.00	0.219
11.00	0.219
28.00	0.219
46.00	0.231
68.00	0.231

Table 20

3.5.4 Preparative-scale periodate oxidation, borohydride reduction and "Smith degradation" of Polysaccharide A. - Preparation of Polysaccharide B.

3.5.4.1 Periodate oxidation of Polysaccharide A.

Polysaccharide A (0.50g) was dissolved in a solution of 0.018 M- sodium meta-periodate (250 ml) and the reaction kept as previously for 67 hours. The rate of oxidation was followed by measuring the periodate content spectrophotometrically.[83] The results are presented in table 21 and fig. 4.

Time (hours)	mol. periodate consumed per 100g carbohydrate
1	0.186
2.5	0.186
25.5	0.192
67.0	0.200

Table 21

After 67 hours the reaction was terminated by the addition of barium carbonate. The solution was filtered and concentrated.

3.5.4.2 <u>Sodium borohydride reduction of oxidised Polysaccharide A.</u>

The polyaldehyde obtained from Polysaccharide A was reduced to the polyalcohol with sodium borohydride and the reduced product (250 mg; 50%) recovered as in section 3.5.2.2.

A portion of reduced-oxidised Polysaccharide A (8 mg) was hydrolysed in $\underline{\underline{N}}$-$H_2SO_4$ at 96°, neutralised (Ba CO_3) and converted to the glycitol acetates. GLC assay of this mixture showed the molar proportions of the monomeric components listed in table 22.

Monomer	Molar proportion %
Glycerol	8
Erythritol ?	1
Rhamnose	0.5
Arabinose	3.5
Galactose	87

Table 22

3.5.4.3 <u>Mild acid hydrolysis of reduced-oxidised Polysaccharide A.</u>

The bulk of reduced-oxidised Polysaccharide A was dissolved in $\underline{\underline{N}}$-$H_2SO_4$ and hydrolysed at 20° for three hours. After

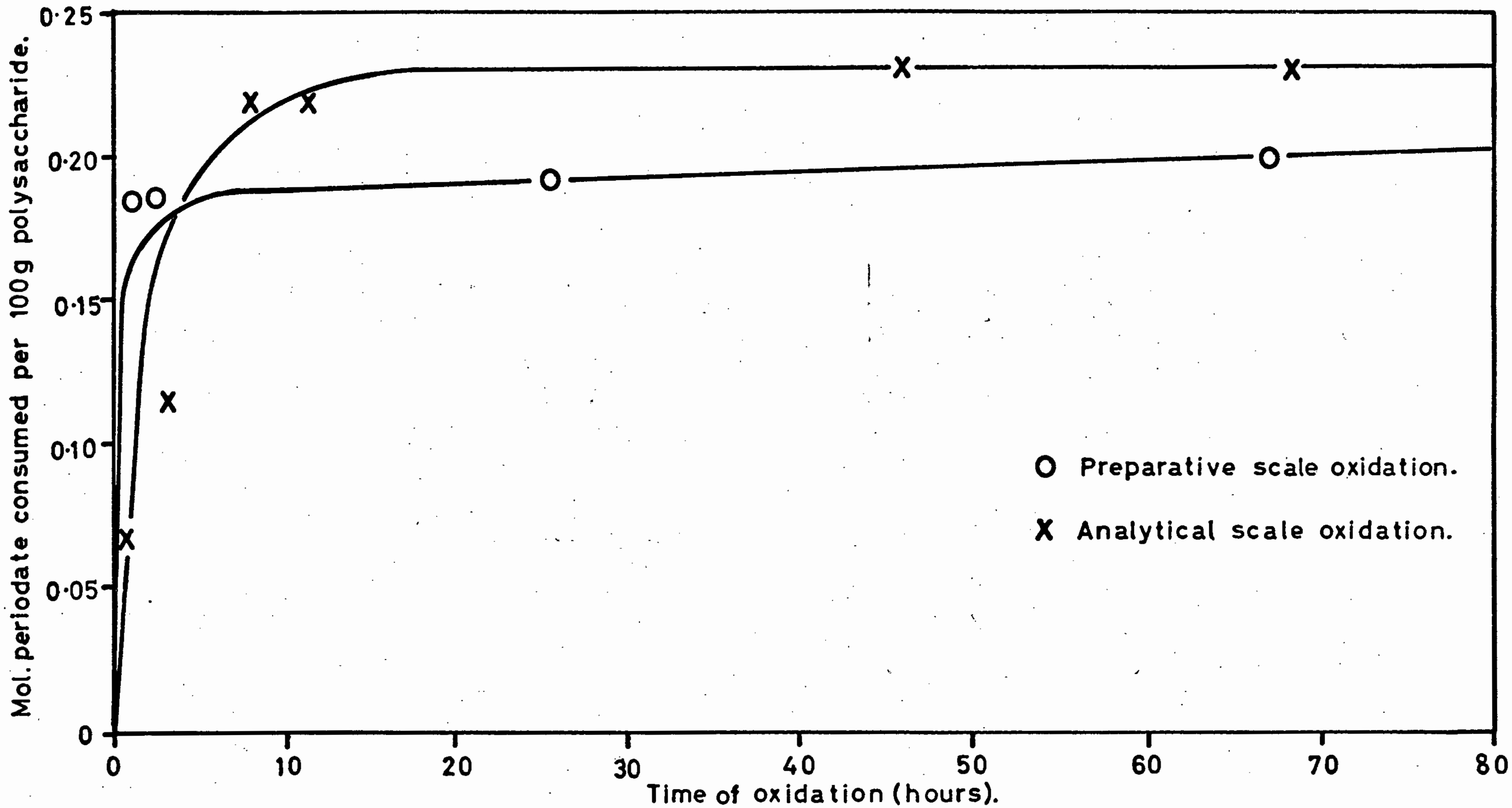


Fig. 4. Periodate oxidation of Polysaccharide A.

neutralisation, filtration and concentration, the product was fractionated to give the following fractions:

(i) A syrup (38 mg), soluble in methanol-acetone (1:1; 100 ml), which on hydrolysis, neutralisation, conversion to the glycitol acetates and GLC assay was shown to be composed of the monomeric components listed in table 23.

Monomer	Molar proportion %
Glycerol	53
Erythritol ?	1.2
Rhamnose	0.8
Arabinose	7.0
Galactose	38

Table 23

(ii) An insoluble residue, Polysaccharide B (224 mg) having $[\alpha]_D$ +40.2° (c 2.51) and approximate molecular weight 1940 (see section 3.6.4). Hydrolysis of a portion, neutralisation and GLC assay of the glycitol acetates showed the molar proportion of monomeric components as listed in table 24.

Monomer	Molar proportion %
Glycerol	3.5
Erythritol ?	tr
Rhamnose	0.7
Arabinose	2.8
Galactose	93

Table 24

Methylation of Polysaccharide B (76 mg) afforded a product (48 mg) having $[\alpha]_D$ +7° (c 2.11 in chloroform) and $[\alpha]_D$ +10.3° (c 2.24 in methanol). Methanolysis and GLC assay showed the methyl ethers and molar proportions as presented in table 25.

O-Methylether	Molar proportion %
2,3,4-Tri-O-methylarabinose	2
3,4-Di-O-methylarabinose	0.3
2,3,4,6-Tetra-O-methylgalactose	8
2,3,6-Tri-O-methylgalactose	1
2,4,6-Tri-O-methylgalactose	54
2,3,4-Tri-O-methylgalactose	7
2,4-Di-O-methylgalactose	5
2,6-Di-O-methylgalactose	21
2-O-methylgalactose*	1
2,3,4-Tri-O-methylglucuronic acid	0.7

Table 25

*shown by paper chromatography.

Hydrolysis of the methylated polysaccharide and paper chromatography in solvent (a) confirmed the above mentioned components and indicated the presence of 2-O-methylgalactose.

3.5.5 Preparative-scale periodate oxidation, borohydride reduction and "Smith degradation" of Polysaccharide B. - Preparation of Polysaccharide C.

3.5.5.1 Periodate oxidation of Polysaccharide B.

Polysaccharide B (128 mg) was dissolved in a solution of 0.015 M-sodium meta-periodate (100 ml) and the reaction kept as previously for 58 hours and the amount of periodate consumed (see table 26) was measured spectrophotometrically.[83]

Time (hours)	mol.periodate consumed per 100g carbohydrate
0.65	0.130
6	0.213
26	0.259
58	0.277

Table 26

3.5.5.2 Sodium borohydride reduction of oxidised Polysaccharide B.

After removal of the excess periodate and iodate ions, the polyaldehyde was reduced to the polyalcohol with sodium borohydride and the product, reduced-oxidised Polysaccharide B (89 mg), recovered as previously. A portion of this product (7 mg) was hydrolysed in N-H_2SO_4, converted to the glycitol acetates and the assay by GLC showed the monomeric components, together with their molar proportions, listed in table 27.

Monomer	Molar proportion %
Glycerol	11
Erythritol ?	0.2
Rhamnose	0.5
Arabinose	4.3
Galactose	84

Table 27

3.5.5.3 Mild acid hydrolysis of reduced-oxidised Polysaccharide B.

The remainder of reduced-oxidised Polysaccharide B was dissolved in N-H_2SO_4 and hydrolysed at 20°C for three hours. The recovered product was fractionated to give:

(i) A syrup (12 mg) soluble in methanol-acetone (1:1; 100 ml), which on hydrolysis and GLC assay of the derived glycitol acetates, was shown to be composed of the monomeric components listed in table 28.

Monomer	Molar proportion %
Glycerol	63
Erythritol ?	0.6
Rhamnose	0.4
Arabinose	7
Galactose	29

Table 28

and (ii) an insoluble fraction, Polysaccharide C, (49 mg; 38% yield) having $[\alpha]_D$ +28^{o} (c 2.47) and approximate molecular-weight 1750 (see section 3.6.4. After hydrolysis, GLC assay of the glycitol acetates showed this residue to be composed of the monomers listed in table 29.

Monomer	Molar proportion %
Glycerol	5.2
Rhamnose	0.3
Arabinose	4.5
Galactose	90

Table 29

Methylation of a portion of Polysaccharide C (8 mg), methanolysis and GLC assay showed the methyl ethers together with their molar proportions, listed in table 30.

O-methyl ether	Molar proportion %
2,3,4,6-Tetra-O-methylgalactose	20
3,4-Di-O-methylarabinose?	3.5
2,3,6-Tri-O-methylgalactose	3.5
2,4,6-Tri-O-methylgalactose	61.5
2,3,4-Tri-O-methylgalactose	2.5
2,6-Di-O-methylgalactose	9

Table 30

3.5.6 Preparative-scale periodate oxidation, borohydride reduction and "Smith degradation" of Polysaccharide C - Preparation of Polysaccharide D.

3.5.6.1 Periodate oxidation of Polysaccharide C.

Polysaccharide C (21 mg) was dissolved in a solution of 0.019 M-sodium meta periodate (25 ml). After 73 hours, when 0.29 mol. periodate was consumed per 100 g carbohydrate, the oxidation was terminated.

3.5.6.2 Sodium borohydride reduction of oxidised Polysaccharide C.

The oxidised product was reduced to the polyalcohol and recovered as previously.

3.5.6.3 Mild acid hydrolysis of reduced-oxidised Polysaccharide C.

The reduced-oxidised polysaccharide was dissolved in N-H_2SO_4 and hydrolysed at 20° for 2 1/2 hours. The recovered product was fractionated to give:

(i) a syrup (6 mg), soluble in methanol-acetone (1:1; 20 ml), which was shown by hydrolysis, conversion to the glycitol acetates and GLC assay to be composed of the monomers listed in table 31.

Monomer	Molar proportion %
Glycerol	48
Erythritol ?	5
Arabinose	10
Galactose	37

Table 31

and (ii) an insoluble residue, Polysaccharide D (10.1 mg) having approximate molecular weight 800 (see section 3.6.4). GLC assay of the glycitol acetates derived from an hydrolysate showed the monomeric components listed in table 32.

Monomer	Molar proportion %
Glycerol	45 ?
Erythritol ?	7
Arabinose	1
Galactose	47

Table 32

3.6 The determination of the molecular weights of Acacia podalyriaefolia gum and its degraded products.

3.6.1 The measurement of the diffusion coefficients.

3.6.1.1 Apparatus

A general view of the apparatus is shown in fig. 5. The water bath, a converted Hilger Watts electrophoresis apparatus

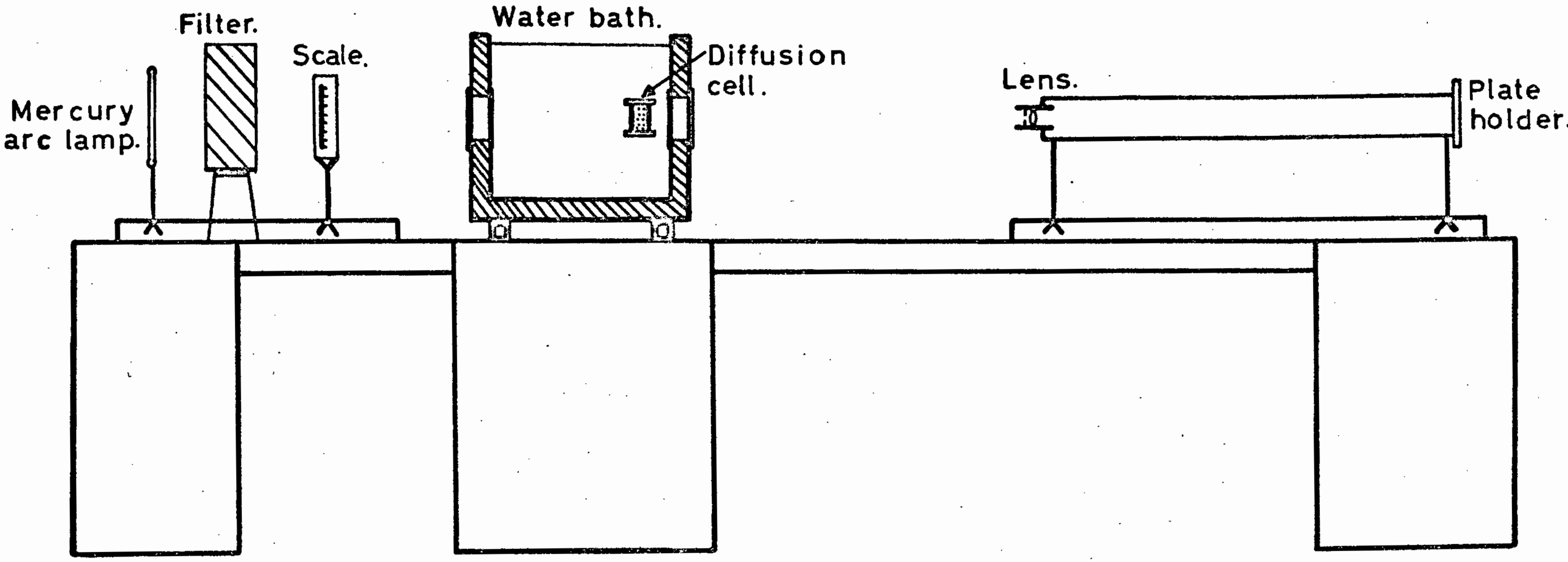


Fig.5. General view of the diffusion apparatus.

fitted with the Lamm scale methods optical arrangement, rests on a central concrete support. Between this block and a concrete block at either end is a rigid iron bar upon which rest two optical benches supporting the other items.

The light source is a low-pressure mercury arc light, mounted vertically behind a Lamm scale.[84] The scale is 10 cm long, has 0.5 mm divisions inscribed onto the flat glass surface, and is mounted vertically. The light is filtered through a nickel sulphate solution, which allows mainly the monochromatic green light ($\approx$ 456mμ) to pass through. The water bath is an insulated vessel, having double plate glass windows at each end.

The diffusion cells are shown in fig. 6. The vertical walls of each cell are of thin glass, and are mounted between two plate glass ends. The dimensions of each cell are 8.5 X 2.5 X 0.3 cm. For ease of diagramatic representation, the thickness of the vertical walls of each cell is not shown in the figure. The diffusion cells are fixed to a brass framework. This framework consists of two platforms connected by four brass rods. The cells rest on the lower platform and are immersed in the waterbath. The upper platform runs freely along two rails so that any one of the cells may be placed in the path of the light. The camera consists of a lens, a long cardboard body and a moveable film-holder.

3.6.1.2 Procedure for the determination of the diffusion coefficients.

The lower ends of the diffusion cells were sealed with plasticine to a height of 2 mm above the glass base, and the bottom was covered with greased cover slips. Each cell was filled with 0.14 N-sodium chloride solution. Each polysaccharide (8-12 mg) was dissolved in 0.14N-sodium chloride solution (0.5 ml) and the solution pipetted cautiously through a fine capillary tube, with a horizontal tip, onto the bottom of a cell, the layer being approximately 1 mm in height.[85]

The cells were sealed with greased cover slips, fixed to the brass framework and immersed in the water bath.

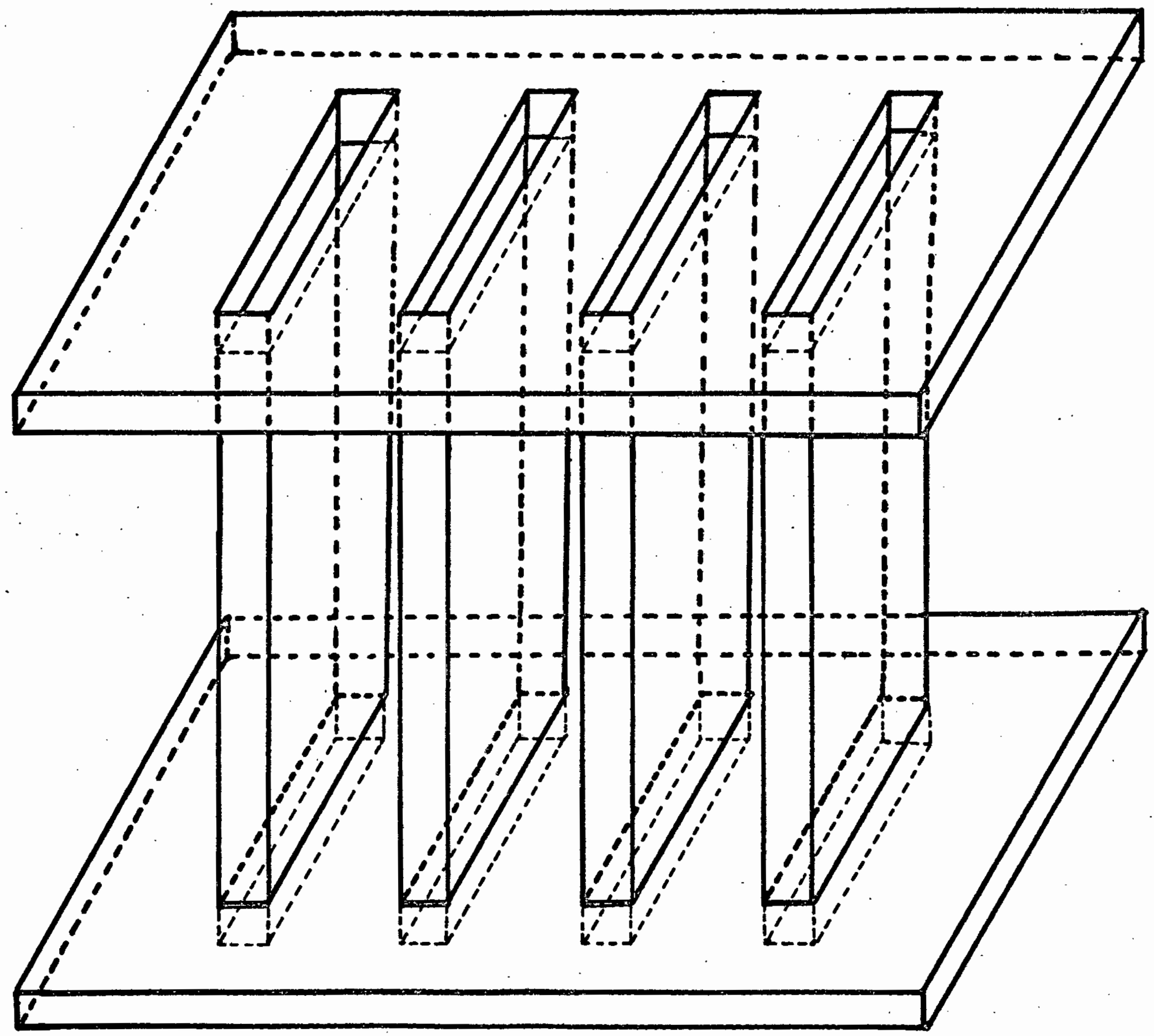


Fig.6. The four diffusion cells.

Photographs were taken at intervals and the diffusion coefficients were calculated as described in section 3.6.1.3.

3.6.1.3 <u>Determination of the diffusion coefficients</u>.

Rays of light passing from the transparent scale through a cell, which contains the diffusing material, are deflected because of the refractive index gradient (which is proportional to the concentration gradient) of the material in the cell. The amount of displacement of the light path was measured by photographing the scale with and without the gradient, under identical conditions. These photographs are the distorted and reference images of the scale respectively. The interval between each division on the distorted photograph was measured by means of a microcomparator to the nearest micron, care being taken to ensure that the axis of the image of the scale was parallel to the movement of the stage of the microcomparator, since a small error leads to a tilt in the base line of the resulting graph.

A standard scale was constructed from the reference photograph. The distance between 50 lines was measured, and when divided by the number of divisions gave an average interval between the lines. The scale was constructed by repeatedly adding this average interval on a calculator.

The reference scale readings were then subtracted from the experimental or distorted scale readings, and each difference (i.e. scale line displacement) was plotted as ordinate against the distorted scale as the abscissa. Figs. 7, 8 and 9 show the curves obtained for <u>A. podalyriaefolia</u> gum, degraded gum B [see section 3.3.1.3] and Polysaccharide A [see section 3.5.2.3(iii)].

The diffusion coefficients, D, were calculated using the inflection point technique from the equation[86],

$$D = \frac{\sigma^2}{2\,t}\left(\frac{\ell - \beta}{\ell \times G}\right)^2$$

where σ is the standard deviation of the frequency curve'

t is the time in seconds

and $\left(\frac{\ell - \beta}{\ell \times G}\right)^2$ is the apparatus constant [87] involving ℓ, the optical

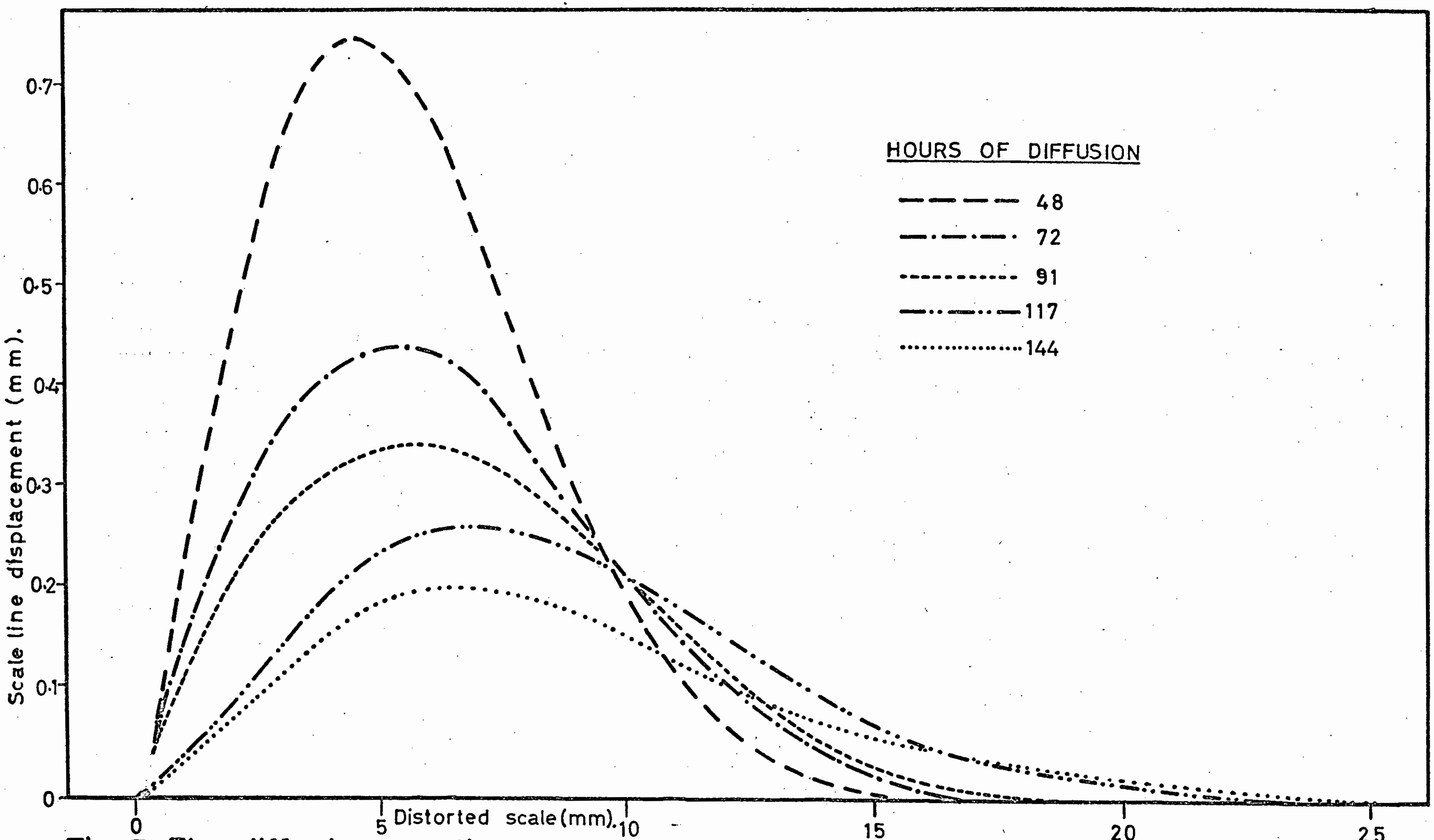


Fig. 7. The diffusion gradient curves of A. podalyriaefolia gum at 20°.

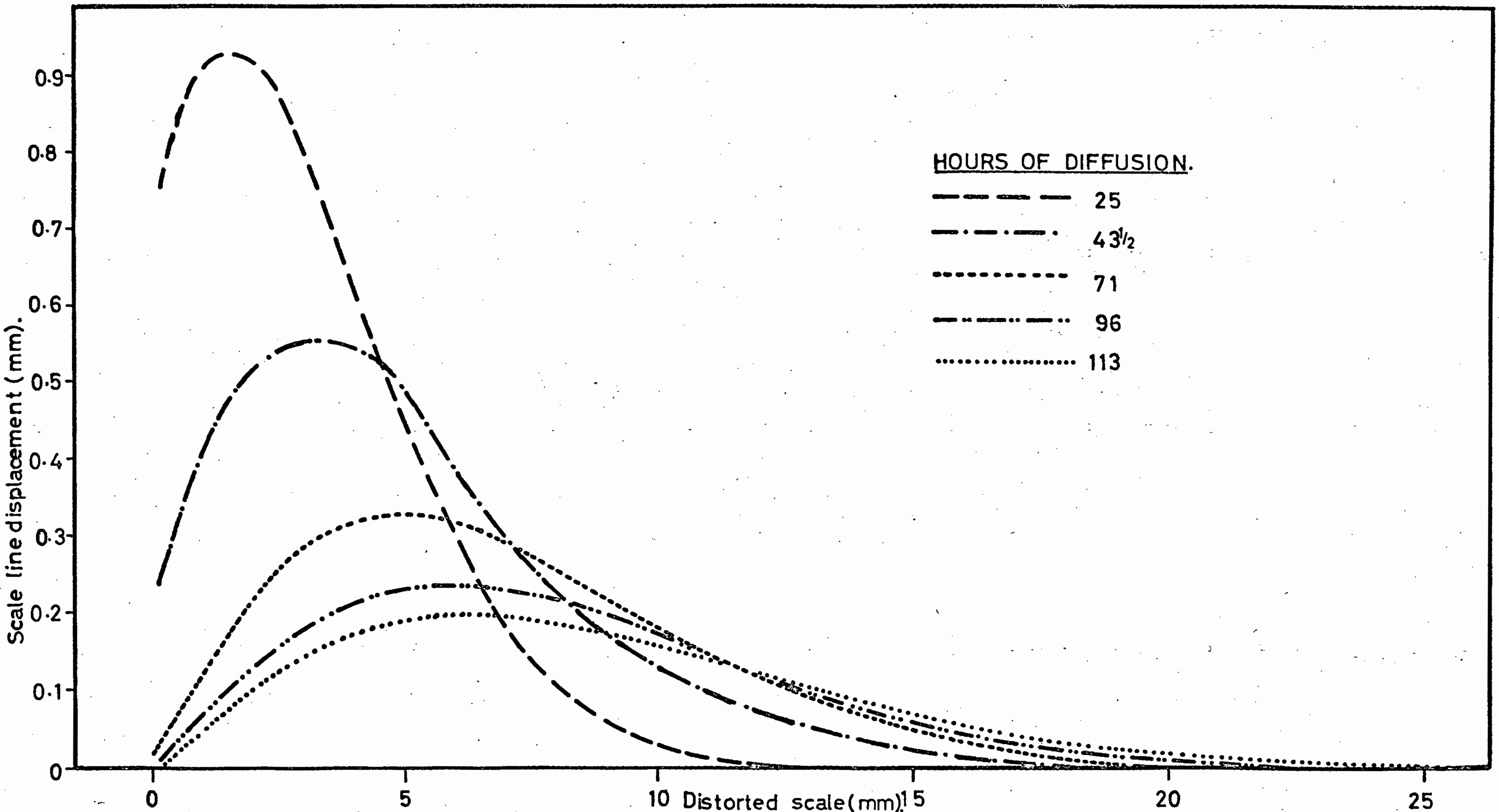


Fig. 8. The diffusion gradient curves of degraded gum B at 20°.

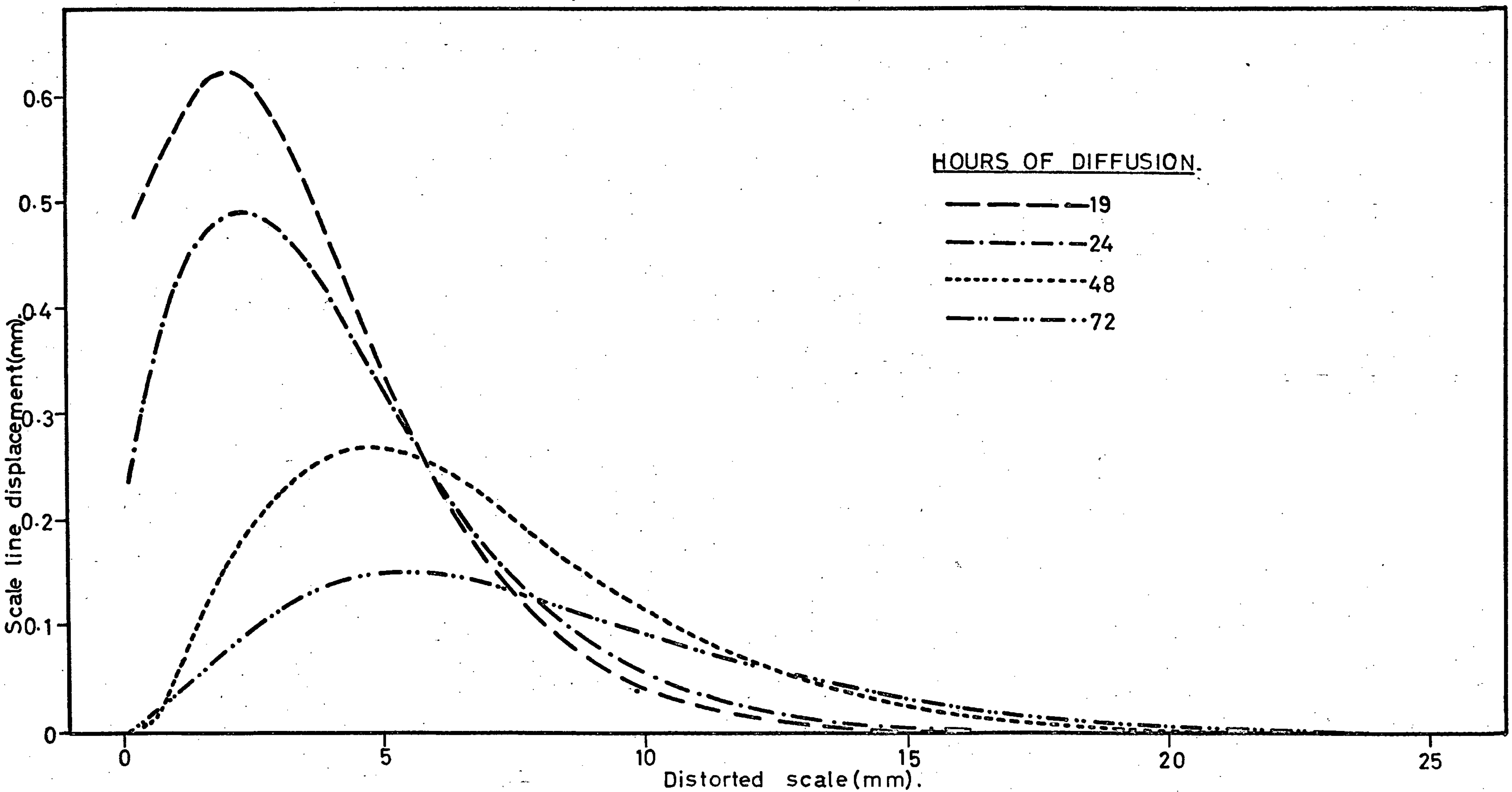


Fig.9. The diffusion gradient curves Polysaccharide A at 20°.

distance from the scale to the centre of the lens,

β, the optical distance from the scale to the centre of the diffusion cell

and G the photographic enlargement.

The value of the apparatus constant $\left(\frac{\ell - \beta}{\ell \times G}\right)^2$ was found by previous workers[88] to be 1.231.

The value of σ for each diffusion gradient curve was obtained as follows: the position of the inflection point, B, was ascertained by measuring the maximum ordinate (Ho) of the curve (see fig.10) and dividing by e (e is the basis of the Napierian logarithms). At this distance (Ho/e) along the maximum ordinate (Ho) from the base line, a line A - B, parallel to the base line, was drawn. The standard deviation is the distance from the maximum ordinate, A, to the point of inflection, B.

The mean values for the diffusion coefficients for A. podalyriaefolia gum and its degraded products are presented in table 33.

Polysaccharide	Diffusion coefficient x $10^7 cm^2 sec^{-1}$ at 20°.
A. podalyriaefolia gum	7.81
Polysaccharide A	15.00
Polysaccharide B	19.15
Polysaccharide C	20.30
Polysaccharide D	31.60
Degraded gum B	10.94

Table 33

If the material undergoing diffusion remains unaltered during the course of the experiment the diffusion coefficient, D, should remain constant. Since $D = \frac{\sigma^2}{2t} \times k$ (where k is a constant), the graph of σ^2 against 2t should show a straight line. That this is so for A. podalyriaefolia gum and its degraded products is

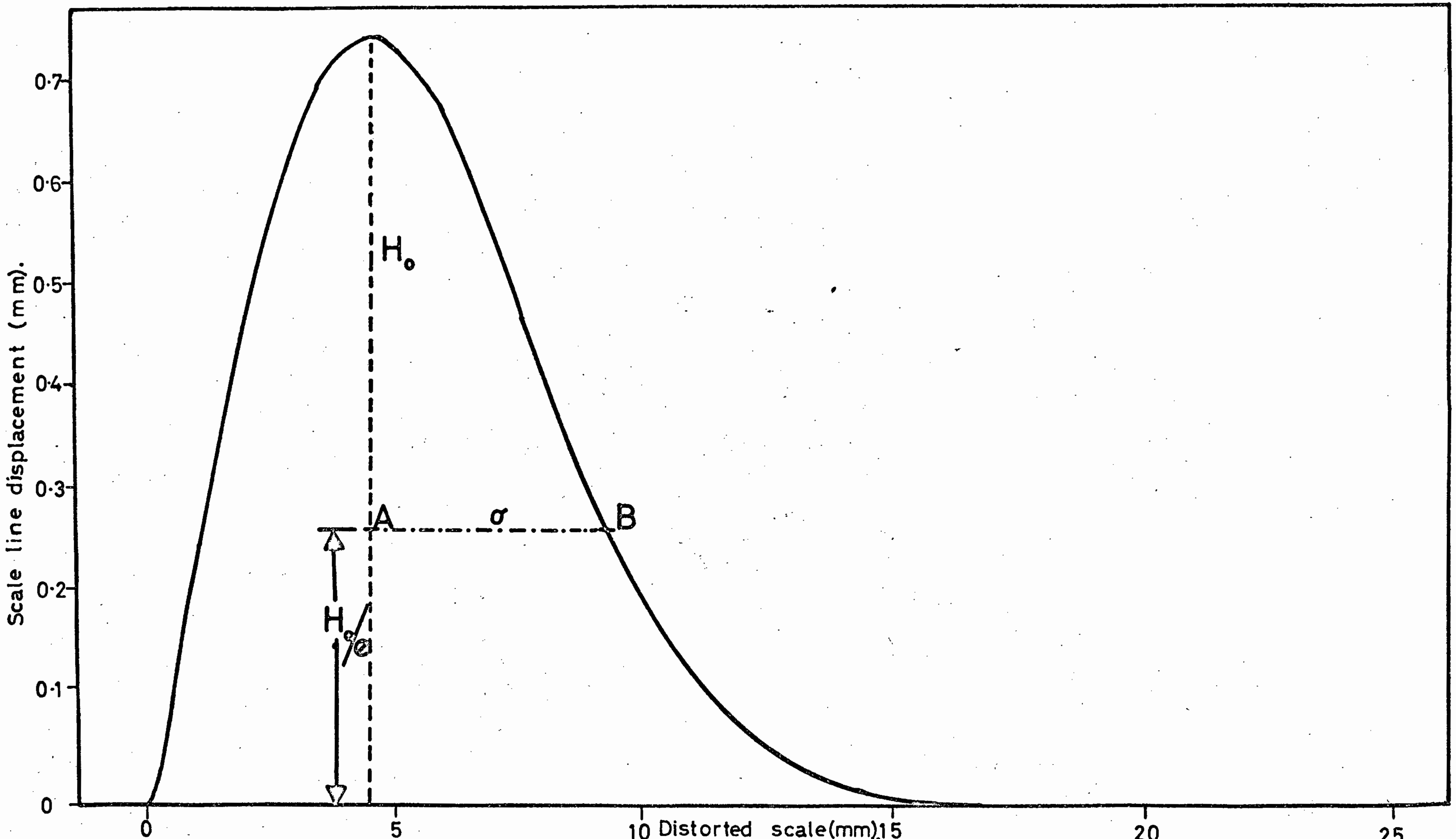


Fig.10. The determination of σ, the standard deviation, from the diffusion gradient curves.

shown in fig. 11 and it may be concluded that these polysaccharides remain stable during the experiment.

3.6.2 The measurement of the sedimentation coefficients at 20° (S_{20w}) for A. podalyriaefolia gum and its degraded products.

The polysaccharide (6 mg) was dissolved in 0.14 N-sodium chloride solution (0.5 ml) and centrifuged in a Spinco Model E ultracentrifuge at 56,100 r.p.m. and at 20°.

A. podalyriaefolia gum was centrifuged in a normal cell, while degraded gum B and Polysaccharide A were centrifuged in a synthetic boundary cell. The concentration gradient was photographed at intervals. These photographs are shown in figs. 12, 13 and 14. The distance of the peak height to the index line was measured on a Projetorscope type microcomparator. The distance of the peak height to the centre of rotation, X, was obtained by dividing the above-mentioned reading by 21, the photographic enlargement factor, and subtracting the result from 7.300, the distance of the index point from the centre of rotation at 56,100 r.p.m. The linear relationship between log X and time for A. podalyriaefolia gum, degraded gum B and Polysaccharide A is shown in fig.15. The sedimentation coefficient at 20° and in water, S_{20w}, was calculated from Svedberg's equation [89]

$$S_{20w} = \frac{2.303 \quad \Delta \quad \log X}{t \quad (2 \quad \pi \quad \frac{r.p.m.}{60})^2}$$

where X is the change in distance of the peak height from the centre of rotation,

t is the time in seconds,

π = 3.142

and r.p.m. = 56,100.

The S_{20w} values obtained for the samples centrifuged are presented in table 34.*

[* Although it is known that the sedimentation coefficient is dependent on concentration to some extent (H.K. Schachman, "Ultracentrifugation in Biochemistry", Academic Press, New York and London, 90,(1959).), the S_{20w} values were not determined at infinite dilution. The relationship of sedimentation coefficient to concentration is sufficiently linear over a wide concentration range (A. Polson, private communication).]

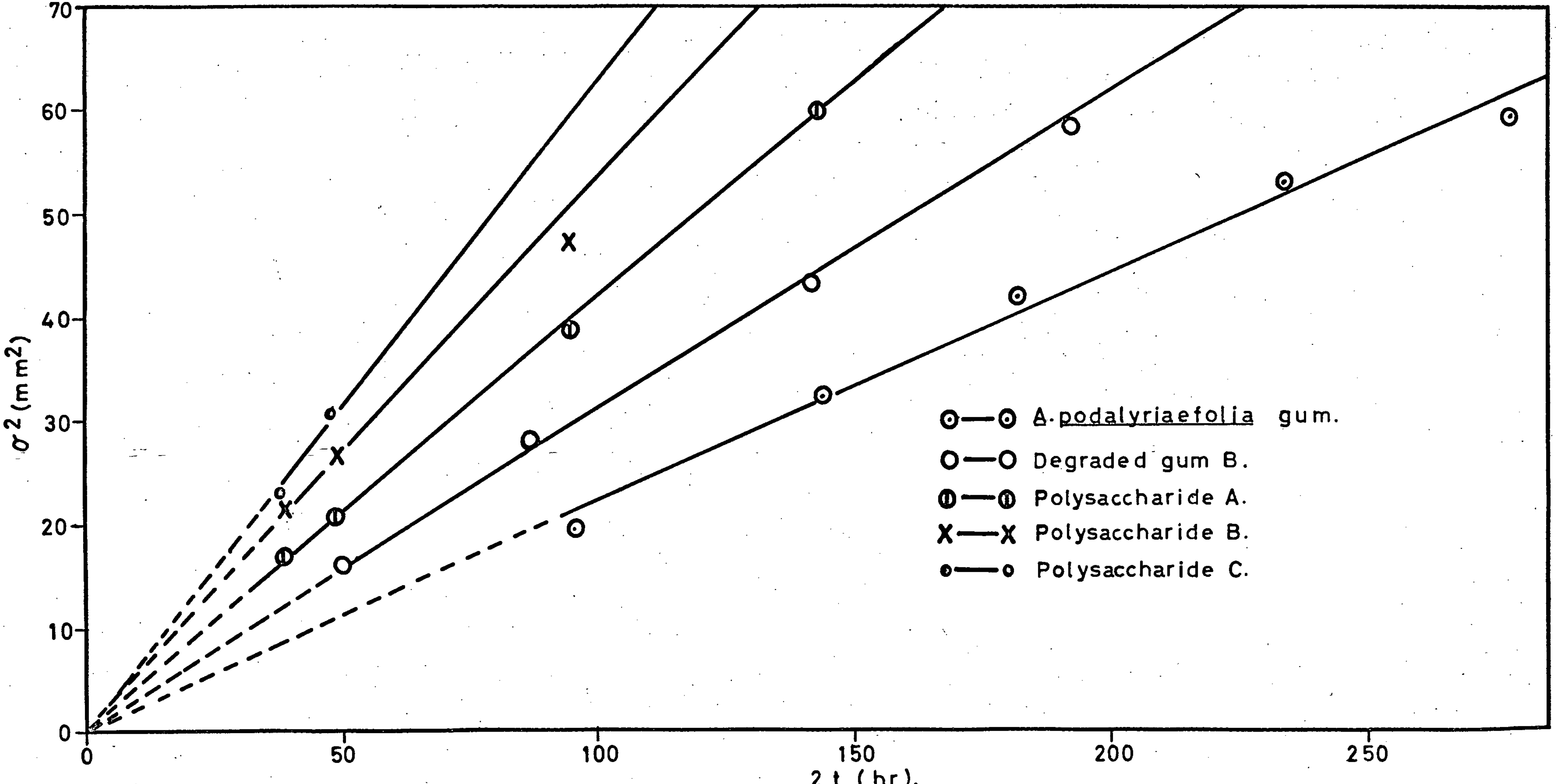


Fig.11. Curves showing the relationship of the square of the standard deviation, σ^2, to time for A. podalyriaefolia gum and some of its degraded products.

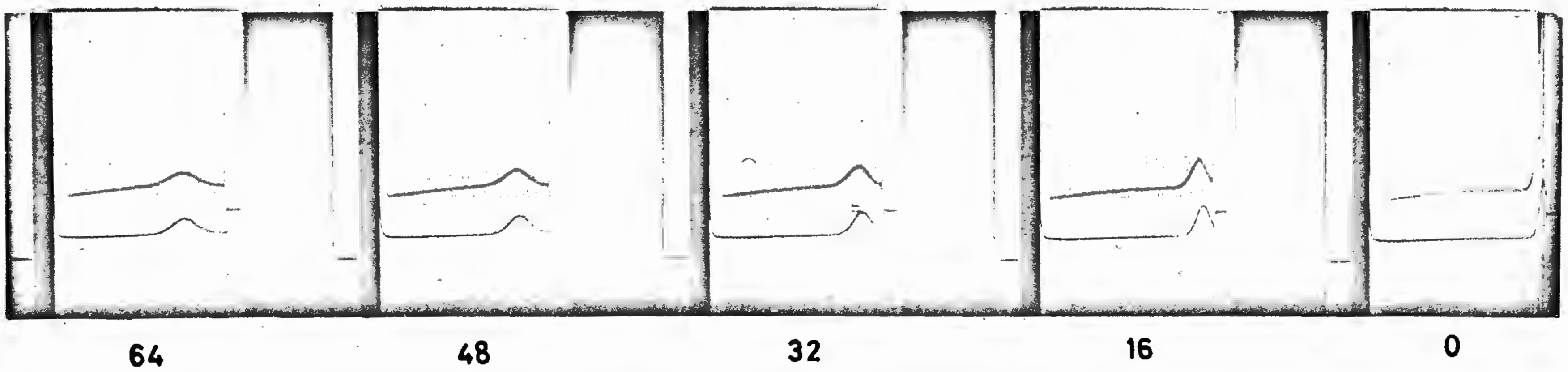


Fig. 12. The sedimentation gradient curves of _A. elata_ (upper curve) and _A. podalyriaefolia_ gums.

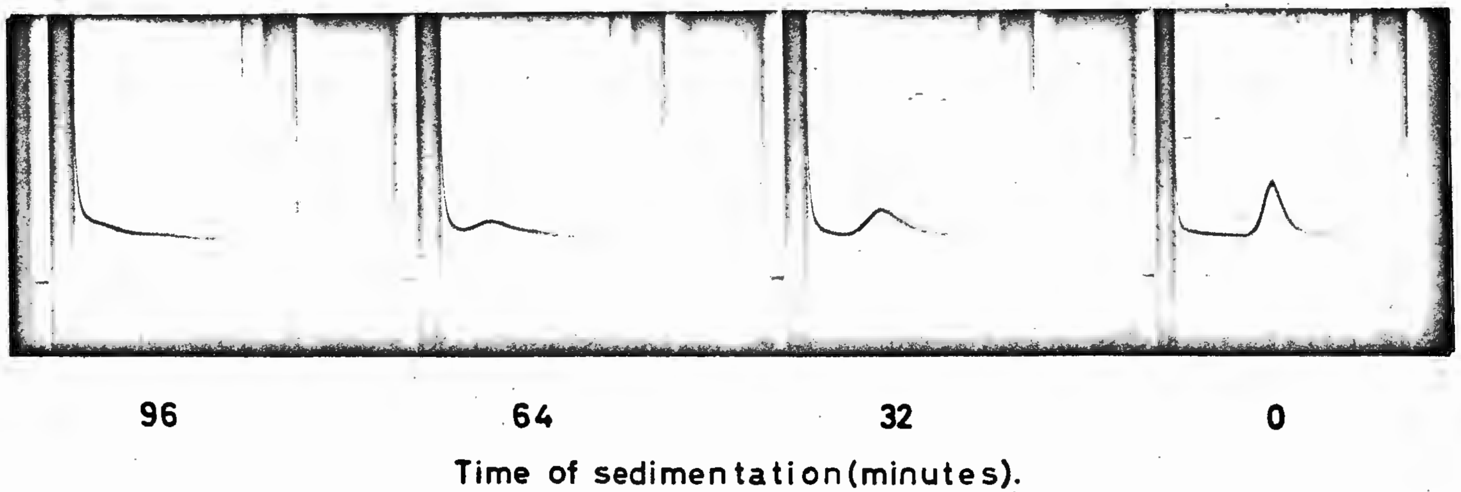


Fig.13. The sedimentation gradient curves of degraded gum B.

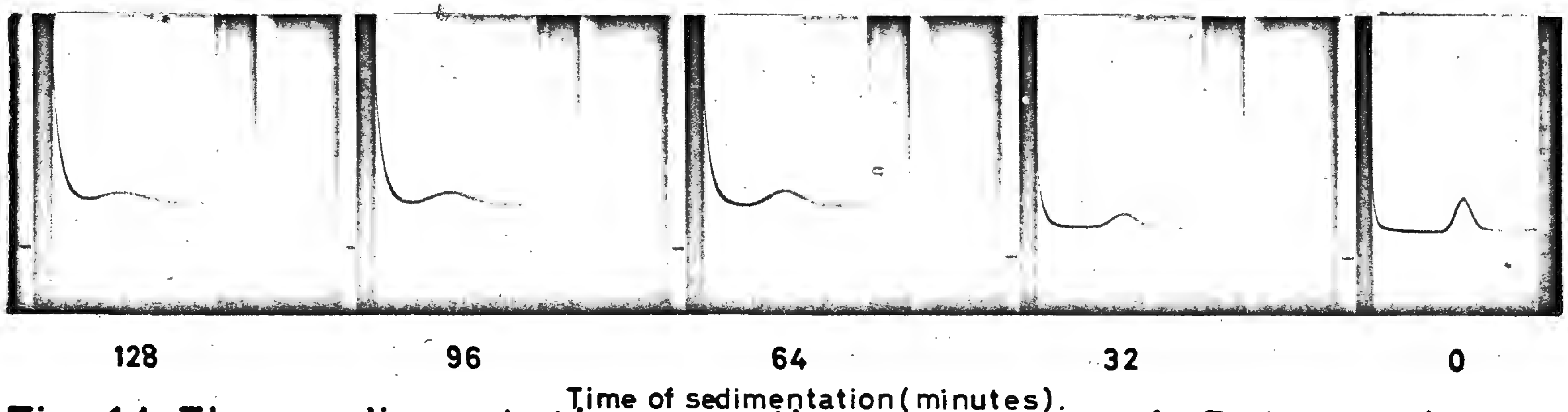


Fig. 14. The sedimentation gradient curves of Polysaccharide A.

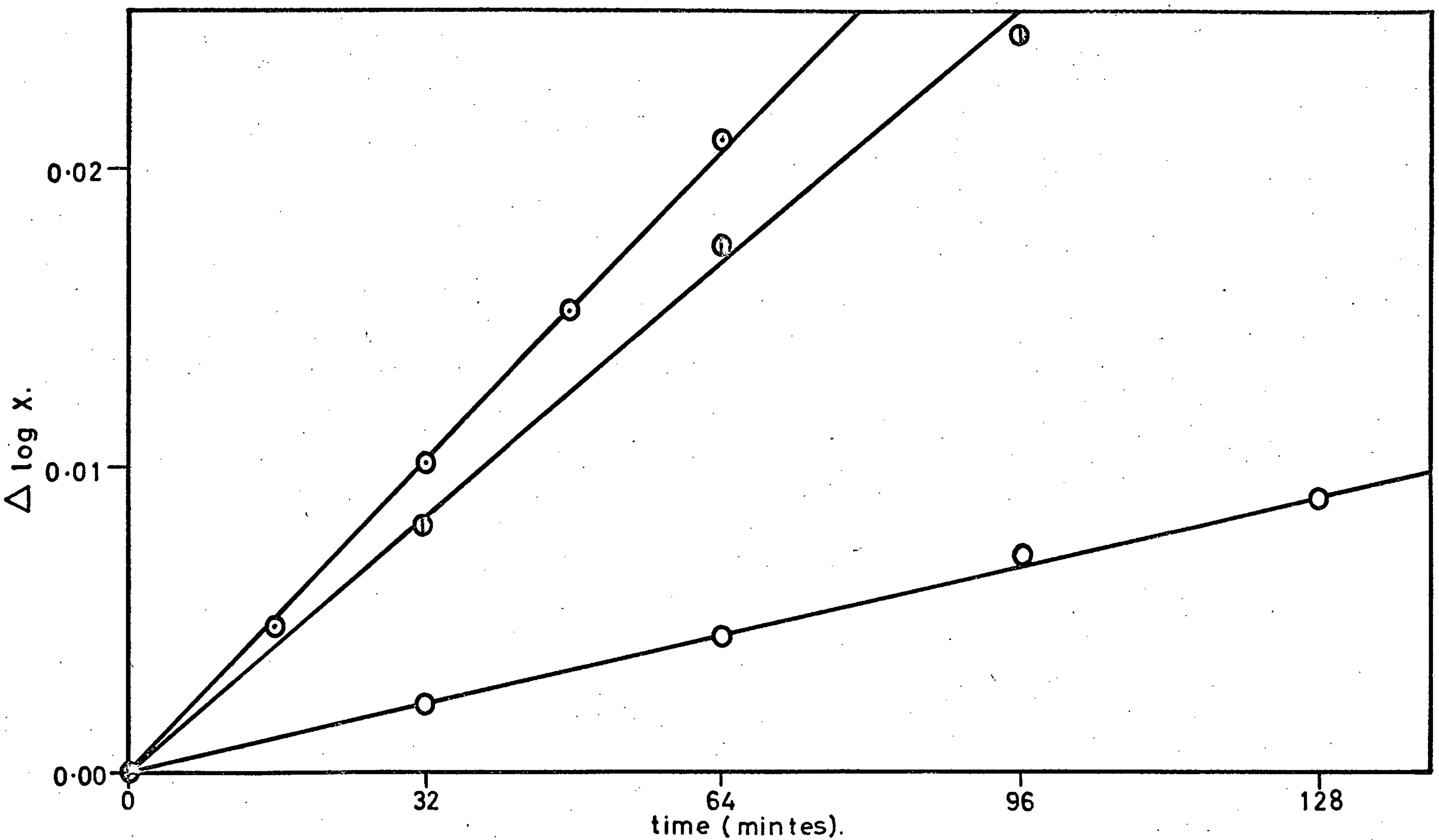


Fig. 15. Curves showing the relationship of logX to time for
A.podalyriaefolia gum ⊙—⊙, Polysaccharide A o—o and degraded gum B ⦶—⦶.

Polysaccharide	Sedimentation coefficient $X10^{-13}$ cm sec^{-1} $dyne^{-1}$ g^{-1}	Partial specific volume $cm^3 g^{-1}$
A. podalyriaefolia gum	3.684	0.657
Degraded gum B	2.873	0.653
Polysaccharide A	0.646	0.648

Table 34.

3.6.3 Measurement of the partial specific volume of A. podalyriae folia gum, degraded gum B and Polysaccharide A.

The partial specific volume is the reciprocal density. The densities of those polysaccharides centrifuged in section 3.6.2 were measured. The volume of a specific gravity bottle was determined by weighing the bottle empty, then with xylene, and dividing the mass of xylene by its density. The polysaccharide was weighed in the bottle; the bottle was then filled with xylene and re-weighed. The volume of xylene added was calculated and the difference in volume was a measure of the volume of the polysaccharide. The partial specific volumes are listed together with the sedimentation coefficients in table 34.

3.6.4 Calculation of the weight-average molecular weights.

The weight-average molecular weights, $\bar{M}_w$ of A. podalyriaefolia gum, degraded gum B and Polysaccharide A were calculated from the equation

$$M = \frac{RT\,S_{20w}}{D(1 - Ve)}$$

where $R = 8.314 \times 10^7$ ergs degree $^{-1}$ mole $^{-1}$,

T = absolute temperature = 293° K,

S_{20w} = sedimentation coefficient at 20° in water $X10^{-13}$ cm sec^{-1} $dyne^{-1} g^{-1}$,

D = diffusion coefficient cm^2 sec^{-1},

V = partial specific volume cm^3 g^{-1}

and e = density of the solvent = 1.00 g cm^{-3}.

The experimentally determined results are presented in table 35.

Polysaccharide	Molecular weight
A. podalyriaefolia gum	33,500
Degraded gum B	18,650
Polysaccharide A	2,985

Table 35.

Polysaccharides B, C and D were too small for the accurate measurement of their sedimentation coefficients and a value for the intrinsic viscosity could not be determined due to the small quantities available. Estimates of their molecular weights were obtained from the curve showing the relationship between the diffusion coefficients and molecular weights of some published results [90] and results obtained by the author. These estimated molecular weights, presented in table 36, were derived from fig.16.

Polysaccharide	Estimated molecular weight
Polysaccharide B	1940
Polysaccharide C	1750
Polysaccharide D	800

Table 36.

The relationship between the sedimentation and diffusion coefficients of the gums of *A. podalyriaefolia*, *A. elata* and the polysaccharides obtained after a Smith degradation of each gum is presented in fig. 17. That the values obtained for degraded gum B do not fall on this curve has suggested that this degraded product is polydisperse.

3.7 The determination of the weight response factors, K, of some glycitol acetates and the GLC analysis of polysaccharides as their derived glycitol acetates.

3.7.1 Source of standards used in the calibration experiments.

(a) tri-*O*-acetyl-glycerol, analar grade, was redistilled and

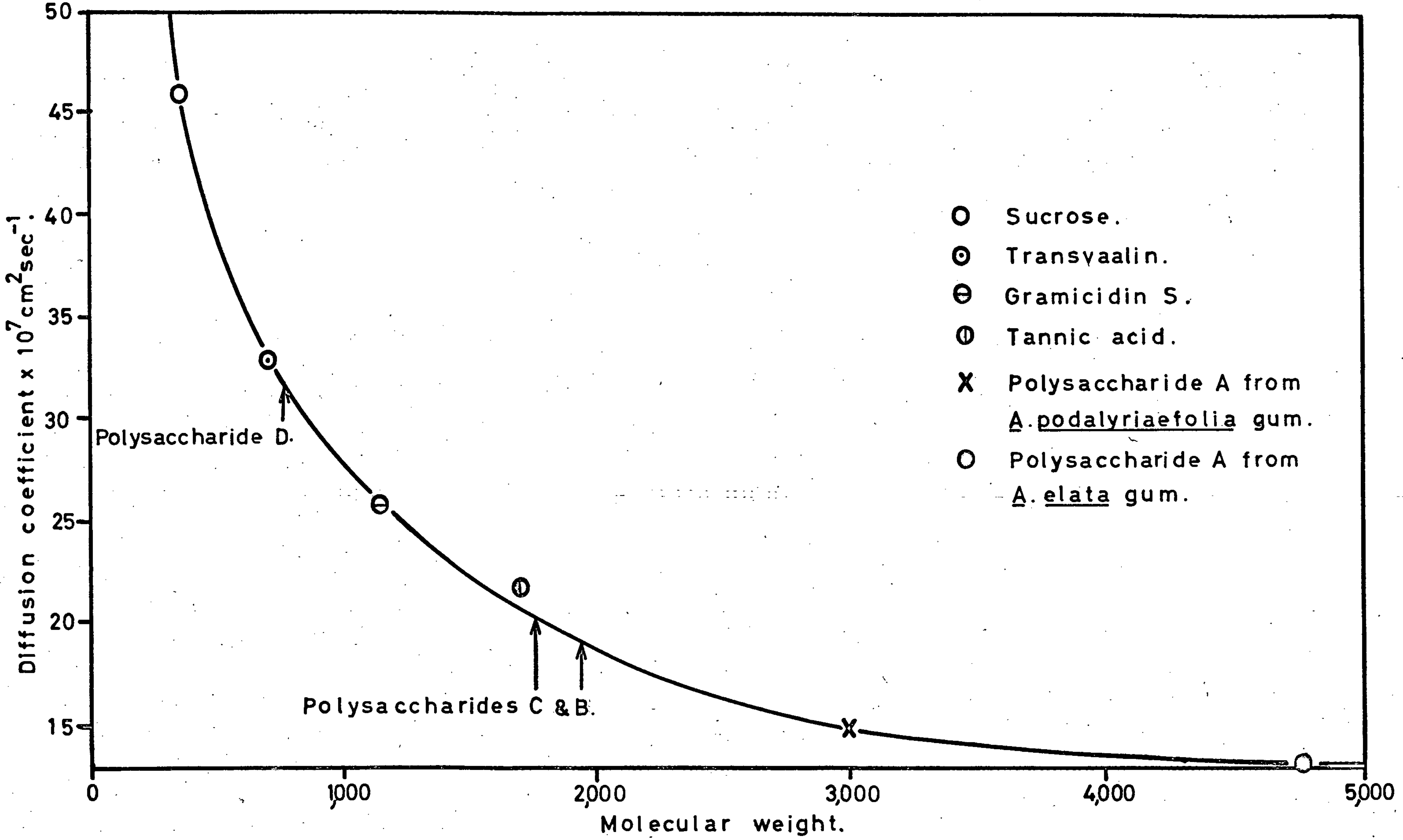


Fig. 16. Curve showing the relationship of molecular weight to the diffusion coefficient.

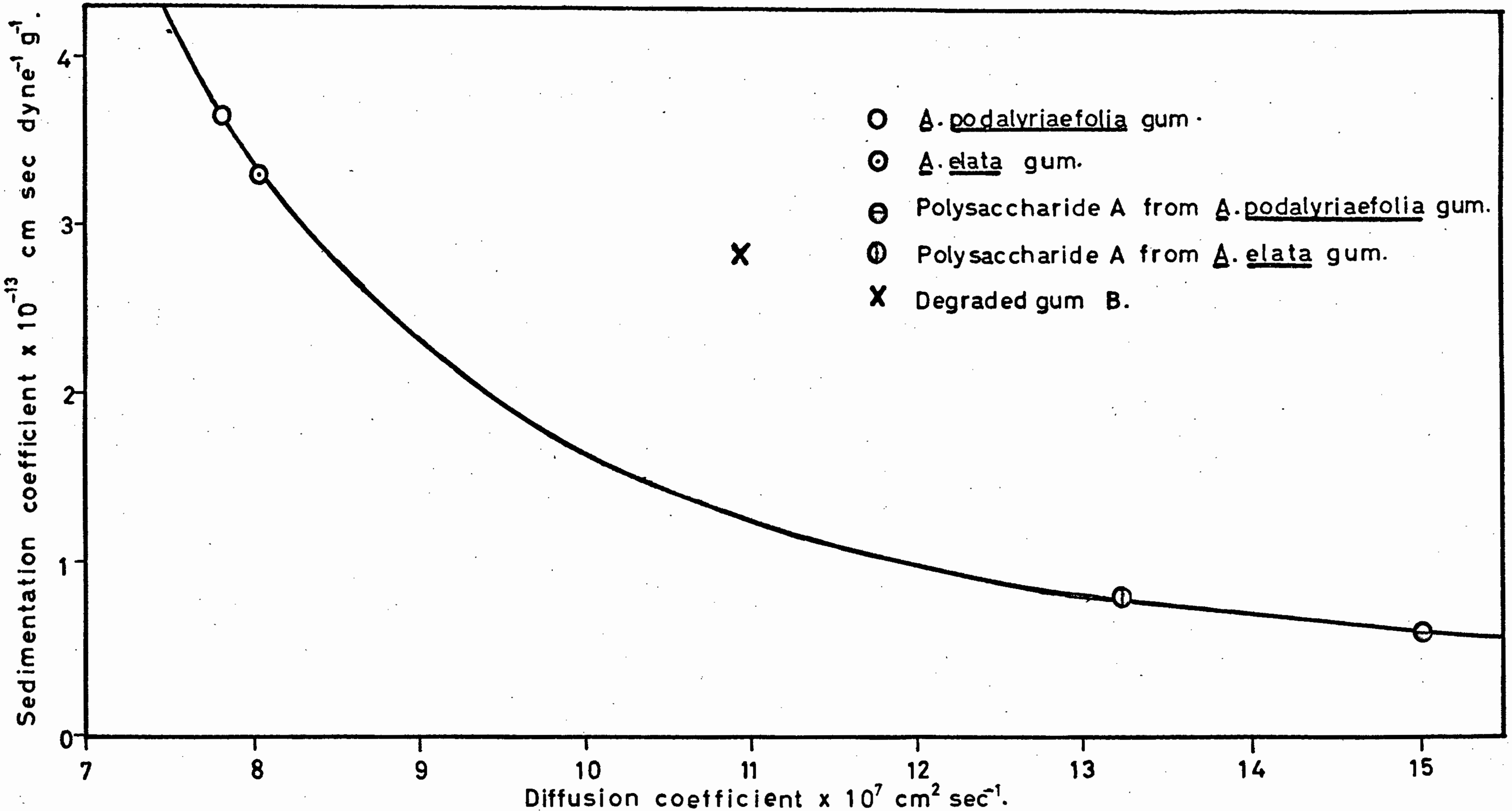


Fig. 17. Curve showing the relationship between the diffusion and sedimentation coefficients of some *Acacia* polysaccharides.

the fraction having b.p. 258-260° was collected.

(b) tetra-O-acetylerythritol was prepared from erythritol and had m.p. 83-84°.

(c) penta-O-acetyl-L-rhamnitol, a syrup, was prepared from L-rhamnose.

(d) penta-O-acetyl-L-arabinitol, prepared from L-arabinose, had m.p. 75-77°.

(e) penta-O-acetyl-D-xylitol, prepared from D-xylose, had m.p. 61-62°.

(f) hexa-O-acetyl-D-mannitol, m.p. 123-124°, was obtained on repeated recrystallisation of a commercial preparation, and

(g) hexa-O-acetyl-D-galactitol, prepared from D-galactose, had m.p. 169-170°.

3.7.2 Preparation of standard solutions for the determination of the weight response factors (K values) and for the determination of retention times (T values).

Between 4 - 15 mg of each of the above glycitol acetates was weighed accurately and the mixture was dissolved in chloroform. Approximately 2 - 5 micro-litres of the solution was injected into the gas chromatograph and separated on ECNSS-M-coated column under the conditions listed in section 3.1.

The weight response factor K was calculated from the equation

$$K = \frac{\text{peak area of 1g of glycitol acetate}}{\text{peak area of 1 g of mannitol acetate}},$$

and the retention times were measured relative to hexa-O-acetyl mannitol.

Three solutions of the glycitol acetates were prepared and a total of eighteen separations were performed. The retention times, the mean K values and the standard deviation of the K values are presented in table 36.

Acetate of	Retention time T	Mean weight response factor K.	Standard deviation
glycerol	0.029	1.19	0.093
erythritol	0.111	1.11	0.086
L-rhamnitol	0.222	1.06	0.075
L-arabinitol	0.380	0.97	0.049
D-xylitol	0.546	0.98	0.060
D-mannitol	1.00	1.00	-
D-galactitol	1.159	1.19	0.077

Table 36.

3.7.3 Preparation of the glycitol acetates of the neutral sugars derived from A. podalyriaefolia gum and its degraded products.

Portions of *A. podalyriaefolia* gum, degraded gums A and B and all fractions obtained during the sequential Smith degradations of the whole gum were hydrolysed in N-H_2SO_4, neutralised ($BaCO_3$) and the monomeric sugars were reduced to the glycitols with sodium borohydride. Excess borohydride was decomposed and the cations were removed with Amberlite IR-120(H^+) resin. The borate was removed on evaporation of methanol from the solution, and the product was acetylated in acetic anhydride containing anhydrous sodium acetate. Excess acetic anhydride was removed at reduced pressure, and 3 - 5 micro-litres of a chloroform solution of the product was separated by GLC. Each glycitol acetate mixture was separated at least three times. The results are presented in the relavent sections of the Experimental.

PART II

4 INTRODUCTION

4.1 The separation of monosaccharides and disaccharides by gas-liquid chromatography.

The technique of gas-liquid chromatography (GLC) was first described by James and Martin[91] in 1952, and soon this method of analysis was applied to the analysis of a wide range of organic chemicals.

The first recorded GLC analysis of monosaccharides was published in 1958 by Bishop and co-workers.[92] The successful separation of methyl 2,3,4-tri-O-methyl-β-D-xyloside, methyl 2,3,4-tri-O-methyl-β-L-arabinoside, methyl 2,3,4,6-tetra-O-methyl α-D-mannoside, methyl 2,3,4,6-tetra-O-methyl α-D-glucoside and methyl 2,3,4,6-tetra-O-methyl-α-D-galactoside was achieved on a column of Apiezon"L" grease at 170°. Helium was used as carrier gas, and the methyl glycosides were detected using a katharometer. The methyl glycosides could be recovered quantitatively and unchanged from the effluent gas stream, showing that no anomerization, hydrolysis or degradation of these derivatives had occurred in passing through the gas chromatograph.

The ease of separation of both fully and partially methylated methylglycosides has been shown by a number of workers; these include Bishop and his collaborators [93,94,95,96], Kircher[97], Barter and Klein[67], Baxter and Perlin [98], Walker and co-workers [99,100,101], Aspinall [102] and more recently by Stephen and co-workers.[75]

The separation of fully acetylated methyl glycosides has been reported by Gunner and co-workers [103], Kircher [104] and van den Heuvel and Horning.[105] Glycose acetates have been separated on Apiezon greases by both Ferrier [106] and Gunner et al.[103,107] Bishop and Cooper [93] have separated the four mono-O-methyl-glucopyranoses by reduction to the glucitols and GLC analysis of the penta-O-acetyl derivatives. Hause et al.[108] have separated mannitol and sorbitol hexa-O-acetyl derivatives and Gunner, Jones and Perry[103], have separated the fully acetylated glycitols. The O-acetyl derivatives of a number of glycitols have been separated by Sawardeker and co-workers [66], and this method has been extended to the separation of both fully and partially methylated glycitol acetates.[68] Williams and Jones have separated the O-acetyl derivatives of both the fully and partially methylated methyl arabinosides and of the fully and partially methylated arabinitols.[109] Jones[110] has separated all of the methyl ethers of D-glucopyranose, both as their acetylated glucopyranosides and as their acetylated alditols.

A number of other derivatives have been separated by GLC, these include acetals, ketals O-ethyl, benzylidene, O-vinyl and trimethyl sily ether derivatives of monosaccharides.[111,112,113,114]

Perry[115] has developed a method for the separation and characterisation of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose.

Anderson et al.[116] have successfully separated methyl 3,5-di-O-acetyl-2-deoxy-2-(ethylthio)-β-L-arabinofuranoside from methyl 2,5-di-O-acetyl-3-deoxy-3-(ethylthio)-β-D-xylofuranoside.

The isopropylidine derivatives of L-arabinitol and ribitol have been separated by Foster et al.[117]

Methylated mono-, di- and trisaccharides have been separated by Gee and Walker[99] while the separation of disaccharide acetates on lightly loaded columns containing fluoroalkyl silicone [105] and on mixed liquid phases on glass beads as solid support[118] have been reported.

4.2 Gas-liquid chromatographic analysis of oligosaccharides and polysaccharides.

The qualitative and quantitative analysis of organic compounds is a problem which besets all aspects of organic chemistry, whether the problems involve the isolation and structural analysis of natural products, the synthesis of compounds or the study of organic reactions.

In the field of polysaccharide chemistry, considerable information is obtained when the polysaccharide is converted to a derivative (e.g. methyl ether), the glycosidic bonds are cleaved and the monosaccharide mixture is analysed and each monosaccharide present is characterised. An analysis of the results show the ring sizes of the sugar constituents, the position of the linkages and which sugars are present as end-groups and where branching occurs in the molecule. This technique, when coupled with periodate oxidation of the polysaccharide followed by reduction and controlled acid hydrolysis, is valuable in the structural elucidation of the polysaccharide.

The classical methods for the separation of the fully and partially methylated monosaccharides are lengthy and tedious and require macro-amounts of the methylated polysaccharide.

Gas-liquid chromatography offered a rapid method of analysis, and the advantages were soon realised and applied to structural analyses in carbohydrate chemistry.

Bishop and co-workers [119] have carried out extensive investigations of polysaccharides using GLC analysis. An arabinogalactan from maple-sap[96] was hydrolysed and the ratio of sugar residues was determined after separation of their fully methylated glycosides. The polysaccharide was then methylated and subjected to methanolysis. The resulting fully and partially methylated glycosides were studied qualitatively and quantitatively to determine the amount and nature of end-groups and branch-points in the original polysaccharide. Although, in this case, the ratio of sugar residues from the hydrolysate of the unmethylated and methylated polysaccharide did not agree exactly, the method possessed so many advantages over conventional methods, that Bishop extended the studies to include a glucomannan from Jack pine[94] and a mannan from _Candida albicans_.[95] The quantitative analysis of the mannan, however, showed that the amount of non-reducing end-groups was slightly less than that required to account for the number of branch-points in the polysaccharide.

Kircher[97] has used this method to analyse the methyl glycosides from Guar gum and a partially methylated dextran NRRL - B - 512. The methyl glycosides obtained from the dextran, methyl 2,3,4,6-tetra-O-methyl-α- and β-D-glucopyranosides, methyl 2,3,4-tri-O-methyl-α-and β-D-glucopyranosides and 2,4-di-O-methyl-α-and β-D-glucopyranosides, were identified and quantitatively evaluated. These components were collected from the effluent gas stream and characterised.

Further application of GLC for the identification of methyl glycosides obtained from methylated oligosaccharides and polysaccharides by Bishop et al.[120,121,122], Neely, Nott and Roberts[123], Aspinall and co-workers [102, 124, 125, 126, 127,128,129] and Stephen and co-workers[7,75] has clearly shown the usefulness of this method of analysis.

GLC has been applied to the analysis of pyrolitic products[130] and in the analysis of the hydrolysis products of periodate oxidised and reduced polysaccharides.[94,131]

A recent improvement has been the separation of both fully and partially methylated glycitols, obtained on reduction of the hydrolysates of methylated polysaccharides, as the fully acetylated derivatives.[68,110,132] This method has the advantages that each reducing sugar gives rise to only one glycitol and is thus characterised by a single peak on GLC, thus simplifying the chromatogram, and the full range of glycitols (di-O-acetyl-tetra-O-methylhexitols through to hexa-O-acetylhexitol) can be analysed in a single separation.

5. METHODS AND RESULTS

5.1 G.L.C. conditions.

The ECNSS-M (Applied Science Laboratories, State College, Pa.) was coated on Chromosorb Q (100 - 120 mesh) and packed in

12 foot, 1/8 inch O.D. copper columns. Analyses were performed on a Beckman GC-4 instrument fitted with dual columns and dual flame ionization detectors, and operated at 175°, using helium as carrier gas.

5.2 Qualitative analysis.

A number of methylated and partially methylated sugars were available by synthesis or by hydrolysis of methylated *Acacia* and related gums. Where possible sugars were converted to the glycitols and these were purified before acetylation. In some cases, where limited amounts of the sugars were available, the sugar was weighed together with 2,3,4,6-tetra-*O*-methyl-D-galactitol, reduced with sodium borohydride, and the recovered products acetylated directly. See experimental, section 7, for the physical constants and sources of the sugars and glycitols. In all cases, the methylated glycitols were acetylated together with 2,3,4,6-tetra-*O*-methyl-D-galactitol in acetic anhydride - anhydrous sodium acetate mixtures at 100° for 1 hour. The acetic anhydride was removed at reduced pressure and acetone solutions were analysed.

The retention times (*T* values) of the various methylated glycitol acetates, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol (*T* = 1.00, time of elution *ca.* 4 minutes), are shown in table 37.

T values were found to be reproducible to within 2% under these conditions, the percentage error increasing as the column aged. Columns were replaced when the time of elution of the standard decreased to three minutes.

5.3 Quantitative analysis.

The relative molar response (R values) for each glycitol acetate was calculated from the equation

$$R = \frac{\text{peak area of 1 mole of glycitol acetate}}{\text{peak area of 1 mole of internal standard.}}$$

An accurately weighed amount of the glycitol together with a similar weight of 2,3,4,6-tetra-*O*-methyl-D-galactitol

Acetylated Methylated Alditol	Retention time	Molar response
1,4-Di-O-acetyl-2,3,5-tri-O-methyl-L-arabinitol	0.42	0.90
1,2,4-Tri-O-acetyl-3,5-di-O-methyl-L-arabinitol	0.79	0.82
1,2,5-Tri-O-acetyl-3,4-di-O-methyl-L-arabinitol	1.13	0.98
1,3,4-Tri-O-acetyl-2,5-di-O-methyl-L-arabinitol	0.87	0.83
1,4,5-Tri-O-acetyl-2,3-di-O-methyl-L-arabinitol	1.07	-
1,2,3,4,5-Penta-O-acetyl-L-arabinitol	2.69	1.06
1,5-Di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol	0.39	0.93
1,2,4,5-Tetra-O-acetyl-3-O-methyl-L-rhamnitol	1.49	1.04
1,2,3,4,5-Penta-O-acetyl-L-rhamnitol	1.63	1.16
1,5-Di-O-acetyl-2,3,4,6-tetra-O-methyl-D-galactitol	1.00	1.00
1,3,5-Tri-O-acetyl-2,4,6-tri-O-methyl-D-galactitol	1.77	0.95
1,4,5-Tri-O-acetyl-2,3,6-tri-O-methyl-D-galactitol	1.86	0.98
1,5,6-Tri-O-acetyl-2,3,4-tri-O-methyl-D-galactitol	2.62	1.16
1,2,3,5-Tetra-O-acetyl-4,6-di-O-methyl-D-galactitol	2.80	1.02
1,3,4,5-Tetra-O-acetyl-2,6-di-O-methyl-D galactitol	2.76	0.83
1,3,5,6-Tetra-O-acetyl-2,4-di-O-methyl-D-galactitol	4.81	1.06
1,4,5,6-Tetra-O-acetyl-2,3-di-O-methyl-D-galactitol	4.24	-
1,2,3,4,5-Penta-O-acetyl-6-O-methyl-D-galactitol	3.68	1.06
1,2,3,5,6-Penta-O-acetyl-4-O-methyl D-galactitol	7.62	1.11
1,3,4,5,6-Penta-O-acetyl-2-O-methyl-D-galactitol	5.88	1.18
1,2,3,4,5,6-Hexa-O-acetyl-D-galactitol	7.93	1.01

Table 37.

was acetylated in acetic anhydride-anhydrous sodium acetate at 100° for 1 hour. For reducing sugars, an accurately weighed amount of the sugar, together with a similar weight of 2,3,4,6-tetra-_O_-methyl-_D_-galactitol, was reduced with sodium borohydride for 2 days, and the product, after removal of the cations with IR-120 (H^+) resin and borate on evaporation of methanol from the residue, was acetylated as above. After removal of the acetic anhydride, an acetone solution was subjected to GLC separation.

The measurement of the peak areas was accomplished using the triangulation method. The R values, calculated using this method, are presented in table 37 and are the means of at least three determinations.

5.4 Analysis of methylated polysaccharides.

Methylated _Acacia podalyriaefolia_ gum used in this investigation was the sample prepared in section 3.4. All other methylated polysaccharides were those investigated by Kaplan and Stephen.[7,75]

Approximately 20-25 mg of the methylated polysaccharide was hydrolysed with _N_-H_2SO_4 containing 98% formic acid, in a sealed tube, at 96° for 15 hours. The solution was neutralised ($BaCO_3$), filtered, concentrated and reduced with sodium borohydride for 2 days. Cations were removed with Amberlite IR-120(H^+) resin, and the borate was removed on evaporation of methanol from the residue. The mixture of partially methylated glycitols was acetylated with acetic anhydride-anhydrous sodium acetate. Acetone solutions, after evaporation of the acetic anhydride, were analysed by GLC. The separations of the methylated glycitol acetate mixtures were performed at least three times, and the results presented in table 38 are therefore a means of at least three determinations.

All peaks were identified by a comparison of their _T_ values with those obtained for the standards (see section 5.2), and the molar proportions of the sugars were obtained by dividing

Polysaccharide from	3R?	234R	235A	23A	25A	34A	35A	2346G	234G	236G	246G	23G	24G	26G	2G	4G	6G	G	234GlA[133]	23 GlA[133]
Acacia																				
cyanophylla	2?	1	8	2	6	-	1	4	5	2	2	tr	23	1	2	1	5	-	5	20
decurrens		2	51	4	5	-	1	5	2	1	2	-	12	5	7	-	-	-	-	3
elata		3	11	-	2	-	1	30	4	4	4	0.5	32	2	6	1	-	-	-	-
giraffae	1?	2	12	1?	1	?	1	6	3	12	4	0.5	27	5	6	0.5	-	-	17	1
karroo	0.5?	1	16	2	15	9	10	2	1	5	2	-	27	0.5	2	-	-	-	3	4
mearnsii	tr	1	53	5	6	2	1	4	2	1	1	0.5	6	3	3	0.5	-	-	6	5
podalyriaefolia	1?	2	15	-	1	-	1	22	5	7	2	1	28	2	10	1	-	-	2	tr
pycnantha		3	24	-	5	-	-	30	4	2	3	-	19	5	2	1	-	-	2	tr
Agave americana		13	34	1	1	-	0.5	9	5	1	2	-	5	6	2	2	-	-	9	10

Table 38.

The numbers shown are in units of mol. %

R=L-rhamnitol, A=L-arabinitol and G=D-galactitol the peak area by the appropriate R value. The separations of the acetylated methylated glycitols are shown in figs. 18 to 26.

6. DISCUSSION

6.1 GLC conditions

The separations of the methyl glycosides obtained on methanolysis of methylated polysaccharides has been extensively applied to structural investigation of polysaccharides. In this field of GLC analysis, both polar and non-polar phases are used, polar phases showing greater selectivity. This method of analysis has the following disadvantages:

(a) as many as four methyl glycosides can be produced from each sugar, increasing the complexity of the chromatogram,

(b) methyl di-O-methylhexosides have retention times of two to two and a half hours and the peaks are broad, decreasing the accuracy of quantitative determinations, and

(c) methyl-mono-O-methylhexosides, methyl pentosides and methyl hexosides are not eluted.

Conversion of the free hydroxyl groups of methyl O-methyl glycosides[103,104,105] and methyl O-methyl glycoses to the more volatile acetates results in complete separation of all sugars with decreased retention times, but anomeric mixtures are obtained.

The analysis of reducing sugars and their derivatives has been simplified by the development of methods for their reduction, conversion to volatile esters (or ethers) and GLC. Bishop and Cooper have separated the mono-O-methylglucitols as their penta-O-acetyl derivatives[93], and more recently the process has been made quantitative for fully acetylated glycitols[66] and extended to include the identification, by their retention times, of the acetates of partially methylated glycitols.[68,109,110]

The acetylated methylated glycitols derived from the hydrolysate of nine methylated polysaccharides have been examined on ECNSS-M-coated columns at 175°, using a carrier flow rate of

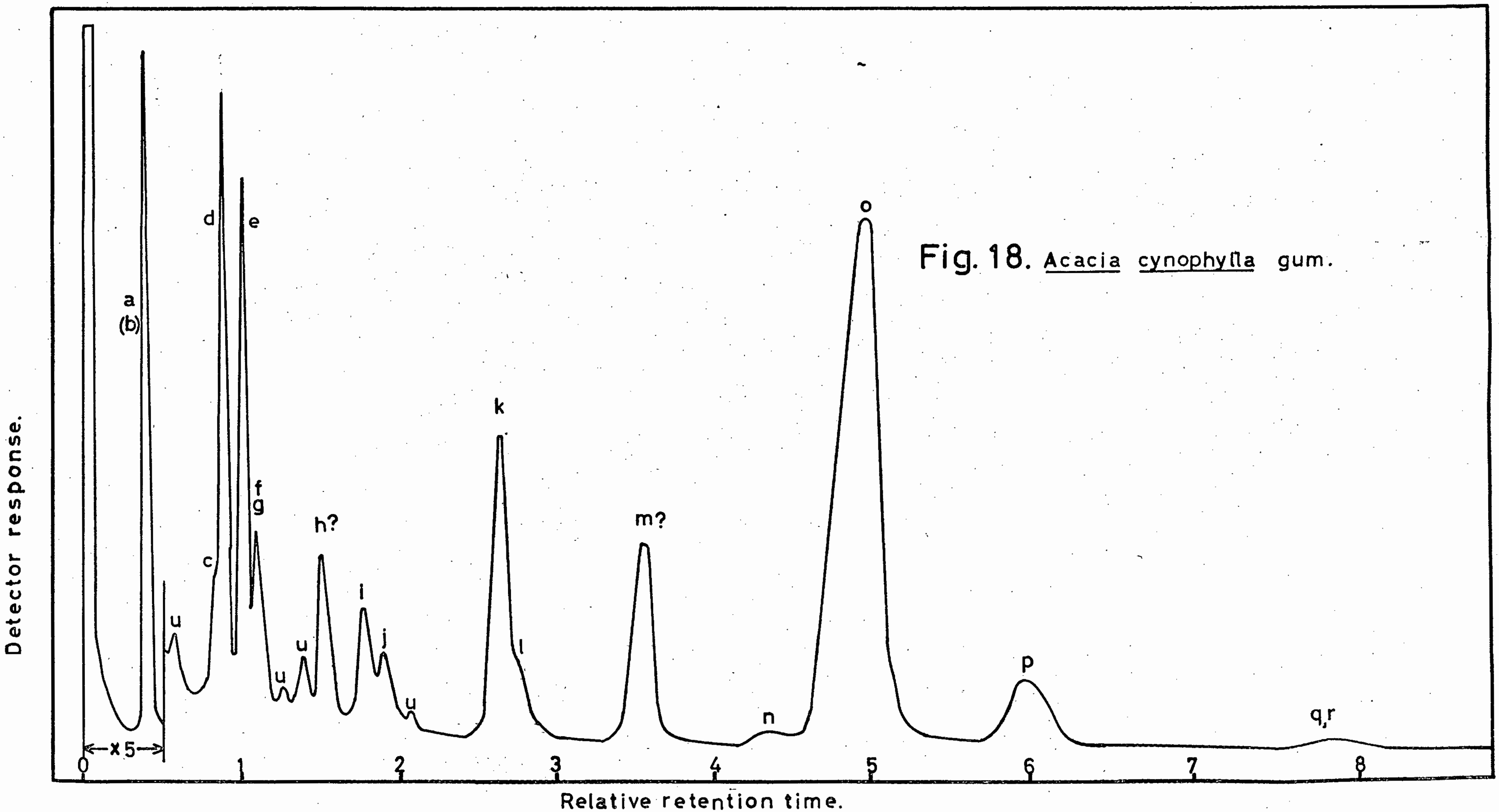


Fig. 18. Acacia cynophylla gum.

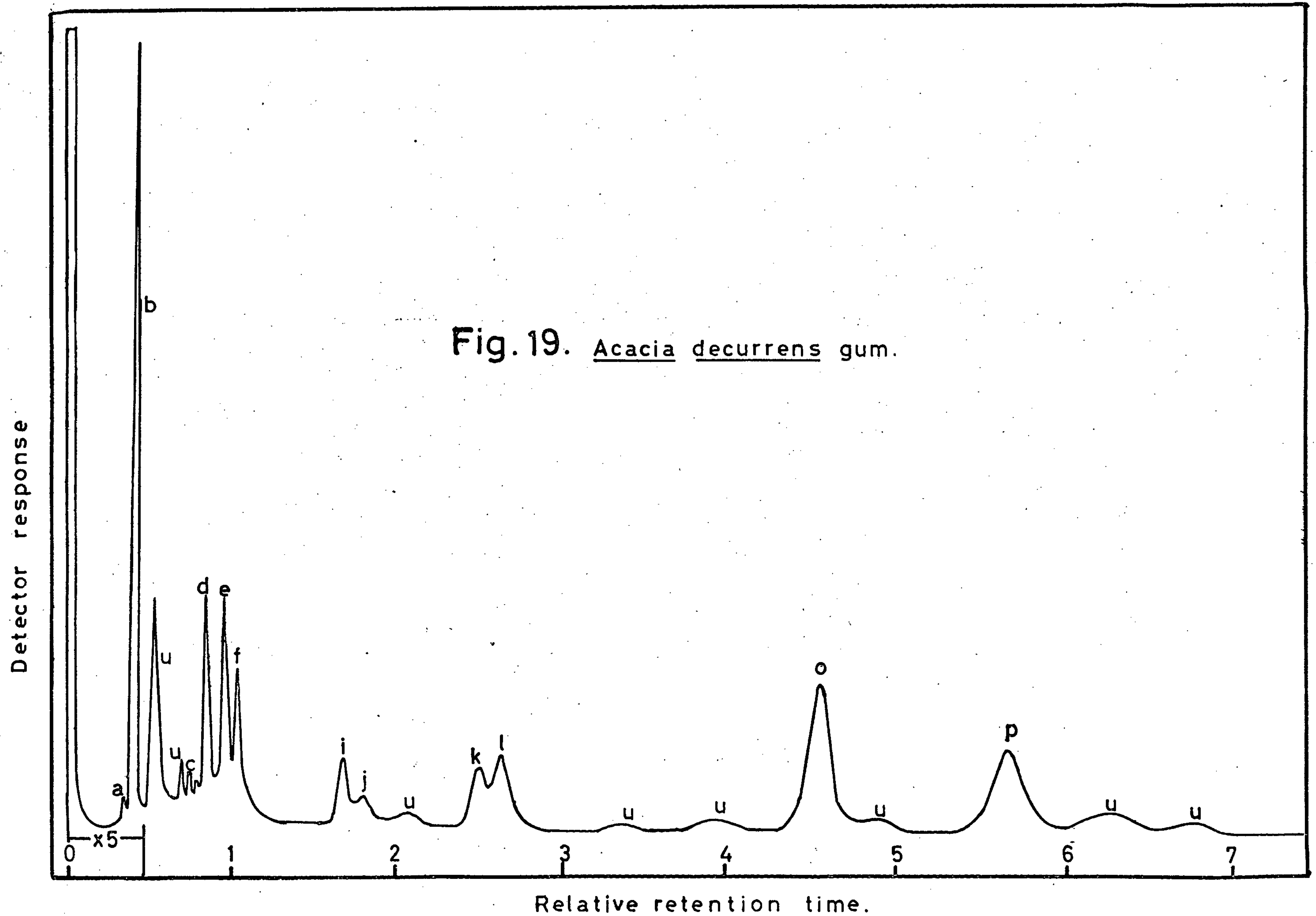


Fig. 19. Acacia decurrens gum.

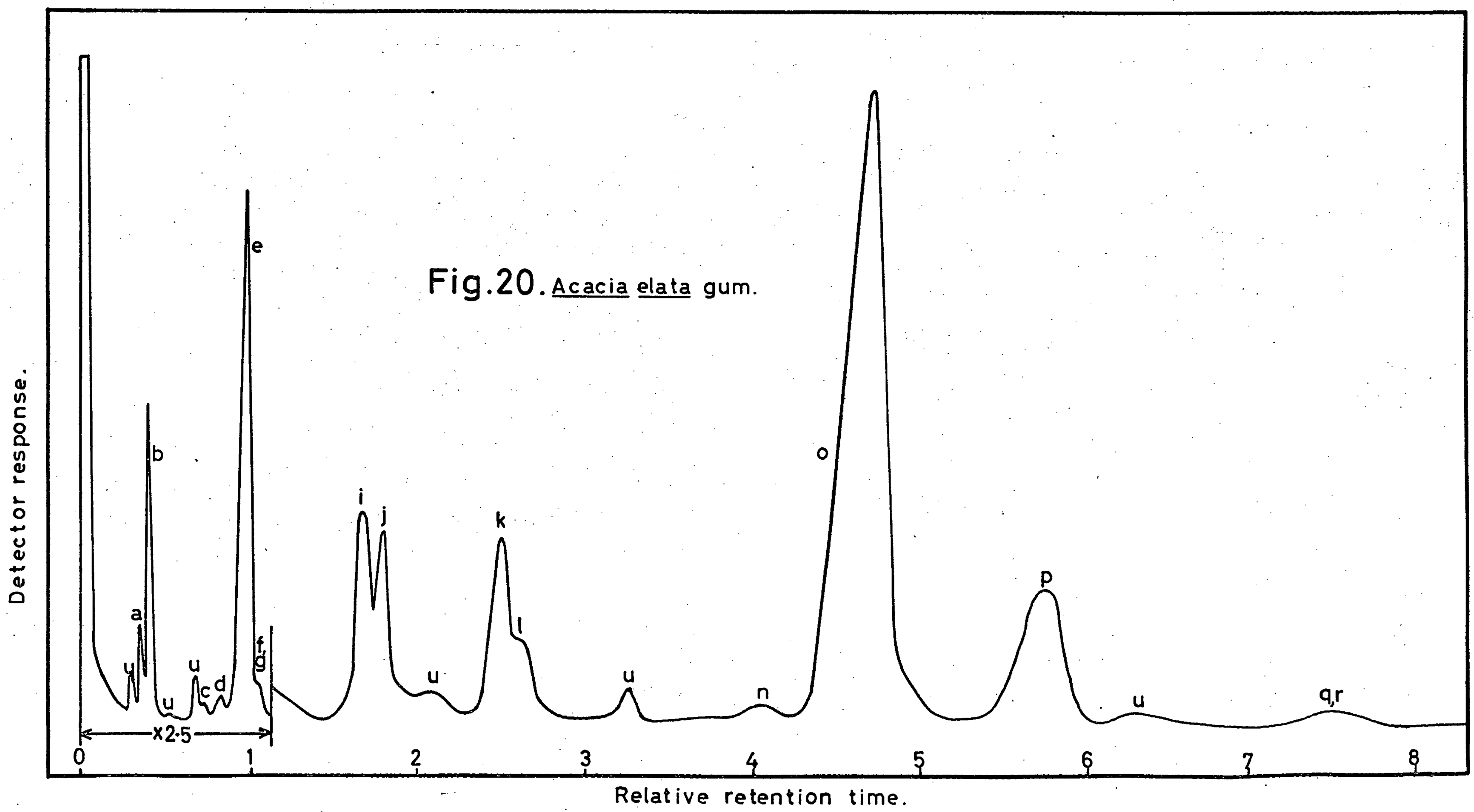


Fig.20. <u>Acacia</u> <u>elata</u> gum.

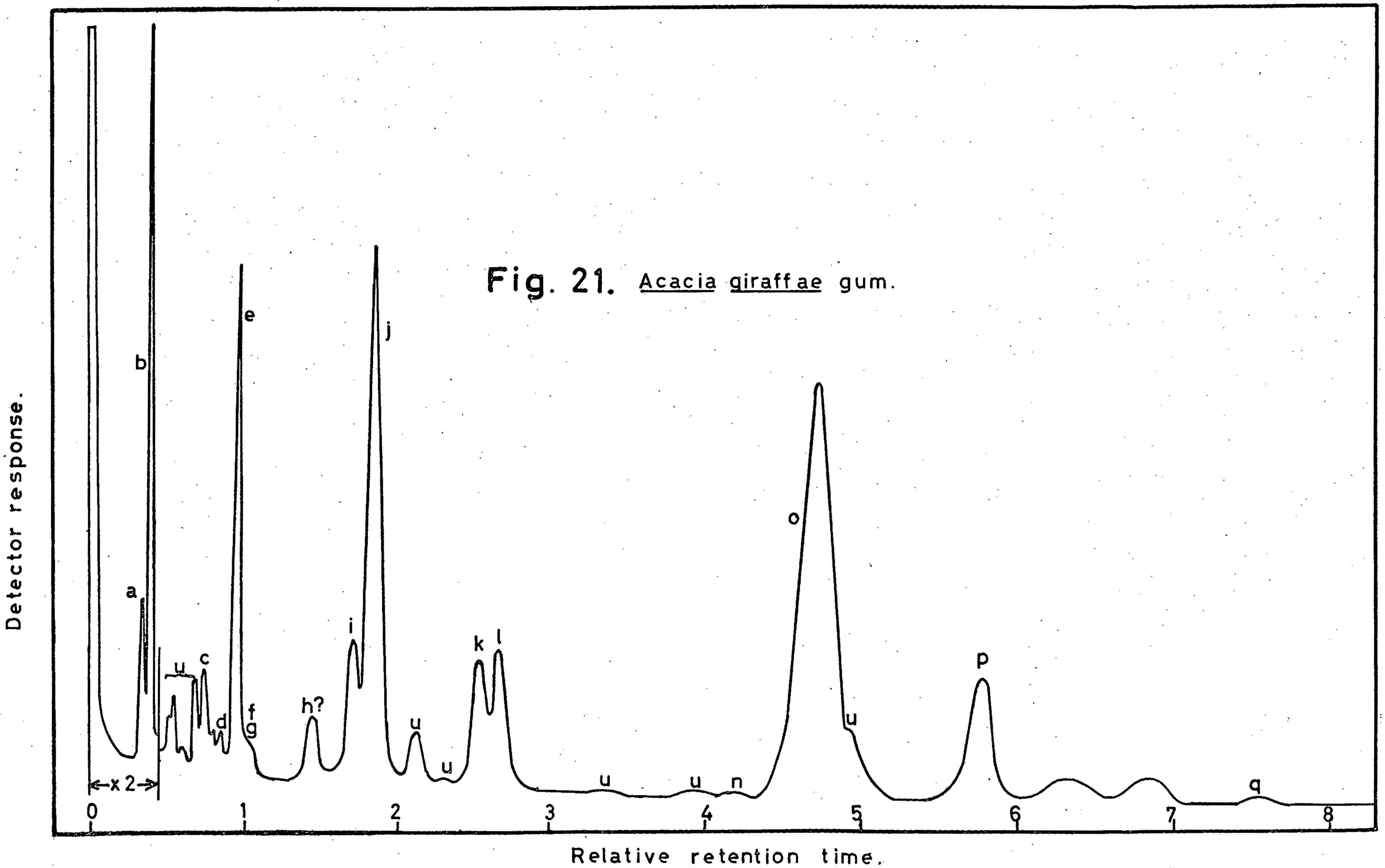


Fig. 21. Acacia giraffae gum.
Detector response.
Relative retention time.
a
b
u
c
d
e
f
g
h?
i
j
u
u
k
l
u
u
n
o
u
p
q
×2
0
1
2
3
4
5
6
7
8

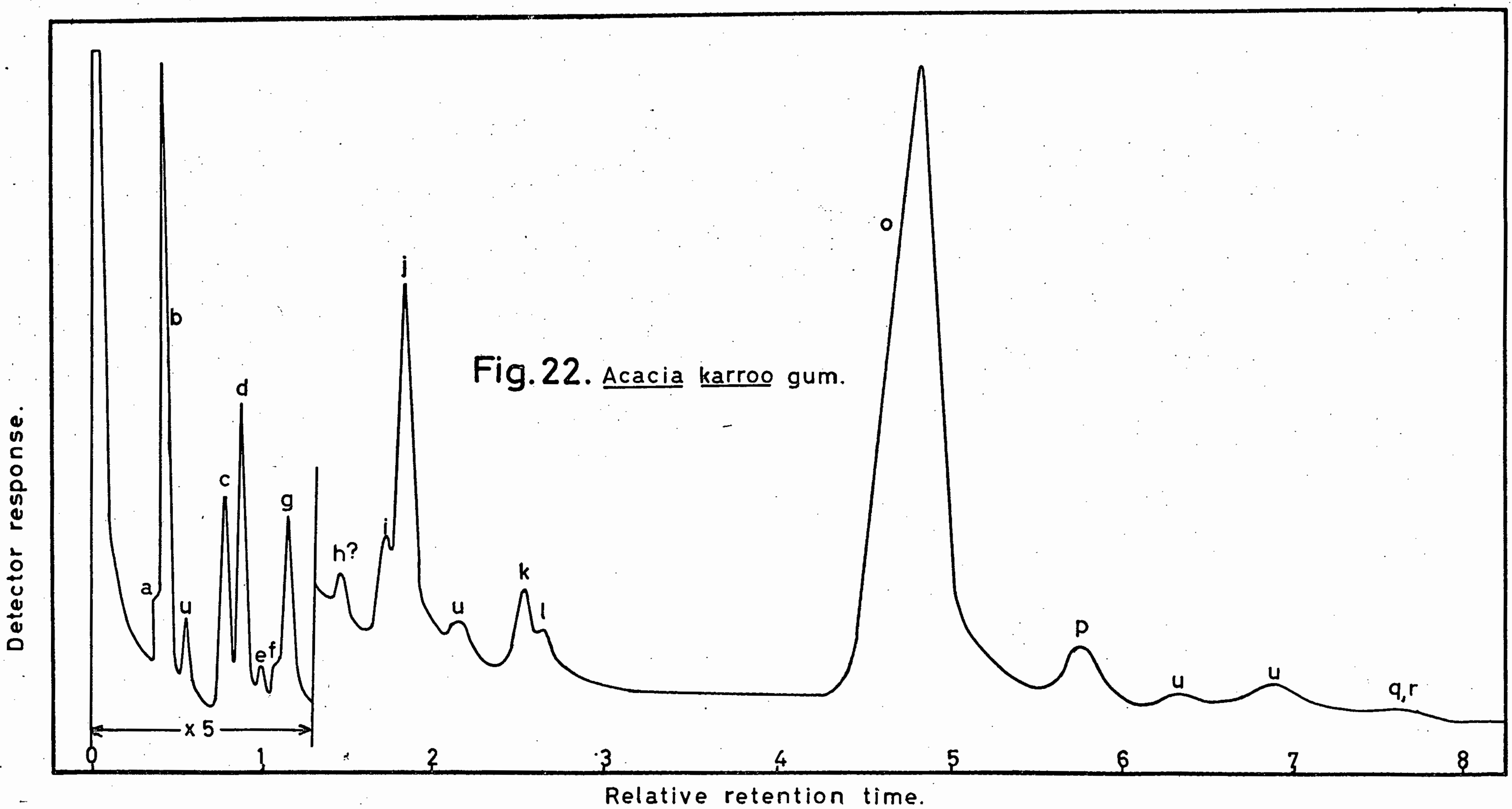


Fig. 22. Acacia karroo gum.

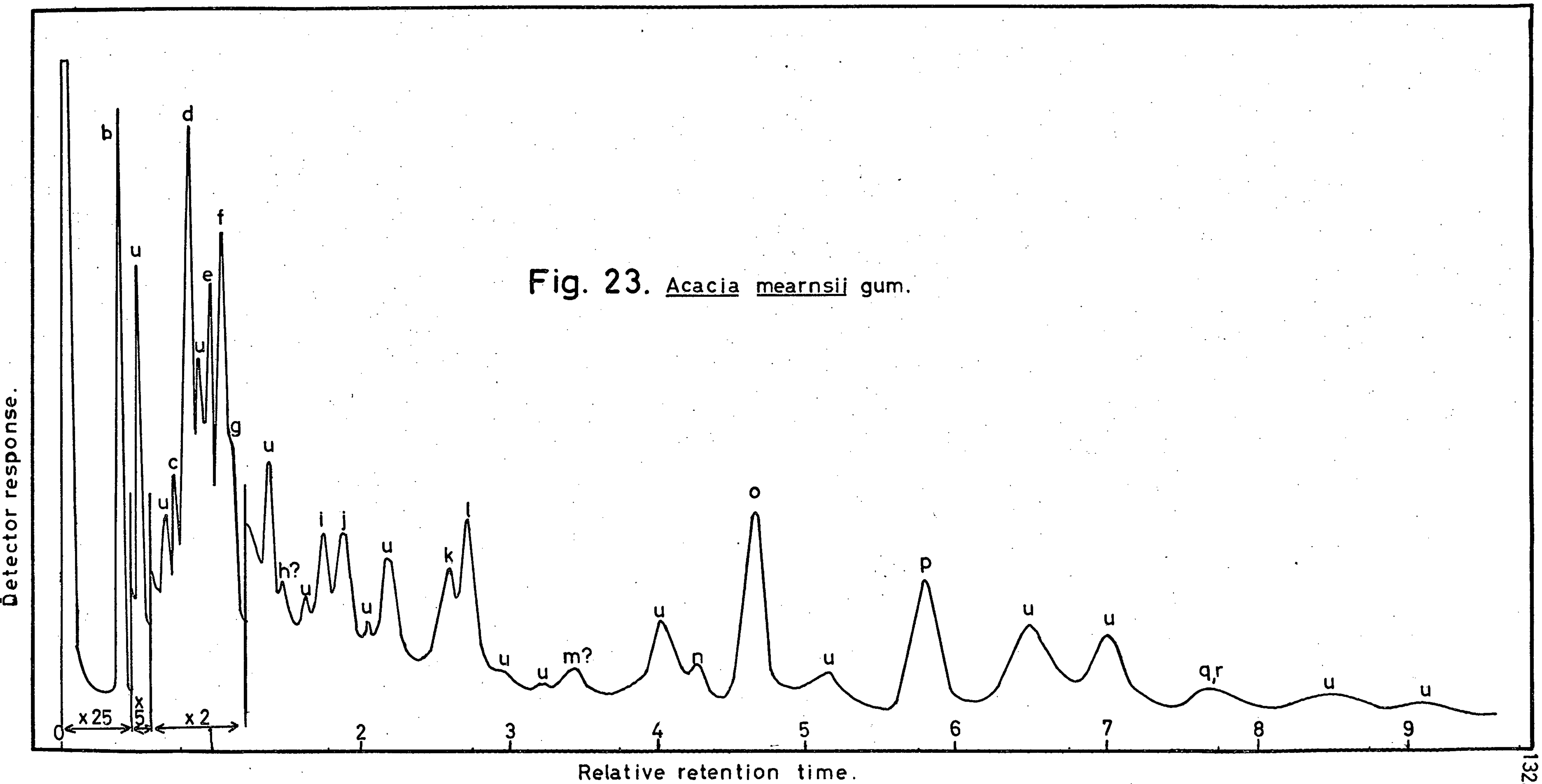


Fig. 23. Acacia mearnsii gum.

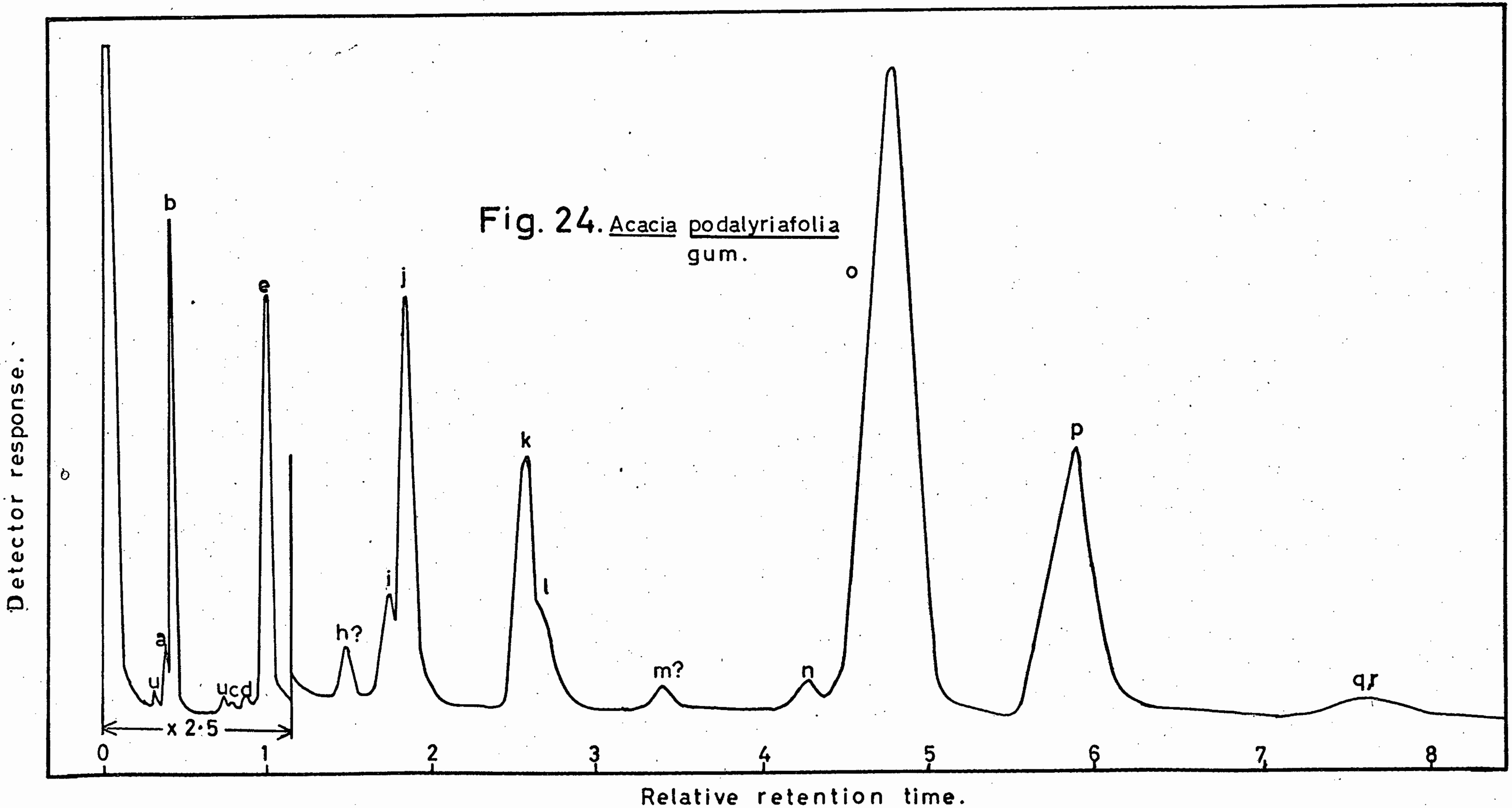


Fig. 24. Acacia podalyriafolia gum.

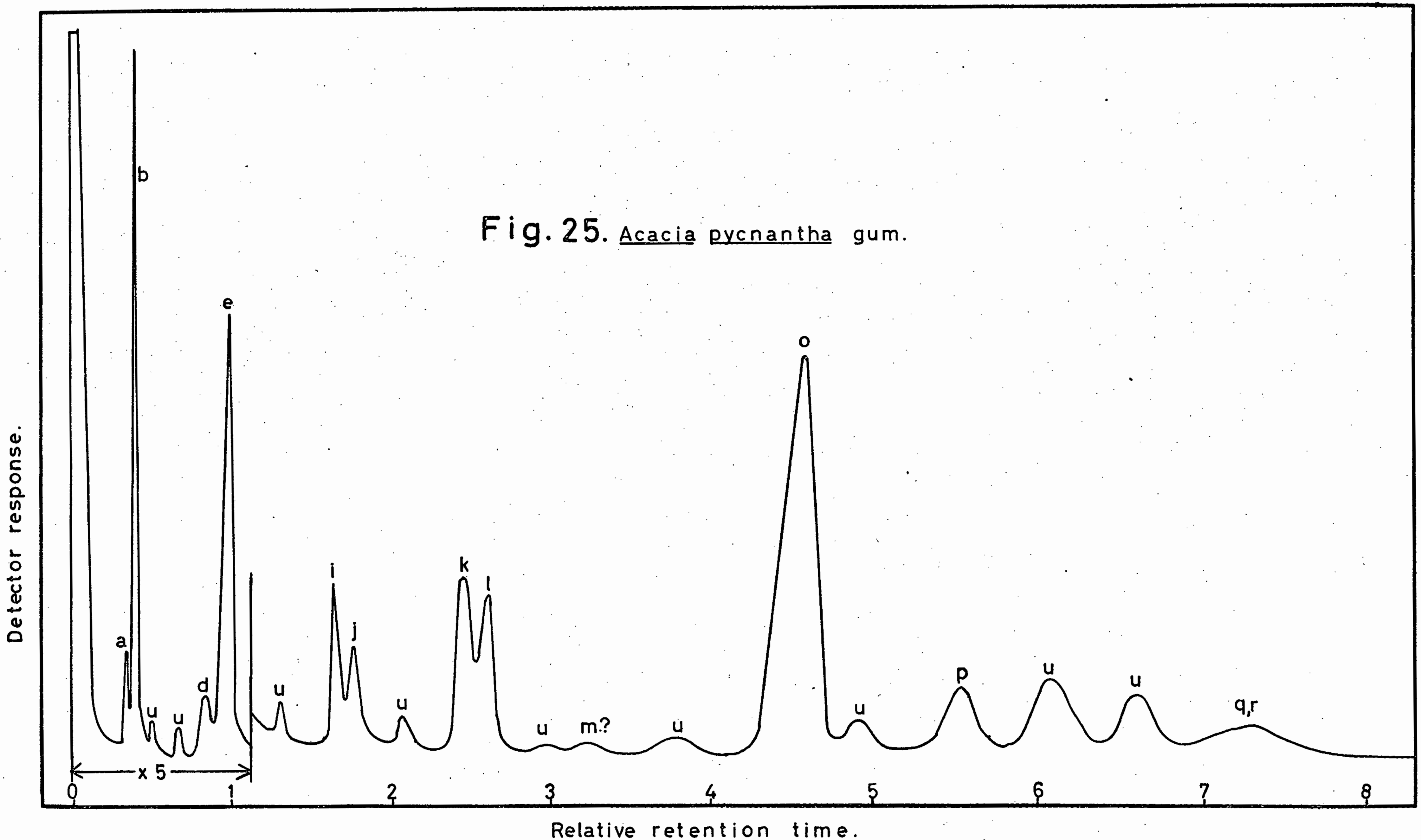


Fig. 25. Acacia pycnantha gum.

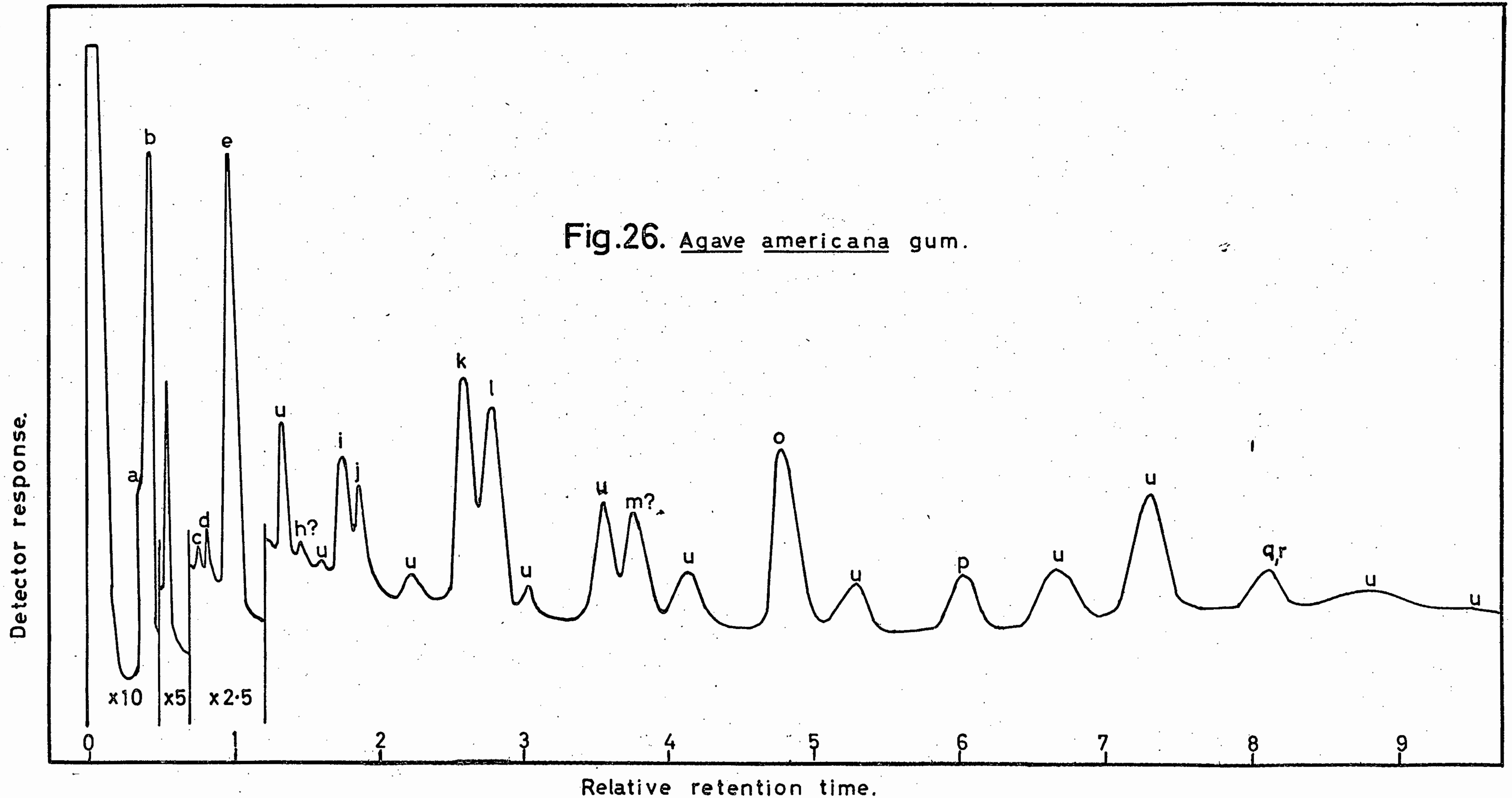


Fig.26. Agave americana gum.

35 ml per minute. Each glycitol acetate mixture was separated at least three times, each separation being completed in 30 - 40 minutes.

Under these conditions peaks were well separated, and it was possible, using only one liquid phase, to estimate quantitatively from the chromatogram, all of the O-methyl sugars present in the hydrolysates of the methylated gums.

Columns deteriorated rapidly with use and noticeable changes in the retention times resulted. This deterioration was not entirely due to column bleed, as the reference column, which had been subjected to the same conditions of carrier flow and temperature as the operating column, was found to separate mixtures perfectly after the operating column had ceased to function.

Although difficulties were encountered in the measurement of the areas of the peaks having retention times of less than 1.00, it was decided not to use a temperature program, as this would have affected the reproducibility of the quantitative analyses. All samples were therefore analysed at an isothermal temperature of 175°.

6.2 Structure and retention time.

The logarithm of retention time, T', of acetylated methylated D-galactitols may be expressed by the equation

$$T' = R + X$$

where R is a constant for all D-galactitols, and may be compared with r_n, the relative retention time of the steroid nucleus, in the steroid series[134],

and

X is the sum of the interactions (expressed in the same terms as R) of the various O-acetyl and O-methyl groupings along the D-galactitol chain.

The various interactions, A - H, are as follows:

A. O-acetyl and O-acetyl terminal groups,

B. O-acetyl and O-methyl terminal groups,

C. O-acetyl and O-acetyl groups cis,

D. O-acetyl and O-acetyl groups trans,

E. O-acetyl and O-methyl groups cis,

F. O-acetyl and O-methyl groups trans,

G. O-methyl and O-methyl groups cis and

H. O-methyl and O-methyl groups trans.

The logarithm of retention time of each D-galactitol could be expressed in terms of R and 4 of the A - H values. From the twelve equations it was possible to calculate seven interaction variations, for example A - B, which represented an alteration of O-acetyl / O-acetyl terminal groups to O-acetyl / O-methyl terminal groups. It was found that the values of these interaction variations depended largely upon whether or not the D-galactitol carried a methoxy group at the $C_{(4)}$ position.

In view of the complexity of this approach, a simpler method was adopted. This involved the determination of the incremental decrease in T' for the replacement of acetoxy groups at each position along the D-galactitol chain.

The incremental decrease in T' when the acetoxy group at $C_{(2)}$ is replaced by a methoxy group can be determined from the difference in the T' values of a number of D-galactitols. The simplest example is $T'_0 - T'_2$, the difference between the log retention times of the acetates of D-galactitol and 2-O-methyl-D-galactitol. The incremental decrease in the log retention times due to the replacement of acetoxy groups at the $C_{(2)}$, $C_{(3)}$, $C_{(4)}$ and $C_{(6)}$ positions of D-galactitol are presented in table 39.

It was found that the incremental decrease in the log retention times of D-galactitols carrying an acetoxy group at $C_{(4)}$ were different from those of D-galactitols carrying a methoxy group at $C_{(4)}$. The number of methoxy groups already present in the D-galactitol also affected the increments in the log retention times for the replacement of the acetoxy group at $C_{(4)}$ by a methoxy group. The value was at its lowest when the acetoxy group at $C_{(4)}$ of D-galactitol was replaced by a methoxy group and at its highest when the acetoxy group of 2,3,6-tri-O-methyl-D-galactitol was replaced by a methoxy group.

Position of replacement of acetoxy group by methoxy group.	Log retention time difference	T' increment for D-galactitols carrying an acetoxy group at $C_{(4)}$	Mean T' increment	T' increment for D-galactitols carrying a methoxy group at $C_{(4)}$	Mean T' increment
2	$T_0' - T_2'$	0.120			
	$T_6' - T_{26}'$	0.125	0.122		
	$T_4' - T_{24}'$			0.200	
	$T_{46}' - T_{246}'$			0.200	0.200
3	$T_2' - T_{23}'$	0.142			
	$T_{26}' - T_{236}'$	0.171	0.157		
	$T_{24}' - T_{234}'$			0.264	
	$T_{246}' - T_{2346}'$			0.248	0.256
4	$T_0' - T_4'$			0.017	0.017
	$T_2' - T_{24}'$			0.087	
	$T_6' - T_{46}'$			0.119	0.103
	$T_{23}' - T_{234}'$			0.209	
	$T_{26}' - T_{246}'$			0.193	0.201
	$T_{236}' - T_{2346}'$			0.270	0.270
6	$T_0' - T_6'$	0.334			
	$T_2' - T_{26}'$	0.329			
	$T_{23}' - T_{236}'$	0.358	0.340		
	$T_4' - T_{46}'$			0.435	
	$T_{24}' - T_{246}'$			0.434	
	$T_{234}' - T_{2346}'$			0.418	0.429

Table 39.

The T' values calculated for the acetylated methylated D-galactitols, using the T' increments, are presented in table 40.

Position of O-methyl group	T'	Calculated T' values (a)*	(b)*	(c)*	(d)*
-	0.899	0.899			
2	0.769	0.777			
3	-	0.742			
4	0.882	0.882			
6	0.566	0.559			
23	0.627	0.620			
24	0.682	0.683	0.674		
26	0.441	0.437			
46	0.447	0.453	0.456		
234	0.418	0.427	(e)*0.418	0.419	
			(f)*0.440		
236	0.270	0.280			
246	0.248	0.253	(e)*0.245	0.236	
			(g)*0.257		
2346	0.000	-0.003	(e)*-0.013	(h)*-0.010	0.011
			(f)*-0.011	(i)*-0.020	
			(g)*-0.001	(j)*-0.002	

*The acetoxy groups were considered to have been replaced in the following order :

(a) G ⟶ 4G ⟶

(b) G ⟶ mono methyl G having a 4-acetoxy-group ⟶ dimethyl G having a 4-methoxy group.

(c) G ⟶ dimethyl G having a 4-acetoxy-group ⟶ trimethyl G having a 4-methoxy group.

(d) G ⟶ trimethyl G having a 4-acetoxy group ⟶ tetramethyl G having a 4-methoxy group.

(e) G $\rightarrow$ 2G $\rightarrow$ 24G $\rightarrow$

(f) G $\rightarrow$ 3G $\rightarrow$ 34G $\rightarrow$

(g) G $\rightarrow$ 6G $\rightarrow$ 46G $\rightarrow$

(h) G $\rightarrow$ 23G $\rightarrow$ 234G $\rightarrow$

(i) G $\rightarrow$ 26G $\rightarrow$ 246G $\rightarrow$

(j) G $\rightarrow$ 36G $\rightarrow$ 346G $\rightarrow$ where G represents D-galactitol and the numbers preceeding G represent the positions along the D-galactitol chain carrying methoxyl groups.

Table 40.

The theoretical T' values for the various acetylated methylated D-galactitols agree favourably with the experimentally determined values.

From table 39 it is apparent that the character of the $C_{(3)}$ methoxy group is different from that at $C_{(4)}$, despite the symmetry in the molecule. Only in the conversion of 2,3,6-tri-O-methyl-D-galactitol to 2,3,4,6-tetra-O-methyl-D-galactitol does the T' increment at $C_{(4)}$ approximate to that at $C_{(3)}$.

It is not clear why two series of T' values, depending on whether or not the $C_{(4)}$ position of the D-galactitol carries a methoxy group, are obtained. This variation could arise from acetoxy-methoxy interactions; it seems more likely that the acetates of those D-galactitols carrying a methoxy group at $C_{(4)}$ are less volatile than the corresponding D-galactitol carrying an acetoxy group at $C_{(4)}$.

6.3 Errors in GLC analysis.

The sources of errors in qualitative and quantitative analyses of acetylated methylated glycitol mixtures by GLC are described under the headings (i) Experimental and (ii) Chemical errors.

(i) Experimental errors include those errors which arise from the measurement of peak areas and from changes in the GLC conditions.

The acetates of tri-O-methyl--L-rhamnitol, 2,3,5-tri-, 2,3-, 2,5-, 3,4- and 2,5-di-O-methyl-L-arabinitol, and 2,3,4,6-tetra-O-methyl-D-galactitol have retention times of 1.00 and less and the peaks on the GLC traces are sharp. The retention times of 2,4-di and 2- and 4-O-methyl-D-galactitol are large and the peaks are consequently broad. In both cases errors are to be expected; the response factors for the acetates of 2,3,5-tri-O-methyl L-arabinitol and 4-O-methyl-D-galactitol, for example, were reproducible to within 5%.

More serious errors resulted from the deterioration of the columns. This had the effect of reducing the T values and distortion of the peaks resulted. The response factor for the acetate of 2,3,4-tri-O-methyl-D-galactitol varied by as much as 14%.

(ii) Chemical errors include those which arise from

(a) the partial failure of the methylating agents to methylate all free hydroxyl groups in the polysaccharides,

(b) de-O-methylation during the hydrolysis of the O-methyl derivatives of the polysaccharides

(c) incomplete hydrolysis of methylated acidic polysaccharides

(d) incomplete reduction of the methyl ethers derived from methylated polysaccharides and

(e) de-O-acetylation.

(a) The occurrence of 2,6-di-O-methyl-D-galactose and 2-O-methyl-D-galactose in the hydrolysates of methylated polysaccharides have often been regarded as resulting from under methylation of the axial 4-hydroxyl group of some of the D-galactopyranose residues in the polysaccharide.[33]

(b) De-O-methylation is known to result during acid hydrolysis of methylated polysaccharides.[135,136,137] Chanda *et al.*[135] have found that the treatment of 2,3-di- and 2,3,4-tri-O-methyl-xylose with 1% methanolic hydrogen chloride at 100° for 7-8 hours,

~~followed by hydrolysis with 0.5N-HCl for 6-8 hours resulted~~ in 0.3% and 2% de-*O*-methylation respectively.

(c) Incomplete hydrolysis of uronic acid-sugar bonds in methylated acidic polysaccharides produces methylated aldobiouronic acids, which are not eluted from the polar phase. This results in low apparent values for the sugars concerned.

(d) Previous workers[66,68] reduced the sugar to the glycitols with borohydride for 10 - 15 minutes. To ensure complete reduction of all sugar residues in the hydrolysate of methylated polysaccharides, the samples were reduced for two days.

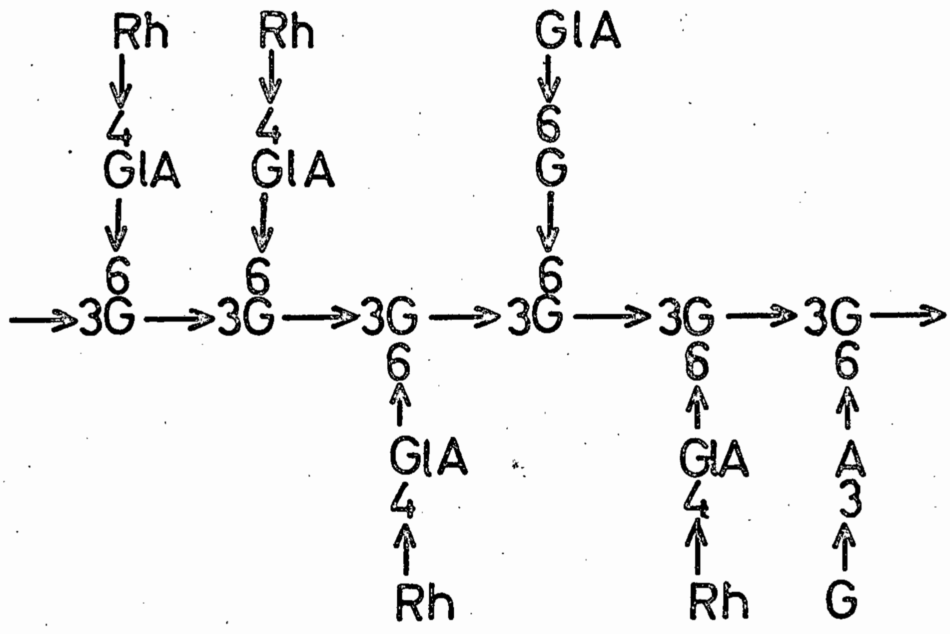
(e) GLC analyses of the acetone solutions of the methylated alditol acetates were found to give different proportions of the components on storage. Re-acetylation and GLC analysis of the stored solutions gave results identical with those obtained when the freshly prepared acetates were first separated. In an attempt to minimise the effects of de-*O*-acetylation, acetate solutions were analysed by GLC within 1-2 hours of their preparation.

6.4 The structures of *Acacia* gums.

In this work the acetylated methylated glycitols derived from eight methylated *Acacia* gums have been examined by GLC. Possible structural models for six polysaccharides, based on a framework in which the main chain consists entirely of 1→3 or 1→6-linked-*D*-galactopyranose residues are proposed. As far as possible, the structural features of the side-chains are presented in the light of the results of previous investigations of *Acacia* gums (see Part I section I).

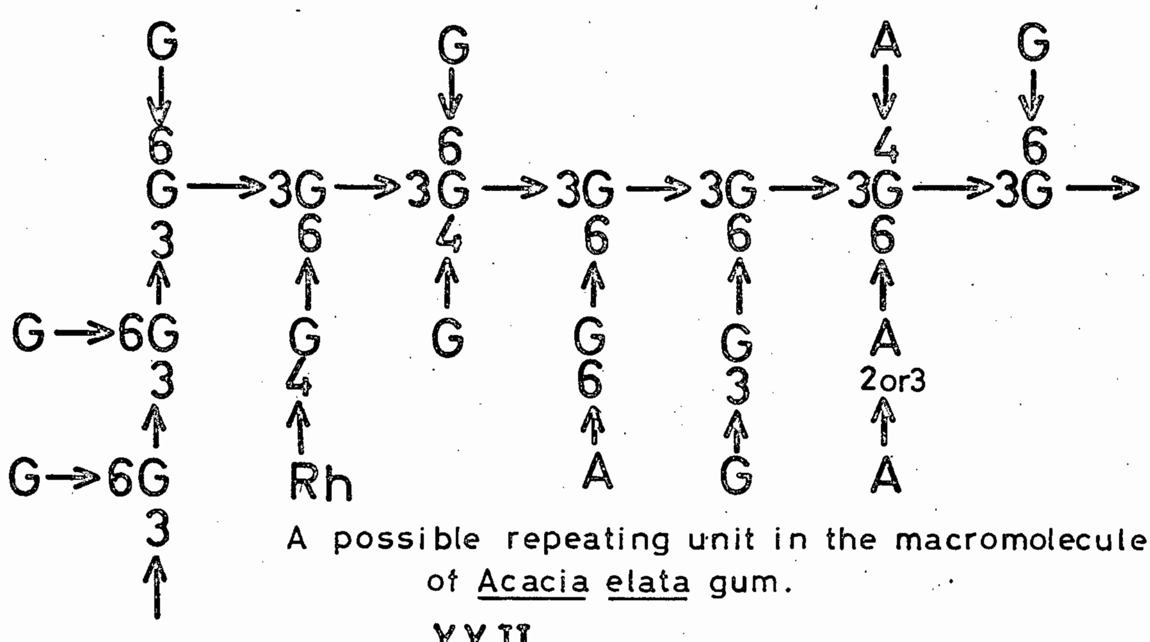
As the GLC results do not provide information as to the exact mode of linkage of the *D*-galactopyranose residues in the main chain, and of the side-chains to the main chain, the linkages shown are entirely arbitrary.

The proposed structures of six of these gums are shown by structures XXI - XXVI, and are based on the GLC results obtained from table 38. The tables accompanying the structures compare



A possible repeating unit in the macromolecule of *Acacia cyanophylla* gum.

XXI.

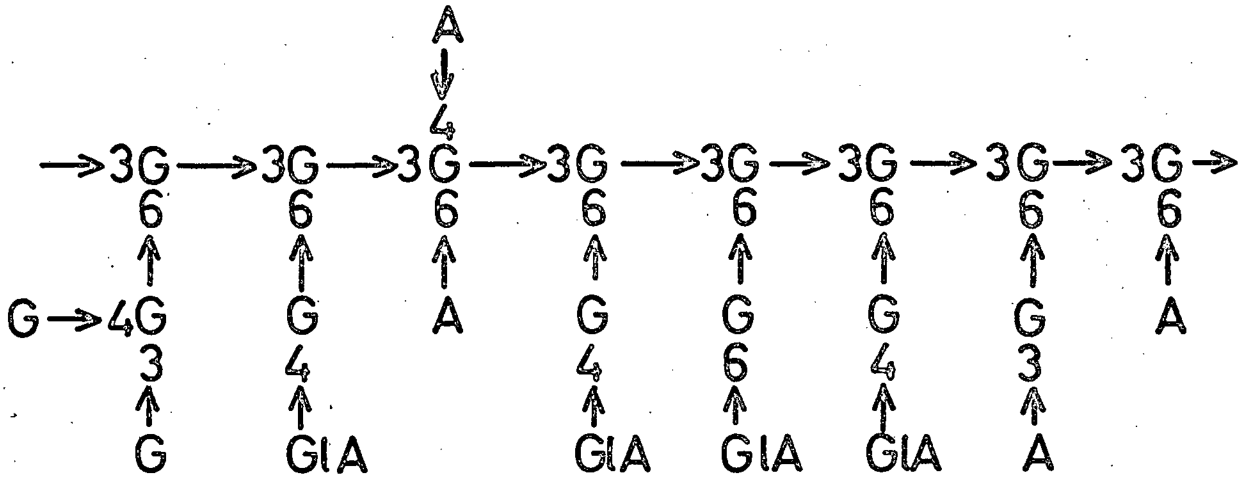


A possible repeating unit in the macromolecule of *Acacia elata* gum.

XXII.

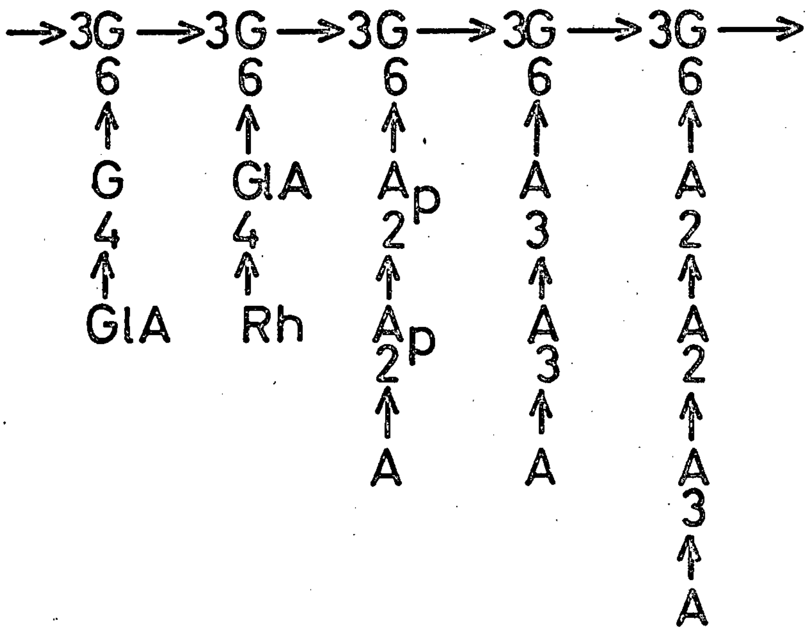
	XXI		XXII			XXI		XXII	
Sugar	GLC	Model	GLC	Model	Sugar	GLC	Model	GLC	Model
234Rh	18	22	3	4	246G	2	—	4	4
235A	?	—	11	12	24G	23	32	32	30
23A	2	—	—	—	26G	1	—	2	—
25A	6	6	2	} 4	2G	2	—	6	8
35A	1	—	1		4G	1	—	1	—
2346G	4	6	30	30	6G?	5	—	—	—
234G	5	6	4	4	234GlA[7]	(5)	6	—	—
236G	2	—	4	4	23GlA[7]	(20)	22	—	—

G = D-galactopyranose, GlA = D-glucopyranosyluronic acid.
A = L-arabinofuranose and Rh = L-rhamnopyranose.



A possible repeating unit in the macromolecule of
Acacia giraffae gum.

XXIII.

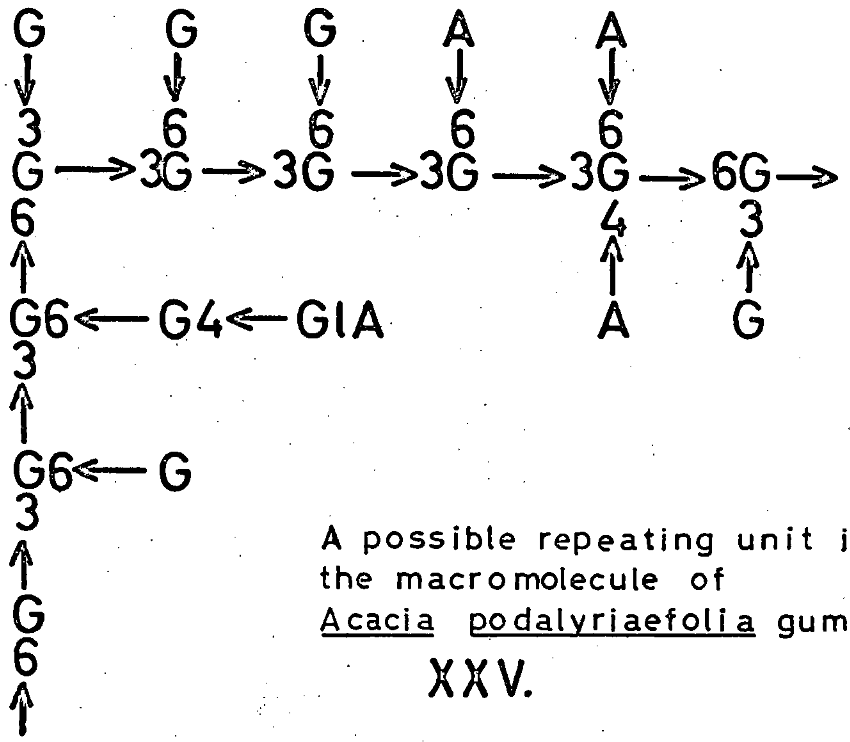


A possible repeating unit in the macromolecule of
Acacia karroo gum.

XXIV.

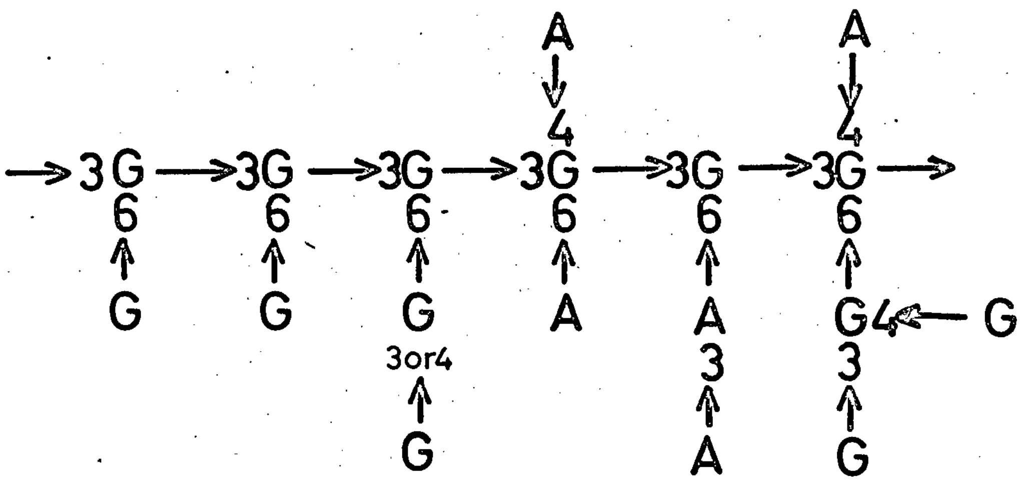
	XXIII		XXIV			XXIII		XXIV	
Sugar	GLC	Model	GLC	Model	Sugar	GLC	Model	GLC	Model
234Rh	2	—	1?	5	246G	4	4	2	—
235A	12	17	16	16	23G	0·5	—	—	—
23A	1?	—	2	—	24G	27	29	27	27
25A	} 2	—	15	16	26G	5	4	0·5	—
35A			10	10	2G	6	4	2	—
34A	—	—	9	10	4G	0·5	—	—	—
2346G	6	4	2	—	234GlA[7]	(17)	17	(3)	5
3R	1?	—	0·5?	—	23GlA[7]	(1)	—	(4)	5
234G	3	4	1	—					
236G	12	13	5	5					

G = D-galactopyranose, GlA = D-glucopyranosyluronic acid,
A = L-arabinofuranose, A_p = L-arabinopyranose, and
Rh = L-rhamnopyranose.



A possible repeating unit in the macromolecule of <u>Acacia</u> <u>podalyriaefolia</u> gum

XXV.



A possible repeating unit in the macromolecule of <u>Acacia</u> <u>pycnantha</u> gum.

XXVI.

	XXV		XXVI			XXV		XXVI	
Sugar	GLC	Model	GLC	Model	Sugar	GLC	Model	GLC	Model
234Rh	2	—	3	—	23G	1	—	—	—
235A	15	16	24	22	24G	28	37	19	22
25A	1	—	5	6	26G	2	—	5	6
35A	1	—	—	—	2G	10	5	2	11
2346G	22	27	30	27	4G	1	—	1	—
234G	5	5	4	—	234GIA[7]	(2)	5	(2)	—
236G	7	5	2	} 6					
246G	2	—	3						

G = $\underline{\underline{D}}$-galactopyranose, A = $\underline{\underline{L}}$-arabinofuranose.
GlA = $\underline{\underline{D}}$-glucopyranosyluronic acid. Rh = $\underline{\underline{L}}$-rhamnopyranosyl.

the GLC results with the theoretical proportions of the partially methylated monosaccharides obtained from the models.

The proportions of the constituent sugars determined by GLC, from the models and from published work, as well as the total number of end-groups, chain-units and branch points are shown in table 41.

The results have supported the observation by Kaplan and Stephen [7] that *A. elata* gum has the simplest structure and that the gums of *A. podalyriaefolia* and *A. pycnantha* are the closest related structurally. *A. elata* and *A. podalyriaefolia* gums contain the highest proportion of D-galactose, viz. 80% and *A. karroo* gum contains the highest proportion of chain-linked arabinose residues. Although periodate degradation studies have not been undertaken, the model structure of *A. karroo* gum suggests that the arabinose-containing side-chains could be at least five units in length. Only in the cases of *A. elata* and *A. giraffae* gums were the proportions of end-groups approximately equal to the proportions of branch points. The proportions of branch points exceeded the proportions of end-groups in the gums of *A. cyanophylla*, *A. karroo* and *A. podalyriaefolia*, while the reverse was the case for *A. mearnsii*, *A. decurrens* and *A. pycnantha*.

The total proportions of the sugars, as analysed by GLC, compares favourably with published results. The total proportions of arabinose was high in the cases of the gums from *A. karroo*, *A. decurrens* and *A. mearnsii*, and the total proportions of galactose for these gums were correspondingly low (table 41). Good agreement is generally observed between the proportions of the individual methylated sugars obtained by GLC and the proportions calculated from the model structures. As was the case in the analysis of the methyl glycosides obtained from these methylated gums, good agreement was obtained between the total galactose, arabinose and rhamnose content of the gum from GLC results and from the models, despite the poor agreement between the end-groups and branch-points, Comparison of the GLC and published results

Polysaccharide from	Galactose			Arabinose			Rhamnose			Uronic acid			End-groups		Branch points		Chain units	
	Lit.	GLC	model	Lit.	GLC	model	Lit.	GLC	model	Lit.	GLC	model	GLC	model	GLC	model	GLC	model
Acacia cyanophylla[5,7]	48	45	44	9	9	6	22	20	22	22	-	28	25	33	39	33	36	33
decurrens[7]	47	34	-	45	61	-	4	2	-	3	-	-	54	-	29	-	17	-
elata[7]	80	83	79	17	14	17	3	3	4	-	-	-	41	42	45	42	14	15
giraffae[7]	66	63	66	11	15	17	1	2	-	18	-	17	36	40	42	40	22	20
karroo[7,38,41]	50	39	32	36	52	53	2	1	5	12	-	10	22	26	30	26	48	48
mearnsii[7,36]	38	21	-	46	67	-	8	1	-	8	-	-	62	-	16	-	22	-
podalyriaefolia[7]	79	78	79	15	17	16	1	2	-	5	-	5	34	45	52	45	13	10
pycnantha[7,35]	65	66	72	27	29	28	1	3	-	5	-	-	57	45	29	45	14	10
Agave americana[7]	52	32	-	16	36	-	10	13	-	19	-	-	63	-	18	-	19	-

Table 41

The numbers shown are in units of mol. %

as analysed by GLC. The results obtained for methylated *A. podalyriaefolia* gum, for example, suggest that no de-*O*-methylation of the arabinose methyl ethers had occurred during hydrolysis; only traces of di-*O*-methyl-L-arabinitols and no arabinitol were detected. As no de-*O*-methylation was detected for the methylated arabinitols, it seems unlikely that de-*O*-methylation would have occurred in the methylated galactitols, and the poor agreement between end-groups and branch points cannot be due entirely to de-*O*-methylation during the hydrolysis of the methylated gums.

Each methylated gum was hydrolysed, reduced and acetylated twice and the two series of analyses agreed within experimental error showing that the techniques of hydrolysis, reduction, acetylation and GLC assay were reproducible. The low proportion of branch-points to end-groups in *A. mearnsii* and *A. pycnantha* gums suggests that either some degradation had occurred during methylation, or that 2,6-, 2,4-di and 2-*O*-methyl-D-galactitols were not fully acetylated. The latter could arise from either under acetylation or de-*O*-acetylation. That under acetylated products were present was indicated by the appearance of unidentified peaks at *T* 5.00 - 5.14, 6.37 - 6.56, 6.77 - 7.10, 8.45 - 8.57 and 8.95 - 9.11 in the acetylated methylated alditol mixtures of some of the gums.

The molar proportions of 2,3,4-tri-*O*-methyl-D-glucuronic acid and 2,3-di-*O*-methyl-D-glucuronic acid presented in table 38 are the values obtained for the methyl glycosides obtained from these gums by Kaplan and Stephen.[7] The assay of the partially methylated acids could not be effected on the polar liquid-phase.

6.5 The structure of *Agave americana* gum.

The GLC analysis of the acetates of the methylated **glycitols** derived from *Agave americana* showed poor agreement between the proportions of end-groups and branch points. As in the cases of the gums of *Acacia mearnsii* and *Acacia decurrens*, where poor agreement was also found, no structure can be proposed.

Conclusion.

The qualitative and quantitative analysis of the fully and partially methylated reducing sugars as their derived glycitol acetates by GLC on an ECNSS-M-coated column give results which are comparable with those obtained from other analyses, such as GLC analysis of the methanolysates of the gums.

This technique has the advantage of analysing all sugars including the fully acetylated glycitols. All components are eluted from the column in 40 minutes, thus minimising the effects of band-spreading. Each sugar gives only one glycitol acetate, thus the analysis is greatly simplified. Errors in quantitative analysis occur as those sugars which have small _T_ values, are eluted as sharp peaks, increasing the difficulty of area measurement.

In the quantitative analysis of methylated acidic polysaccharides, incomplete hydrolysis of the glycosidic bond between the acid and certain sugars occurs, resulting in low apparent proportions for those sugars concerned. Perhaps a better approach to the analysis of the methylated acidic gums would be the investigation of the carboxyl-reduced methylated gums. This would result in the quantitative analysis of the fully and partially glucuronic acids as the corresponding partially methylated glucitols. Also the hydrolysis of the carboxyl-reduced methylated gum would not result in low apparent proportions of some sugars on GLC analysis.

7. EXPERIMENTAL

7.1 Source of standards used in calibration experiments.

a. 2,3,4,6-tetra-O-methyl-D-galactitol, methylated *Acacia podalyriaefolia* hydrolysate fraction 3, m.p. 68-69°, $[\alpha]_D$ +9.1° (*c* 1.51 in ethanol), R_f 0.47 (TLC, solvent (*f*)).

b. 2,4,6-tri-O-methyl-D-galactitol, methylated *Virgilia oroboides* gum hydrolysate fraction 6;[77] the sugar was separated from 2,3,6-tri-O-methyl-D-galactose by TLC after periodate oxidation of the borohydride product; an oil was dried *in vacuo* 3 hours.

c. 2,3,6-tri-O-methyl-D-galactitol, methylated *Acacia podalyriaefolia* gum hydrolysate fraction 7, m.p. 86-87°, R_f 0.32 (TLC, solvent (*f*)).

d. 2,3,4-tri-O-methyl-D-galactitol, methylated *Acacia podalyriaefolia* gum fraction 10, m.p. 122-123°, R_f 0.29 (TLC, solvent (*f*)).

e. 4,6-di-O-methyl-D-galactose, a gift from Dr.H.Parolis, Rhodes University, Grahamstown.

f. 2,6-di-O-methyl-D-galactitol, methylated *Acacia elata*[60] gum fraction 10, was reduced with borohydride, and the product, an oil, dried *in vacuo*.

g. 2,4-di-O-methyl-D-galactitol, methylated *Acacia podalyriaefolia* gum, fraction 15, m.p. 136-137°, $[\alpha]_D$ +27.8° (*c* 2.28 in methanol), R_f 0.16 (TLC solvent (*f*)).

h. 2,3-di-O-methyl-D-galactitol, methylated *Khaya senegalensis*, reduced acids fraction J[138], reduced with borohydride and the oil dried *in vacuo*.

i. 6-O-methyl-D-galactitol, prepared by Mr.E.R. Rathbone, Chemistry Department, University of Cape Town, m.p. 142-143°.

(j) 4-O-methyl-D-galactose, a gift from Dr.H.Parolis, Rhodes University, Grahamstown.

(k) 2-O-methyl-D-galactitol, methylated Acacia podalyriaefolia gum hydrolysate fraction 16, m.p. 105°, $[\alpha]_D$ +4.8° (c 1.04 in methanol), R_f 0.06 (TLC, solvent (f)).

(l) D-galactitol, borohydride reduced D-galactitol, m.p. 186-187°.

(m) 2,3,5-tri-O-methyl-L-arabinose, methylated Acacia podalyriaefolia gum hydrolysate fraction 2, $[\alpha]_D$ -24.3° (c 2.34).

(n) 2,3-di-O-methyl-L-arabinose, methylated Acacia Karroo gum hydrolysate fraction 9.[41]

(o) 2,5-di-O-methyl-L-arabinose, methylated Watsonia pyramidata gum hydrolysate fraction 6.[139]

(p) 3,4-di-O-methyl-L-arabinose, methylated Acacia karroo gum fraction 11.[41]

(q) 3,5-di-O-methyl-L-arabinose. A mixture of 2,5-di-O-methyl-L-arabinose and 3,5-di-O-methyl-L-arabinose derived from methylated Watsonia pyramidata gum hydrolysate fraction 5.[139]

(r) L-arabinitol, borohydride reduced L-arabinose, m.p. 100-101°.

(s) 2,3,4-tri-O-methyl-L-rhamnose, methylated Acacia Karroo gum hydrolysate fraction 1.[41]

(t) 3-O-methyl-L-rhamnitol, borohydride reduction of 3-O-methyl L-rhamnose.

(u) L-rhamnitol acetate, an oil, prepared from L-rhamnose.

7.2 Preparation of standards and methylated polysaccharides.

(a) Glycitols

Approximately 1-4 mg of the alditol was accurately weighed out together with a similar weight of 2,3,4,6-tetra-*O*-methyl *D*-galactirol and the mixture was acetylated in acetic anhydride (1 ml) containing anhydrous sodium acetate (20 mg) at 96° for 1 hour. Excess acetic anhydride was removed under reduced pressure. 1-3 Micro-litres of an acetone solution were injected onto the column.

(b) Sugars

Approximately 1-4 mg of the sugar was accurately weighed out together with a similar weight of 2,3,4,6-tetra-*O*-methyl-*D*-galactitol and an aqueous solution was treated with sodium borohydride for 2 days. Excess borohydride was decomposed and the cations were removed with Amberlite IR-120 (H^+) resin. Methanol was evaporated from the residue to remove the borate ions and the product was acetylated as (a) above.

(c) Methylated polysaccharides

Approximately 20-25 mg of the methylated polysaccharide was hydrolysed in *N*-H_2SO_4 (1 ml) containing 98% formic acid (3 drops) in a sealed tube at 96° for 15 hours. The solution was neutralised ($BaCO_3$), reduced and acetylated as in (b) and (a) above respectively.

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