STRUCTURAL INVESTIGATIONS OF THE POLYSACCHARIDE GUM EXUDATES FROM *Brabeium stellatifolium* AND *Grevillea robusta*.

A thesis submited to

THE UNIVERSITY OF CAPE TOWN

for the degree of

D O C T O R O F P H I L O S O P H Y

by

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October, 1971.

CONTENTS

S U M M A R Y

The structures of the polysaccharide gum exudates from Brabeium stellatifolium and Grevillea robusta, both belonging to the family Proteaceae, have been investigated and compared. A general similarity between these two polysaccharide gums, both of high molecular weight, was apparent, although techniques such as methylation analysis, Smith-degradation and partial acid hydrolysis revealed small differences in fine structure.

Brabeium stellatifolium gum was examined in somewhat more detail. In a study of hydrolysis of the gum by acid the degradation process was followed by gel chromatography. The elution curves obtained at different stages of hydrolysis demonstrated the production of fragments of preferred molecular size, and permitted analysis of the degradation pattern in terms of the fission of peripheral and of internal linkages. Hydrolysis of methylated Brabeium stellatifolium gum, followed by separation of the resulting methyl ethers on a cellulose column, gave results in good agreement with those obtained on semi-quantitative GLC analysis of the methanolysate of the methylated gum.

In Smith-degradation studies of both Brabeium stellatifolium and Grevillea robusta gums the conditions generally used for mild acid hydrolysis of the reduced, oxidised polysaccharides were modified to ensure complete cleavage of acetal linkages. Both gums yielded Smith-degradation products exhibiting a high degree of polymolecularity. The relative decreases in weight - average molecular weight $\overline{M}_w$, accompanying successive Smith-degradations were similar for the two gums. A further

point of resemblance was the unusual resistance to periodate oxidation of D-mannose and uronic acid residues in both gums.

Part of the work reported in this thesis has been published, viz;

Acid hydrolysis of the polysaccharide gum exudate from Brabeium stellatifolium - A.M. Stephen and P. van der Bijl, J.S. African Chem. Inst., 24, 103 (1971).

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude to his supervisor, Professor A.M. Stephen, for his guidance, encouragement and interest throughout the course of the work.

Thanks are also due to Dr. S.C. Churms of the C.S.I.R. Carbohydrate Research Unit for help in correcting the final draft of this thesis; Dr. P.I. Bekker* for his practical advice and for many enlightening discussions; Professor E.C. Leisegang for use of the facilities of the Chemistry Department; Dr. K.G. Fuhr for doing the microanalyses and Mr. L.G. Fowle of the Physics Department, U.C.T. for taking X-ray diffraction and electronmicro-photographs.

The South African Council for Scientific and Industrial Research is thanked for financial assistance and granting permission to present the work performed as a thesis.

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A B B R E V I A T I O N S

The following abbreviations are used in this thesis :

Ara	arabinose
b.p.	boiling point
c	concentration
$\overline{DP}$	degree of polymerisation
DMF	dimethyl formamide
eqn.	equation
EtOAc	ethyl acetate
EtOH	ethanol
Fig.	figure
g	gram
GA	glycolaldehyde
Gal	galactose
GlA	glucuronic acid
GLC	gas-liquid chromatography
GPC	gel permeation chromatography
h	hour (s)
IR	infra-red
Kv	kilovolt
Man	mannose
MeOH	methanol
mg	milligram
ml	millilitre
M	molar
mM	millimolar
mm Hg	millimetres of mercury
m.p.	melting point

nm	nanometre
PMR	proton magnetic resonance
PC	paper chromatography
Rham	rhamnose
sec.	second (s)
TLC	thin-layer chromatography
TTC	triphenyl tetrazolium chloride
V	volts
vol.	volume
v/v	volume per volume
wt.	weight
w/w	weight per weight
Xyl	xylose

Abbreviations and symbols omitted from above list are defined in the text.

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1. INTRODUCTION

1.1 Chemical taxonomy of plants

Chemical taxonomic relationships have in recent years proved to be of great value in the botanical classification of plants into particular orders, families, genera and species. In addition to alkaloids, tannins, essential oils and glycosides isolated from various botanical sources, polysaccharide gum exudates from higher plants, especially from the genera Acacia and Prunus, have been widely investigated for taxonomic purposes. Although small differences[1] have been found among gum exudates from plants within the same genus, it has become possible to generalise to a certain extent about the chemical composition to be expected in polysaccharide gums from a certain type of plant.

This approach, however, is limited by the extreme complexities[2] involved in elucidating the structures of the gum exudates. It is doubtful whether even with the methods now available, a structural formula better than Haworth's classical repeating unit, which represents merely the average distribution and position of the sugar residues over a certain number of chain units, can be assigned to a particular polysaccharide gum.

Since relatively little information is available about gum exudates from genera other than those mentioned above, a comparative examination of the polysaccharide gums of two plants of the Proteaceae family, namely Brabeium stellatifolium and Grevillea robusta, was under-

taken, and forms the subject of this thesis. No previous study of the gum of Brabeium stellatifolium has been reported; in the case of the Grevillea robusta exudate only a preliminary investigation of the hydrolysis products has been published.[3]

1.2 The occurrence, function and origin of plant gums.

Polysaccharides are natural macromolecules of almost universal occurrence in living organisms, where they perform a variety of functions, many of which are not fully understood.[4] Their occurrence and importance in nature are well documented,[5,6] as is the value of this group of natural products in industry.[7]

In higher plants and seaweeds, polysaccharides act as skeletal substances in the cell walls, provide reserve food supplies and function as protective agents in the form of exudate gums, sealing off sites of injury from invasion by micro-organisms.[4]

The origin of these gums is uncertain; various theories have been suggested. The gums are considered by some to be products of normal plant metabolism,[5,7,8] whereas others suggest that they arise from pathological conditions of trees.[5] Infection of plants, by bacterial or fungal micro-organisms is also known to have caused gummosis.[5,7,9]

Investigations of the biosynthesis of polysaccharides have shed new light on the enzyme-catalysed reactions involved in the interconversion of sugar units and the polymerisation of these units.[4,10] Knowledge of the actual

processes involved is at present fragmentary, but the most general route for polysaccharide synthesis in nature appears to involve transfer of units from sugar nucleotides[11,12] to growing polysaccharide chains either directly or via an intermediate "carrier." It is believed that the initial synthesis of chains from sugar nucleotides is followed by redistribution of the linkages, under the influence of another enzyme to give branched chains.[13] Recently it has been discovered that in some cases where the polymer contains different sugar units in a fixed sequence, repeating along the chain, the units are transferred from nucleotides to the growing chains, not directly but via the "carrier" mentioned before.[14]

In this way many interconversions take place very readily and efficiently in the living cell. These are at present under extensive investigation.

1.3 Literature survey

In structural polysaccharide chemistry it is customary to compare exudate gums from plants of similar botanical classification.[15] Interest has been largely confined to gums collected from trees of the families Leguminosae and Rosaceae,[16] and among the Leguminosae especially those belonging to the genus Acacia; exudates from some thirty species of Acacia have now been chemically investigated.[15] Little attention has been paid to gums from Proteaceae, the only investigations published being those of exudates from Hakea acicularis[17] (examined in some detail) and Grevillea robusta[3] (a preliminary study only.) Since the Proteaceae family is taxonomically isolated comparisons made in this

thesis between the two Proteaceae gums investigated and other polysaccharide gums, having similar chemical characteristics but originating from plants of different families, must necessarily be of chemical interest rather than taxonomic.

From the chemical point of view, the gums of Brabeium stellatifolium and Grevillea robusta are comparable with other polysaccharide gums of the type usually described by Aspinall[18] as "glucuronomannans." Previous studies of such gums are reviewed below.

1.3.1 Hakea acicularis gum

The gum from Hakea acicularis[17] (family Proteaceae) was shown on analysis to contain D-galactose (58%), L-arabinose (19%), D-xylose (8%), D-mannose (7%) and D-glucuronic acid (8%). Titration gave a value of ca. 2 000 for the equivalent weight of the gum, but this value must be regarded as approximate, owing to the low solubility of the alcohol-precipitated gum in water. The specific rotation $[\alpha]_D$, was -13^0 (c 0.8 in dilute alkali) and the ash content < 1%.

Partial acid hydrolysis[17] (5mM H_2SO_4, 68h, 96^0) released most of the arabinose and some xylose and galactose. Solvent fractionation with methanol afforded the above sugars (ca. 12% by weight), a degraded polysaccharide and a partially soluble oligosaccharide.

Further hydrolysis (0.5 M H_2SO_4, 10h, 96^0) of the degraded polysaccharide afforded, after fractionation, a neutral and an acidic fraction.[17] The latter was shown,

by paper chromatography, to contain glucuronic acid, an aldobiouronic acid and galactose. The aldobiouronic acid was isolated and identified as 2-O-(β-D-glucopyranosyluronic acid)-D-mannose, the mannose almost certainly being linked to galactose in the polysaccharide. The presence of mannose and glucuronic acid in approximately equimolar amounts in the gum suggests that all the mannose is linked through C-2 to glucuronic acid.

Periodate oxidation of the gum resulted in the liberation of acid, the amount per equivalent of polysaccharide being similar to that found in the case of gum ghatti.[17] After partial acid hydrolysis the consumption of periodate per equivalent of polysaccharide was less, but a relatively higher yield of acid was obtained. This suggests the presence of a high proportion of (1→6)-linked galactose units in the backbone of the polysaccharide gum molecule.

1.3.2 Virgilia oroboides gum

The very complex gum of Virgilia oroboides (family Leguminosae) has been investigated in considerable detail by Stephen and his collaborators.[19-24] After purification by precipitation with ethanol from an acidified aqeous solution the gum had $[\alpha]_D$ + 35° and an equivalent weight of 2 100.[19] Acid hydrolysis of the gum showed that the main components were D-galactose (45%), L-arabinose (38%), D-mannose (6%) and D-glucuronic acid (8%). In addition smaller amounts of D-xylose and 4-O-methyl-D-glucuronic acid were found on more detailed examination.

Partial hydrolysis[21] of the gum afforded a large number of oligosaccharides, which were isolated and identified. Among these were 5-O-α-L-arabinopyranosyl-L-arabinose, 5-O-α-L-arabinofuranosyl-L-arabinose, an α-(1→5)- linked L-arabinotriose, 6-O-β-D-xylopyranosyl-L-arabinose, 6-O-β-D-galactopyranosyl-D-galactose and the corresponding β-(1→6)- linked galactrotriose and galactotetraose, 3-O-β-D-galactopyranosyl-D-galactose, O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→3)-D-galactose, 2-O-(β-D-glucopyranosyluronic acid)-D-mannose, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and an aldobiouronic acid composed of 4-O-methylglucuronic acid and galactose. A small amount of 3-O-α-L-arabino-furanosyl-L-arabinose was also isolated.

Methylation analysis[22,23] showed the main framework of the gum to consist of β-(1→6)-linked-D-galactopyranose units, approximately half of these joined (through C-3 and C-4) to short side-chains, composed principally of (i) D-glucuronic acid end-groups β-linked to D-galactopyranose at C-6 or to D-mannopyranose at C-2, and (ii) L-arabino-furanose or L-arabinopyranose end-groups α-(1→5)-linked to L-arabinofuranose. The side-chains are joined to the main chains either directly or through 3- or 6- linked D-galactose or 5- linked L-arabinose units. It was found that the D-mannose residues are subsidiary branch-points.[22,23] Minor structural features include D-xylopyranose end-groups replacing some of the L-arabinopyranose, and D-glucuronic acid residues to which are attached methyl groups or other sugars

at C-4.

Periodate oxidation studies[24] according to the Smith procedure[25] of oxidation and then borohyride reduction followed by controlled hydrolysis of the reduced, oxidised polysaccharide, substantiated previous conclusions regarding the molecular structure of Virgilia oroboides gum and indicated the distribution of periodate-resistant and periodate-vulnerable galactose residues in this polysaccharide.

A structural fragment (I) proposed for Virgilia oroboides gum is shown.

1.3.3 Damson (Prunus insitia) gum

Damson gum (family Rosaceae), which has $[\alpha]_D$ -26° and an equivalent weight of ca. 1 100, is an acidic polysaccharide resembling gum arabic and cherry gum in the viscosity of its aqueous solutions.[26] Examination of the sugars obtained on hydrolysis of the gum[26] showed the presence of: D-glucuronic acid (1 part), D-mannose (1 part), D-galactose (2 parts), L-arabinose (3 parts) and D-xylose (ca. 2%). It is of interest that this investigation is the first in which the aldobiouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was isolated and identified in an hydrolysate from a polysaccharide.[26]

Hydrolysis of the fully methylated derivative of damson gum,[27,28] followed by isolation and identification of the resulting methyl ethers allowed the approximate proportions of O-methyl sugars in the methylated gum to be estimated as follows: 2,3,5-tri-O-methyl-L-arabinose (8 parts), 2,3-di-O-methyl-L-arabinose (4 parts), 2,4,6-tri-O-methyl-D-galactose (3 parts), 2,4-di-O-methyl-

D-galactose (3 parts), 2-O-methyl-D-galactose (1 part), 4(?)-O-methyl-D-galactose (1 part), 2,3,4-tri-O-methyl-D-glucuronic acid (2 parts) and 2,3-di-O-methyl-D-glucuronic acid (2 parts). O-Methyl derivatives of mannose and xylose were also detected.

Methylation of the arabinose-free autohydrolysate of damson gum followed by hydrolysis of the fully methylated product, gave the following methylated sugars: 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-glucuronic acid, and an unidentified derivative of D-mannose, with a trace of 4,6-di-O-methyl-D-galactose. Since the 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose did not occur among the hydrolysis products of the methylated whole gum, it was inferred that the arabinose in the gum occurs in the furanose form and is attached to galactose. It is these galactose residues which give rise to the 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose detected in the hydrolysate of the methylated derivative of the degraded, arabinose-free gum.

Since autohydrolysis of damson gum[28] resulted in the removal of L-arabinose residues only and hydrolysis of the fully methylated undegraded gum afforded 2,3,5-tri-O-methyl-L-arabinose and 2,3-di-O-methyl-L-arabinose in the ratio 2:1, it was suggested that in each repeating unit, composed of

arabinose (3 parts), galactose (2 parts), mannose (1 part) and glucuronic acid (1 part), there are two side chains consisting entirely of L-arabinose, linked to C-3 or C-6 of the galactose residues comprising the main chain of the molecule. It seems unlikely that any of the D-glucuronic acid residues are linked to arabinose, since the relative proportions of 2,3,4-tri-O-methyl-and 2,3-di-O-methyl-D-glucuronic acid in the hydrolysate of the methylated polysaccharide appear to be unaffected by prior autohydrolysis of the gum. It appears, however, that L-arabinose residues are attached to those galactose residues which give rise to mono-O-methyl-D-galactoses on hydrolysis of the methylated gum since 2- and (possibly) 4-O-methyl-D-galactose (the latter assignment is uncertain) were found to be present in the products of hydrolysis of the methylated whole gum, but not in those of the methylated degraded gum. It is however possible that the occurrence of these sugars was due to undermethylation, but it is believed that they are in fact structurally significant.

1.3.4 Cherry (Prunus cerasus) gum

The gum exudate from the cherry tree (family Rosaceae) has been found[29] to be very similar to damson gum. The gum has $[\alpha]_D$ -28^0 and an equivalent weight of ca. 1 500. Quantitative analysis of a hydrolysate showed the polysaccharide to consist of the following sugars in the proportions indicated: D-glucuronic acid (1 mol), D-galactose (2 mol), D-mannose (1 mol) and L-arabinose (6 mol). A small amount (ca. 1.5%) of D-xylose was also found to be present. The biouronic acid, 2-O-(β-D-glucopyranosyluronic acid)-D-

mannose yielded by damson gum, was obtained from cherry gum and from the hydrolysate of the degraded, arabinose-free polysaccharide.

Hydrolysis of the fully methylated derivative of cherry gum[30] yielded the following methylated sugars: 2,3,5-tri-O-methyl-L-arabinose, 2,5-di-O-methyl-L-arabinose, 2,4,6-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3-di-O-methyl-D-glucuronic acid. Two unidentified sugars, probably derivatives of D-mannose were also separated.

Partially degraded, arabinose-free cherry gum was also methylated and then hydrolysed.[31] The resulting methylated sugars, separated by cellulose column chromatography, were identified as: 2,3,4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose, 2,6-di-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3-di-O-methyl-D-glucuronic acid.

These results permit general conclusions to be drawn about the structure of cherry gum, as follows. Degraded cherry gum (arabinose-free) is a branched-chain polysaccharide containing terminal residues of D-galactopyranose and D-xylopyranose; the arabinose in the whole gum is presumably linked to these residues. The presence of both (1→3)- and (1→6)- linkages between D-galactopyranose residues has been demonstrated. The occurrence of 2,4-di-O-methyl-D-xylose in the methylated derivative of the degraded gum (and probably also in that of the undegraded gum) indicates that the D-xylopyranose residues are substituted through C-3, and not through C-2 as in some plant mucilages.

Since the methylated derivatives of both the degraded and the undegraded gums gave on hydrolysis, 2,3,4-tri-O-methylglucuronic acid and 2,3-di-O-methylglucuronic acid it appears that these residues are not linked to L-arabinofuranose residues.

Periodate oxidation[31] of cherry gum liberated approximately 2 molecules of formic acid per equivalent of the gum, but in view of the occurrence of glucuronic acid residues as end groups, the structural significance of this result is doubtful. The proportion of galactose was virtually unaffected on oxidation (dropping slightly from 25.6% to 24.5%), but the xylose was completely removed and the arabinose content fell from 52.2% to 20.2%. The fate of the mannose and uronic acid was uncertain. These results confirm that most of the galactose residues present in the gum and approximately 50% of the arabinose are periodate-invulnerable. The proportion of periodate-resistant arabinose residues is in accordance with that indicated by the yield of 2,5-di-O-methyl-L-arabinose obtained on hydrolysis of the fully methylated gum.

1.3.5 Gum ghatti (from Anogeissus latifolia)

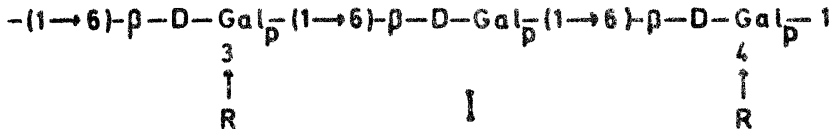
Gum ghatti,[32] from Anogeissus latifolia (family Combretaceae), having $[\alpha]_D$ -50^0 and equivalent weight 1 600, is composed as follows: L-arabinose (5 mol), D-galactose (3 mol), D-mannose (1 mol), D-xylose (0.5 mol), D-glucuronic acid (1 mol) and traces (< 1%) of methylpentose. Two aldobiouronic acids, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose,

were isolated and identified after graded hydrolysis of the gum.

On hydrolysis of the fully methylated gum,[33] followed by separation and characterisation of the resulting methyl ethers, the following O-methyl sugar residues were found to be present in the methylated gum, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose, 2-O-methyl-D-galactose, 4-O-methyl-D-mannose, 2,3,4-tri-O-methyl-D-glucuronic acid, 2,3-di-O-methyl-D-glucuronic acid, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5-tri-O-methyl-L-arabinose, 2,3-di-O-methyl-L-arabinose, 2,4-di-O-methyl-L-arabinose, 2,5-di-O-methyl-L-arabinose and 3,5-di-O-methyl-L-arabinose.

The methylated derivative of the autohydrolysed gum contained all the above O-methyl sugars and in addition 2,3-di-O-methyl-D-galactose, 3,4,6-tri-O-methyl-D-mannose and 2,3,4-tri-O-methyl-D-xylose.

Partial hydrolysis of the gum gave rise to two series of neutral oligosaccharides[34] (see structures II and III). It was suggested at first that these oligosaccharides arose from the basal chains of the polysaccharide, and that the two aldobiouronic acids also produced on hydrolysis were attached as side-chains. However, Smith-degradation and subsequent partial hydrolysis of the product yielded the following four disaccharides: 3-O-(β-D-galactopyranosyl)-D-galactose, 6-O-(β-D-galactopyranosyl)-D-galactose, 3-O-(β-D-galactopyranosyl)-L-arabinose and 3-O-(L-arabinopyranosyl)-D-mannose.[34] These results implied that D-mannose residues are present in the molecular core; chains of (1→6)-linked β-D-galactopyranose residues are believed to be joined



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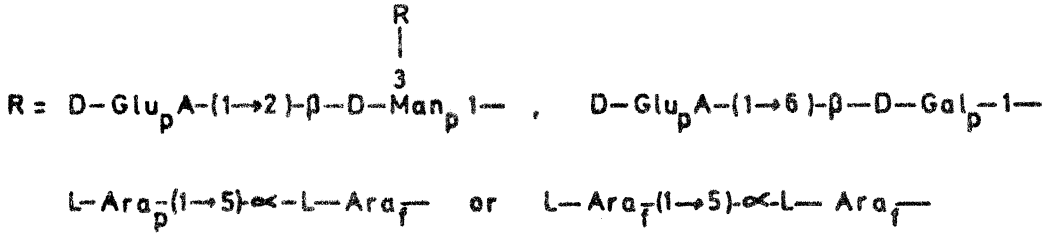
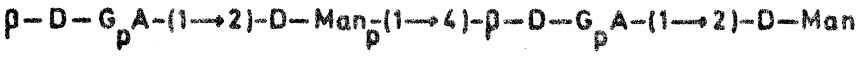


II

III



IV

through L-arabinopyranose to D-mannose residues in the basal chain of the polysaccharide.[35] Isolation of an acidic oligosaccharide[36] (structure IV) in an hydrolysate provided evidence for the sequence of the sugar residues in the interior of the molecule, and a partial structure (V) for the gum was postulated.

Little information is as yet available on certain structural aspects of the gum, e.g. the position of some of the L-arabinose residues. The largest proportion of these occur as furanose end-groups,[33] attached as side chains to the (1→6)-linked β-D-galactopyranose residues, but some nonterminal units, e.g. 2-, 3- and 5-O- substituted L-arabinofuranose as well as 3- and 4-O- substituted L-arabinopyranose, have been recognised.[33] Only some of these have been placed (see structure V); the others probably occur in peripheral chains. Uncertainty also exists about the positions of some (1→3)-linked β-D-galactopyranose chains and of the aldobiouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose. Since D-glucuronic acid end-groups occur in the gum, the possibility exists that these aldobiouronic acid units terminate some of the (1→6)-linked chains of β-D-galactopyranose residues, as shown in structure V.

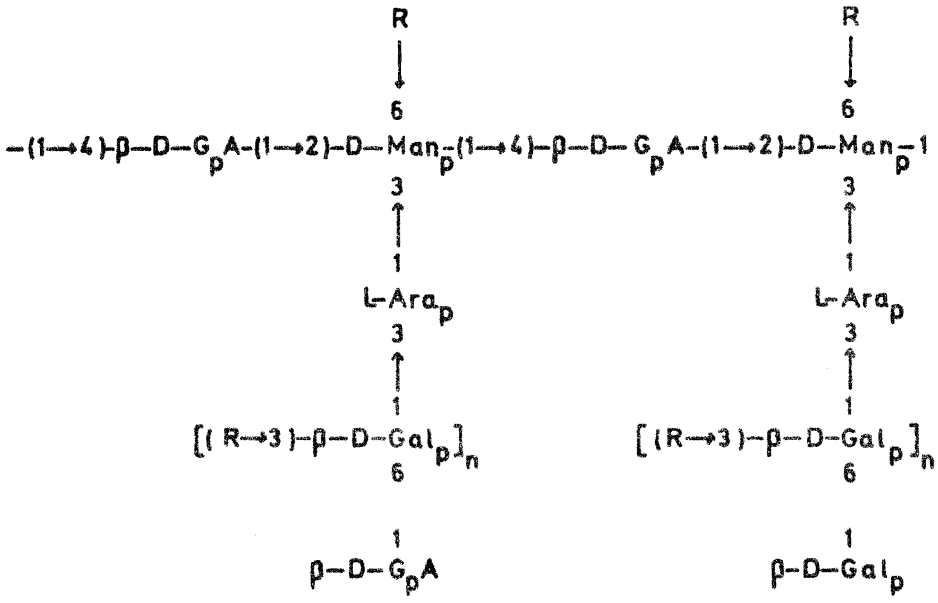
1.3.6 Anogeissus leiocarpus (formerly Anogeissus schimperi) gum

The gum exudate from Anogeissus leiocarpus (family Combretaceae) was found, in a preliminary investigation,[37] to contain the following sugars: L-arabinose (32%),

D-xylose (12%), D-galactose (5%), D-mannose (2%) and traces of rhamnose, ribose and fucose. The uronic anhydride content was ca. 22% and a mixture of acidic oligosaccharides (20%) was obtained on hydrolysis. The two biouronic acids, 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and 6-O-(β-D-glucopyranosyluronic acid)-D-galactose present in gum ghatti were detected in this hydrolysate.

More recent investigations[36,38] have shown that two distinct but structurally related polysaccharides were present in the gum. The major component has been designated leiocarpan A, the minor component leiocarpan B. Leiocarpan A has been studied in detail.[36,38,39] Its glucuronomannan accounts for over 50% of the polysaccharide; most of the attached side-chains consist of single sugar residues. This is in contrast to the situation in gum ghatti, in which the glucuronomannan chain constitutes a relatively small proportion of the polysaccharide structure. When fully methylated leiocarpan A was carboxyl-reduced and then hydrolysed,[36,38,39] and the resulting methylated sugars were characterised, the major components found were 2,3,4-tri-O-methyl-D-xylose, 3,4,6-tri- and 3,4-di-O-methyl-D-mannose and 2,3-di-O-methyl-D-glucose. Minor products detected were 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-D-galactose, 2,3,4-tri-, 2- and 3-O-methyl-D-glucose, 4-O-methyl-D-mannose, 2,3,6-tri-, 2,6-di- and 2-O-methyl-D-galactose, 2,3,4-tri-, 2,4-di- and 2-O-methyl-L-arabinose.

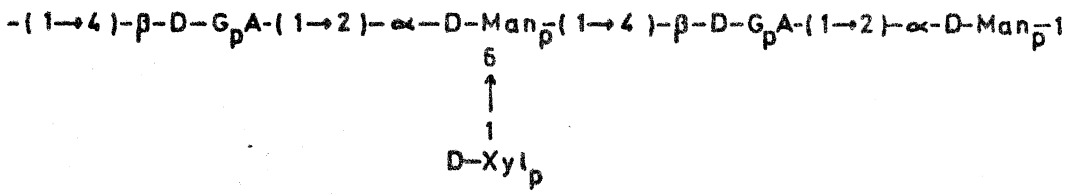
A partial structure VI has been proposed for the degraded polysaccharide obtained on controlled acid



V

R= L-Araf-(1, or, less frequently, L-Araf-(1→2)-L-Araf-(1,

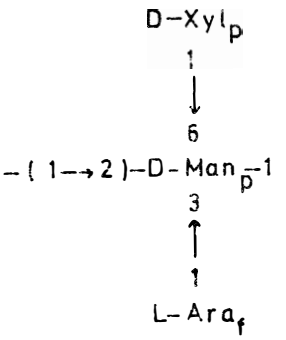
L-Araf-(1→3)-L-Araf-(1, or L-Araf-(1→5)-L-Araf-(1.



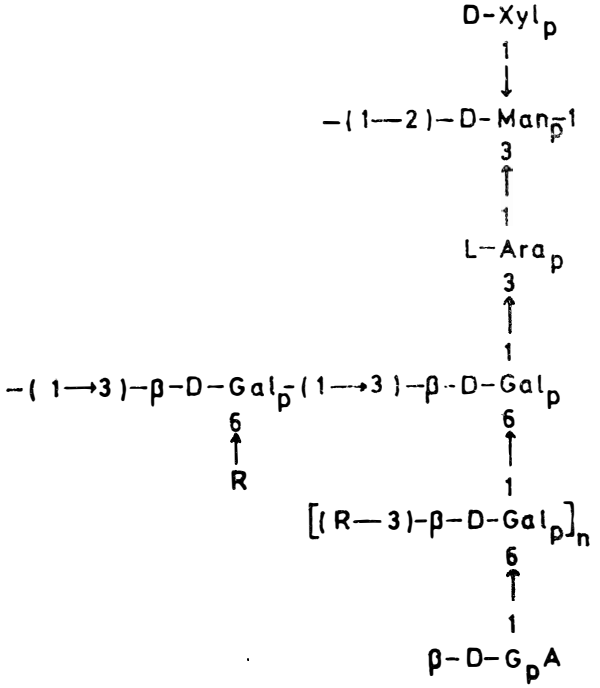
VI

hydrolysis of leiocarpan A, on the basis of methylation data and the identification of the acidic tetrasaccharide (structure IV)[36] in the hydrolysate. Acetolysis of carboxyl-reduced leiocarpan A afforded the oligosaccharides 2-O-β-D-glucopyranosyl-D-mannose, 4-O-α-D-mannopyranosyl-D-glucose and a series of higher oligosaccharides containing alternating residues of D-glucose and D-mannose.[39] From these results it was inferred that the basal chains of leiocarpan A consist largely, if not exclusively, of alternating 4-O-substituted β-D-glucuronic acid and 2-O-substituted α-D-mannopyranose residues. Gum ghatti and leiocarpan A can thus be termed true glucuronomannans.

As a result of further studies, it has become possible to propose partial structures VII and VIII for the polysaccharide leiocarpan A.[38] Examination of the degraded polysaccharide produced on Smith-degradation of the carboxyl-reduced leiocarpan A[36,38,39] has enabled the structure of the side-chains to be postulated. A large proportion of L-arabinofuranose residues possibly occur as single-unit side-chains linked (1→3) to D-mannopyranose residues as in VII. These side-chains are joined through L-arabinopyranose "link" units to core residues of D-mannose (see Structure VIII). Residues of D-galactose, a minor constituent of leiocarpan A, are believed to be linked similarly to those in gum ghatti. Differences in length and degree of branching of the galactan side-chains are suspected. Partial characterisation[36] of a trisaccharide in an hydrolysate as



VII



VIII

R = L-Ara f-(1, or L-Ara f-(1→3)-L-Ara f-(1.

O-(D-glucopyranosyluronic acid)-(1→6) O-D-galactopyranosyl-(1→3)-L-arabinose indicates that some side-chains may contain a single D-galactopyranose residue terminated by a D-glucuronic acid residue, while others may consist of two or more D-galactopyranose residues. When the Smith-degraded carboxyl-reduced leiocarpan A was subjected to another Smith-degradation the unprotected (1→6)-linked β-D-galactopyranose residues were removed, while those involved in (1→3)-linkages remained, the suggestion was made[38] that the latter are attached as branches to the innermost D-galactopyranose residues in structure VII.

Residues of D-mannopyranose are mainly doubly branched, and they probably also carry single D-xylopyranose residues at C-6. Further peripheral L-arabinofuranose residues are located at the end of some galactan chains as shown in VII.

1.3.7 Encephalartos longifolius gum.

The gum exudate from Encephalartos longifolius (family Cycadaceae), having $[\alpha]_D$ -28° and equivalent weight 600, may contain more than one molecular species and is the first polysaccharide reported to contain 3-O-methyl-L-rhamnose as a significant constituent.[40,41] Partial acid hydrolysis (0.25 M H_2SO_4, 11h, 96°) released the following monosaccharides in the proportions indicated (approximate percentages by weight of the total carbohydrate content of the gum): 3-O-methyl-L-rhamnose (3), L-rhamnose (14), L-fucose (0.2), D-xylose (4), L-arabinose (8), D-mannose (0.5) and D-galactose (8). The amount of D-mannose released on hydrolysis is very small and it is believed that most of the D-mannose

is linked to D-glucuronic acid; this may account for the low mannose content of the hydrolysate described above. Of the D-galactose residues released, approximately one-third or the total D-galactose are presumably end-groups, chain units or branch points to which uronic acid residues are not attached.

On partial hydrolysis with acid as described above, three aldobiouronic acids were also released; these were characterised as 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose. In addition, a mixture of higher oligosaccharides containing residues of D-glucuronic acid and D-mannose, and probably consisting of the tetrasaccharide IV and higher homologues was isolated.[41] To confirm the presence of this repetitive sequence of D-glucuronic acid and D-mannose residues, the fourth member of the homologous series identified was methylated and hydrolysed; the major neutral product was 3,4,6-tri-O-methylmannose. Methanolysis of the methylated oligosaccharide yielded large amounts (as methylglycosides) of 2,3,4-tri-O-methylglucuronic acid, 2,3-di-O-methylglucuronic acid and 3,4,6-tri-O-methylmannose, and a small proportion of 2,3,4-tri-O-methylgalactose.

From the above results it is apparent that the chains of alternating D-glucuronic acid and D-mannose residues which are found in the polysaccharide leiocarpan A from Anogeissus leiocarpus[36,38,39] and in gum ghatti[35] may be present in the gum from Encephalartos longifolius. The major part of this polysaccharide, however consists of D-glucuronic acid bound to a galactan framework with peripheral sugars attached.

Methylation analyses of the fully methylated derivative of Encephalartos longifolius gum were complicated by the number and nature of the methylated sugars present.[40] A tentative assignment[40,41] was made, however; the methylated gum is believed to contain residues of 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5-tri-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,4-tri-O-methyl-D-glucuronic acid, 2,3,4-tri-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose, 2,5-, 2,3- and/or 2,4-di-O-methyl-L-arabinose, 2,3-di-O-methyl-D-glucuronic acid, 2,4-di-O-methyl-D-galactose, 4,6-di-O-methyl-D-mannose and 2-O-methyl-D-galactose. This suggests the presence in the gum of end-groups of 3-O-methyl-L-rhamnopyranose, L-rhamnopyranose, and L-arabinofuranose with L-arabinopyranose and (probably) D-xylopyranose residues also in the periphery; D-galactopyranose residues probably occur as chain units linked through C-3 or C-6, or branch points linked through C-3 and C-6 while D-mannopyranose residues are present partly as end-groups but also as branch points linked through C-2 and C-3.[41] The large proportion D-glucopyranuronic residues are probably present as end-groups or chain-units linked through C-4 to D-mannose as in some of the other gums of the glucuronomannan group[18] or to L-rhamnose as in *Acacia*[42] gums.

1.3.8 General comparison of the gums discussed in sections 1.3.1-1.3.7

The aldobiouronic acid 2-O-(β-D-glucopyranosyluronic

acid)-D-mannose, first isolated as a product of partial acid hydrolysis of damson[26] and cherry[29] gums, has now been shown to be an integral unit of many polysaccharide gums notably those from plants of the Prunus species.[18] In a series of investigations of gums from various types of cherry tree[43,44,45] as well as blackthorn[46] and apricot[47] trees it was found that partial acid hydrolysis furnished tri- and higher oligosaccharides consisting of residues of D-glucuronic and D-mannose, together with other products. Not all the gums containing 2-O-(β-D-gluco-pyranosyluronic acid)-D-mannose have been examined in detail; the foregoing discussion has therefore been confined to those gums for which some structural conclusions have been drawn and which bear a chemical resemblance to the gums of Brabeium stellatifolium and Grevillea robusta.

The information available at present does not permit generalisation about the location of the 2-O-(β-D-gluco-pyranosyluronic acid)-D-mannose in these gums. Evidence that the aldobiouronic acid units are located in interior chains within the molecular framework of the polysaccharide, exists in the cases of gum ghatti[35] and Anogeissus leiocarpus gum,[39] where alternating units of D-glucuronic acid and D-mannose, mutually linked, have been found. In the gum of Virgilia oroboides,[19-24] however, units of the aldobiouronic acid appeared to be linked to D-galactose in terminal positions (see structure I). Another problem is possible; heteropolymolecularity of some of the gums

e.g. that of Encephalartos longifolius,[40,41] in which the glucuronomannan chains, if present probably constitute a relatively small proportion of the polysaccharide material, which is difficult to fractionate.

The gums which have been discussed contain similar sugar residues, Encephalartos longifolius[40,41] gum containing in addition fucose and a significant amount of 3-O-methyl-L-rhamnose. Other common features include the presence of D-galactopyranose chains of different lengths, the proportion of β-(1→3) and β-(1→6)-linkages between these residues varying. Residues of L-arabinose occur in the gums mainly as furanose end-groups and to a lesser extent in short chains or as isolated "link" units. The four types of nonterminal units of arabinose found in these gums are (1→2)-, (1→3)-linked L-arabinofuranose, (1→4)-linked L-arabinopyranose or (1→5)-linked L-arabinofuranose residues or both, and (1→3)-linked L-arabinopyranose residues as shown by the presence of 3,5-, 2,5-, 2,3-and 2,4-di-O-methylarabinose in hydrolysates of the methylated gums.

The aldobiouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose has been isolated from gum ghatti,[35] and the gums from Anogeissus leiocarpus,[36,38,39] Virgilia oroboides,[19-24] and Encephalartos longifolius,[40,41] as well as in gums from certain plants of the Prunus species,[18] more recently investigated.

Specific rotations of the gums discussed were negative in most cases while their equivalent weights seemed to lie in the ranges 500 - 3 000.

D I S C U S S I O N

2. Brabeium stellatifolium gum

2.1 The occurrence and characteristics of Brabeium stellatifolium

Brabeium stellatifolium, also known as the Wild Almond, has the distinction of being the first indigenous tree to be cultivated in South Africa.[48] This tree will always be romantically linked with history, since in 1661 Jan van Riebeeck planted a hedge of wild almonds, both as a boundary to the Cape Colony and to prevent Hottentots from stealing cattle. What remains of this historic hedge, still to be seen in the Botanical Gardens at Kirstenbosch C.P., is now protected as a National Monument.[48,49]

This very large, spreading, evergreen tree, belonging to the order Proteales, family Proteaceae and tribe Persoonioideae, grows wild in the South-Western Cape near rivers and moist places. The tree can grow to a height up to 7,5 metres and has a dense, light green foliage, the pointed leaves being arranged in whorls at intervals around the stem.[48,49] Tanning material, about 13%, is found in the thick, greyish-brown bark of the tree.[50] Fragrant, white, fluffy flowers appear in summer and the fruits, resembling almonds in shape and size, ripen in autumn, but are poisonous since they contain hydrocyanic acid.[50] When the tree is injured and favourable climatic conditions prevail, a relatively small quantity of polysaccharide gum is exuded.

Gum exudate from a tree grown in Kirstenbosch, C.P., and identified as Brabeium stellatifolium by courtesy of the

Compton Herbarium (National Botanical Gardens, Kirstenbosch, C.P.) was used in the investigations described in this thesis.

2.2 Purification and characteristics of Brabeium stellatifolium gum

When Brabeium stellatifolium gum was vigorously stirred in cold water an extremely viscous suspension was formed (see section 4.2.1.1). Titration afforded a clear aqueous solution, which was deionised with Amberlite IR-120(H^+) and freeze-dried. The white fluffy product thus obtained was used, without further purification, throughout this investigation.

The low solubility of the freeze-dried gum in water necessitated the use of dilute alkali as solvent for the measurement of the specific rotation $[\alpha]_D$, which had a small negative value *i.e.* -6^0 (*c* 0.17, M NaOH). Analysis of a portion of the gum showed C,36.8;H,6.7; N<0.3; S,<0.2; OMe, 0.8 and ash 1.8%. The 4-*O*-methyl derivative of *D*-glucuronic acid has been found to be present in a number of polysaccharides of plant origin;[51] this may account for the methoxyl content of Brabeium stellatifolium gum.

The equivalent weight of the gum was found to be *ca.* 2 500 (corresponding to 8 mol % of uronic anhydride) of an electrodialysed solution of the freeze-dried gum in water. This value is, however, approximate owing to the low solubility of the gum in water.

Prolonged acid hydrolysis (0.5 M H_2SO_4, 96^0, 6h), followed by reduction, acetylation and quantitative gas-liquid chromatography of the products gave the relative amounts of neutral

sugar residues present in the gum (see Fig. 17). Isolation of these sugars by preparative paper chromatography,[52] measurement of their optical rotations and transformation of the sugars into suitable derivatives provided the necessary identification of the residues constituting the polysaccharide. The presence of residues of L-arabinose (52), D-galactose (37), D-xylose (7), D-mannose (3) and (probably) L-rhamnose (<1) has been established in this manner, figures in parenthesis giving percentages on a molar basis.

A biouronic acid, identified as 2-O-(β-D-glucopyranosyluronic acid)-D-mannose, and a uronic acid, identified as D-glucopyranuronic acid were isolated by chromatography of the hydrolysis products on ion-exchange resins Amberlite IR-45(OH^-) and Amberlite IR-120(H^+) followed by preparative paper chromatography.[52] Very faint traces of another acid, possibly 4-O-methyl-glucopyranuronic acid on the basis of a comparison with standard substances, were also observed on paper chromatography.

Attempts to determine the molecular weight of Brabeium stellatifolium gum by chromatography on Bio-Gel P-300 were unsuccessful, since the only peak observed in the elution curve was obtained at the void volume of the column, indicating total exclusion of the molecules from the matrix of this gel, and hence a weight-average molecular weight $\bar{M}_w$, exceeding 80 000, the upper limit of the polysaccharide fractionation range of the gel used. Chromatography on the agarose gel Sagavac 6F which has a much higher exclusion limit than Bio-

Gel P-300, showed Brabeium stellatifolium gum to have a weight-average molecular weight $\bar{M}_w$, of 560 000. Although the elution curve (see later) showed only one peak, polymolecularity was later demonstrated in a study of the hydrolysis of the gum by acid (see section 2.3).

Electron microscopy of the Brabeium stellatifolium gum polysaccharide revealed long fibres of variable thickness interspersed with a number of small spherical particles. Some branches in the fibres were observed, but unfortunately the resolution of the instrument used was insufficient to permit finer details of the molecular structure to be displayed.

A diffuse X-ray diffraction pattern was obtained; this is often indicative of a highly branched structure.[4,53]

2.3 Acid hydrolysis of Brabeium stellatifolium gum[54]

In an attempt to investigate the mode of depolymerisation of the gum in a quantitative manner and to obtain more information about its complex molecular structure, the hydrolysis of the gum by acid was studied in detail, the degradation process being followed by gel chromatography. The extent of depolymerisation of the polysaccharide in hydrolysate samples removed after various time intervals and the average rate of hydrolysis over each of these time intervals were determined, and the molecular-weight distribution of these hydrolysate samples were found from the elution patterns obtained on gel chromatography.

A similar study had previously been conducted on the gum exudate from stems of Acacia podalyriaefolia;[55] this permitted comparison of such data as rates of hydrolysis, mode of depolymerisation etc., for the two gums in relation to their individual structures. However, in contrast to those of Acacia podalyriaefolia gum,[55] the molecular-weight distributions of the degradation products of Brabeium stellatifolium gum were complicated by polymolecularity of the parent polysaccharide (see later).

The gum was hydrolysed in two stages, as described below in sections 2.3.1 and 2.3.2.

2.3.1 Partial hydrolysis in 5mM acid.

On hydrolysis of the gum in 5mM acid it was found (paper chromatography) that arabinose was released within 2h, the quantity released increasing with time of hydrolysis. After 5h traces of galactose and xylose were detected, in addition to six neutral oligosaccharides. From the linearity[56] of plot of log $\left[\left(\frac{1}{R_F}\right)-1\right]$ vs. degree of polymerisation (hexose units) for these oligosaccharides and authentic D-galactose and 3-O-β-D-galactopyranosyl-D-galactose (see Fig. 15) and in some cases by comparison of their paper chromatographic mobilities against those of standards, the oligosaccharides were judged to be a series of β-(1→3)-linked D-galactopyranose oligomers. Two further components, later identified as the acids 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and D-glucopyranuronic acid, appeared after hydrolysis for 12h.

A plot of the degree of scission, x, vs. time of

hydrolysis (Fig. 1) shows an initial rapid rise which decreases smoothly, until at x = 0.5 (46h) the increase in x with time is barely perceptible. Most of the arabinoside linkages (comprising approximately 50% of the total) are presumably broken at this stage. There follows a gradual, approximately linear, upward trend to x = 0.64 (96h); during this stage fission of galactopyranoside linkages is predominant.

From Table 6 it is evident that $[\alpha]_D$, initially -6^0, rises rapidly during the first 8h of hydrolysis and levels off abruptly after 12h, reaching $+58^0$ at 96h. The positive increase in rotation is consistent with the scission of arabinofuranoside linkages and is typical of plant gums containing this structural feature. For instance, for Acacia podalyriaefolia gum the rise in optical rotation on hydrolysis has been attributed to fission of α-L-arabinofuranoside bonds.[55]

The first-order hydrolysis rate-constant k, (see Table 6) has, between 2 and 5h, after the commencement of hydrolysis an average value of 18.3×10^{-6} sec^{-1}, which is considerably lower than the value of k (260×10^{-6} sec^{-1}) found[57,58] for methyl α-L-arabinofuranoside in 5 mM acid at 100^0. The hydrolysis of polysaccharides is presumably affected by steric and other factors which do not necessarily influence the rate of hydrolysis of simple glycosides. The value of k found for Brabeium stellatifolium gum during the initial 5h period of hydrolysis is in turn higher than that (1.24×10^{-3} sec^{-1}) found under comparable conditions

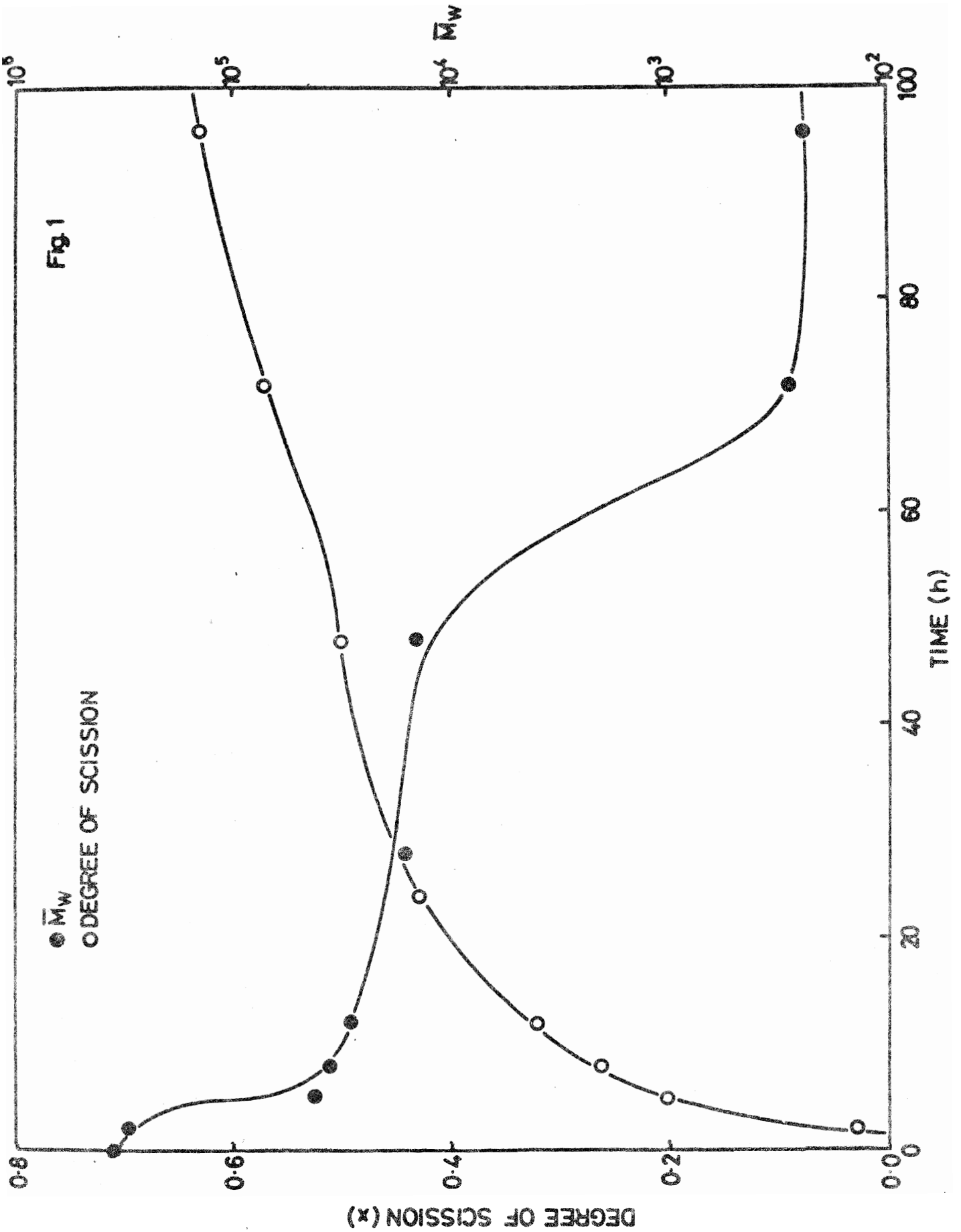


Fig. 1
$\bar{M}_w$
DEGREE OF SCISSION
$\bar{M}_w$
10^5
10^4
10^3
10^2
100
80
60
40
20
0
TIME (h)
0·8
0·6
0·4
0·2
0·0
DEGREE OF SCISSION (x)

for Acacia podalyriaefolia gum,[55] as expected from the higher arabinose content of the former. As the time of hydrolysis increases the value of k decreases; the fission of galactopyranoside linkages in the gum proceeds approximately 10 times more slowly (average $k = 1.8 \times 10^{-6}$ from 48 to 96h) than that of arabinofuranoside. This slower decrease in k for the acid hydrolysis of polysaccharides relative to those of the respective methyl glycosides, was also found for a larch arabinogalactan studied by Bouveng.[59]

2.3.1.1 Molecular weight distribution at successive stages of hydrolysis

The decrease of $\bar{M}_w$ with increasing time of hydrolysis of the gum in 5 mM acid is shown in Fig. 1, where a log scale of $\bar{M}_w$ is used for convenience. The elution patterns from which these $\bar{M}_w$ values were calculated are illustrated in Figs. 3-10.

After 2h ($x = 0.03$) hydrolysis of the gum in 5 mM acid, the elution pattern (Fig. 3), shows that the bulk of the gum still has a high molecular weight; the portion of the hydrolysate of molecular weight below 30 000 consists largely of the monosaccharides revealed by paper chromatography. The appearance in this elution pattern of a peak at molecular weight 1 580 000 which was not evident in that of the undegraded gum (Fig. 2) demonstrates the polymolecularity of the gum; the decrease in the amount of material of high molecular weight after hydrolysis for 2h presumably facilitates resolution of the various components on the gel column.

Legend to Figures 2 - 14

(Elution patterns of whole and partially degraded *Brabeium stellatifolium* gum on various gels.)

Figure 2.

Undegraded Brabeium stellatifolium gum on Sagavac 6F gel. For the undegraded gum $\bar{M}_w$ = 560 000.

For the following diagrams, the time of hydrolysis, the acid concentration, degree of scission (x) and the gel used for obtaining the elution pattern, are given. Also the molecular weights corresponding to peaks as numbered are given.

Figure 3.

Time of hydrolysis : 2 h

Acid concentration : 5 mM H_2SO_4

Degree of scission (x) : 0.03

Gel used : Sagavac 6F

A, 580 000; B, 560 000; C, 222 000; D, 140 000; E, $\leq$ 30 000

Figure 4.

Time of hydrolysis : 5 h

Acid concentration : 5 mM H_2SO_4

Degree of scission (x) : 0.21

Gel used : Sagavac 6F

A, 560 000; B, 390 000; C, 220 000; D, 140 000; E, 89 000; F, 40 000; G $\leq$ 30 000

Figure 9

Time of hydrolysis : 72 h

Acid concentration : 5 mM H_2SO_4

Degree of scission (x) : 0.58

Gel used : Bio-Gel P-10

A, 5 010; B, 3 630; C, 2 290. D, 1 440; E, 1 030; F, 720; G, $\leq$ 260

Figure 10

Time of hydrolysis : 96 h

Acid concentration : 5 mM H_2SO_4

Degree of scission (x) : 0.64

Gel used : Bio-Gel P-10

A, 5 010; B, 3 630; C, 2 290; D, 1 440; E, 1 140; F, 910; G, 720; H, $\leq$ 260

Figure 11

Time of hydrolysis : 1 h

Acid concentration : 50 mM H_2SO_4

Degree of scission (x) : 0.76

Gel used : Bio-Gel P-10

A, 5 010; B, 3 630; C, 2 290; D, 1 440; E, 910; F, $\leq$ 260

Figure 12

Time of hydrolysis : 3 h

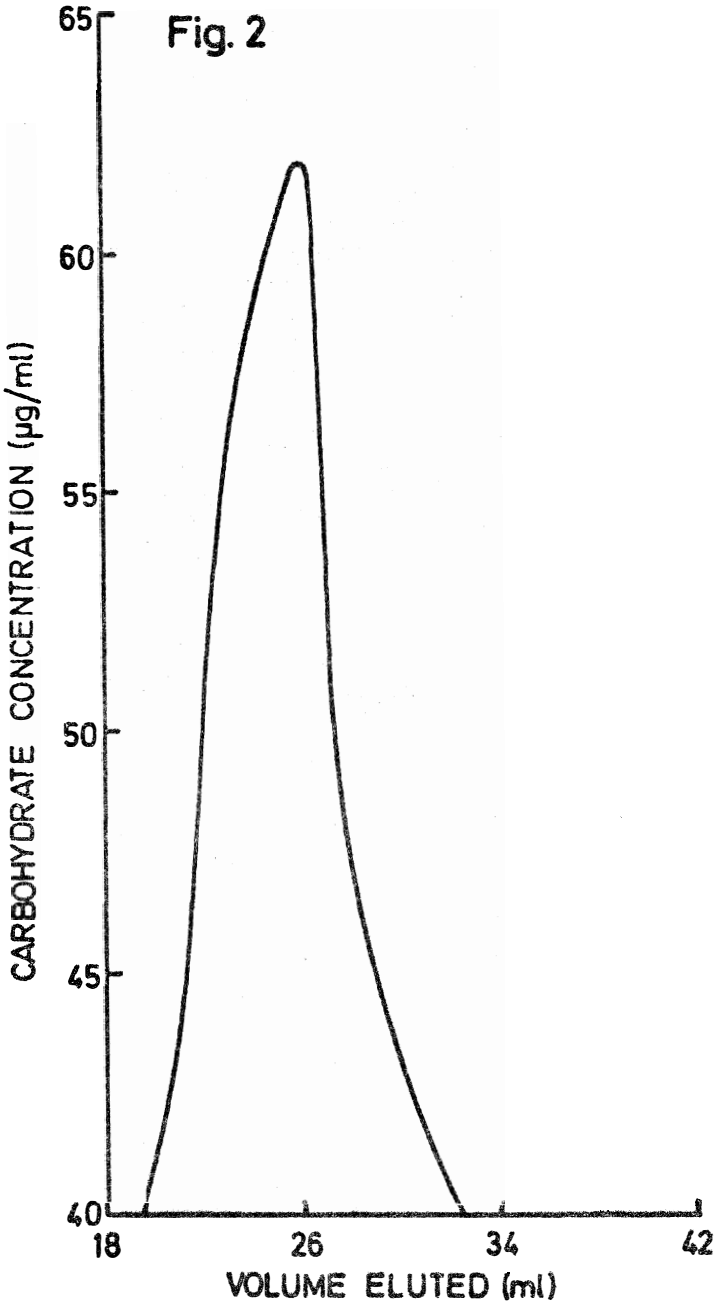
Acid concentration : 50 mM H_2SO_4

Degree of scission (x) : 0.77

Gel used : Bio-Gel P-10

A, 5 010; B, 3 630; C, 2 290; D, 1 440; E, 1 140; F, 720; G, $\leq$ 260

Fig. 2


65
60
55
50
45
40
CARBOHYDRATE CONCENTRATION (µg/ml)
18
26
34
42
VOLUME ELUTED (ml)

After 5h hydrolysis (x = 0.21) the elution pattern (Fig. 4) shows large quantities of material of molecular weight below 390 000, with peaks at regular intervals of elution volume; some of these, e.g. that at molecular weight 140 000, are greatly enhanced in Fig. 5 (8h hydrolysis; x = 0.26).

After 12h (x = 0.32) most of the polysaccharide has molecular weight below 30 000 (Fig. 6), although further components of high molecular weight (1 580 000 and 891 000) are now resolved on the gel. The large decrease in the overall $\bar{M}_w$ (at this stage the value is less than one-tenth of that of the gum) indicates some cleavage of internal linkages in addition to the usual rapid hydrolysis of peripheral arabinofuranoside linkages and selective removal of those galactopyranose residues which occur as end-groups.[55] Fig. 7 (24h hydrolysis; x = 0.43) which represents elution from Bio-Gel P-300 instead of Sagavac 6F gives information on the material of molecular weight below 30 000 which comprises the bulk of the sample. The overall $\bar{M}_w$ (20 500) is about $\frac{1}{15}$ of the theoretical value (300 000) calculated on the assumption of hydrolysis of peripheral linkages only; this suggests that, on the average, approximately 14 internal linkages per molecule have been broken, at this stage.

It is significant that among the numerous peaks in Fig. 7 there are several pairs representing molecular weights in the ratio 1:2, viz. Q and H, O and G, N and F, M and D, K and C, C and B. The molecules of preferred size that are grouped at peaks B, C, D, F, G and H apparently tend to undergo preferential fission near the centre. Fig. 8 (48 h

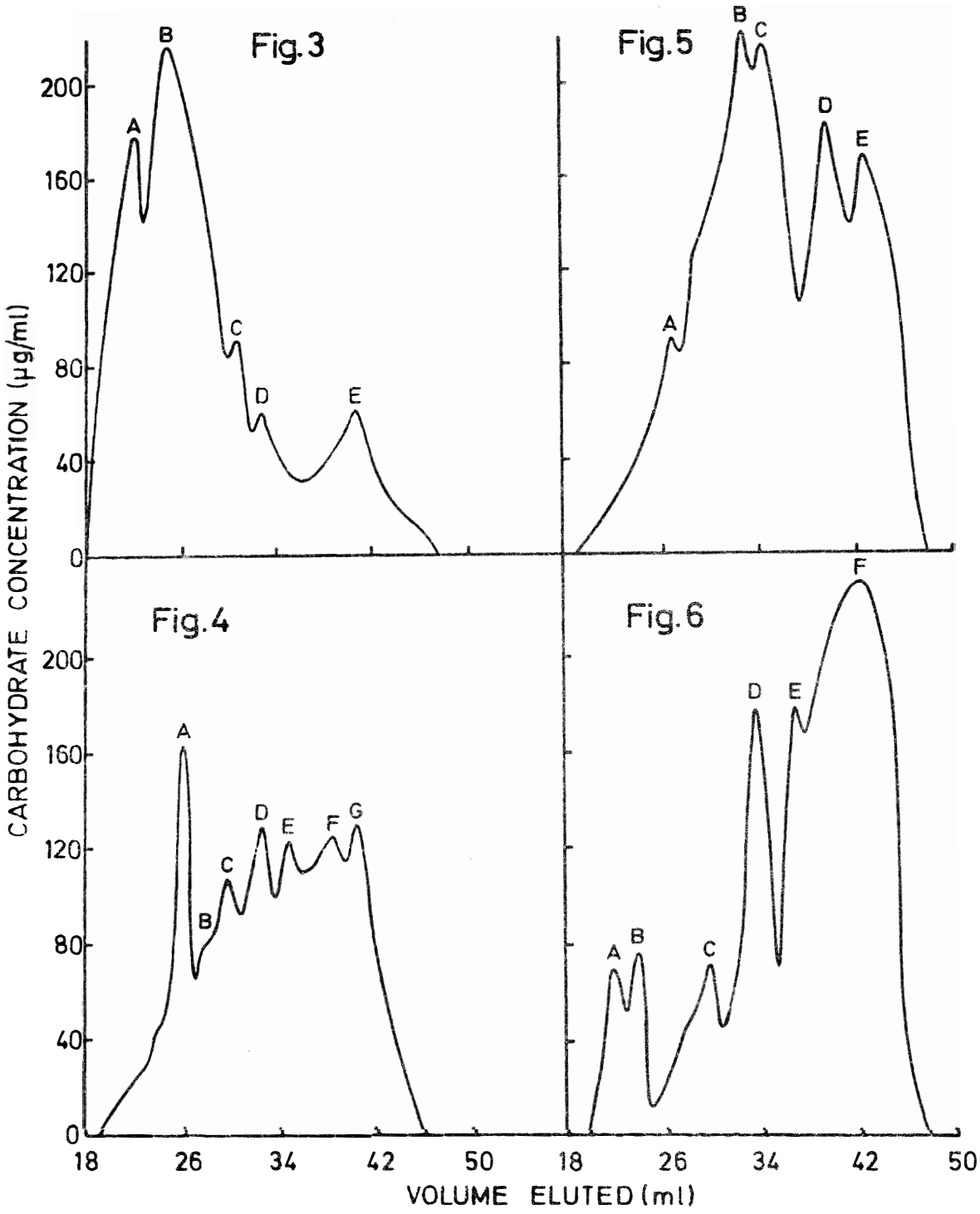


Fig.3
Fig.5
Fig.4
Fig.6
CARBOHYDRATE CONCENTRATION (µg/ml)
VOLUME ELUTED (ml)
0
40
80
120
160
200
18
26
34
42
50
A
B
C
D
E
F
G

hydrolysis; x = 0.51) consists of seven peaks corresponding to molecular weights below 80 000, all of which coincide with major peaks in Fig. 7 and which again illustrate the existence of pairs of peaks representing molecular weights in the ration 1:2. This persistence of certain peaks in the elution patterns obtained at different stages of hydrolysis, which demonstrates the production of fragments of preferred molecular size, was also noted in the case of hydrolysates from Acacia podalyriaefolia gum,[55] and has recently been demonstrated in a similar study of the acid hydrolysis of Acacia elata gum.[60] The 1:2 molecular-weight relationship between pairs of peaks in the elution patterns of hydrolysates has also been observed in recent studies of other gums.[55,60,61]

A rapid fall in $\overline{M}_w$ from 18 200 to 420 occurs during the next 24h of hydrolysis (48 - 72h). Fig. 9 (72h; x = 0.58), obtained by chromatography on Bio-Gel P-10, shows that a high proportion of the hydrolysate is, at this stage, of molecular weight $\leq$ 260, though there are several peaks at roughly equal intervals of elution volume which show the presence of residual material of $\overline{DP}$ as high as 30. The pattern is little changed during the following 24h of hydrolysis (see Fig. 10). A possible explanation for the rapid fall in molecular weight observed after hydrolysis for 48h is that the flexibility of uronic acid-containing chains at this stage is such that interaction of carboxylic acid groups with glycosidic bonds may be possible, hydrolysis being accelerated thereby. Such large and unexpected

Fig.7

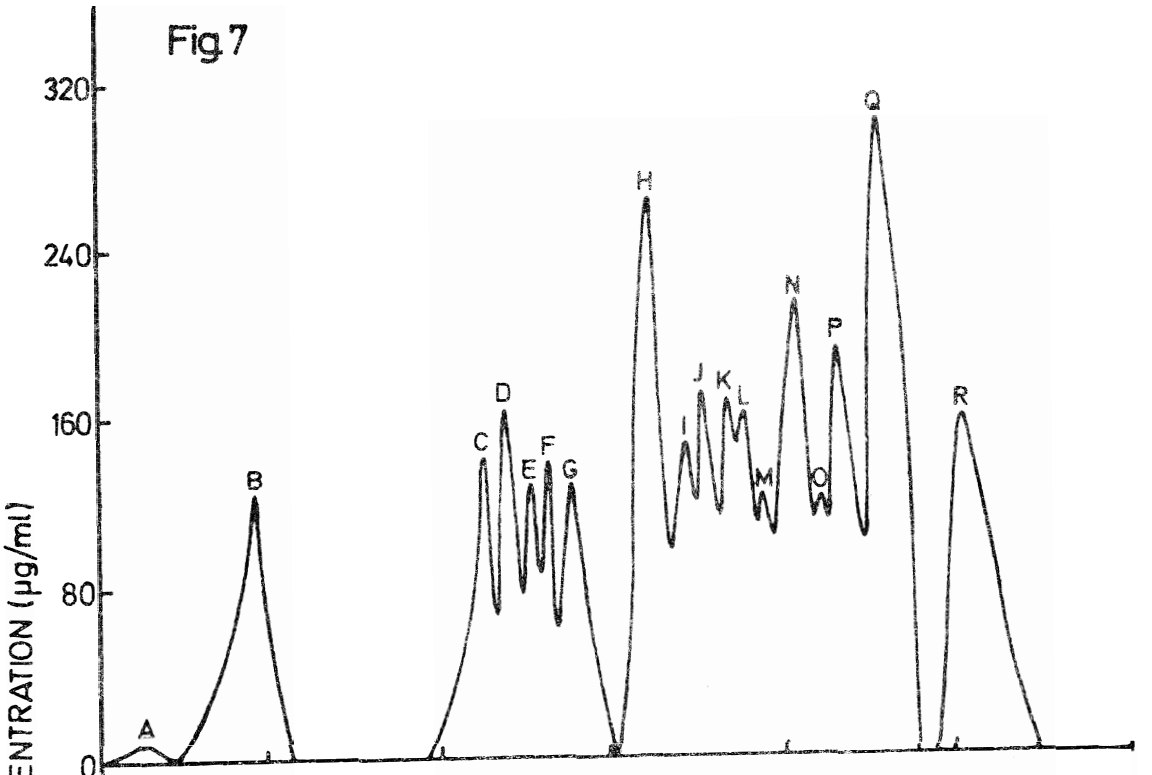
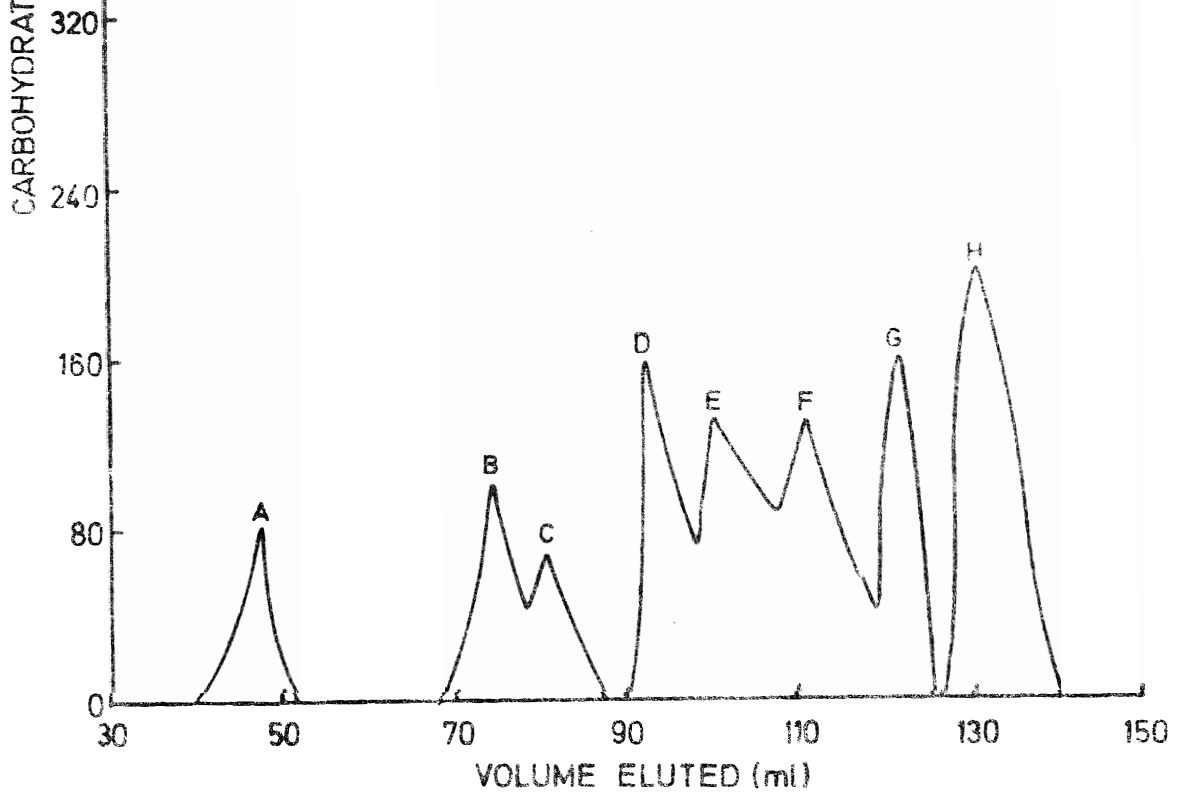

320
240
160
80
0
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R

Fig.8


CARBOHYDRATE CONCENTRATION (µg/ml)
320
240
160
80
0
A
B
C
D
E
F
G
H
30
50
70
90
110
130
150
VOLUME ELUTED (ml)

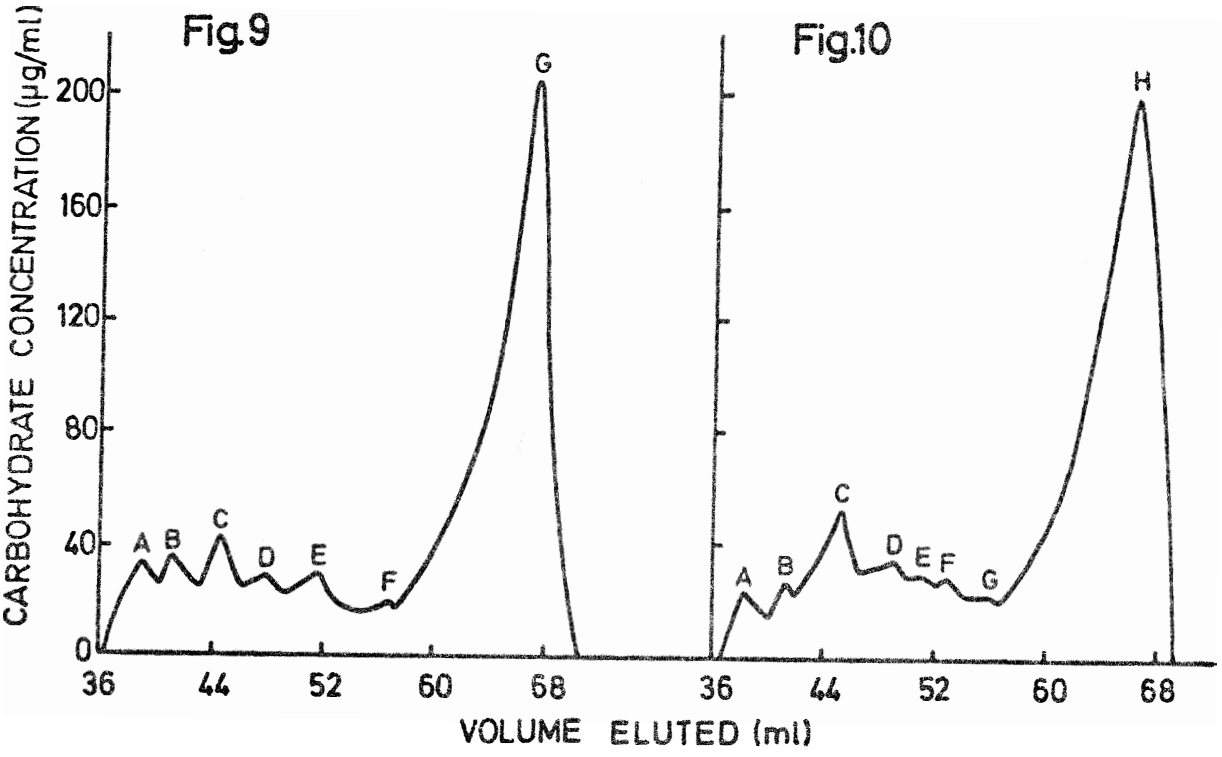


Fig.9
Fig.10
CARBOHYDRATE CONCENTRATION (µg/ml)
200
160
120
80
40
0
A
B
C
D
E
F
G
H
36
44
52
60
68
VOLUME ELUTED (ml)

decreases in molecular weight have been observed during the hydrolysis of other acid-containing gums, e.g. those of Virgilia oroboides[23] and Acacia senegal,[62] and the direct intervention of acid groups in this way has been considered.[62,63]

2.3.2 Further hydrolysis in 50 mM acid

A further decrease in $\bar{M}_w$ occurs on continuation of hydrolysis in 50 mM acid under which conditions the rate-constants (see Table 7) are considerably higher than those observed in the later stages of hydrolysis in 5 mM acid. Fig. 11 (1h in 50 mM acid; x = 0.76) shows all the peaks observed in Fig. 10, but reduced in size. Figs. 12 and 13 show further diminution of the peaks corresponding to molecular weights above 260 as the time of further hydrolysis in 50 mM acid increases to 8h, until finally, after 23h, the elution curve (Fig. 14) shows only one peak, at $\bar{M}_w \leq 260$. Hydrolysis in 50 mM acid thus accomplishes the final degradation of galactose oligomers remaining after the first (5 mM acid) hydrolysis stage. However, the biouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was still detected on paper chromatography; its presence is possibly the reason for the slight dissymmetry of the peak in Fig. 14. The resistance to hydrolysis of aldobiouronic acids is well known.[57,64]

2.3.3 Statistical aspects of the depolymerisation of Brabeium stellatifolium gum.

Statistical methods[65,66,67,68] were employed to obtain some information on the degree of randomness of the depolymerisation of Brabeium stellatifolium gum.

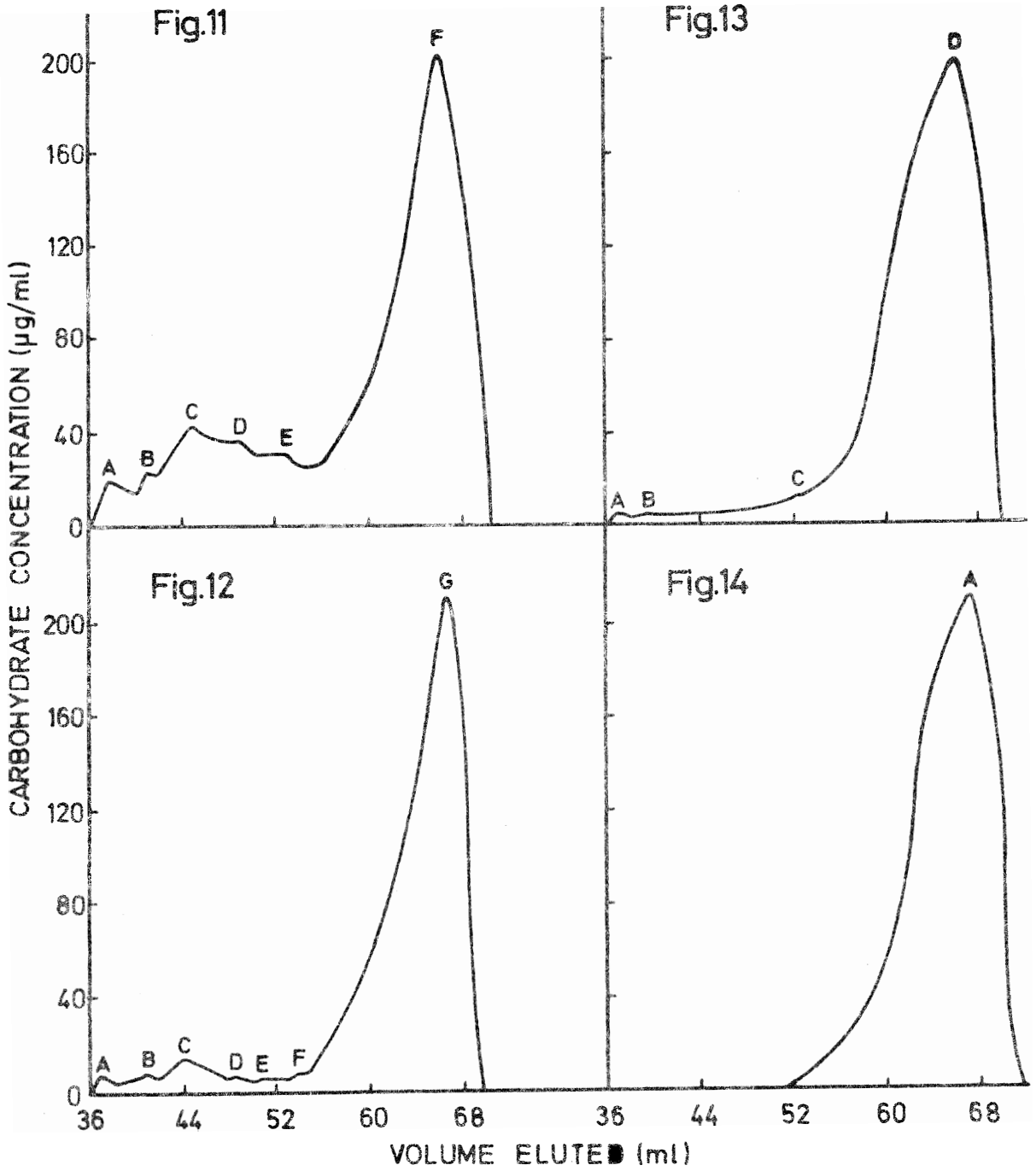


Fig.11
Fig.13
Fig.12
Fig.14
CARBOHYDRATE CONCENTRATION (µg/ml)
VOLUME ELUTED (ml)
0
40
80
120
160
200
36
44
52
60
68
A
B
C
D
E
F
G

From Fig. 16 it can be seen that the experimentally obtained values of $\frac{\overline{M}_w}{M_o}$ deviate increasingly from curve (1), the theoretical curve for exclusively peripheral hydrolysis, as the degree of scission (x) increases and cleavage of peripheral arabinofuranoside linkages, tending to completion, plays a lesser part in the overall hydrolysis. However, the experimental curve lies above curve (3), the theoretical curve for random hydrolysis of galactopyranoside linkages, over much of its length, the two coinciding at values of $x \geq 0.35$. It may therefore be concluded that the hydrolysis of this polysaccharide is non-random, even after the removal of most of the arabinofuranoside. Reasons for this non-random depolymerisation include the more rapid hydrolysis of terminal, relative to internal linkages,[57] and of β-(1→3)- to β-(1→6)-linkages,[69] as well as retardation of hydrolysis at branch-points.[70]

Fig. 15 $\log\left[\left(\frac{1}{R_F}\right)-1\right]$ PLOTTED VS. DEGREE OF POLYMERISATION FOR THE HOMOLOGOUS SERIES OF β-(1→3)- LINKED D-GALACTOSE OLIGOMERS, OBTAINED ON PARTIAL HYDROLYSIS OF *Brabeium stellatifolium* GUM.

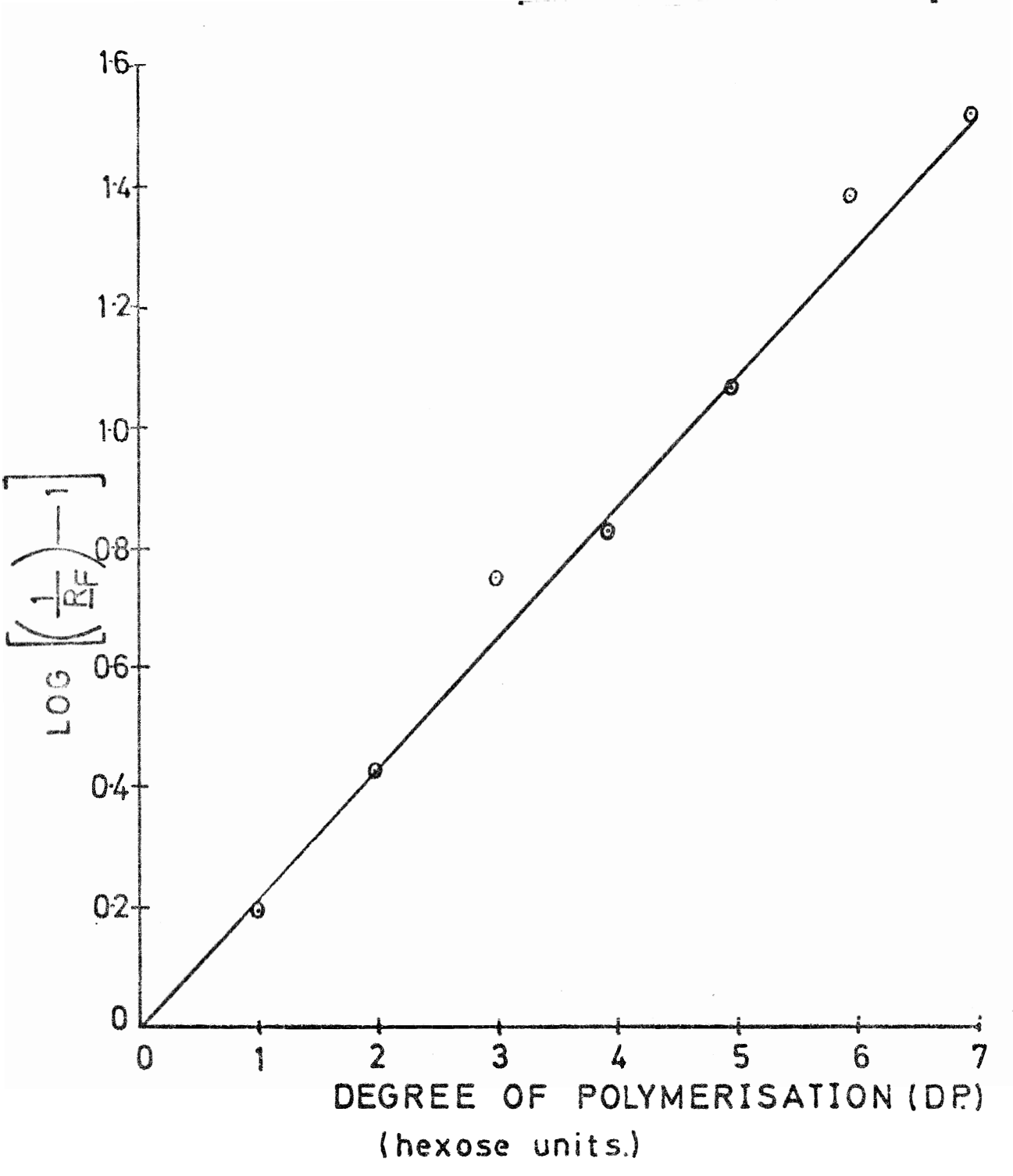
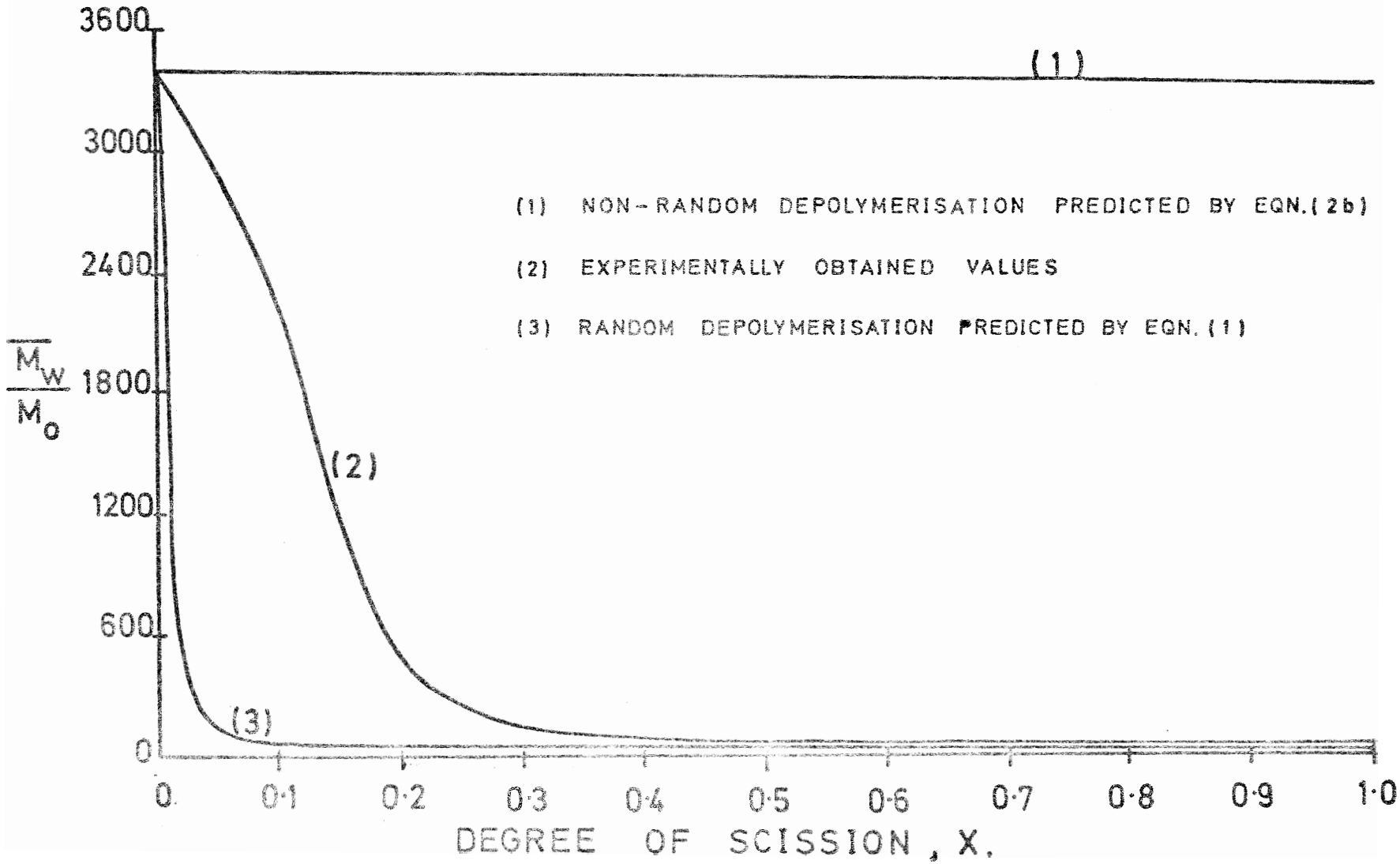


Fig. 16 VARIATION OF $\bar{M}_w / M_o$ WITH DEGREE OF SCISSION DURING PARTIAL HYDROLYSIS OF Brabeium stellatifolium GUM.



Legend to Figure 17

(GLC separation of neutral sugars, as their alditol acetates, obtained from the hydrolysate of Brabeium stellatifolium gum.)

Peak	Acetate of:
a	Rhamnitol
b	Arabinitol
c	Xylitol
d	Mannitol
e	Galactitol

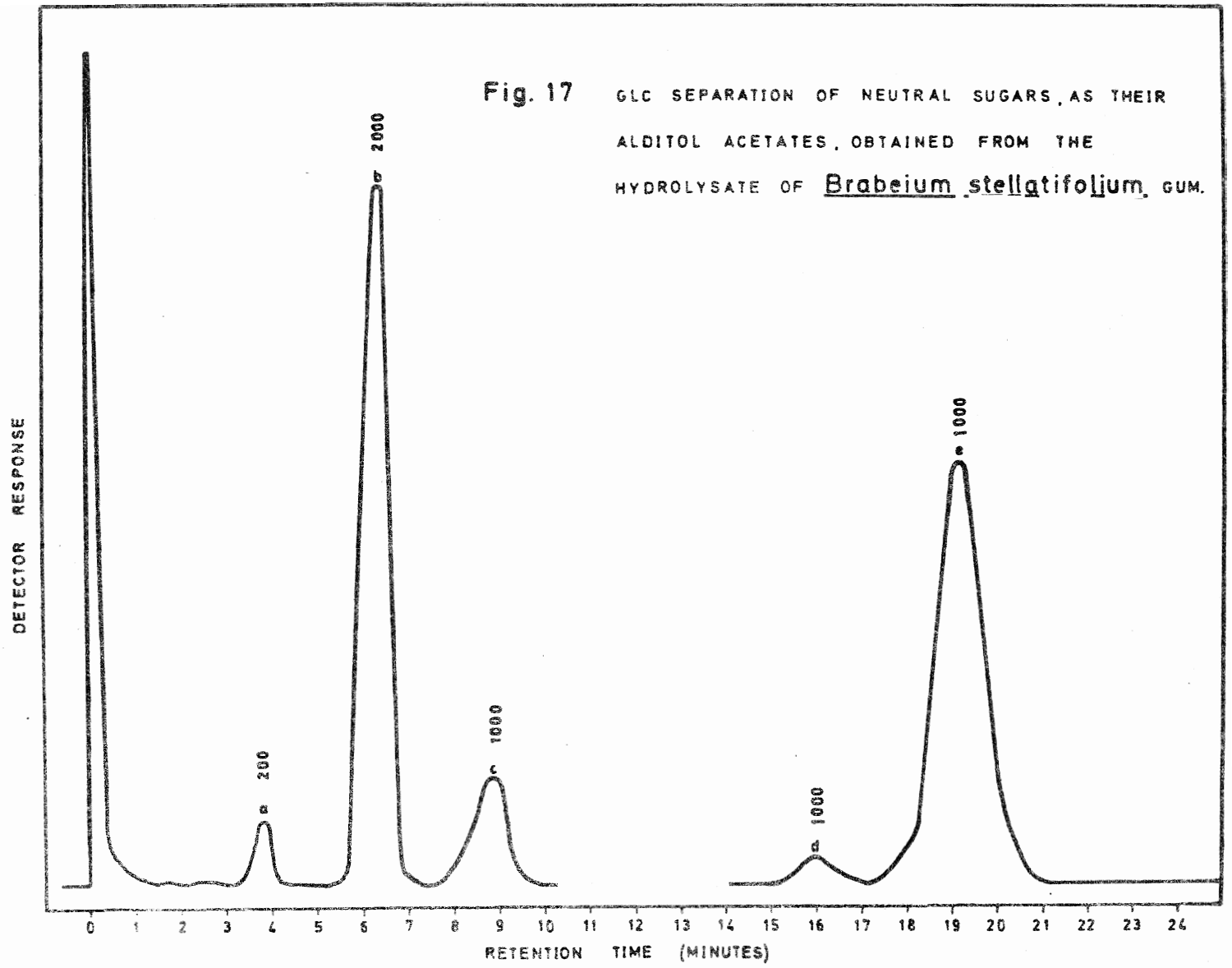


Fig. 17 GLC SEPARATION OF NEUTRAL SUGARS, AS THEIR ALDITOL ACETATES, OBTAINED FROM THE HYDROLYSATE OF *Brabeium stellatifolium* GUM.

2.4 Methylation analysis of Brabeium stellatifolium gum

Two samples of Brabeium stellatifolium gum were methylated by the procedures of Haworth[71] (once), Kuhn[72] (twice) and Purdie[73] (five times), until neither displayed any absorption in the IR region 3 200-3 500 cm^{-1}.

A portion of each methylated gum sample was methanolysed in a sealed tube with 2% methanolic hydrogen chloride, as described in section 4.4. The methanolysates were neutralised, filtered and evaporated to dryness, a drop of methanol was added to each dry residue and the two products were analysed semi-quantitatively by GLC[74,75] (see Fig. 18). Average values of the relative molar proportions of the various O-methyl sugar residues present in the methylated gum, as determined by this procedure, are recorded in Table 1.

Methylated gum from the larger sample was hydrolysed with 98% formic acid and 0.5 M H_2SO_4, as described in section 4.4. The hydrolysate, after neutralisation with $BaCO_3$, filtration and concentration to a small volume, was placed on a cellulose column. Separation of this mixture of methylated sugars and sugar acids into its components was effected by gradient elution of the cellulose column with mixtures of petroleum ether and water-saturated butan-1-ol, ethanol/water mixtures and finally water only (see fig. 19).

Fractions from the column were combined according to evidence obtained from paper and thin-layer chromatography

Legend to Figure 18

(GLC separation of methylated sugars, as their methyl glycosides, obtained from the methanolysate of methylated Brabeium stellatifolium gum.)

Peak	Methyl glycoside of:
(a)	2,3,4-tri-O-methylxylose and 2,3,4-tri-O-methylrhamnose (?)
(b)	2,3,5-tri-O-methylarabinose and 2,3,4-tri-O-methylxylose
(c)	2,3,5-tri-O-methylarabinose
(d)	3,4-di-O-methylrhamnose (?)
(e)	3,5-di-O-methylarabinose
(f)	2,3-di-O-methylarabinose and 2,5-di-O-methylarabinose
(g)	2,3-di-O-methylarabinose and 2,5-di-O-methylarabinose
(h)	2,3,4-tri-O-methylglucuronic acid and 3,4,6-tri-O-methylmannose
(i)	2,3,4-tri-O-methylglucuronic acid and 3,4,6-tri-O-methylmannose
(j)	2,4,6-tri-O-methylgalactose
(k)	2,4,6-tri-O-methylgalactose
(l)	2,3,4-tri-O-methylgalactose
(m)	2,6-di-O-methylgalactose and 2,3-di-O-methylglucuronic acid
(n)	2,6-di-O-methylgalactose

Peak	Methyl glycoside of:
(o)	2,6-di-O-methylgalactose
(p)	2,6-di-O-methylgalactose
(q)	2,4-di-O-methylgalactose
(r)	2,4-di-O-methylgalactose

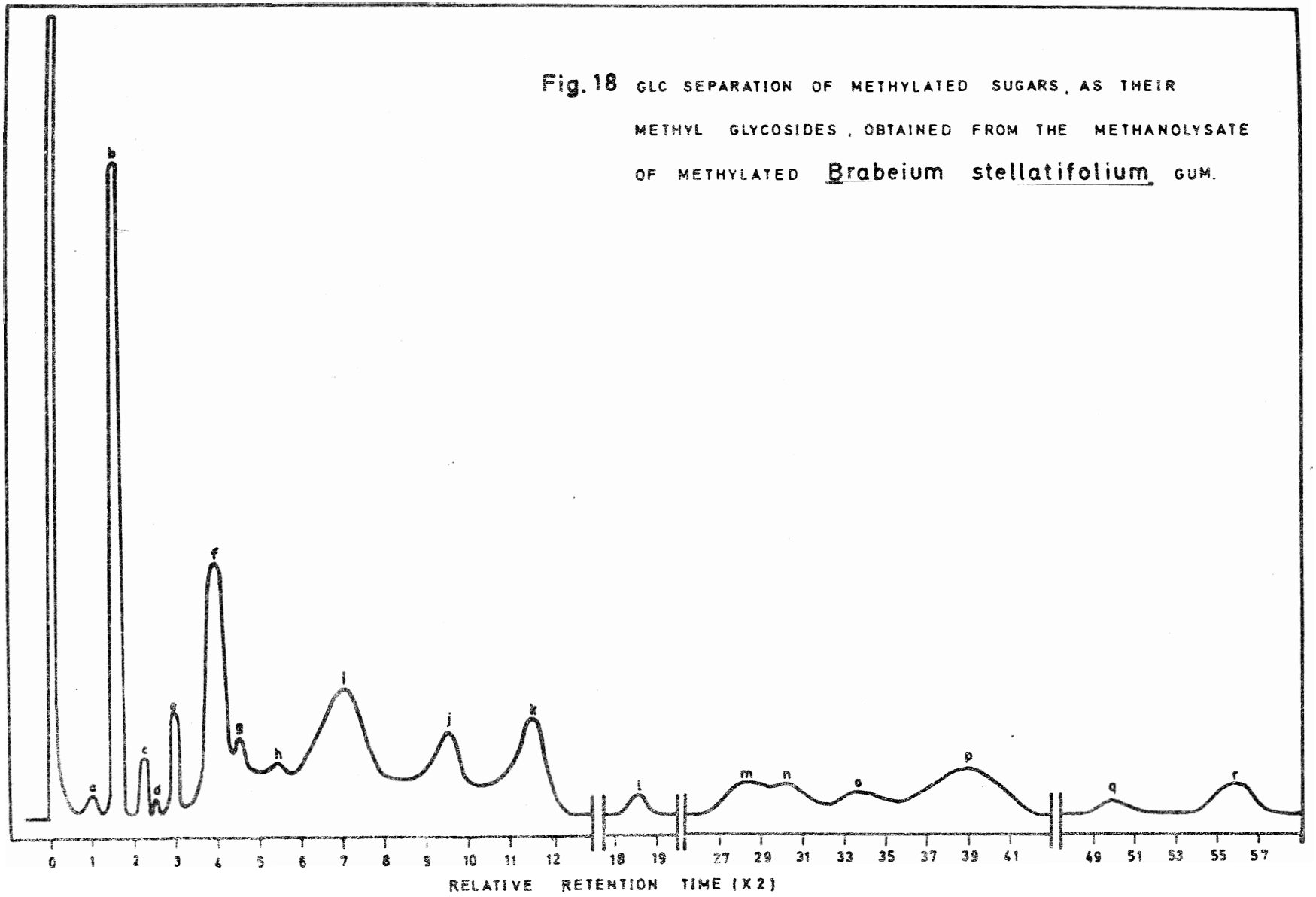


Fig. 18 GLC SEPARATION OF METHYLATED SUGARS, AS THEIR METHYL GLYCOSIDES, OBTAINED FROM THE METHANOLYSATE OF METHYLATED Brabeium stellatifolium GUM.

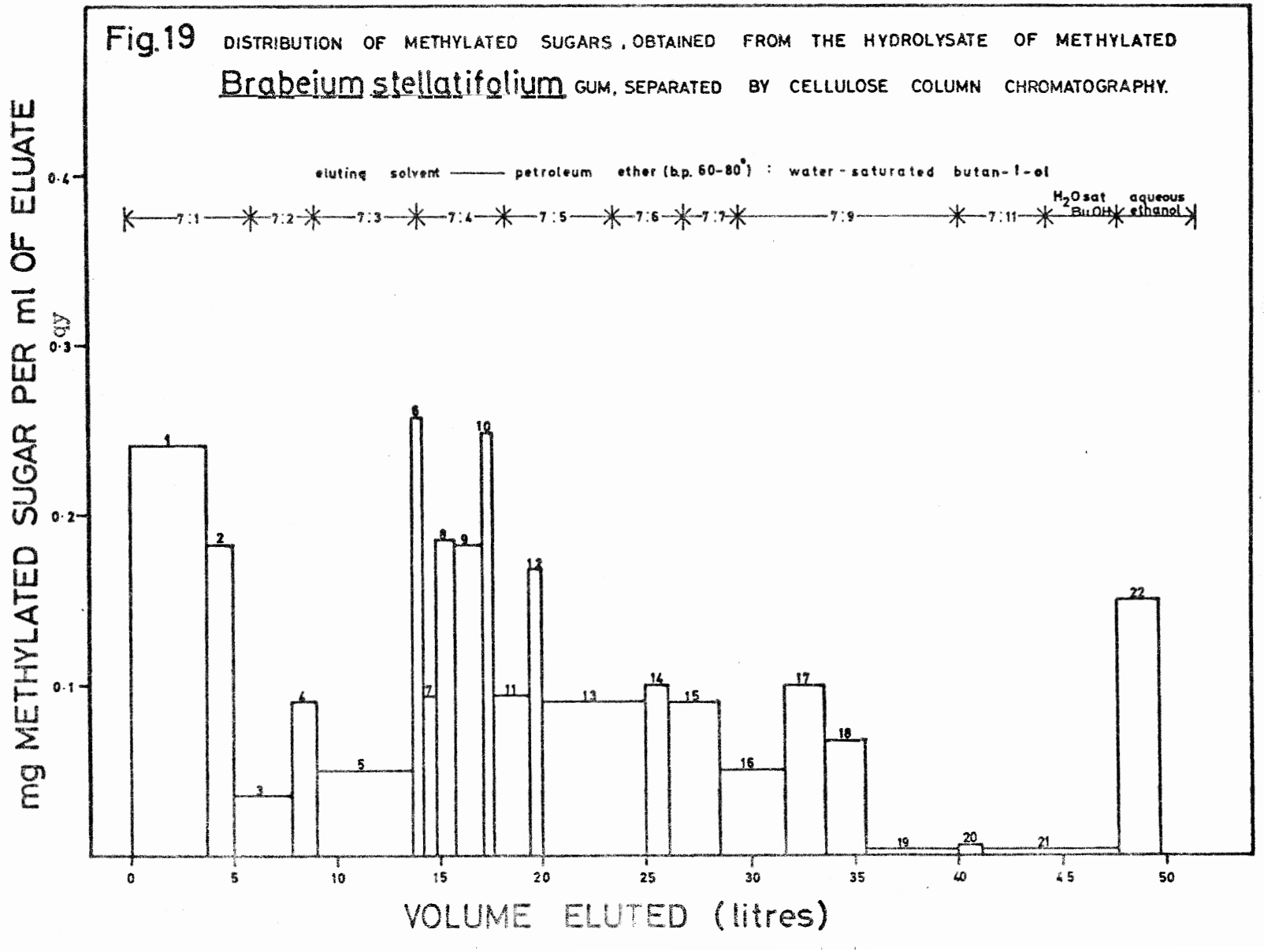


Fig.19 DISTRIBUTION OF METHYLATED SUGARS, OBTAINED FROM THE HYDROLYSATE OF METHYLATED
Brabeium stellatifolium GUM, SEPARATED BY CELLULOSE COLUMN CHROMATOGRAPHY.
eluting solvent —— petroleum ether (b.p. 60-80°) : water-saturated butan-1-ol
7:1 7:2 7:3 7:4 7:5 7:6 7:7 7:9 7:11 H2O sat BuOH aqueous ethanol
mg METHYLATED SUGAR PER ml OF ELUATE
0·4
0·3
0·2
0·1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
0 5 10 15 20 25 30 35 40 45 50
VOLUME ELUTED (litres)

and examined by a variety of different methods. Fractions containing sugar components which lend themselves, as their methyl glycosides, to GLC analysis,[74,75] namely tetra-, tri- and di-O-methylhexoses, as well as tri-, di- and some mono-O-methylpentoses and tri- and di-O- methyl derivatives of glucuronic acid, were all methanolysed and examined semi-quantitatively in this manner.

Unknown peaks present on GLC charts of these fractions were ascribed to the butan-1-ol used as eluant, since the same peaks were observed on GLC of a sample obtained by evaporation of a portion of this butan-1-ol and methanolysis thereof.

It was not possible to correct the weights of the sugar fractions by using these peaks as a measure of impurity, since their nature was not known and therefore no molar response factors could be calculated. The weights of pure carbohydrate material in the fractions were determined as follows:

A weighed portion of each fraction was dissolved in water and repeatedly extracted with petroleum ether. Both water and petroleum ether layers were evaporated to dryness and each residue was weighed. The brown material obtained on evaporation of the petroleum ether extracts was in each case shown to contain no carbohydrate by qualitative screening using the phenol/sulphuric acid method.[76] The

proportion of pure carbohydrate in each fraction was therefore calculated from the weight of the water-soluble portion relative to that of the sample solvent-extracted and the total weight of the fraction was corrected accordingly to obtain a more accurate value for the weight of carbohydrate present. The specific rotations reported in section 4.4 are those of the purified, water-soluble portions of the various fractions.

Table 1 shows the relative proportions of methylated sugars released on hydrolysis of methylated Brabeium stellatifolium gum, as determined by the procedures discussed above. These results are in fairly good agreement with those obtained by semi-quantitative GLC analysis of the methanolysate of the methylated gum.

The molar proportions of the component sugars in the gum, calculated from the data obtained on cellulose column chromatography of the hydrolysate of the methylated gum, were: L-arabinose (45), D-galactose (42), D-xylose (2), D-mannose (4) and D-glucuronic acid (7). There is, in general good agreement between these values and those obtained on analysis of an hydrolysate of the gum itself (see section 2.2) the only exceptions being the somewhat lower proportion of arabinose and much lower xylose content indicated by methylation analysis. In the first instance losses of the relatively volatile 2,3,5-tri-O-methyl-arabinose, and possibly hydrolysis of acid-labile arabino-

TABLE 1 Methylated sugars obtained from methylated Brabeium stellatifolium gum:

(a) by hydrolysis and (b) by methanolysis

Methylated sugar	(a) Cellulose column chromatography of hydrolysate Wt. (mg) recovered	mol %*	(b) GLC of methanolysate mol %
2,3,5-Tri-O-methylarabinose	991	23.2	24
2,3-Di-O-methylarabinose	297	8.0	10
3,5-Di-O-methylarabinose	152	4.3	12
2,5-Di-O-methylarabinose	63	2.1	
3-O-methylarabinose	209	6.4	
Arabinose	10	0.8	
2,3,4,6-Tetra-O-methylgalactose	144	3.2	
2,3,4-Tri-O-methylgalactose	277	6.1	5
2,3,6-Tri-O-methylgalactose	349	7.5	14
2,4,6-Tri-O-methylgalactose	152	3.5	
2,6-Di-O-methylgalactose	566	12.9	24
2,4-Di-O-methylgalactose	279	6.7	
2-O-methylgalactose	61	1.9	
Galactose	15	0.9	
3,4,6-Tri-O-methylmannose	168	3.9	4
2,3,4-Tri-O-methyxylose	45	1.5	trace
2,3,4-Tri-O-methylglucuronic acid	171	3.8	4
2,3-Di-O-methylglucuronic acid	129	3.1	3

* Calculated for each fraction by GLC analysis.

furanose linkages during methylation of the gum, may be responsible for the discrepancy. A possible reason for the low xylose content is that 2,3,4-tri-O-methylxylose has a paper chromatographic mobility similar to that of 2,3,5-tri-O-methylarabinose, and the GLC retention times of the methyl glycosides of these two methylated sugars are also almost identical.[75] This makes the quantitative determination of 2,3,4-tri-O-methylxylose somewhat difficult in the presence of large quantities of 2,3,5-tri-O-methylarabinose.

The results of this methylation analysis suggest that the polysaccharide has a highly branched structure (the ratio of branch points to chain units being ca. 1:1.1). Chain units of (1→3)-, (1→4)- and (1→6)- linked D-galactopyranose are present, in a ratio of ca. 1:2.1:1.7 End-groups of L-arabinofuranose predominate over those of other sugars, an observation consistent with the mode of degradation observed during partial hydrolysis (see section 2.3). However, some of the arabinose residues in the gum are present as chain units; (1→2)- and (1→3)- linked L-arabinofuranose, (1→5)- linked L-arabinofuranose and/or -pyranose and (1→4)-linked L-arabinopyranose chain units have been detected. Residues of 2,5- and 3,5-di-O-methyl-L-arabinose have been found to be present in the methylated derivatives of a variety of gums, especially those of the genus Acacia,[15] and where the proportions are high these O-methyl sugar residues have been regarded as arising from non-terminal

units occurring in side chains which consist exclusively of arabinose. These units are resistant to periodate oxidation, and may therefore be expected to persist in the polysaccharide products of successive Smith-degradations of the gum (see section 2.5).

Minor structural features include end-groups of D-galactose and D-xylose. D-Glucuronic acid is present in the gum both as end-group and as a chain unit. D-Mannose occurs as a chain unit, linked through C-2, mainly (or perhaps exclusively) to D-glucuronic acid on the evidence of the presence of the biouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose in an hydrolysate of the gum.

Hydrolysis studies have shown that L-rhamnose occurs as a minor consituent in the gum but, since no methyl ethers of L-rhamnose were detected on methylation analysis, the location of these residues must remain uncertain. The 2,3,4-tri-O-methyl-rhamnose which would be produced if the rhamnose were present as end groups (highly likely by analogy with other gums) is volatile, and may have been lost on concentration of the fractions.

Incomplete methylation of sterically hindered hydroxyl groups[77] and, possibly, some de-O-methylation during hydrolysis[78] may account for the small amounts of galactose and arabinose found in the hydrolysate of the methylated gum.

2.5 Smith degradation studies on Brabeium stellatifolium gum

To substantiate and expand conclusions drawn from the partial hydrolysis studies and methylation analysis, Brabeium stellatifolium gum was further examined by periodate oxidation. A sample of the gum was subjected to five sequential Smith-degradations and portions of all degradation products were examined by GLC analysis and paper chromatography. Molecular-weight distributions of the degraded polysaccharides, obtained by chromatography on appropriate gels were used[79,80] in conjunction with other data to establish an average pattern for the breakdown of the gum under these conditions.

Aqueous solutions of Brabeium stellatifolium gum and Smith-degraded polysaccharides derived therefrom were oxidised with sodium metaperiodate under the usual Smith-degradation conditions.[25] Periodate uptake was measured spectrophotometrically[81] and, at a later stage in the investigation, also by the 2,4,6-tri-2-pyridyl-S-triazine (TPTZ) method[82] as a check on the results obtained by the former procedure. The amounts of polysaccharide taken, the concentration and volume of the periodate solutions used and the amounts of periodate consumed, both in analytical and in preparative oxidations are given in Table 8. In each case when the periodate consumed reached a constant value (see Figs. 22-26) oxidation of the polysaccharide was terminated by destruction of the excess periodate, either by the addition of ethylene glycol or barium carbonate or by the simultaneous reduction of the periodate and the oxidised polysaccharide (see section

4.6.2). After removal of ions, ethylene glycol and other material of low molecular weight by various methods (section 4.6.2), oxidised polysaccharides were reduced with sodium borohydride to give the corresponding polyols, or reduced, oxidised polysaccharides (see Table 10).

The next step in a Smith-degradation stage involves cleavage of acetal linkages in the reduced, oxidised polysaccharide (see Fig. 41). Incomplete hydrolysis of acetal linkages in reduced, oxidised polysaccharides, resulting in high glycerol: sugar ratios in the Smith-degraded polysaccharides,[61,83,84] necessitated modification of the conditions recommended by Smith,[25] who used 0.2 M HCl and 0.5 M HCl for 6 and 8h respectively, at room temperature, in this acetal hydrolysis step.

To ensure complete cleavage of acetal linkages in the reduced, oxidised polysaccharides, the progress of acetal hydrolysis (in 0.5 M H_2SO_4, at room temperature) was monitored[61,83,84] by periodate oxidation of samples removed at intervals during the hydrolysis step and subsequent determination, by the chromotropic acid method,[85] of the formaldehyde released on oxidation of the glycerol and (if present) glycolaldehyde produced on hydrolysis of the acetal linkages; the amount of formaldehyde liberated tends to a maximum as acetal cleavage approaches completion. Alternatively after hydrolysis of the acetal linkages in the reduced, oxidised polysaccharide for 48h, followed by solvent fractionation (described later), a small sample (ca. 2 mg) of the recovered

polysaccharide was placed in 0.5 M H_2SO_4 for another 24h, after which time the solution was assayed for glycerol or glycolaldehyde as described. In this manner further acetal cleavage was detected and, if necessary, the bulk of the polysaccharide material was subjected to another acid treatment. This procedure was repeated (see Fig. 41) until no further formaldehyde was released on hydrolysis of the reduced, oxidised polysaccharide.

That acetal cleavage was, in fact, complete in the Smith-degraded polysaccharides is evident from their low glycerol contents (commensurate with the occurrence of glycerol solely as nonreducing end-group) and the absence of glycolaldehyde (see Table 11). However, some fission of glycosidic linkages occurred; this has also been reported by Shaw,[86] who found that arabinose was liberated on treatment of the reduced, oxidised polysaccharide gum of Watsonia pyramidata with acid for periods longer than 4h. In the present work, cleavage of glycosidic linkages was indicated by slight increases in reducing power,[87,88] and by the presence of some arabinose and galactose on paper chromatography, during the first and second stages in the Smith-degradation of Brabeium stellatifolium gum. The extent of hydrolysis of glycosidic linkages in reduced, oxidised polysaccharides did however, not exceed 4% as calculated from the increases in reducing power, during acetal hydrolysis.

In each case, after complete cleavage of acetal

linkages had been achieved, the solution of the degraded polysaccharide in 0.5 M H_2SO_4 was neutralised, filtered and evaporated to dryness. The products were then fractionated into :

(a) a fraction soluble in methanol-acetone (1:1 v/v),

(b) a methanol-soluble fraction, and

(c) an insoluble residue (Smith-degraded polysaccharide)

The two fractions (a) and (b) were combined in all cases, the product being described as the alcohol-soluble fraction (see Table 9).

Solvent extraction as described above of the material obtained after the first Smith-degradation stage, showed that all of the xylose and rhamnose and most of the arabinose, together with some uronic acid, had been removed from the gum. The insoluble residue, polysaccharide A, ca. 20% by weight of the starting material, was shown by gel chromatography to consist of a variety of components of different molecular weight ($\bar{M}_w$ 156 000). The insoluble products of the four subsequent treatments (yields respectively 8, 1.3, 0.6 and 0.2% by weight of the starting material) were also polymolecular, having $\bar{M}_w$ 15 800, 3 690, 3 200 and 3 000 respectively (Figs. 29, 30, 31 & 32 and Table 11). Glycerol constituted a large proportion of the soluble material produced at each stage, in addition to some arabinose and galactose, and glycosides of these sugars. The presence of threitol in both soluble and insoluble material confirmed the existence of (1→4)- linked galactopyranose residues in

the gum. Some arabinose persisted in the insoluble product after the third Smith-degradation, but this component was absent from the degraded polysaccharides obtained thereafter in the sequence. The presence of 2,5- and 3,5-di-O-methyl-arabinose in an hydrolysate of fully methylated Brabeium stellatifolium gum provides an explanation; these two O-methyl sugars arise from (1→3)- and (1→2)- linked arabinofuranose residues which are periodate-resistant chain-units in the gum. On successive Smith-degradations removal of protecting side-chains exposes the above two residues to periodate oxidation, hence the absence of arabinose in the degraded polysaccharides after the third Smith-degradation.

Uronic acid residues, in decreasing amounts, were present in the insoluble fractions obtained after each of the first four Smith-degradation stages; in theory all uronic acid should have been removed from the gum during the first Smith-degradation. Incomplete oxidation, due to steric hindrance, of uronic acid residues containing α-glycol groups,[89] and the resistance to hydrolysis of acetals involving α-hydroxy acids linked to aldehyde groups,[90] have been observed before. The latter situation facilitates hemiacetal formation between the aldehydic group of the oxidised acid and a free hydroxyl on a neighbouring molecule, so that both residues involved resist oxidation on subsequent periodate treatment. In view of the presence of the biouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose in an hydrolysate of the gum, it is possible that such interaction between uronic acid and

D-mannose may account for the persistence, not only of uronic acid, but also of D-mannose residues through several Smith-degradations (see Table 11).

Consideration of the values of $\overline{M}_w$ and the periodate uptakes (Tables 11 and 8) of the insoluble products (polysaccharides A-E) of successive Smith-degradations of this gum leads to the following conclusions. The first Smith-degradation stage results in a decrease in $\overline{M}_w$ from 560 000 to 156 000 (a drop of ca. 72%). The latter is approximately half of the $\overline{M}_w$ value (332 000) predicted for polysaccharide A if it is assumed that only end-groups are removed during this degradation; this suggests cleavage of one internal linkage per average molecule. Polysaccharide A is highly branched, having on the average 287 (ca. 30%) unprotected nonreducing end-groups or periodate-vulnerable chain units and 675 periodate-resistant sugar residues per molecule.

A further decrease in $\overline{M}_w$, from 156 000 to 15 800 (a drop of ca. 90%) occurs during the second Smith-degradation stage. Exclusive removal of end-groups would result in an insoluble product having $\overline{M}_w$ 109 000; on the average, therefore, 6 internal linkages (exposed after removal of protecting side chains) are broken at this stage. The degree of branching (ca. 28%) in polysaccharide B is similar to that in polysaccharide A; an average molecule contains 28 nonreducing end-groups and 69 periodate-resistant chain units [though the degree of polymerisation here ranges from ca. 40 to 184 sugar units (Fig. 29)].

The decrease in $\overline{M}_w$, from 15 800 to 3 690 (*ca.* 75%), accompanying the third Smith-degradation indicates fission of a further two internal linkages per average molecule, since $\overline{M}_w$ for polysaccharide C is approximately $\frac{1}{3}$ of the value (10 600) predicted on the assumption of peripheral cleavage only.

A less drastic decrease in $\overline{M}_w$, from 3 690 to 3 200 (ca. 13%), accompanies the fourth Smith-degradation. This is consistent with the removal of end-groups only at this stage. The same applies to the fifth Smith-degradation, during which $\overline{M}_w$ decreases by only 200, indicating the removal of one end-group per average molecule.

It has thus been established from these Smith-degradation studies that the gum polysaccharide of Brabeium stellatifolium has a periodate-resistant galactomannan core having $\overline{M}_w$ *ca.* 3 000 (approximately 20 hexose units per average molecule). A high degree of polymolecularity is exhibited, however, and therefore meaningful conclusions regarding the structure of this galactomannan must await preparative-scale fractionation and further study of the components thus separated.

2.6 Summary of the structural features of Brabeium stellatifolium gum

The results of all the investigations performed on *Brabeium stellatifolium* gum exudate indicate that this polysaccharide consists of a basal structure of D-galactose and D-mannose, the latter probably linked (through C-2) to D-glucuronic acid residues. The average size of the periodate-

resistant galactomannan core is ca. 20 hexose units. Various side chains consisting mainly of D-galactose, are attached to this galactomannan skeleton. L-arabinose residues are present in high proportion, mainly as arabinofuranose end-groups, although chain units of arabinose have also been shown to be present. D-Xylose residues replace arabinose as end-groups in some cases. The location of L-rhamnose, known to be present as a minor constituent, is uncertain.

3. Grevillea robusta gum

3.1 The occurrence and characteristics of Grevillea robusta

Grevillea robusta A. Cunn. (family Proteaceae) better known as the silk oak, is a robust tree which is sometimes small and slender, sometimes tall (24-90 metres). The leaves, which are spinnate, are silky beneath, and golden yellow flowers sometimes appear, as well as a broad-shaped fruit.[91] The tree is indigenous to Australia, where it grows in the coastal regions of Queensland and New South Wales. The wood is commercially used in Ceylon, California (U.S.A.) and South Africa.[92,93]

When injured or diseased, Grevillea robusta exudes a reddish-brown gum. The fact that healthy trees contain very little gum supports the suggestion that plant gums are usually pathological products of plant metabolism.[3] This tree is, however, believed to be very susceptible to gummosis.[3]

Gum exudate from a tree growing near Stellenbosch, C.P., which was identified as Grevillea robusta by courtesy of the Bolus Herbarium (University of Cape Town, Rondebosch, C.P.) was used in the investigations described in this thesis.

3.2 Purification and characteristics of Grevillea robusta gum

A sample of Grevillea robusta gum was vigorously stirred with ca. 20 times its weight of water for 2h, to form a milky colloidal suspension (see section 4.2). Filtration

through a fine nylon mesh was followed by acidifying to a pH of ca. 1 with 10 M H_2SO_4. On addition of 8 volumes of cold ethanol to this acidified solution, a white solid was precipitated; this was washed with a little ethanol, re-dissolved in water and freeze-dried. The product thus obtained was used without further purification, for all investigations described.

This material had a negative specific rotation $[\alpha]_D$ -10^0 (c, 0.48), and analysis showed C, 38.7; H, 6.7; N<0.3; S, nil; OMe, 0.6 and ash 2.0%. As suggested in the case of Brabeium stellatifolium gum, the methoxyl content of Grevillea robusta gum is probably due to the presence of the 4-O-methyl derivative of D-glucuronic acid (see section 2.2).

Titration of an electrodialysed solution of the gum gave a mean value of ca. 1 300 for the equivalent weight, corresponding to a uronic anhydride content of ca. 11% (molar proportion).

The neutral sugars present in the gum, and their relative molar proportions, were determined by acid hydrolysis (0.5 M H_2SO_4, 96^0, 6h), followed by reduction, acetylation and quantitative gas-liquid chromatography, of the sugars produced (see Fig. 20). Isolation of the various components on Whatman No. 3 MM chromatographic paper, measurement of their optical rotations and preparation of suitable derivatives provided further identification of these sugars. The sugar composition of the gum (molar percentages in parenthesis) was as follows: L-arabinose (55), D-galactose (37),

Legend to Figure 20

(GLC separation of neutral sugars, as their alditol acetates, obtained from the hydrolysate of Grevillea robusta gum.)

Peak	Acetate of:
a	Rhamnitol
b	Arabinitol
c	Xylitol
d	Mannitol
e	Galactitol

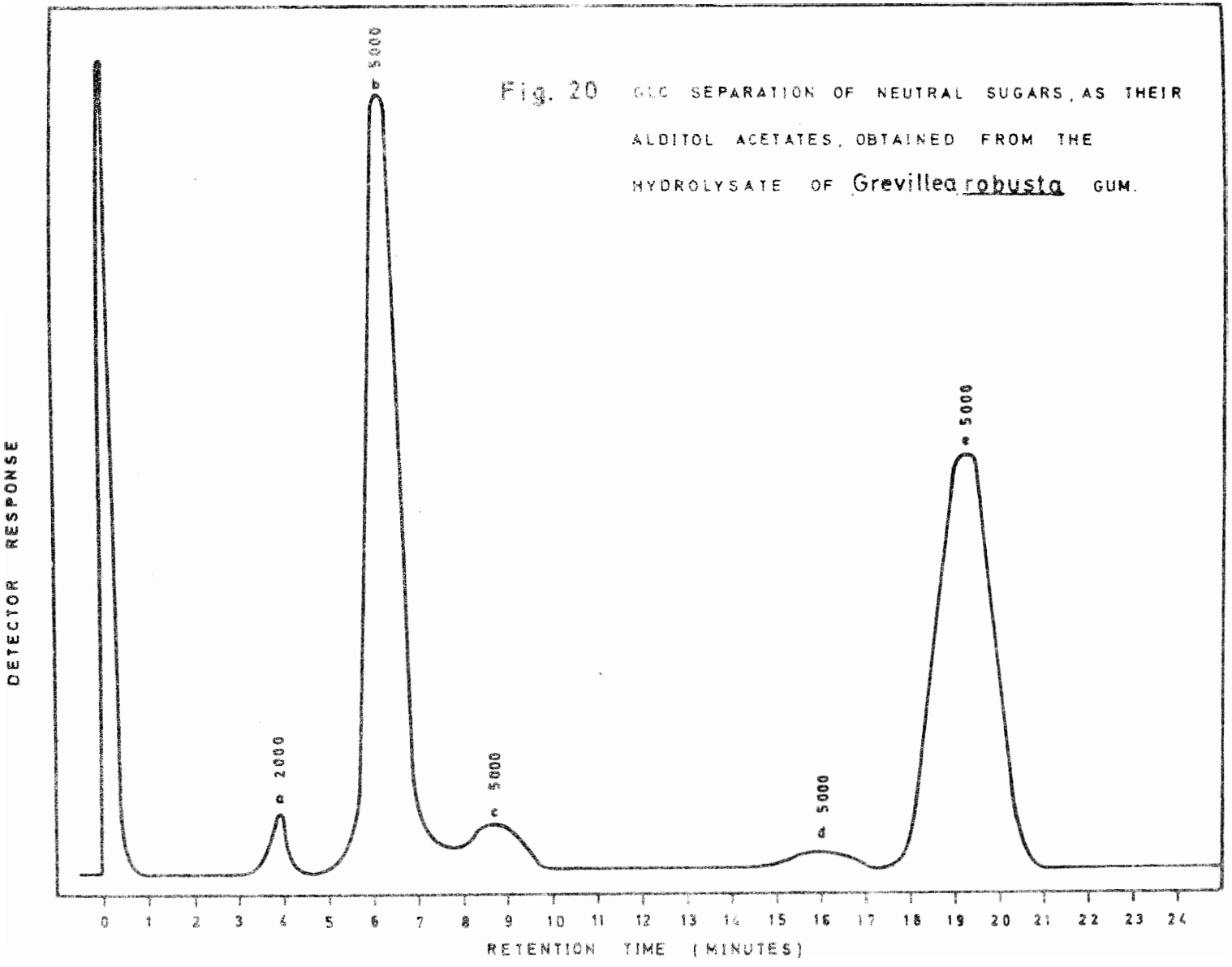


Fig. 20 GLC SEPARATION OF NEUTRAL SUGARS, AS THEIR ALDITOL ACETATES, OBTAINED FROM THE HYDROLYSATE OF *Grevillea robusta* GUM.

D-xylose (5), probably D-mannose (1) and L-rhamnose (2).

Three acidic components D-glucopyranuronic acid, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and 2-O-(β-D-glucopyranosyluronic acid)-D-mannose were identified by paper chromatography against standards, the first component being isolated and characterised in addition.

Molecular weight determinations by gel chromatography on Sagavac 6F showed a weight-average molecular weight, $\bar{M}_w$ of ca. 1 500 000.

It is of interest to compare the results of a previous investigation[3] of the composition of Grevillea robusta gum, collected from trees growing in a very different geographical location, i.e. Phoenix (Arizona, U.S.A.), with those reported above, since significant differences in properties have been found among samples of different origin in the case of gums from Acacia species.[94] The main chemical and physical properties of the two different samples of Grevillea robusta gum are tabulated in Table 5.

Anderson and Harris[3] found that the gum exuded by Grevillea robusta trees growing in Phoenix (Arizona, U.S.A.) was only partially soluble in water and consisted of the calcium and magnesium salts of a polyuronide composed of D-glucuronic acid combined with D-galactose and L-arabinose only. Mild acid hydrolysis liberated all of the L-arabinose, suggesting that most of it was attached in the furanose form on the periphery of the polysaccharide molecule; some D-galactose was also liberated. The polysaccharide residue

remaining after hydrolysis consisted of a polyuronic acid combined with D-galactose. Prolonged acid hydrolysis left a simple adlobiouronic acid composed of D-glucuronic acid linked to D-galactose.

It is interesting to note that no D-mannose, D-xylose or L-rhamnose was reported to be present in the gum investigated by Anderson and Harris,[3] in contrast to that used in the present investigation, in which all of these sugar residues are present, D-xylose to the extent of ca. 5 mol %, the others in smaller proportions. Tests for methoxyl groups were negative in the case of the gum sample from Phoenix, indicating the absence of any 4-O-methylglucuronic acid, possibly present in the sample used here.

In the present study the structure of Grevillea robusta gum has been examined in more detail by methylation analysis and sequential Smith-degradations.

3.3 Methylation analysis of Grevillea robusta gum

A sample of Grevillea robusta gum was methylated according to the procedures of Haworth[71] (once), Kuhn[72] (once) and Purdie[73] (five times) to yield a clear yellow glass, as described in section 4.5. A portion of this product was methanolysed with methanolic hydrogen chloride and after neutralisation, filtration and concentration the resulting mixture of methyl glycosides was analysed semi-quantitatively by GLC[74,75] (see Fig. 21).

The results obtained on methylation analysis of Grevillea robusta gum (see Table 2) indicate that this polysaccharide consists of a highly complex array of D-galactopyranose

Legend to Figure 21

(GLC separation of methylated sugars, as their methyl glycosides, obtained from the methanolysate of methylated Grevillea robusta gum.)

Peak	Methyl glycoside of:
(a)	2,3,4-tri-O-methylxylose and 2,3,4-tri-O-methylrhamnose
(b)	2,3,5-tri-O-methylarabinose and 2,3,4-tri-O-methylxylose
(c)	2,3,5-tri-O-methylarabinose
(d)	3,4-di-O-methylrhamnose (?)
(e)	2,3-di-O-methylarabinose and 2,3,4,6-tetra-O-methylgalactose
(f)	2,3-di-O-methylarabinose and 2,5-di-O-methylarabinose
(g)	3,4,6-tri-O-methylmannose and 2,3,4-tri-O-methylglucuronic acid
(h)	3,4,6-tri-O-methylmannose and 2,3,4-tri-O-methylglucuronic acid
(i)	2,4,6-tri-O-methylgalactose
(j)	2,4,6-tri-O-methylgalactose
(k)	2,3,6-tri-O-methylgalactose
(l)	2,3,4-tri-O-methylgalactose
(m)	2,3,4-tri-O-methylgalactose
(n)	2,3-di-O-methylglucuronic acid and 2,6-di-O-methylgalactose
(o)	2,3-di-O-methylglucuronic acid and 4,6-di-O-methylmannose

(p) 2,6-di-O-methylgalactose

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

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

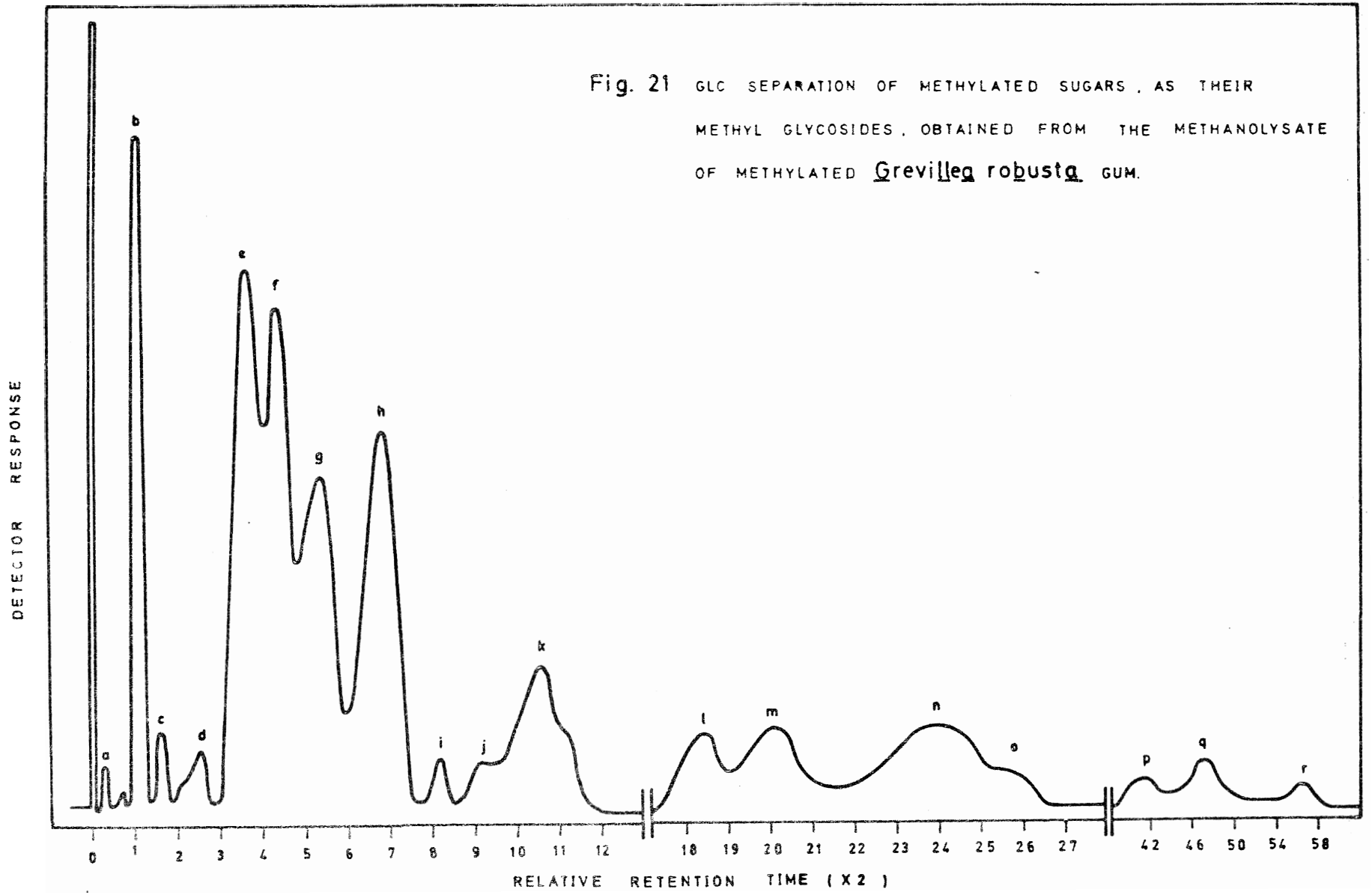


Fig. 21 GLC SEPARATION OF METHYLATED SUGARS, AS THEIR METHYL GLYCOSIDES, OBTAINED FROM THE METHANOLYSATE OF METHYLATED Grevillea robusta GUM.

Table 2 Methylated sugars, as their methyl glycosides, found by GLC in the methanolysate of methylated Grevillea robusta gum

Methylated sugar	Mol %
2,3,5-Tri-O-methylarabinose	30
2,5-Di-O-methylarabinose	3
2,3-Di-O-methylarabinose	trace
2,3,4-Tri-O-methylxylose	5
2,3-Di-O-methylxylose	trace
2,3,4-Tri-O-methylrhamnose	trace
2,3,4,6-Tetra-O-methylgalactose	3
2,3,4-Tri-O-methylgalactose	13
2,3,6-Tri-O-methylgalactose 2,4,6-Tri-O-methylgalactose	trace
2,6-Di-O-methylgalactose 2,4-Di-O-methylgalactose	22
3,4,6-Tri-O-methylmannose	5
4,6-Di-O-methylmannose	trace
2,3,4-Tri-O-methylglucuronic acid	7
2,3-Di-O-methylglucuronic acid	8

units, mainly (1→6)- linked, though (1→3)- and (1→4)-linkages between D-galactose units are also present. Residues of L-arabinofuranose occur in the gum mainly as end-groups; however, the presence of methylglycosides of 2,5- and 3,5-di-O-methylarabinose in the methanolysate suggests that some of the L-arabinose residues in this polysaccharide are chain units. That D-xylose residues are present in the gum mainly as end-groups, is shown by the proportion of the methyl glycoside of 2,3,4-tri-O-methylxylose in the methanolysate of the methylated gum, but a trace of that of 2,3-di-O-methylxylose was also detected, indicating the occurrence of some chain-units of D-xylose in the gum. The L-rhamnose residues present in small proportion occur exclusively as end groups.

It is interesting to note that the methanolysate of the methylated gum contains the methylglycosides of 2,3,4-tri-O-methylglucuronic acid and 3,4,6-tri-O-methylmannose in a molar ratio of *ca.* 7:5. This, taken in conjunction with the detection of 2-O-(β-D-glucopyranosyluronic acid)-D-mannose in an hydrolysate of the gum, suggests that D-glucuronic acid and D-mannose residues may be mutually linked to some extent. Residues of D-glucuronic acid and D-galactose, mutually joined to form 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, the other biouronic acid produced on hydrolysis, may terminate some side-chains in the polysaccharide, as in the case of the gum from Anogeissus leiocarpus.[38] In addition, these biouronic acid units may

be present as bridges between side-chains, possible evidence for this type of arrangement being the presence in the methanolysate of the methylglycosides of 2,3-di-O-methyl-glucuronic acid, which indicates the occurrence of glucuronic acid as a chain unit in the gum.

3.4 Smith-degradation studies on Grevillea robusta gum

Smith-degradation studies on a sample of Grevillea robusta gum were performed under the experimental conditions which were the same as those used in a similar study of the Brabeium stellatifolium gum exudate (see section 2.5).

When a sequence of four successive Smith-degradation stages was performed on Grevillea robusta gum, the degradation pattern was found to resemble closely that observed with Brabeium stellatifolium gum. To facilitate comparison, data on: (a) periodate uptake of Grevillea robusta gum and the degraded polysaccharides derived therefrom, (b) the alcohol-soluble fractions obtained at each stage, (c) the reduced, oxidised polysaccharides and (d) the degraded polysaccharides A'-D' have been tabulated similar to the corresponding data for Brabeium stellatifolium gum and its products, in tables 12, 13, 14 and 15 respectively.

After the first Smith-degradation stage, solvent-fractionation of the degraded material showed that all the xylose and ca. $\frac{2}{3}$ of the arabinose residues initially present in the gum had been removed together with some glucuronic acid. The Smith-degraded polysaccharide A' (ca. 59% by weight of the original gum) consisted of a variety of components of different molecular size (gel chromatography; see Fig. 37). The weight-average molecular weight, $\bar{M}_w$, for polysaccharide A' was found to be 468 000. The polysaccharide products B', C' and D' of each of the successive stages, (yields respectively 11, 1.9 and 0.9% by weight of the starting material) had $\bar{M}_w$

50 200, 2 000 and 1 900 respectively (see Table 15). The alcohol-soluble fractions (see Table 13) in each case consisted largely of glycerol, together with some arabinose, galactose and mannose, and glycosides of these sugars. Some threitol was also present in both alcohol-soluble and alcohol-insoluble fractions, indicating the existence of (1→4)-linked galactopyranose residues in the gum.

As in the case of Brabeium stellatifolium gum, residues of D-mannose, in relatively high proportion, persisted in the degraded polysaccharides throughout the sequence of Smith-degradations. L-Arabinose in decreasing proportion was also present in all of the polysaccharides A'-D', probably due to the same reason as given for a similar situation found for Brabeium stellatifolium gum (see section 2.5). D-Glucuronic acid persisted in polysaccharides A'-C', but was not detected in polysaccharide D'.

A decrease in $\bar{M}_w$ from 1 500 000 to 468 000 (ca. 69%) accompanied the first Smith-degradation stage. Comparison of $\bar{M}_w$ for polysaccharide A' with the value (713 000) predicted, from the periodate uptake of the gum, on the assumption that only peripheral sugar residues are removed, indicates fission of one internal linkage in most of the polysaccharide molecules during the first Smith-degradation stage. Polysaccharide A' is highly branched, having on the average 1 169 (ca. 40%) nonreducing end-groups or periodate-vulnerable chain units and 1 720 periodate-resistant sugar residues per molecule.

The decrease in $\bar{M}_w$, from 468 000 to 50 200 (ca. 89%), occurring during the second degradation stage suggests an average of 5 internal breaks per molecule, since $\bar{M}_w$ for polysaccharide B' is approximately $\frac{1}{6}$ of the value (280 000) predicted if the second Smith-degradation removed only end-groups. Polysaccharide B' is also fairly extensively branched, containing on the average 102 (ca. 33%) unprotected nonreducing end-groups or (1→6)- linked chain units and 207 periodate-resistant sugar residues per molecule.

During the third degradation stage $\bar{M}_w$ decreased from 50 200 to 2 000 (ca. 96%). Removal of end-groups only would give a product having $\bar{M}_w$ 33 740; on the average, therefore, 16 internal fissions per molecule occur at this stage. This large increase in the number of internal linkages broken is possibly due to the exposure of periodate-vulnerable sugar residues as a result of the removal of protecting branches during the preceding degradation stages.

The small decrease in $\bar{M}_w$, from 2 000 to 1 900, accompanying the fourth degradation stage is consistent with the removal of one end-group only from each molecule; this is substantiated by the periodate uptake of polysaccharide C'. No further internal fissions occur at this stage.

It is evident from the above results that a periodate-resistant galactomannan core, having $\bar{M}_w$ ca. 2 000 (approximately 12 hexose units per average molecule) remains after four successive Smith-degradations of Grevillea robusta gum.

3.5 Summary of the structural features of Grevillea robusta gum

The general structure of this polysaccharide is very similar to that of Brabeium stellatifolium gum. The results discussed above indicate that this polysaccharide also has a basal galactomannan structure, the average size of the periodate-resistant core being ca. 12 hexose units. Various types of side chain are attached to this basal chain; these consist mainly of D-galactose and are terminated by residues such as L-arabinose (mainly), D-xylose and L-rhamnose. As in the case of Brabeium stellatifolium gum, there is evidence that the D-mannose residues are linked (through C-2) to D-glucuronic acid. However, the detection of the aldobiouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose in an hydrolysate of Grevillea robusta gum, suggests that in the gum some of the D-glucuronic acid residues are linked to D-galactose.

3.6 Comparison between the gums of Brabeium stellatifolium and Grevillea robusta

On the evidence obtained from all the investigations conducted on the gums of Brabeium stellatifolium and Grevillea robusta, the physical and chemical properties and mode of reaction of the two gums appear to correspond in many respects. The main physical and chemical characteristics of these gums are compared in Table 3, from which a remarkable similarity in sugar composition is clearly evident. The equivalent weights of the two gums differ appreciably, but these were found by titration and the values reported are approximate, owing to the low solubilities of the gums

Table 3 A comparison between the main physical and chemical characteristics of Brabeium stellatifolium and Grevillea robusta gums

	Brabeium stellatifolium gum	Grevillea robusta gum
$[\alpha]_D$	-6^0	-10^0
$\overline{M}_w$	560 000	ca. 1 500 000
Equivalent weight	ca. 2 500	ca. 1 300
Hence uronic anhydride (%)	8	11
Constituent sugars (mol %)		
Arabinose	53	55
Galactose	37	37
Xylose	7	5
Mannose	3	1
Rhamnose	trace	2

Note: In order to show the similar proportions of neutral sugar residues in the gums as found by GLC, the sugar compositions have not been corrected for the acid content of the gums, found from their equivalent weight.

in water. Gel chromatography showed both gums to have high molecular weights, $\bar{M}_w$ of Grevillea robusta gum being ca. twice that of Brabeium stellatifolium gum. Both gums formed solutions of high viscosity in water; this behaviour was consistent with their high molecular weights.

Both the gum of Brabeium stellatifolium and that of Grevillea robusta resemble gums of the glucurono-mannan group,[18] in producing the biouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-mannose on hydrolysis. Grevillea robusta gum also yields the biouronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, as do many of the gums of this group.

Methylation analysis shows both gums to have molecular structures containing mainly D-galactose units β-(1→3)-, β-(1→4) and β-(1→6)- linked to each other. In both cases the L-arabinose residues (ca. 50 mol %) appear to be present mainly as nonreducing arabinofuranose end-groups. Residues of D-xylose and L-rhamnose replace L-arabinose as end-groups, in some branches of both gums.

The similarity in the breakdown patterns obtained during successive Smith-degradations of each of the two gums is even more striking. This similarity is clearly evident from a plot of $\bar{M}_w$ (a log scale used for convenience) against the number of Smith-degradations performed (see Fig. 42). $\bar{M}_w$ which initially differs considerably for the two gums, decreases greatly during the first two Smith-

degradations of each gum. After the third Smith-degradation, the curves flatten, indicating that most of the periodate-vulnerable sugar units have been removed at this stage, leaving a periodate-resistant galactomannan core of ca. 12 to 20 sugar units for each gum.

Incomplete oxidation of uronic acid residues during Smith-degradation[89,90] demonstrated by the presence of such residues in the polysaccharide products of several successive Smith-degradations, and the persistence of D-mannose, in relatively high proportion, in the degraded polysaccharides throughout the sequential Smith-degradations, are further points of resemblance between the two gums.

The results discussed above strongly suggest close similarity in structure between the gums of Brabeium stellatifolium and Grevillea robusta, both of which have characteristics of gums of the glucuronomannan group.[18]

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

4 EXPERIMENTAL

4.1 General experimental conditions

Paper chromatography

Paper chromatography was carried out using the following solvent systems (all v/v):

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

(b) ethyl acetate-pyridine-water (10:4:3),

(c) butan-1-ol-acetic acid-water (2:1:1),

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

(e) ethyl acetate-acetic acid-formic acid-water (18:3:1:4) and

(f) butan-1-ol-acetic acid-water (4:1:5, upper layer).

Sugars, methylated sugars and glycitols were chromatographed on Whatman No. 1, 4, 3 MM and 17^{52} chromatographic paper. In order to reveal the spots the paper chromatograms were sprayed with one or more of the following reagents:

(i) p-anisidine hydrochloride,

(ii) aqueous ammoniacal silver nitrate,

(iii) periodate followed by benzidine and

(iv) aniline hydrochloride.

R_{gal} and R_G refer to rates of movement relative to galactose and 2,3,4,6-tetra-O-methylglucose respectively on Whatman No. 1 chromatographic paper in the solvent system specified.

Thin-layer chromatography

Thin-layer chromatography was carried out on glass plates coated with silica gel G (Merck) using solvent system (g), chloroform-methanol (5:1 v/v). Compounds separated by TLC were revealed by spraying the plates with M H_2SO_4 and heating in an oven at 120^0.

Paper electrophoresis

Paper electrophoresis was conducted for 4-5h at 2.5 V/cm in 0.1 M borate buffer.[95] M_g refers to the rate of movement of compounds relative to glucose on Whatman No. 1 chromatographic paper. Sugars were revealed by spraying the air-dried papers with p-anisidine hydrochloride solution containing 1% (v/v) glacial acetic acid.

Gas-liquid chromatography

GLC analyses of mixtures of methyl glycosides were carried out on a Beckman GC-2A chromatograph [nitrogen carrier gas, 91.5 cm (3-foot) x 0.63 cm (¼ inch) outer diameter copper column, packed with 14% (w/w) ethylene glycol succinate polyester on Chromosorb W (80-100 mesh, acid washed) at 155°, flame ionisation detector].[74,96] Retention times (T-values) were measured relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside (actual retention time, ca. 4 minutes).[75]

Analyses of glycitol acetate mixtures were performed using a Beckman GC-4 chromatograph [helium carrier gas (60 ml/min), dual glass columns 126 cm x 0.4 cm inner diameter, packed with 5.23 g of 3% ECNSS-M on Gas-Chrom Q (100-120 mesh) at 180°, dual flame ionisation detectors].[97] Retention times (T-values) were measured relative to hexa-O-acetyl-D-mannitol. The on-column injection technique was used to avoid decomposition of the glycitol acetates in the inlet port. Molar ratios of glycitol acetates, and hence the relative molar proportions of sugars in mixtures, were calculated using the detector response values ($\bar{K}$-values) for each sugar. These $\bar{K}$-values had previously been determined by calibration of

the flame ionisation detectors, by injecting several glycitol acetate mixtures of known composition into the chromatograph.[83]

Gel chromatography

Gel chromatography was carried out on the agarose gel Sagavac 6F* (0.9 x 60 cm column) and on the two polyacrylamide gels Bio-Gel+ P-300 (100-200 mesh)(1.5 x 90 cm column) and Bio-Gel P-10 (100-200 mesh)(1.25 x 60 cm column).

Samples loaded on the Sagavac 6F and Bio-Gel P-300 columns were in the concentration range 3-10 mg/ml, but concentration-dependence[98] of elution volumes in the more tightly cross-linked Bio-Gel P-10 necessitated the restriction of sample concentrations to the range 2-3 mg/ml, i.e. that used in the calibration of the column (see below). Fractions were eluted from the columns using M sodium chloride.[99] Fractions (1ml) of the column effluent were collected using an Isco Model 270 fraction collector, and were screened for carbohydrate by the phenol-sulphuric acid method,[76] absorbances being measured at 490 nm on a Unicam SP 600 spectrophotometer.

Molecular weights corresponding to peaks in the elution diagrams were found from calibration plots relating peak elution volume to log $\bar{M}_w$ (weight average molecular weight), which were obtained by observing the elution volumes of dextrans of known $\bar{M}_w$ and of sugar standards on the same column.[79,80]

* Seravac Laboratories, Cape Town

+ Bio-Rad Laboratories, Richmond, California (USA)

Electron microscopy

Samples of the gum to be examined, were deposited on carbon film supports by evaporation of suitable solutions in appropriate solvents. The samples were shadowed with platinum-carbon at an oblique angle of 15^0 and examined under a Philips 300 (80 Kv) electron microscope. Selected areas were photographed to give an overall linear magnification of 210 000 on the prints.

X-ray diffraction

X-ray diffraction photographs were taken using a Philips Model PW 1050 diffractometer (Cu-Kα radiation) and a Cambridge X-ray diffraction camera. Exposures lasted for ca. 15h.

General conditions

Infrared and ultraviolet spectra were obtained with a Perkin-Elmer 237 and a Beckman DB spectrophotometer respectively. Specific rotations, unless otherwise stated, were equilibrium values for aqueous solutions at ca. 20^0 and were measured using a Bellingham and Stanley Model A polarimeter.

Unless otherwise stated, solutions were concentrated at $40-45^0$ and 20 mm Hg in rotary evaporators. Melting points were determined with a Fischer-Johns apparatus and are uncorrected.

The methylated sugar fractions obtained from the cellulose column as described in section 2.4 were weighed, after being dried *in vacuo*, and identified (against standard substances) by a variety of procedures such as the measurement of $[\alpha]_D$, paper chromatography in solvent system (a),

paper electrophoresis,[97] TLC, de-O-methylation with HBr[100] and BCl_3,[101] periodate oxidation[102] and (in some cases) P M R spectroscopy[103] (Varian A-60 spectrometer, D_2O solutions). In all cases methylated sugars were converted to their methyl glycosides by heating in 2% methanolic HCl for 6h (in sealed tubes at 96°) and examined by GLC using standard sugars for comparison.

4.2 Purification and analysis of gums

4.2.1 Purification and characteristics of gums

4.2.1.1 Brabeium stellatifolium gum

All the investigations reported here were performed on a sample of the gum collected from a tree growing in the National Botanical Gardens, Kirstenbosch, C.P., in January 1969.

The gum sample was vigorously stirred for 24h in cold water to form an extremely viscous suspension containing jelly-like lumps. After filtration, to remove these lumps and some small pieces of bark, the clarified solution was deionised with Amberlite IR-120(H^+) and neutralised with $BaCO_3$. This solution was further filtered to remove any insoluble barium salts, deionised with Amberlite IR-120 (H^+), shell-frozen and freeze-dried to give a white, fluffy product.

The jelly-like material was transferred from the filter to a flask and dried by azeotropic (ethanol/benzene (1:1 v/v)) distillation to give a greyish, flaky solid. The sugar compositions of the soluble and insoluble fraction, the

latter of which dissolved during hydrolysis in the dilute acid, were found to be alike, however, and therefore only the water-soluble fraction was used in subsequent investigations.

The main physical and chemical characteristics of the gum sample are shown in Table 4. The weight-average molecular weight, $\bar{M}_w$, was determined by gel chromatography on the agarose gel Sagavac 6F. The equivalent weight was found by titration with 0.01 M NaOH using phenolphthalein as indicator, of an electrodialysed solution of the gum. Sugar compositions were determined by prolonged acid hydrolysis (0.5M H_2SO_4, 96^0, 6h) followed by reduction, acetylation and then quantitative gas-liquid chromatography,[97] of the glycitol acetate mixture (see Fig. 17). Values obtained in this manner are presented as molar proportions (mol %) of the constituents present (see Table 4).

Table 4 The main physical and chemical characteristics of Brabeium stellatifolium gum.

Date collected	Jan. 1969
$[\alpha]_D$ (c. 0.17 M NaOH)	-6^0
$\overline{M}_w$	560 000
N, (%)	< 0.3
Ash, (%)	1.8
Equivalent weight *	ca. 2 500
Hence uronic anhydride (%)	8
Arabinose	48
Galactose	34
Xylose	7
Mannose	3
Rhamnose	trace

* This value is approximate owing to the low solubility of the gum in water.

Note: The proportions of neutral sugars in this table have been corrected for the acid content of the gum, found from the equivalent weight.

4.2.1.2 Grevillea robusta gum

A sample of Grevillea robusta gum, collected from a tree growing in Stellenbosch, C.P., was purified by the methods used by previous workers,[3] with slight modifications.* The reddish-brown gum was vigorously stirred with 20 times its weight of water for 2h to form a milky colloidal suspension. This suspension was filtered through a fine nylon mesh to remove pieces of bark and other undissolved solids, and the clear solution was acidified with 1OM H_2SO_4 to a pH of ca. 1. Addition of 8 volumes of cold ethanol caused precipitation of a white solid; this was washed with some ethanol and redissolved in water, and the solution was deionised with Amberlite IR-120(H^+). Neutralisation with $BaCO_3$ and filtration to remove any insoluble barium salts was followed by deionisation with Amberlite IR-120(H^+). The resulting solution was shell-frozen and freeze-dried to give a white, amorphous product.

Analyses showed the ethanol-soluble material and freeze-dried gum to contain the same sugars. Only the ethanol-precipitated, freeze-dried polysaccharide was used in further investigations.

The main physical and chemical characteristics of Grevillea robusta gum as determined by the author and by Anderson and Harris,[3] are summarised in Table 5. Weight-average molecular weight ($\bar{M}_w$), equivalent weight and sugar

* e.g., by dissolving the gum in cold water instead of hot, to prevent autohydrolysis and subsequent loss of L-arabinofuranose.

composition were determined as described for Brabeium stellatifolium gum (see section 4.2.1.1) (see Fig. 20).

Table 5 The main physical and chemical characteristics of Grevillea robusta gum

Gum sample	Author	Anderson and Harris [3]
Date collected	April 1968	-
$[\alpha]_D$ in H_2O	-10^0 (c,0.48)	-
$\overline{M}_w$	ca. 1 500 000	-
N,(%)	0.3	-
Ash,(%)	2.0	0.22
Equivalent weight	1 300	1 176
Hence uronic anhydride (%)	11	16
Arabinose	48	32
Galactose	33	52
Xylose	5	-
Mannose	1	-
Rhamnose	2	-

Note: The proportions of neutral sugars in this table have been corrected for the acid content of the gum, found from the equivalent weight.

4.2.2 Separation and characterisation of the neutral and acidic components released on hydrolysis of Brabeium stellatifolium gum

Brabeium stellatifolium gum (5.0g) was hydrolysed (M H_2SO_4, 24h, 96^0) and the cooled solution neutralised ($BaCO_3$) and filtered through Celite 535. Concentration in vacuo gave a syrup (4.4g) which was separated into a neutral (3.2g) and an acidic fraction (0.91g) by chromatography on

Amberlite ion exchange resins IR-120(H^+) and IR-45(OH^-).

Examination of the neutral fraction

Chromatography of a portion (2.1g) of this fraction on Whatman No. 17 chromatographic papers[52] in solvent system (f) gave three sugar-containing fractions:

(i) A syrup (755 mg) which crystallised after seeding with a crystal of D-galactose and standing at 0^0 for several days. The crystalline material had $[\alpha]_D$ + 81^0 (c 1.02), m.p. and mixed m.p. with an authentic sample of D-galactose, $163-165^0$ and its paper chromatographic mobility in solvent systems (a), (b) and (c) was indistinguishable from that of D-galactose. A portion (271mg) of this fraction was oxidised to mucic acid (301mg) which, after purification by precipitation from alkaline solution by the addition of acid, had m.p. and mixed m.p. with a genuine sample of mucic acid of $210-212^0$ (with decomposition) [Lit. value $212-213^0$].[104]

(ii) A syrup (753mg), which crystallised after seeding with a crystal of L-arabinose and storage at 0^0 for several days. The crystalline material had $[\alpha]_D$ + 97^0 (c 1.13), m.p. and mixed m.p., with a sample of pure L-arabinose, $155-157^0$. Its mobility on paper chromatography [R_{gal} 1.48, 1.39 and 1.23 in solvent systems (a), (b) and (c) respectively] was the same as that of L-arabinose. From a portion (212mg) of the mixture most of the arabinose was removed as the benzoylhydrazone derivative[105] (266mg), which after recrystallisation from ethanol had m.p. $190-202^0$.[105] The mother

liquor remaining was concentrated to a syrup which on paper chromatography was found to contain mannose. The presence of D-mannose was confirmed by the preparation from this syrup of the phenylhydrazone derivative[105] of D-mannose (23mg), which after recrystallisation from ethanol had m.p. 185-190° (with decomposition) and $[\alpha]_D$ + 25° (c 1.3).[107]

(iii) A syrup (639mg), having $[\alpha]_D$ + 61° (c 0.98), which on paper chromatography in solvent systems (a), (b) and (c) was found to contain components having the same mobility as arabinose [R_{gal} 1.48, 1.39 and 1.23 in solvents (a), (b) and (c), respectively] and xylose [R_{gal} 1.86, 1.60 and 1.24 respectively]. From a portion (307mg) of the syrup most of the arabinose was removed as the benzoylhydrazone derivative[105] (263mg) which had the same characteristics as that obtained in (ii) above. Concentration of the mother liquor gave a thin syrup which on chromatography was found to contain mainly xylose. The presence of D-xylose was confirmed by the preparation, from this syrup, of the dimethyl acetal of dibenzylidene xylose[108] (119mg) which after recrystallisation from chloroform-petroleum ether (b.p. 40-60°), had m.p. 210-211° and $[\alpha]_D$ - 8°.[108] (c 0.99 in chloroform).

Examination of the acidic fraction

A portion (200mg) of the acidic fraction was separated by preparative paper chromatography[52] on Whatman No. 1 paper into two components:

Fraction (i). A syrup (68mg as Ba-salt), having $[\alpha]_D$ -31° (c 1.3), R_{gal} 0.56 in solvent system (c) and M_g 0.70. Paper chromatographic and electrophoretic mobilities were indistinguishable from those of 2-O-(β-D-glucopyranosyluronic acid)-D-mannose. A sample (20mg) of this syrup was methylated by the procedure of Haworth[71] and Kuhn.[72] GLC following methanolysis gave peaks at T 3.72s and 2.78m, corresponding to 2,3,4-tri-O-methylglucopyranuronic acid and 3,4,6-tri-O-methylmannose. After saponification with sodium methoxide and neutralisation (solid CO_2), GLC showed only one peak, at T 3.72s. Since the molar proportions calculated from the peak areas before and after saponification were equal, the presence of 2-O-(β-D-glucopyranosyluronic acid)-D-mannose was confirmed.

Fraction (ii). Small granular crystals (32mg as the Ba-salt), having $[\alpha]_D$ + 14° (c, 1.3), R_{gal} 0.89 in solvent system (c) and M_g 1.20. Paper chromatographic and electrophoretic mobilities were indistinguishable from those of β-D-glucopyranuronic acid. After methylation, as for fraction (i), followed by methanolysis, GLC showed two peaks, at T 3.72s and 2.78m, corresponding to 2,3,4-tri-O-methylglucopyranuronic acid. GLC following saponification as for fraction (i) showed no peaks indicating the presence of D-glucopyranuronic acid only.

4.2.3 Separation and characterisation of the neutral and acidic components released on hydrolysis of Grevillea robusta gum

Grevillea robusta gum (1.0g) was hydrolysed (M H_2SO_4, 24h, 96°) and the cooled solution was neutralised ($BaCO_3$) and filtered through Celite 535. Concentration in vacuo gave a syrup (800mg), which was separated into six fractions on Whatman No. 3 MM chromatographic papers in solvent system (c).

Fraction (i) A syrup (57mg), having $[\alpha]_D$ + 8° (c 1.3). Paper chromatography in solvent systems (c) and (e) showed the presence of components having mobilities indistinguishable from those of 6-O-(β-D-glucopyranosyluronic acid)-D-galactose and traces of D-galactose. After further acid hydrolysis (M H_2SO_4, 24h, 96°) D-glucuronic acid, D-galactose and unchanged biouronic acid were detected on paper chromatography.

Fraction (ii) A syrup (8mg). Paper chromatography in solvent systems (c) and (e) showed the presence of components having mobilities indistinguishable from those of 2-O-(β-D-glucopyranosyluronic acid)-D-mannose and D-galactose.

Fraction (iii) Small crystals (20mg) having $[\alpha]_D$ + 12° (c, 0.98). Paper chromatography in solvent systems (c) and (e) showed this fraction to consist mainly of D-glucuronic acid, together with some D-galactose. Isolation of the acidic component by chromatography on Amberlite ion-exchange resins IR-45 (OH^-) and IR-120 (H^+) was followed by concentration in vacuo to a thin syrup (15mg). Methylation of this syrup by the methods of Haworth[71] and Purdie,[73] was followed by methanolysis and GLC. Two peaks at T 3.72s and 2.78m were observed,

both of which disappeared on saponification with sodium methoxide, confirming the presence of 2,3,4-tri-O-methyl-glucopyranuronic acid.

Fraction (iv) A syrup (91mg), which crystallised after seeding with a crystal of D-galactose and storage at 0^0 for several days. The crystalline material had $[\alpha]_D$ + 77^0 (c 1.4), m.p. and mixed m.p., with an authentic sample of D-galactose, $162-164^0$. Oxidation of a portion of the crystals with a 20% (v/v) solution of nitric acid gave a precipitate of mucic acid, which after purification by precipitation from alkaline solution by addition of acid had m.p. $211-213^0$ (with decomposition) [Lit. value $212-213^0$].[104]

Fraction (v) A syrup (192mg), $[\alpha]_D$ + 79^0 (c 1.0). Paper chromatography in solvent system (a) and (c) showed the presence of arabinose (mainly) and some mannose and xylose. The benzoylhydrazone derivative of L-arabinose was successfully prepared despite the presence of the other components.[105] Recrystallisation of this derivative from ethanol gave white needle-like crystals, m.p. $190-200^0$ (with decomposition) [Lit. value $190-202^0$].

Fraction (vi) A syrup (45mg), $[\alpha]_D$ + 57^0 (c 1.5), was shown by paper chromatography in solvents (a) and (c) to consist mainly of xylose together with some arabinose. From this syrup the dimethyl acetal of dibenzylidene xylose[108] was prepared. Recrystallisation from chloroform-petroleum ether (b.p. $40-60^0$) gave a crystalline derivative m.p. $210-212^0$ [Lit. value $211-213^0$], having $[\alpha]_D$ - 9^0 (c 0.8 in chloroform); this confirmed the presence of D-xylose.

4.3 Acid hydrolysis of Brabeium stellatifolium gum[54]

4.3.1 Partial hydrolysis in 5 mM sulphuric acid

Freeze-dried Brabeium stellatifolium gum (1.88g) was heated at 96° in 5 mM sulphuric acid (100ml) for 96h. The pH of the solution was recorded before and after hydrolysis (see Table 6). Samples (5ml) were removed at intervals. Each hydrolysate sample was immediately cooled and its optical rotation measured. After neutralisation with barium carbonate and centrifugation, the samples were examined by chromatography on paper and on an appropriate gel (see section 4.1). The reducing power of each hydrolysate sample was determined by the Nelson modification[87] of the Somogyi method,[88] absorbances being measured at 546 nm on a Unicam SP 600 spectrophotometer; 1ml aliquots were diluted so that the concentration of reducing sugar lay within the range 25-250 µg/ml. The degree of scission (x) of the polysaccharide was calculated in each case from the equation

$$x = \frac{RPi - RPgum}{RPh}$$

where RPi = reducing power (expressed as g reducing sugar/g polysaccharide) of each hydrolysate fraction,

RPh = reducing power of a sample of the gum subjected to prolonged hydrolysis (0.5 M H_2SO_4, 96°, 18h) and

RPgum = reducing power of the undegraded gum.

4.3.2 Further hydrolysis in 50 mM sulphuric acid

The acid concentration in the remaining solution (60ml) was adjusted to 50 mM by the addition of 0.5M sulphuric acid (ca. 8ml). Again the pH was measured before and after hydrolysis (see Table 7). The solution was heated at 96^0 for a further 23h. Samples (5ml) were removed at intervals and were examined as described in section 4.3.1.

4.3.3 Kinetics of hydrolysis

The mean values of the hydrolysis rate constant, k, over the successive time intervals between samples are given in Tables 6 and 7. These values were calculated from the equation

$$k = \frac{1}{t_2 - t_1} \ln \frac{1 - x_1}{1 - x_2}$$

where t_1 and t_2 are the times of hydrolysis, in seconds, corresponding to consecutive measured values, x_1 and x_2, of the degree of scission.

4.3.4 Statistical depolymerisation calculations

Several statistical treatments of the depolymerisation of long-chain molecules have been published.[65,66,67,68] Montrol and Simha[66] considered the decrease of $\bar{M}_w$ with increasing degree of scission (x) in the case of completely random depolymerisation and derived the equation

$$\frac{\bar{M}_w}{M_o} = \frac{nx^2+2(1-x)[(1-x)^n+nx-1]}{nx^2} \quad \ldots\ldots(1)$$

where M_o = molecular weight of one monomer unit

and n = number of monomer units in the undegraded polymer.

Simha[67] subsequently considered the case of preferential scission of terminal linkages, the rate constant for the breaking of internal linkages being regarded as negligible in comparison with that for terminal bonds. Thermal depolymerisation of polystyrene in the liquid phase at 350° was cited as an example of a reaction believed to proceed in this way. For this situation he derived the equation:

$$\frac{\bar{M}_w}{M_o} = 1+e^{-z}\left[(n-1)\frac{z^{n-2}}{(n-2)!} + (n-2z-1+\frac{2z}{n})\frac{z^{n-3}}{(n-3)!} + (1-\frac{z}{n})(n-z-1+\frac{z}{n-z})(e^{-z}-\sum_{r=n-3}^{\infty}\frac{z^r}{r!})\right]+\frac{2R}{n} \quad \ldots\ldots \quad (2a)$$

where $z = 2kt$, k being the hydrolysis rate constant at time t and

$$R = e^{-z}2^{n-2}\sum_{i=n-1}^{\infty}(\frac{z}{2})^i\frac{1}{i!}$$

M_o and n having the same significance as in equation (1).

When n becomes very large and z very small (ie: $z<<1$) equation (2a) can be approximated to :

$$\frac{\bar{M}_w}{M_o} \doteq 1 + \left[(1-\frac{z}{n})(n-z-1+\frac{z}{n-z})\right] \quad \ldots\ldots \quad (2b)$$

Since equations (1) and (2a) or (2b) can be used to calculate the variation of $\frac{\bar{M}_w}{M_o}$ with x^* when the depolymerisation is completely random or non-random respectively, comparison of the actual values of $\frac{\bar{M}_w}{M_o}$ at different x with

the theoretical values calculated from these equations gives some idea of the randomness or non-randomness of depolymerisation of the gum.

In Fig. 16 the experimental values of $\frac{\bar{M}_w}{M_o}$ + for Brabeium stellatifolium gum [curve (2)] are plotted against x on the same axes as the theoretical values from equations (1) [curve (3)] and (2b) [curve (1)]. The correct constraints could be placed upon n and z since these are readily calculated for this particular polysaccharide molecule, from available data.

* $\frac{\bar{M}_w}{M_o}$ can be plotted against any variable proportional to time.

+ $\frac{\bar{M}_w}{M_o}$ has been calculated on a basis of a mean value of 163 for M_o.

TABLE 6

Partial hydrolysis of Brabeium stellatifolium gum in 5 mM sulphuric acid at 96°

Time of hydrolysis (h)	Degree of scission, (x)	$[\alpha]_D^{20}$ (c 1.88, 5 mM H_2SO_4)	$\bar{M}_w$	10^6 k sec^{-1}	pH
0		- 6°	560 000	-	1.83
2	0.03	+ 8°	480 000	*	
5	0.21	+34°	63 000	18.30	
8	0.26	+47°	58 900	6.15	
12	0.32	+53°	53 100	4.44	
24	0.43	+55°	20 500	3.63	
48	0.51	+56°	18 200	1.95	
72	0.58	+57°	420	1.81	
96	0.64	+58°	370	1.70	2.00

TABLE 7

Further hydrolysis of Brabeium stellatifolium gum in 50 mM sulphuric acid at 96°

Time of further hydrolysis (h)	Degree of scission, (x)	$[\alpha]_D^{20}$ (c 1.66, 50 mM H_2SO_4)	$\bar{M}_w$	10^6 k sec^{-1}	pH
0	-	-	-	-	1.25
1	0.76	+58°	330	-	
3	0.77	+59°	305	9.28	
8	0.79	+59°	280	5.75	
23	0.79	+59°	≤260	-	1.45

* Rate constant unreliable because of the initial insolubility of the gum in 5 mM H_2S●$_4$.

4.4 Methylation analysis of Brabeium stellatifolium gum

4.4.1 Methylation of the gum

(a) Bulk methylation

A sample (6.00g) of the gum was methylated by the procedures of Haworth[71] (once), Kuhn[72] (twice) and Purdie[73] (five times). The product (4.80g) showed no absorption in the IR region 3 200-3 500 cm^{-1}, had $[\alpha]_D$ -21^0 (c, 1.93 in $CHCl_3$), (Found: C, 48.5; H, 6.9 and ash 1.2%).

(b) Semi-micro methylation

Another sample (1.00g) of the gum, methylated as in (a) above, yielded a product (620mg) showing no absorption in IR region 3 200-3 500 cm^{-1}, $[\alpha]_D$ -20.7^0 (c, 0.87 in $CHCl_3$), (Found: C, 48.7; H, 6.6 and ash 1.4%).

Methanolysis of the methylated gum

Portions (ca. 500mg) of the methylated gum samples from (a) and (b) above were methanolysed in sealed tubes with 2% methanolic HCl for 6h at 96^0. After neutralisation (Ag_2CO_3), filtration through Celite 535 and evaporation to dryness in vacuo, a drop of methanol was added to each dry residue, and each product was analysed semi-quantitatively by GLC (see Fig. 18). The results are shown in Table 1 (section 2.4).

4.4.2 Separation and identification of the methylated monosaccharides released on hydrolysis of the methylated gum

Hydrolysis of the methylated gum.

Another portion (4.30g) of the methylated gum sample from

(a) above was heated at 96° in 98% formic acid (15ml) for 45 minutes. Water (30ml) was added and the product evaporated to dryness. Hydrolysis was completed by heating in 0.5 M H_2SO_4 (100ml) at 96° for 12h. The hydrolysate, after neutralisation with $BaCO_3$, was filtered through Celite 535, and the filtrate was concentrated to a syrup, which was fractionated by cellulose column chromatography.

Chromatographic separation of the methylated sugars

The above syrup, containing the methyl ethers of the sugars present in the gum, was applied to a water-jacketed (thermostatted at 35°) glass column (120 x 5.8cm) packed with acid-washed (5 M HCl) cellulose and eluted with the following solvent systems: Petroleum ether (b.p. 60-80°) mixed with water-saturated butan-1-ol [proportions of alcohol being increased from 1:7 to 11:7 in 8 steps (total volume 50 litres)]; water-saturated butan-1-ol and ethanol/water (1:1) and finally water (see Fig. 19). Fractions (on the average 40ml each) were collected hourly in beakers, samples being screened by paper chromatography [solvent (a)] and by TLC [solvent (g)]. In this way 22 sugar-containing fractions* were obtained. The R_g values quoted in the description of these fractions below are measured in solvent system (a). The T-values reported are all for the methyl glycosides derived from the sugars.[75]

* These were made up as follows, fraction numbers preceding the numbers of beakers used in the collection: 1, 1-94; 2, 95-114; 3, 115-199; 4, 200-228; 5, 229-342; 6, 343-347; 7, 348-367; 8, 368-391; 9, 392-430; 10, 431-437; 11, 438-486; 12, 487-500; 13, 501-630; 14, 631-655; 15, 656-721; 16, 722-799; 17, 800-848; 18, 849-896; 19, 897-1 016; 20, 1 017-1 040; 21, 1 041-1 203; 22, 1 204-1 254.

Fractions 1-19 were all de-O-methylated with hot aqueous HBr[100] and, where pentose sugars were present, with BCl_3[101] as well. In each case paper chromatography of the product showed the expected series of methyl ethers, identical with those from authentic specimens.

Fraction 1. A syrup (866mg), having $[\alpha]_D$ -29° (c, 1.4), R_g 0.95, T 0.55s and 0.75W. GLC showed this fraction to contain 2,3,5-tri-O-methylarabinose only. Conversion of a portion of the fraction into 2,3,5-tri-O-methyl-L-arabonamide (m.p. 130-132°), [Lit. value 133-135°][22] confirmed this identification.

Fraction 2. A syrup (136mg), having $[\alpha]_D$ -6° (c, 0.98), R_g 0.94, T 0.50m; 0.60s, 0.95w; and 2.00w, containing 2,3,4-tri-O-methylxylose (45mg), 2,3,5-tri-O-methylarabinose (7.5mg) and 2,3,4,6-tetra-O-methylgalactose (16mg).

Fraction 3. A syrup (97mg), $[\alpha]_D$ +31° (c, 1.01), R_g 0.92, T 0.55s, 0.79w; and 1.85m, containing 2,3,5-tri-O-methylarabinose (50mg) and 2,3,4,6-tetra-O-methylgalactose (47mg).

Fraction 4. A syrup (101mg), $[\alpha]_D$ +40° (c, 1.12), R_g 0.88, T 1.95s; and 3.70m, which showed the presence of 2,3,4,6-tetra-O-methylgalactose (66mg) and 3,4,6-tri-O-methylmannose (55mg). Paper electrophoresis showed a spot having the same mobility as 3,4,6-tri-O-methylmannose.

Fraction 5. A syrup (251mg), $[\alpha]_D$ -20° (c, 2.1), R_g 0.89 and 0.85, T 1.27m, 3.77s; 1.94w; 2.25m; and 3.57m, containing 3,5-di-O-methylarabinose (115mg), 2,3,4,6-tetra-O-methylgalactose (15mg), 2,5-di-O-methylarabinose (20mg) and 2,4,6-tri-O-methylmannose (101mg).

Fraction 6. A syrup (41mg), having $[\alpha]_D$ -17° (c, 0.96), R_g 0.83, T 1.20w, 3.77s; 2.15s, 4.15w; 3.57s was found to consist of 3,5-di-O-methylarabinose (15mg), 2,5-di-O-methylarabinose (14mg) and 3,4,6-tri-O-methylmannose (12mg).

Fraction 7. A syrup (73mg), having $[\alpha]_D$ +14° (c, 0.99), R_g 0.84 and 0.75, T 1.52m, 3.55m; 2.12s, 4.80w; 4.00m, 5.20w and 6.20w, corresponding to 3,5-di-O-methylarabinose (22mg), 2,5-di-O-methylarabinose (29mg), and 2,3,6-tri-O-methylgalactose (22mg).

Fraction 8. A syrup (164mg), $[\alpha]_D$ +68° (c, 1.1), R_g 0.78, T 1.95s, 2.54m; 3.40s, 4.10w; and 5.20m, containing 2,3-di-O-methylarabinose (20mg), 2,3,6-tri-O-methylgalactose (113mg) and 2,4,6-tri-O-methylgalactose (31mg). After reduction with sodium borohydride followed by periodate oxidation paper chromatography [solvent (a)] showed mainly 2,3-di-O-methylthreose, while TLC [solvent (g)] showed the presence of the periodate-resistant 2,4,6-tri-O-methylgalactose.

Fraction 9. A syrup (273mg), having $[\alpha]_D$ +76° (c, 2.6), R_g 0.75, T 2.10s, 2.40m; 3.60s, 4.90w; and 5.48m, was shown to consist of 2,3-di-O-methylarabinose (77mg), 2,3,6-tri-O-methylgalactose (165mg) and 2,4,6-tri-O-methylgalactose (31mg). After borohydride reduction of this fraction followed by periodate oxidation paper chromatography [solvent (a)] showed mainly 2,3-di-O-methylthreose, while the periodate-resistant 2,4,6-tri-O-methylgalactose was detected by TLC [solvent (g)].

Fraction 10. A syrup (61mg), $[\alpha]_D$ +80° (c, 1.10), R_g 0.72, T 1.84s, 2.47s; 3.63s, 4.63w; and 5.42s, consisted of 2,3-di-O-methylarabinose (17mg), 2,3,6-tri-O-methylgalactose (38mg) and 2,4,6-tri-O-methylgalactose (6mg). After borohydride reduction and periodate oxidation 2,3-di-O-methylthreose was detected on paper chromatography [solvent (a)], 2,4,6-tri-O-methylgalactose by TLC [solvent (g)].

Fraction 11. A syrup (187mg), $[\alpha]_D$ +79° (c, 1.9), R_g 0.48 (trace), 0.64 and 0.72, T 1.94s, 2.50m; 3.72m, 4.83m; 5.50s; and 8.60w, which showed the presence of 2,3-di-O-methylarabinose (118mg), 2,3,6-tri-O-methylgalactose (11mg), 2,3,6-tri-O-methylgalactose (51mg) and 2,3,4-tri-O-methylgalactose (7mg).

Fraction 12. A syrup (91mg), $[\alpha]_D$ +84° (c, 0.97), R_g 0.48 (trace) 0.64 and 0.72, T 1.84s, 2.37s; 4.70w; 5.25m; and 8.25s, was shown to contain 2,3-di-O-methylarabinose(38mg), 2,4,6-tri-

O-methylgalactose (14mg) and 2,3,4-tri-O-methylgalactose (39mg).

Fraction 13. A syrup (423mg), having $[\alpha]_D$ +73° (c, 2.1), R_g 0.21 (trace), 0.50, 0.65 and 0.72, T 1.82s, 2.40m; 4.75w, 5.40m; 8.56s; and 14.3s, contained 2,3-di-O-methylarabinose (21mg), 2,4,6-tri-O-methylgalactose (13mg), 2,3,4-tri-O-methylgalactose (190mg) and 2,6-di-O-methylgalactose (199mg). Periodate oxidation gave the expected methoxymalondialdehyde (canary-yellow spot on spraying with p-anisidine hydrochloride, having R_f 0.21 in solvent system (a)).

Fraction 14. A syrup (112mg), $[\alpha]_D$ +75° (c, 1.4), R_g 0.57 and 0.66, M_g 0 and 0.53, T 1.70m, 2.29w; 2.37w, 4.30 ; 6.08w; 10.9m; 11.9s, 14.9s, 17.0m and 21.0w, was shown to contain 2,3-di-O-methylarabinose (6mg), 2,4,6-tri-O-methylgalactose (6mg), 2,3,4-tri-O-methylgalactose (41mg) and 2,6-di-O-methylgalactose (59mg). After periodate oxidation paper chromatography showed the expected methoxymalondialdehyde as for fraction 13.

Fraction 15. A syrup (216mg), $[\alpha]_D$ +81° (c, 2.2), R_g 0.55, M_g 0 and 0.53, T 13.1s, 17.2m; 23.7m and 28.0w, contained 2,6-di-O- methylgalactose (203mg) and 2,4-di-O-methylgalactose (13mg). After periodate oxidation paper chromatography showed the same yellow spot as was obtained for fractions 13 and 14.

Fraction 16. A syrup (159mg), $[\alpha]_D$ +87° (c, 1.3), R_g 0.08, 0.21 and 0.52, M_g 0.27, T 2.43w, 5.16s; 12.0s, 16.0m, 20.0 ; and 21.0w which showed the presence of 3-O-methylarabinose (48mg), 2,6-di-O-methylgalactose (105mg) and 2,4-di-O-methylgalactose (6mg). After periodate oxidation paper chromatography showed the same yellow spot as was obtained for the previous three fractions.

Fraction 17. A crystalline component (198mg) $[\alpha]_D$ +84° (c, 1.4), R_g 0.33 (trace) and 0.45, M_g 0, T 4.40w, 6.00w; 21.0s and 25.0s, which showed the presence of 3-O-methylarabinose (20mg) and 2,4-di-O-methylgalactose (178mg). Recrystallisation from chloroform-petroleum ether (b.p. 80-100°) gave crystals, m.p. and mixed m.p. (with authentic 2,4-di-O-methyl-α-D-galactose) 105-106° [Lit. value 106-107°][83]. The aniline derivative had m.p. and mixed m.p. (with authentic 2,4-di-O-methyl-N-phenyl-D-galactosylamine) 213-214°. P.M.R. spectoscopy showed the crystalline sugar to consist of mainly the α-anomer, with methoxyl singlets corresponding to those found for an authentic specimen of 2,4-di-O-methyl-α-D-galactose.

Fraction 18. A syrup (141mg), $[\alpha]_D$ +79° (c, 1.8), R_g 0.35, 0.39 and 0.48, M_g 0.72, T 4.41m, 6.23s, 10.2m, 13.1s; 22.9m and 26.6s, was shown to contain 3-O-methylarabinose (70mg) and 2,4-di-O-methylgalactose (71mg).

Fraction 19. A syrup (82mg) having $[\alpha]_D$ +93° (c, 1.2), R_g 0.22 (trace), 0.39, 0.43, 0.50 (trace) and 0.56 (trace), M_g 0.72, T 4.46m, 6.23s, 10.2m, 13.0m; 21.5w and 25.2m, showed the presence of 3-O-methylarabinose (71mg) and 2,4-di-O-methylgalactose (11mg).

Fraction 20. A syrup (10mg) having $[\alpha]_D$ +80° (c, 0.90), R_g 0-0.03 (trace), 0.09 (trace), 0.21, 0.30 (trace) and 0.40 (trace), M_g 1.3, was judged by paper chromatography to be a mixture of components containing mainly arabinose.

Fraction 21. A crystalline sugar (61mg), having $[\alpha]_D$ +70° (c, 1.0), R_g 0.26 (trace), 0.32 and 0.43 (trace), M_g 0.20, was judged by paper chromatography to consist mainly of 2-O-methylgalactose. Periodate oxidation gave the expected methoxymalondialdehyde (canary-yellow spot, R_f 0.25 in solvent system (a)).

Fraction 22. A syrup (315mg), having $[\alpha]_D$ +25° (c, 3.1), R_g 0.03 and 0.09, T 2.51m, 3.76s, 12.60m and 16.00m, was judged by paper chromatography to consist of a mixture containing ca. 20 parts of the Ba salts of two methylated glucuronic acids (ca. 300mg) and ca. 1 part of galactose (ca. 16mg). GLC showed the mixture of methylated glucuronic acids to consist of 2,3,4-tri-O-methylglucuronic acid (ca. 171mg as the Ba salt) and 2,3-di-O-methylglucuronic acid (ca. 129mg as the Ba salt). An unidentified component (trace) of a polymeric nature was also found to be present.

The syrup was passed through Amberlite IR-120 (H^+), to remove Ba^{2+} ions, concentrated and transferred to a column of Amberlite IR-45 (OH^-). After elution of the neutral materials, which were concentrated to a syrup (10mg), the acidic component was washed from the resin with 0.1M NaOH. After removal of Na^+ ions with Amberlite IR-120 (H^+), the resulting solution was concentrated and heated in a sealed tube with 2% methanolic HCl for 12h. Neutralisation (Ag_2CO_3), filtration through Celite 535 and concentration yielded a thin brown syrup (293mg).

A portion (43mg) of the syrup containing the acidic components, was reduced with $LiAlH_4$ in dry tetrahydrofuran (12ml) for 2h with stirring. After addition of wet ethyl acetate (5ml) and acidification to pH ca. 4, the mixture was extracted with chloroform (3 x 10ml) and dried over anhydrous Na_2SO_4 for 4h. After concentration paper chromatography of the hydrolysed product and GLC analysis of the product itself showed the presence of 2,3,4-tri-O-methylglucose and 2,3-di-O-methylglucose only.

4.5 Methylation analysis of Grevillea robusta gum

4.5.1 Methylation of the gum

A sample (1.3g) of Grevillea robusta gum was methylated according to the procedures of Haworth[71] (once), Kuhn[72] (once) and Purdie[73] (five times) to yield a clear yellow glass (895mg), having $[\alpha]_D$ -40^0 (c 1.79 in chloroform) and showing no absorption in the IR region 3200-3500 cm^{-1}. (Found: C, 49.3; H, 7.3; N, 0.2; OMe, 36.9 and ash, nil).

4.5.2 Identification by GLC of the methylated monosaccharides released on methanolysis of the methylated gum

A portion (100mg) of the methylated gum was methanolysed with 2% (v/v) methanolic hydrogen chloride at 96^0 for 6h. Neutralisation (Ag_2CO_3) was followed by filtration, concentration and GLC analysis[75] (see Table 2 and Fig. 21).

4.6 Smith-degradation conditions

4.6.1 Periodate oxidation

Aqueous solutions of the gums of Brabeium stellatifolium and Grevillea robusta were oxidised with sodium metaperiodate at room temperature and in the absence of light, as described by Smith and co-workers.[25] Each polysaccharide sample was oxidised on both an analytical and a preparative scale, using concentrations of periodate of 0.08 M or less. An excess of at least 100% of periodate ion was maintained, while the polysaccharide concentrations did not exceed 1%.

Aliquots of oxidation mixtures were removed at intervals and assayed spectrophotometrically,[81] for periodate content. Oxidation was continued until flattening was observed in the curve showing the increase, with time, of the periodate uptake

per 100g of polysaccharide monitored in this way (see Figs. 22-26 and 33-36). In some cases the data obtained by the spectrophotometric method were confirmed by using in addition, the 2,4,6-tri-2-pyridyl-*S*-triazine (TPTZ) method[82] for the determination of periodate in aqueous solutions.

4.6.2 Termination of the oxidation reaction

Periodate oxidation reactions were terminated in various ways:

(i) Remaining periodate was destroyed by the addition of excess ethylene glycol to form iodate ions and formaldehyde. Where the polysaccharide material had $\bar{M}_w \geq 10\,000$, these reagents were removed by dialysis in two stages. Initially possible losses of carbohydrate material having low molecular weight were checked by dialysis of the solution against distilled water for 2 days, the water being changed daily. A representative aliquot (*ca.* 300ml) of the combined dialysate was concentrated to a small volume from which the iodate was removed by addition of barium acetate followed by filtration (to remove precipitated barium iodate) and deionisation with Amberlite ion exchange resins IR-120 (H^+) and IR-45 (OH^-). After concentration of the ion-free solution to *ca.* 10ml, the volume of the solution was accurately adjusted to 25ml, and aliquots were assayed for carbohydrate both qualitatively (Molisch test) and quantitatively (phenol-sulphuric acid method[76]). These tests were negative for all dialysates investigated.

Further dialysis (2-3 days) against running tap water removed any remaining ethylene glycol, formaldehyde and iodate

ions; acidified KI solution was used to test qualitatively for iodate.

(ii) Precipitation of iodate and periodate as barium salts by the addition of barium carbonate while stirring, over a period of 3 days, was used as an alternative procedure in cases where the polysaccharide material had $\overline{M}_w$ < 10 000. Excess barium carbonate together with the precipitated barium iodate and barium periodate, was filtered off from the solution through Celite 535. Losses of carbohydrate material on the filter bed were found to be a serious drawback of this method and therefore the procedure described in (i) above was used whenever possible.

(iii) Improvements in the yields of polysaccharide material having $\overline{M}_w$ < 10 000 were obtained by immediate sodium borohydride reduction of the oxidised polysaccharides without prior removal of periodate and iodate ions. In this manner these ions were simultaneously reduced to iodide; the reduced, oxidised polysaccharide was recovered as described in section 4.6.3(ii).

4.6.3 Reduction of the oxidised polysaccharide

Excess sodium borohydride was added in portions with constant stirring, over several days to an aqueous solution of the oxidised polysaccharide until reduction was complete. This was determined by either Fehlings test or the TTC test for aldehydes on an acidified aliquot of the solution. Reduced, oxidised polysaccharides were recovered, in either of two ways:

(i) After destruction of excess borohydride by the addition of glacial acetic acid, with stirring, until evolution of hydrogen ceased, reduced, oxidised polysaccharides of high molecular weight ($\bar{M}_w$ > 10 000) were recovered by dialysis of the reduction mixtures against distilled water for several days, the water being changed daily, until an acidified aliquot of the dialysate gave a negative flame test for borate. A sample from the combined dialysates was acidified, concentrated and deionised with Amberlite ion-exchange resins IR-120 (H^+) and IR-45 (OH^-) and evaporated to dryness. After removal of borate by repeated evaporation with methanol the residue was dissolved in water and the solution made up to a known volume. Aliquots from this solution were checked for carbohydrate material as described in section 4.6.2(i). No carbohydrate was found in the dialysates from the reduced, oxidised polysaccharides treated in this way.

(ii) Polysaccharides of lower molecular weight ($\bar{M}_w$ < 10 000) were treated differently. In this case excess sodium borohydride was decomposed by the addition of an ion-exchange resin, Amberlite IR-120 (H^+), until the evolution of hydrogen ceased. Filtration to remove the ion-exchange resin was followed by evaporation of the filtrate to dryness. Repeated evaporation to dryness with methanol removed all borate and afforded the reduced, oxidised polysaccharide, which was re-dissolved in water, tested for the presence of iodate, iodide and borate, and finally freeze-dried. A sample of each reduced,oxidised polysaccharide was hydrolysed and qualitat-

ively examined by PC. Reduction and acetylation of a portion of the hydrolysate was followed by GLC analysis of the glycitol acetate mixture to determine the composition of the reduced, oxidised polysaccharide (see Tables 10 and 14).

4.6.4 Hydrolysis of the acetal linkages

Hydrolysis of acetal linkages in reduced, oxidised polysaccharides was effected in either of two ways. Initially solutions (1-5%) of the reduced, oxidised polysaccharide in 0.5 M H_2SO_4 were allowed to stand at room temperature and the progress of acetal cleavage was monitored by removal of aliquots at various times, periodate oxidation of the glycerol (and glycolaldehyde, if present) and determination, using the chromotropic acid method,[85] of the formaldehyde thus produced. When the yield of formaldehyde became constant, the hydrolysis was terminated either by neutralisation with $BaCO_3$ followed by filtration through Celite 535, or by neutralisation with a hot, saturated solution of barium hydroxide followed by centrifugation. Since improved yields were obtained with the latter method, it was more frequently employed. The solutions obtained by either method were concentrated to a small volume and deionised with ion-exchange resins Amberlite IR-120 (H^+) and IR-45 (OH^-). These deionised solutions were evaporated to dryness to afford the Smith-degradation products.

Since the process of monitoring acetal cleavage by taking aliquots at various times, oxidising these and determining the HCHO produced was found to be tedious, reduced, oxidised polysaccharides were at a later stage allowed to stand in 0.5 M H_2SO_4 at room temperature for 48h, after which Smith-degra-

dation products were recovered as before. After solvent-fractionation of the mixture of Smith-degraded products (see section 4.6.5) and recovery of these, ca. 2mg of the alcohol-insoluble fraction was dissolved in 0.5 M H_2SO_4 and allowed to stand for a further 24h, any further acetal cleavage at this stage being detected as before. If by this method acetal hydrolysis was shown to be incomplete after the initial 48h treatment, the bulk of the alcohol-soluble fraction was given acid treatment for a further 24h. This procedure was continued until no further acetal cleavage was observed (see Fig. 41).

The reducing power[87,88] of the polysaccharide material was determined before and after acid treatment, to ascertain whether any glycosidic bonds were broken during the acetal hydrolysis process. It was found that on the average 0.032g reducing sugar per g of polysaccharide was released per 24h period of acetal hydrolysis during the first two Smith-degradations of Brabeium stellatifolium and Grevillea robusta gums. This places some limitation upon the above method of achieving complete acetal cleavage by repeated, prolonged treatment with acid.

4.6.5 Solvent-fractionation of the Smith-degradation products.

Separation of the Smith-degradation products into fractions of low and higher molecular weight was effected by extraction of the residue obtained after evaporation, using large volumes of methanol: acetone (1:1 v/v), followed by methanol. The soluble fractions were filtered through glass-wool and evapor-

ated to constant weight. Any solid carbohydrate material left on the glass-wool filter, was washed thoroughly with methanol, dissolved in water and combined with the methanol-insoluble material.

Any free glycerol present in the insoluble fractions was effectively removed by redissolving these fractions in water and adding ethanol to precipitate the polysaccharide material of higher molecular weight. This polysaccharide was collected by centrifugation and washed with ethanol on a sintered-glass filter, and the washings were combined with the centrifugate, affording another soluble fraction.

After paper chromatography had shown all the soluble fractions from the same mixture of degradation products to be similar, these were combined and evaporated to constant weight. In each case this combined fraction was hydrolysed, and after reduction and acetylation of the hydrolysate, the resulting glycitol acetate mixture was analysed by GLC (see Tables 9 and 13).

A portion of each alcohol-insoluble fraction was also hydrolysed and the hydrolysate, after examination by PC, was reduced and acetylated. GLC analysis of the derived glycitol acetate mixture afforded a quantitative estimate of the sugars present in the degraded polysaccharide (see Tables 11 and 15).

4.7 Smith-degradations of Brabeium stellatifolium gum

Details of the periodate oxidation of Brabeium stellatifolium gum and degraded polysaccharides A, B, C, D and E are

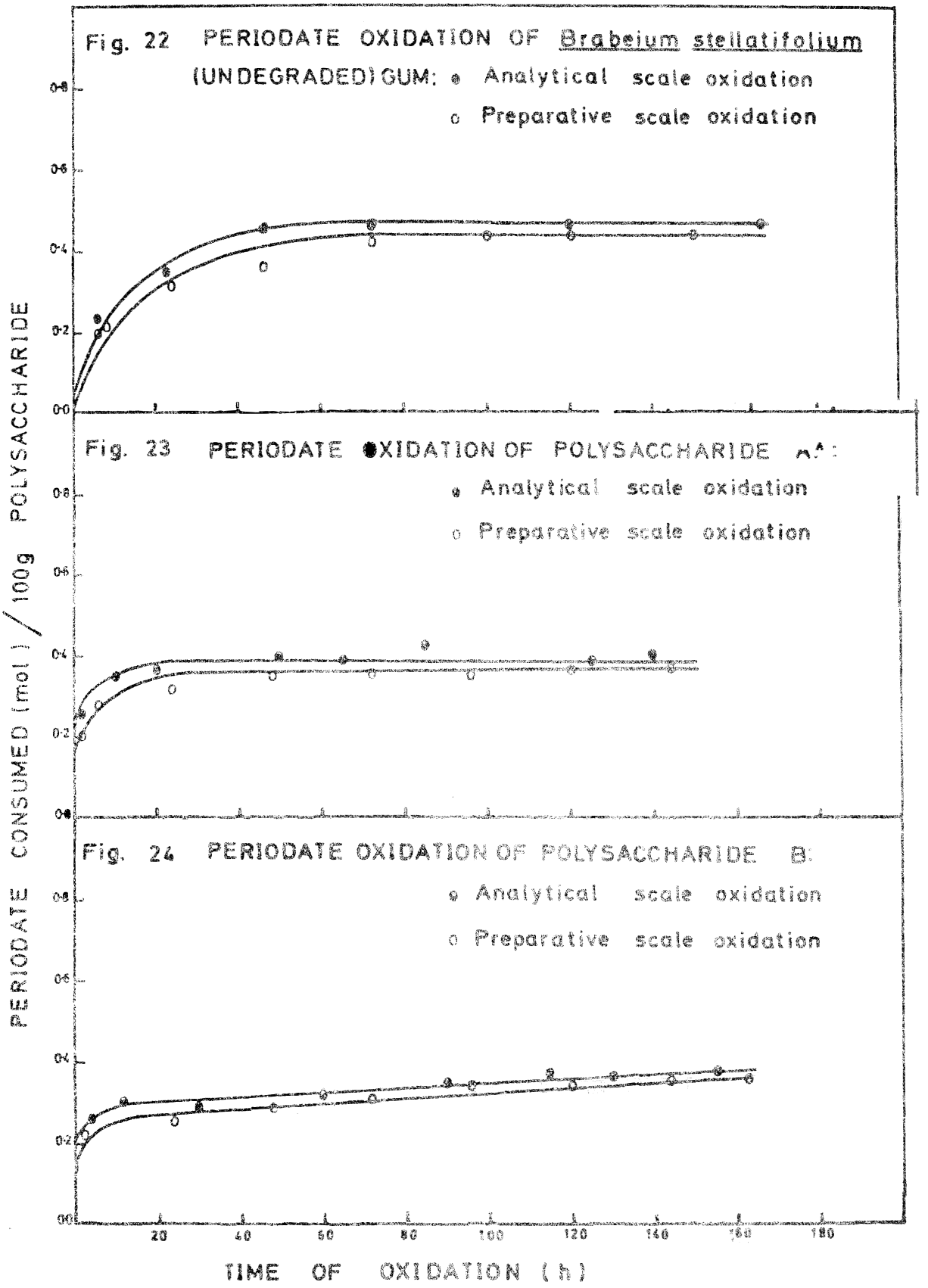


Fig. 22 PERIODATE OXIDATION OF Brabeium stellatifolium (UNDEGRADED) GUM: • Analytical scale oxidation
o Preparative scale oxidation
Fig. 23 PERIODATE OXIDATION OF POLYSACCHARIDE A:
• Analytical scale oxidation
o Preparative scale oxidation
Fig. 24 PERIODATE OXIDATION OF POLYSACCHARIDE B:
• Analytical scale oxidation
o Preparative scale oxidation
PERIODATE CONSUMED (mol) / 100g POLYSACCHARIDE
0·8
0·6
0·4
0·2
0·0
20
40
60
80
100
120
140
160
180
TIME OF OXIDATION (h)

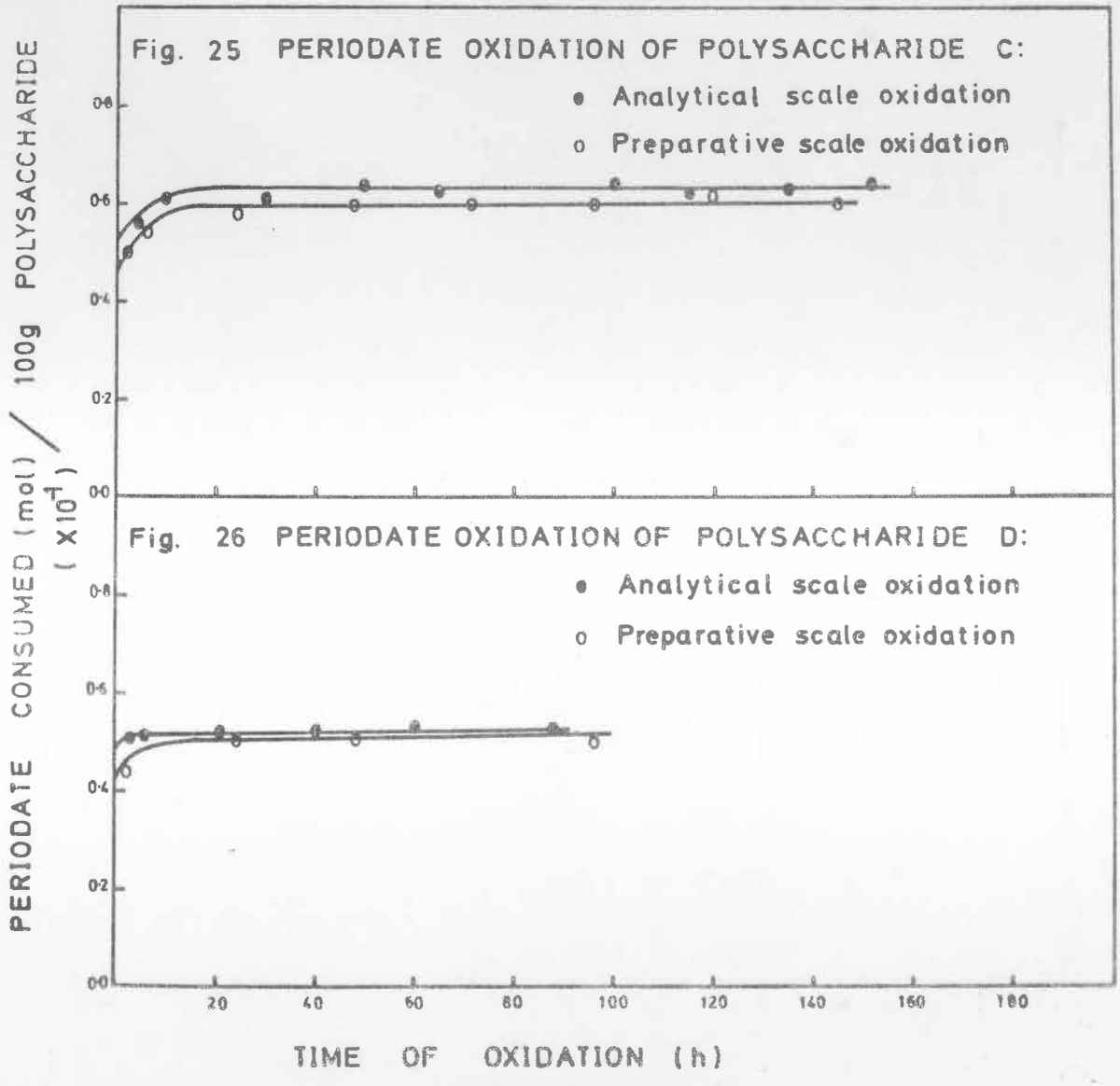


Fig. 25 PERIODATE OXIDATION OF POLYSACCHARIDE C:
• Analytical scale oxidation
o Preparative scale oxidation
Fig. 26 PERIODATE OXIDATION OF POLYSACCHARIDE D:
• Analytical scale oxidation
o Preparative scale oxidation
PERIODATE CONSUMED (mol) / 100g POLYSACCHARIDE
(X10⁻¹)
0·0
0·2
0·4
0·6
0·8
20
40
60
80
100
120
140
160
180
TIME OF OXIDATION (h)

Legend to Figures 27 - 32

(Elution patterns of Smith-degraded polysaccharides of Brabeium stellatifolium gum on various gels.)

For the following diagrams the polysaccharide, the gel used in obtaining the elution pattern and the molecular weights corresponding to peaks as numbered are given.

Figure 27

Polysaccharide A

Gel used : Sagavac 6F

A, 250 000

Figure 28

Polysaccharide A

Gel used : Bio-Gel P-300

A, $\geq$ 80 000; B, 79 000

Figure 29

Polysaccharide B

Gel used : Bio-Gel P-300

A, 30 000; B, 26 500; C, 24 000; D, 19 000; E, 17 380; F, 15 850; G, 14 450; H, 13 180; I, 12 590; J, 11 480; K, 11 220; L, 9 550; M, 8 710; N, $\leq$ 8 000

Figure 30

Polysaccharide C

Gel used : Bio-Gel P-10

A, 30 000; B, 27 500; C, 12 590; D, 4 570; E, 3 630; F, 2 290; G, 1 820; H, 1 440; I, 1 000; J, 912

Figure 31

Polysaccharide D

Gel used : Bio-Gel P-10

A, 30 000; B, 27 500; C, 10 000; D, 3 630; E, 2 880; F, 2 290; G, 1 000; H, 323

Figure 32

Polysaccharide E

Gel used: Bio-Gel P-10

A, 30 000; B, 27 500; C, 10 000; D, 2 880; E, 2 290; F, 980; G, 323

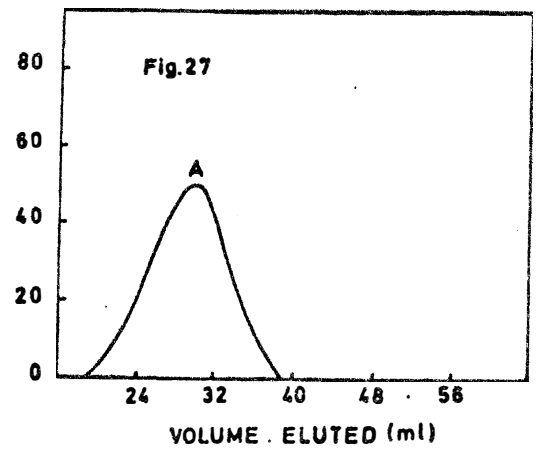


Fig.27
A
80
60
40
20
0
24
32
40
48
56
VOLUME ELUTED (ml)

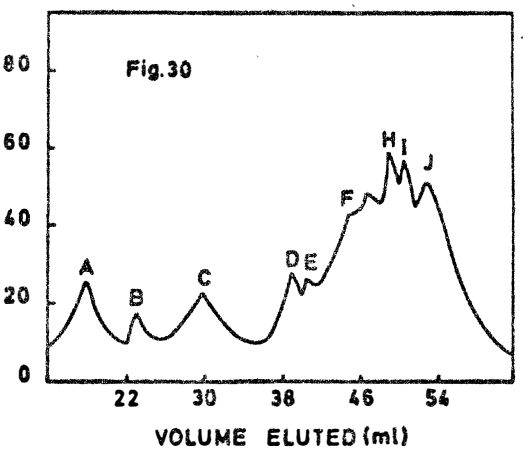


Fig.30
A
B
C
D
E
F
H
I
J
80
60
40
20
0
22
30
38
46
54
VOLUME ELUTED (ml)

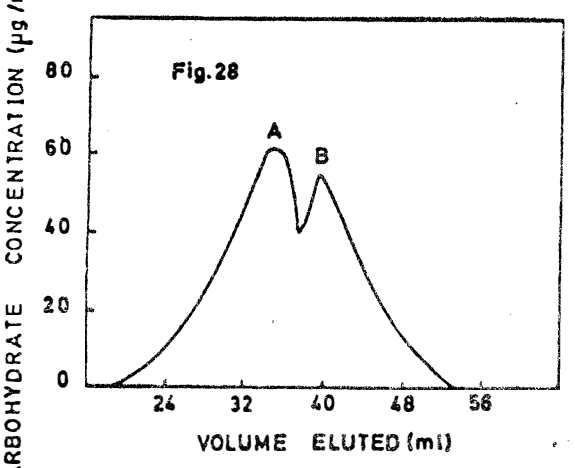


Fig.28
A
B
CARBOHYDRATE CONCENTRATION (µg/ml)
80
60
40
20
0
24
32
40
48
56
VOLUME ELUTED (ml)

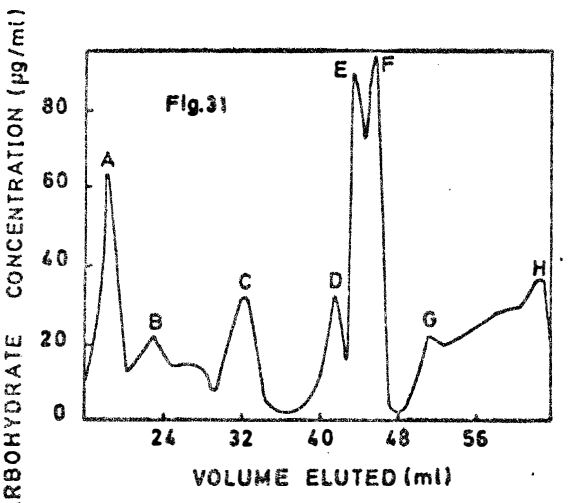


Fig.31
A
B
C
D
E
F
G
H
CARBOHYDRATE CONCENTRATION (µg/ml)
80
60
40
20
0
24
32
40
48
56
VOLUME ELUTED (ml)

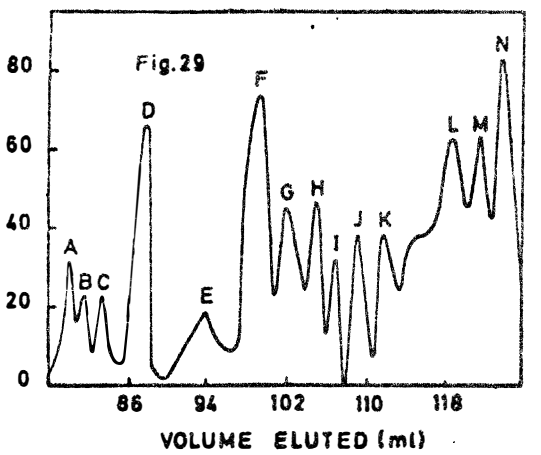


Fig.29
A
B
C
D
E
F
G
H
I
J
K
L
M
N
80
60
40
20
0
86
94
102
110
118
VOLUME ELUTED (ml)

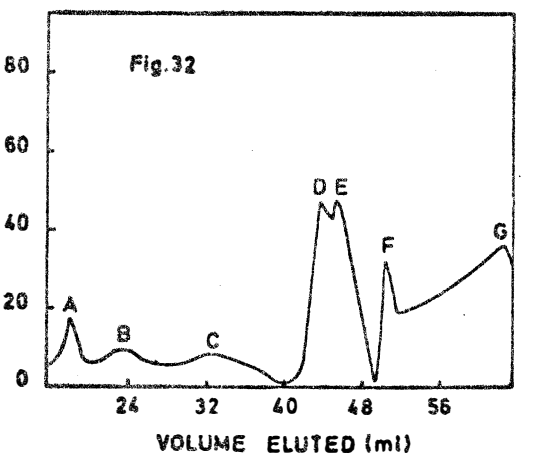


Fig.32
A
B
C
D
E
F
G
80
60
40
20
0
24
32
40
48
56
VOLUME ELUTED (ml)

given in Table 8 (see Figs. 22-26). Analyses of the reduced, oxidised polysaccharides, together with their weights, are presented in Table 10.

Mild acid hydrolysis (0.5 M H_2SO_4 at room temperature) of the reduced, oxidised polysaccharides was continued until acetal cleavage was complete and terminated with the methods described in section 4.6.4. Filtration, evaporation to dryness and solvent-fractionation as described in section 4.6.5, afforded an alcohol-soluble and an alcohol-insoluble fraction in each case. Details of these two fractions are given in Tables 9 and 11 respectively. Polysaccharide E was oxidised on an analytical scale only to determine its period uptake.

4.8 Smith-degradations of Grevillea robusta gum

Smith-degradation studies on Grevillea robusta gum were conducted under identical conditions to those used for Brabeium stellatifolium gum. Details of the periodate oxidation of the gum and of the degraded polysaccharides A', B' and C' are given in Table 12 (see Figs. 33-36) and the properties of the reduced, oxidised polysaccharides and of the alcohol-soluble and alcohol-insoluble fractions obtained on solvent-fractionation of the degradation products are shown in Tables 13 and 15 respectively.

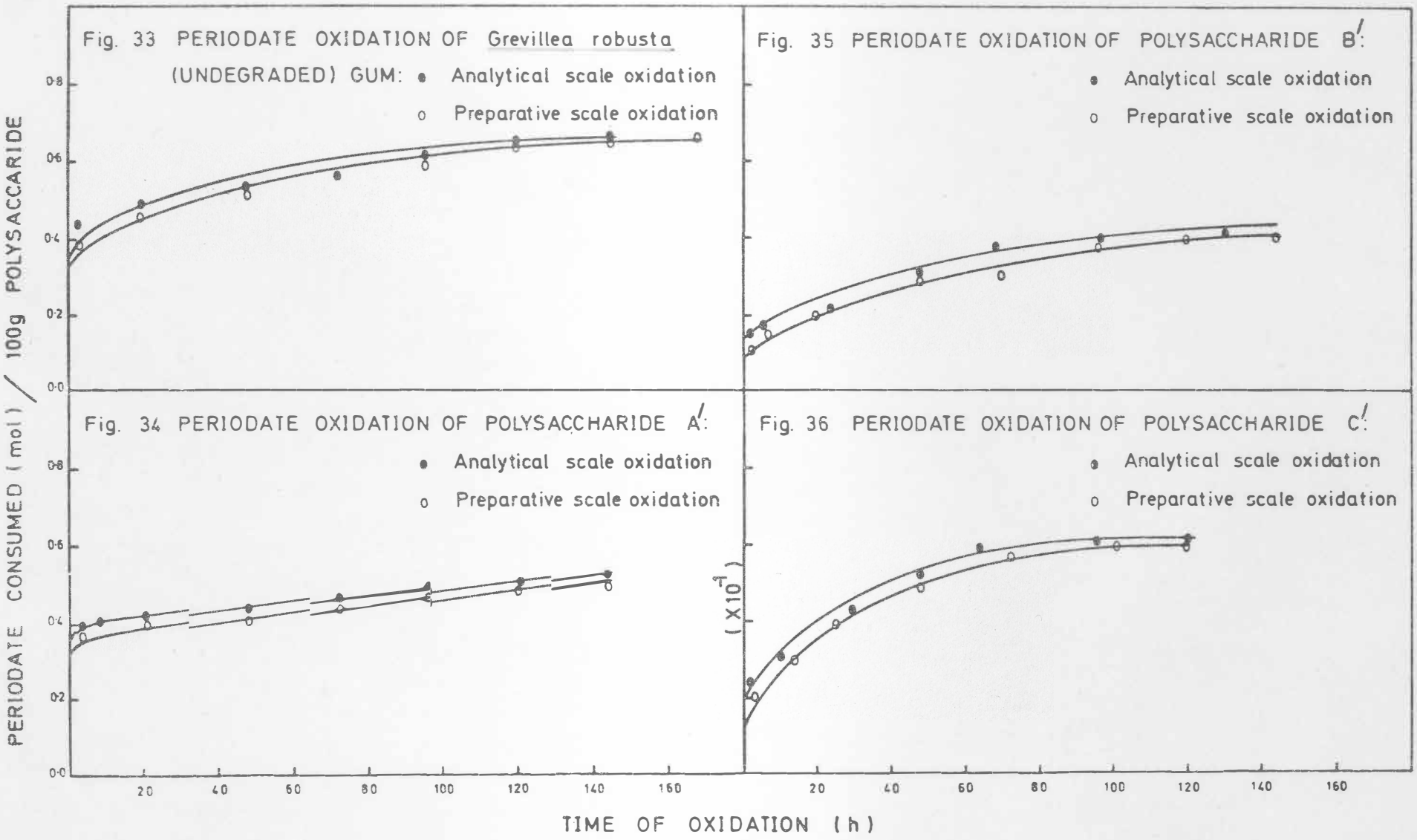


Fig. 33 PERIODATE OXIDATION OF Grevillea robusta (UNDEGRADED) GUM: • Analytical scale oxidation o Preparative scale oxidation
Fig. 34 PERIODATE OXIDATION OF POLYSACCHARIDE A': • Analytical scale oxidation o Preparative scale oxidation
Fig. 35 PERIODATE OXIDATION OF POLYSACCHARIDE B': • Analytical scale oxidation o Preparative scale oxidation
Fig. 36 PERIODATE OXIDATION OF POLYSACCHARIDE C': • Analytical scale oxidation o Preparative scale oxidation
PERIODATE CONSUMED (mol) / 100g POLYSACCARIDE
(X10⁻⁴)
0·0
0·2
0·4
0·6
0·8
20
40
60
80
100
120
140
160
TIME OF OXIDATION (h)

Legend to Figures 37 - 40

(Elution patterns of Smith-degraded polysaccharides of *Grevillea robusta* gum on various gels.)

For the following diagrams the polysaccharide, the gel used in obtaining the elution pattern and the molecular weights corresponding to peaks as numbered are given.

Figure 37

Polysaccharide A'

Gel used : Sagavac 6F

A, 1 500 000; B, 1 000 000; C, 794 000; D, 398 000

E ≤ 158 000

Figure 38

Polysaccharide B'

Gel used : Bio-Gel P-300

A, ≥ 80 000; B, 78 000; C, 75 000; D, 69 000; E, 66 000;

F, 63 100; G, 60 200; H, 50 100; I, 45 700; J, 41 600;

K, 34 600; L, 30 200; M, 26 300; N, 25 100; O, 21 800;

P, 19 900; Q, 16 600; R, 15 800; S, 13 800; T, 11 400.

U, ≤ 10 400

Figure 39

Polysaccharide C'

Gel used : Bio-Gel P-10

A, ≥ 31 000; B, 19 900; C, 12 500; D, 6 900; E, 5 000;

F, 1 900; G, 1 000; H, 630; I ≤ 300

Figure 40

Polysaccharide D'

Gel used : Bio-Gel P-10

A, ≥ 31 000; B, 19 900; C, 12 500; D, 69 000; E, 5 000;

F, 1 900; G, 1 000; H, 620; I, ≤ 300

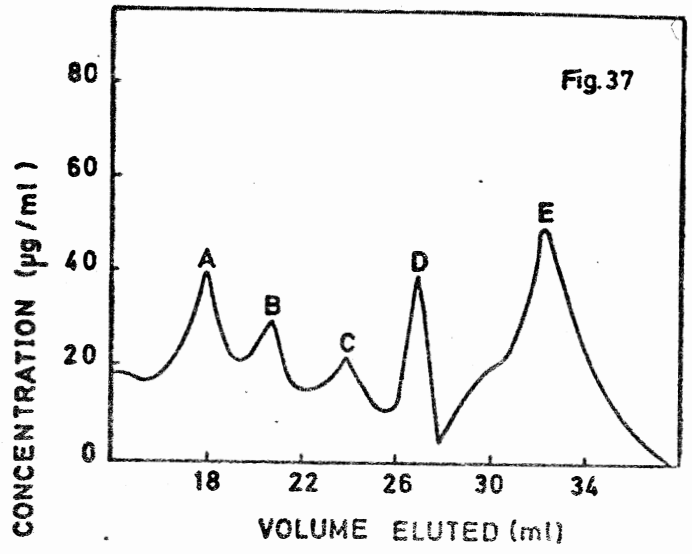


Fig.37
CONCENTRATION (µg/ml)
80
60
40
20
0
A
B
C
D
E
18
22
26
30
34
VOLUME ELUTED (ml)

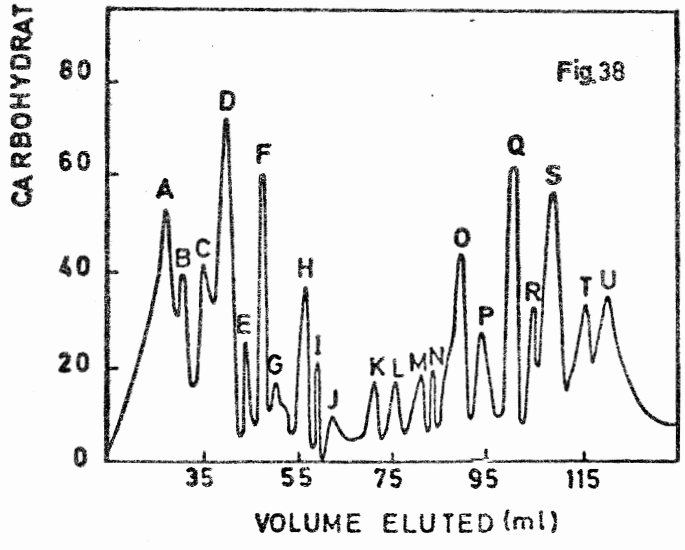


Fig.38
CARBOHYDRATE
80
60
40
20
0
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
35
55
75
95
115
VOLUME ELUTED (ml)

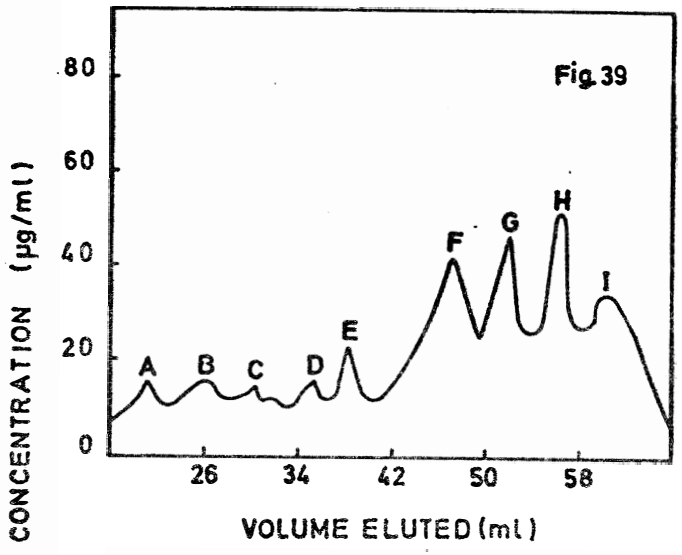


Fig.39
CONCENTRATION (µg/ml)
80
60
40
20
0
A
B
C
D
E
F
G
H
I
26
34
42
50
58
VOLUME ELUTED (ml)

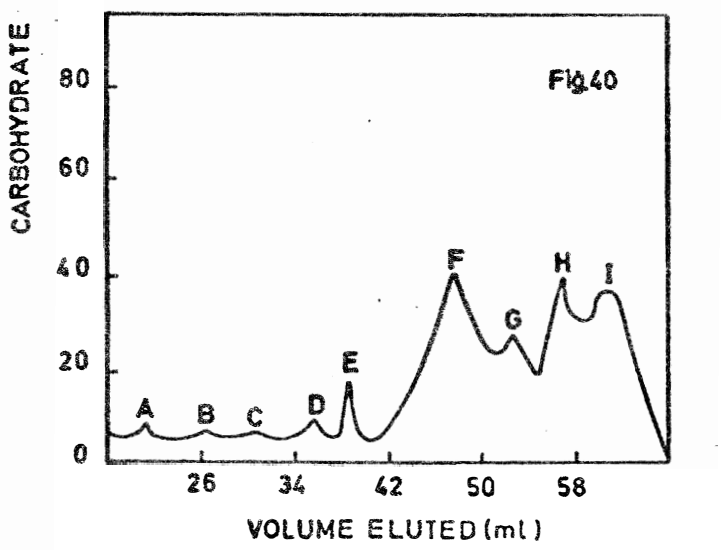
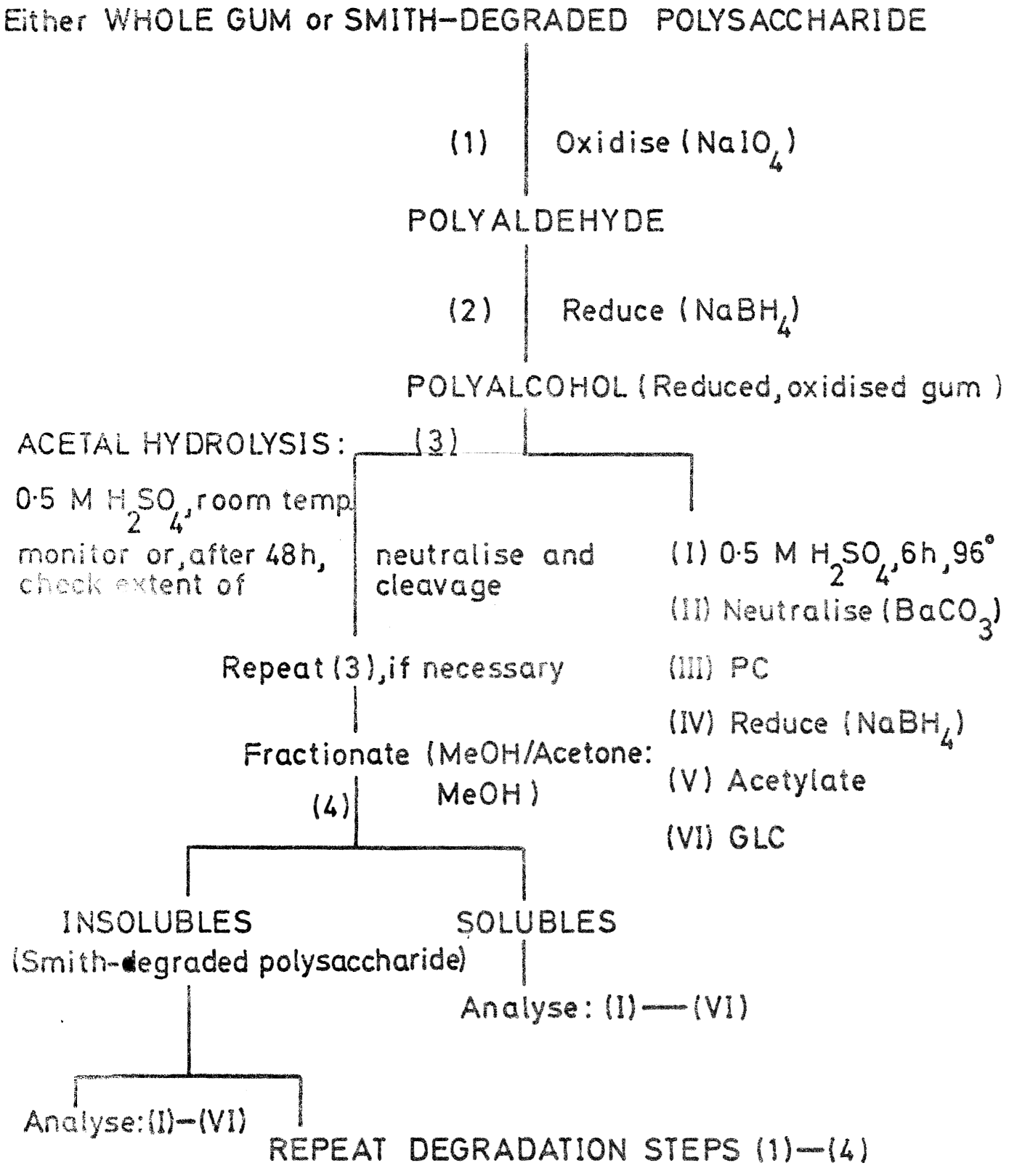


Fig.40
CARBOHYDRATE
80
60
40
20
0
A
B
C
D
E
F
G
H
I
26
34
42
50
58
VOLUME ELUTED (ml)

Fig.41 SCHEMATIC REPRESENTATION OF THE VARIOUS STEPS IN THE SMITH–DEGRADATION AS USED FOR *Brabeium stellatifolium* AND *Grevillea robusta* GUMS



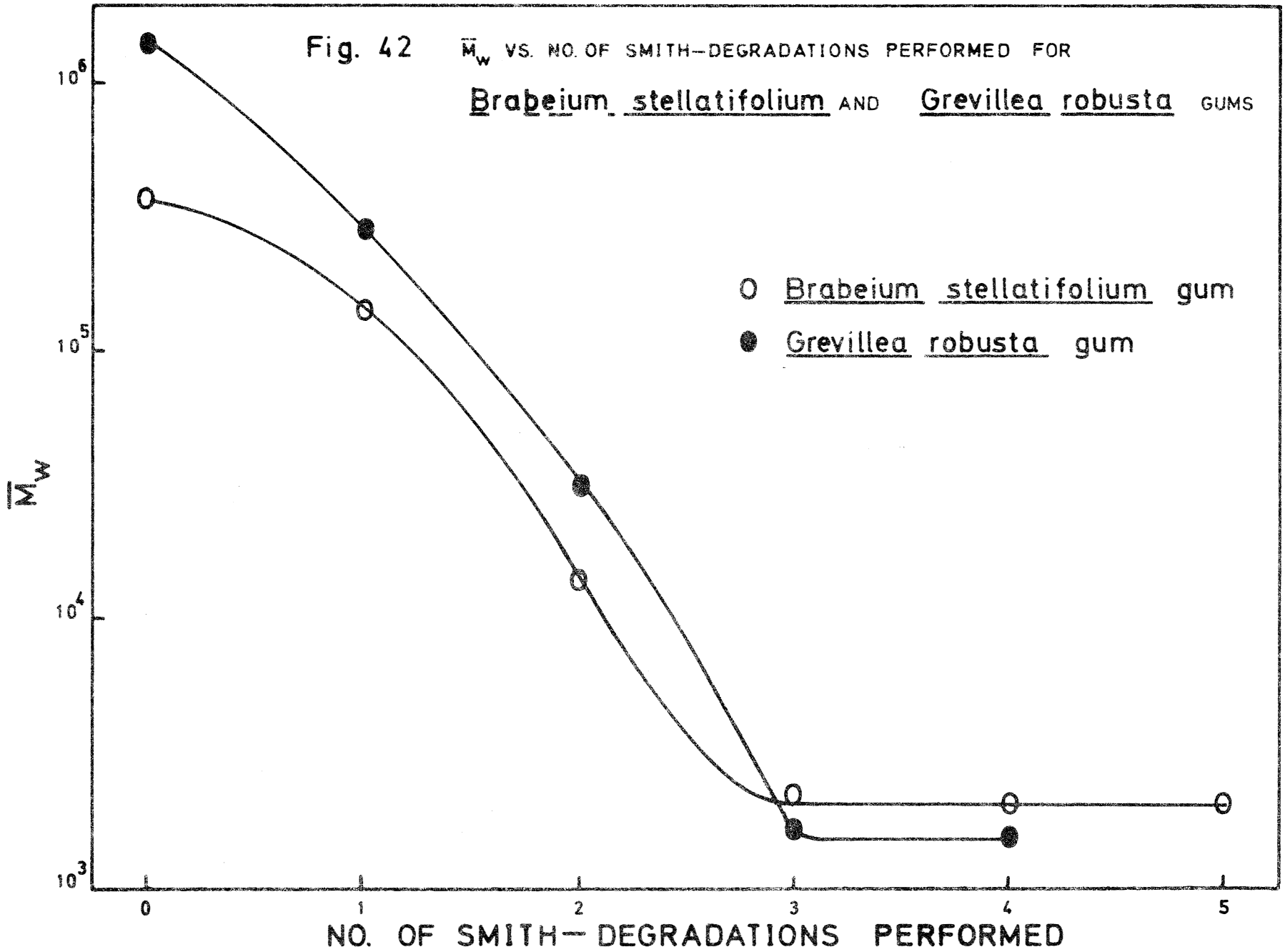


Fig. 42 $\bar{M}_w$ VS. NO. OF SMITH-DEGRADATIONS PERFORMED FOR
Brabeium stellatifolium AND Grevillea robusta GUMS
O Brabeium stellatifolium gum
● Grevillea robusta gum
$\bar{M}_w$
10^6
10^5
10^4
10^3
0
1
2
3
4
5
NO. OF SMITH-DEGRADATIONS PERFORMED

TABLE 8 Periodate oxidation of Brabeium stellatifolium gum and Smith-degraded polysaccharides derived therefrom

Polysaccharide	Whole gum		A		B		C		D		E
Wt.(mg)gum oxidised	5 000		990		381		58		24		6
Vol.(ml) periodate solution used	500		100		400		60		30		10
Molarity of periodate solution	0.05		0.05		0.04		0.02		0.02		0.015
Periodate consumption mol. IO_4^-/100g of polysaccharide with time (h) of oxidation	Analytical scale oxidation		Preparative scale oxidation		Analytical scale oxidation		Preparative scale oxidation		Analytical scale oxidation		Preparative scale oxidation		Analytical scale oxidation		Preparative scale oxidation		Analytical scale oxidation	
	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-	Time	IO_4^-
	6	0.241	6	0.200	2	0.255	2	0.211	4	0.265	2	0.230	4	0.056	2	0.050	3	0.051	2	0.044	2	0.041
	23	0.355	8	0.244	10	0.350	6	0.283	12	0.305	6	0.249	10	0.061	6	0.058	21	0.052	6	0.050	6	0.042
	46	0.462	24	0.321	20	0.370	24	0.320	30	0.290	24	0.259	30	0.061	24	0.060	40	0.053	24	0.051	24	0.041
	72	0.460	46	0.360	50	0.401	48	0.351	60	0.320	48	0.290	50	0.064	48	0.060	60	0.053	48	0.051	48	0.042
	120	0.461	72	0.425	65	0.394	72	0.355	90	0.355	72	0.311	65	0.063	72	0.060	88	0.052	96	0.051	96	0.043
	166	0.465	100	0.433	85	0.433	96	0.357	115	0.370	96	0.342	100	0.064	96	0.060						
			120	0.440	125	0.390	120	0.365	130	0.364	120	0.341	105	0.062	120	0.062						
			130	0.449	140	0.400	144	0.369	155	0.369	144	0.351	135	0.063	145	0.060						
											162	0.352	152	0.063								

TABLE 9 The alcohol-soluble fractions of the Smith-degradation products of Brabeium stellatifolium gum

Alcohol-soluble fraction	A		B		C		D		E	
Wt. (mg) of reduced, oxidised polysaccharide before acid treatment	4 265		573		188		93		34	
Wt. (mg) of solubles	1 620		19		31		8		3	
Compositions of soluble fractions (mol %)	GLC	PC	GLC	PC	GLC	PC	GLC	PC	GLC	PC
Galactose	33.3	+++	8.3	++	16.2	++	20.4	++	16.3	++
Arabinose	13.0	++	3.8	+	1.7	+	-	-	-	-
Xylose	1.3	tr.	-	-	-	-	-	-	-	-
Mannose	2.0	tr.	3.2	+	5.9	+	18.0	++	12.2	+
Threitol	6.1	+	4.0	+	11.0	++	4.2	+	14.7	++
Glycerol	45.1	+++	80.9	++++	65.0	+++	57.3	+++	56.9	+++
GA *	-	+	-	+	-	+	-	+	-	+

* Glycolaldehyde observed on paper chromatography in solvent system (a) only. Reduction of portions of the soluble fractions followed by paper chromatography in solvent (a) showed: threitol, R_{gal} 2.9 (identical to erythritol); glycerol, R_{gal} 4.6 and ethylene-glycol (reduced glycolaldehyde), R_{gal} 6.2. The characteristic streaking of GA was absent.

TABLE 10 Reduced, oxidised polysaccharides from Brabeium stellatifolium gum

Reduced, oxidised polysaccharide	A		B		C		D		E	
Wt. (mg) of reduced, oxidised polysaccharide recovered	4 200		508		157		51		19	
Yield (%) (i)	84		10		3.1		1.0		0.4	
Composition of reduced, oxidised polysaccharides (mol %)	GLC	PC	GLC	PC	GLC	PC	GLC	PC	GLC	PC
Galactose	37.5	+++	34.2	+++	17.8	++	25.8	++	23.4	++
Arabinose	12.5	++	3.0	+	4.1	+	-	-	-	-
Xylose	0.5	tr.	-	-	-	-	-	-	-	-
Mannose	5.0	+	12.3	++	27.2	+++	22.0	++	21.1	++
Threitol	7.5	+	18.0	++	9.8	+	11.1	+	15.2	++
Glycerol	37.5	+++	32.4	+++	41.2	+++	41.1	+++	40.3	+++
GA (ii)	-	+	-	+	-	+	-	+	-	+

(i) Yields have been calculated relative to the amount of gum taken for the first stages of the sequence of Smith-degradations (i.e. 5g).

(ii) Glycolaldehyde, streaking along the paper chromatogram, in solvent system (a).

TABLE 11 Smith-degraded polysaccharides from Brabeium stellatifolium gum

Polysaccharide	A		B		C		D		E	
Wt.(mg) of Smith-degraded polysaccharide	1 002		400		64		33		11	
Yield (%) (i)	20		8		1.3		0.6		0.2	
$[\alpha]_D$	+ 47°		+49°		+51°		+50°		+52°	
$\bar{M}_w$ (ii)	156 000		15 800		3 690		3 200		3 000	
Composition of Smith-degraded polysaccharides (iii) (mol %)	GLC	PC	GLC	PC	GLC	PC	GLC	PC	GLC	PC
Galactose	59.3	+++	61.5	+++	36.6	+++	49.1	+++	48.9	+++
Arabinose	29.4	+++	6.3	+	3.8	+	-	-	-	-
Mannose	4.7	++	24.9	+++	52.7	+++	44.5	+++	48.1	+++
Threitol	-	-	2.0	+	3.2	+	2.8	+	1.9	+
Glycerol	1.0	+	1.6	+	1.4	+	1.3	+	1.0	+
Glucuronic acid (iv)	5.8	+	3.8	+	2.4	+	1.9	+	-	-

(i) Yields have been calculated relative to the amount of gum taken for the first stage of the sequence of Smith-degradations (i.e. 5g).

(ii) Determined from the peak areas under each peak in the gel chromatography elution pattern (see Fig. 27-32).

(iii) Corrected for glucuronic acid content where necessary.

(iv) Obtained from the equivalent weight found by titration.

TABLE 12 Periodate oxidation of Grevillea robusta gum and Smith-degraded polysaccharides derived therefrom

Polysaccharide	Whole gum		A'		B'		C'		D'
Wt.(mg) gum oxidised	5 000		2 780		375		90		6
Vol.(ml) periodate solution used	500		290		380		95		10
Molarity of periodate solution	0.05		0.05		0.02		0.02		0.015
Periodate consumption mol IO_4^-/100g of polysaccharide with time (h) of oxidation	Analytical scale oxidation	Preparative scale oxidation	Analytical scale oxidation	Preparative scale oxidation	Analytical scale oxidation	Preparative scale oxidation	Analytical scale oxidation	Preparative scale oxidation	Analytical scale oxidation
	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-	Time IO_4^-
	3 0.435	3 0.421	2 0.399	2 0.390	2 0.151	3 0.113	2 0.024	3 0.022	2 0.016
	20 0.489	20 0.465	8 0.402	21 0.400	6 0.174	7 0.152	12 0.030	14 0.030	10 0.021
	48 0.533	48 0.527	20 0.410	48 0.403	24 0.210	20 0.200	30 0.042	25 0.039	24 0.030
	72 0.571	96 0.590	48 0.431	72 0.466	48 0.312	48 0.296	48 0.051	48 0.049	48 0.041
	96 0.605	120 0.650	72 0.472	96 0.477	69 0.379	70 0.308	64 0.060	72 0.057	64 0.045
	120 0.659	144 0.651	96 0.495	120 0.485	97 0.400	96 0.382	96 0.060	101 0.060	96 0.050
	144 0.659	168 0.654	120 0.504	144 0.499	100 0.412	120 0.401	120 0.061	120 0.060	120 0.050
			144 0.508		130 0.410	144 0.405			

TABLE 13 The alcohol-soluble fractions of the Smith-degradation products of Grevillea robusta gum

Alcohol-soluble fraction	A'		B'		C'		D'	
Wt. (mg) of reduced, oxidised polysaccharide before acid treatment	4 685		798		248		95	
Wt. (mg) of solubles	1 210		112		43		21	
Compositions of soluble fractions (mol %)	GLC	PC	GLC	PC	GLC	PC	GLC	PC
Galactose	7.6	+(+?)	30.3	+++	9.0	+	40.4	+++
Arabinose	2.8	+	4.3	+	14.0	++	3.4	+
Mannose	4.5	+	46.3	+++	10.9	++	38.6	+++
Threitol	-	-	2.2	+	16.0	++	6.7	+
Glycerol	85.1	+++	16.8	++	50.0	+++	10.9	++
GA *	-	+	-	+ ?	-	+ ?	-	+ ?

* Glycolaldehyde observed on paper chromatography in solvent system (a) only. Reduction of portions of the soluble fractions followed by paper chromatography in solvent (a) showed: threitol, R_{gal} 2.9 (identical to erythritol); glycerol, R_{gal} 4.6 and ethylene glycol (reduced glycolaldehyde), R_{gal} 6.2. The characteristic streaking of GA was absent.

TABLE 14 Reduced, oxidised polysaccharides from Grevillea robusta gum

Reduced, oxidised polysaccharide	A'		B'		C'		D'	
Wt. (mg) of reduced, oxidised polysaccharide recovered	4 630		778		213		78	
Yield (%) (i)	92		15		4.2		1.5	
Composition of reduced, oxidised polysaccharides (mol %)	GLC	PC	GLC	PC	GLC	PC	GLC	PC
Galactose	29.0	+++	16.8	++	45.7	+++	48.3	+++
Arabinose	35.0	+++	14.1	++	10.4	++	2.8	+
Mannose	12.0	++	39.1	+++	33.4	+++	39.5	+++
Threitol	18.7	++	7.8	+	3.9	+	3.2	+
Glycerol	57.5	+++	31.2	+++	7.5	+	6.4	+
GA (ii)	-	+	-	+ ?	-	+ ?	-	+ ?

(i) Yields have been calculated relative to the amount of gum taken for the first stage of the sequence of Smith-degradations (i.e. 5g)

(ii) Glycolaldehyde, streaking along the paper chromatogram, in solvent system (a).

TABLE 15 Smith-degraded polysaccharides from Grevillea robusta gum

Polysaccharide	A'	B'	C'	D'
Wt. (mg) of Smith-degraded polysaccharide	2 930	560	97	46
Yield (%) (i)	59	11	1.9	0.9
$[\alpha]_D$	+ 35°	+ 37°	+ 36°	+ 38°
$\overline{M}_w$ (ii)	468 000	50 200	2 000	1 900
Composition of Smith-degraded polysaccharides (mol %) (iii)				
Galactose	35.0	32.4	51.4	40.9
Arabinose	16.8	8.3	5.6	2.1
Mannose	42.5	53.9	39.2	35.2
Threitol	0.4	1.4	1.0	0.9
Glycerol	1.3	0.8	0.8	0.9
Glucuronic acid (iv)	4.2	3.3	2.3	-

(i) Yields have been calculated relative to the amount of gum taken for the first stage of the sequence of Smith-degradations (i.e. 5g)

(ii) Determined from the peak areas under each peak in the gel chromatography elution pattern (see Fig. 37-40)

(iii) Corrected for glucuronic acid content where necessary

(iv) Obtained from the equivalent weight found by titration

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