"THE POLYSACCHARIDES OF

Opuntia ficus-indica (L.) Mill.

AND

Opuntia aurantiaca Lindl."

by

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A Thesis

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List of abbreviations

Ara	arabinose
Araf	arabinofuranose
Arap	arabinopyranose
CTAB	cetyltrimethylammonium bromide
DEAE cellulose	diethylaminoethyl cellulose
DMF	dimethyl formamide
DMSO	dimethyl sulphoxide
Fuc	fucose
Fucp	fucopyranose
Gal	galactose
Gal A	galacturonic acid
Galp	galactopyranose
Galp A	galactopyranosyluronic acid
g.l.c.	gas liquid chromatography
Glc	glucose
Glc A	glucuronic acid
Glcp	glucopyranose
Glcp A	glucopyranosyluronic acid
LAH	lithium aluminium hydride
Man	mannose
Manp	mannopyranose
m.s.	mass spectrometry
Rha	rhamnose
Rhap	rhamnopyranose
Xyl	xylose
Xylp	xylopyranose

CONTENTS

SUMMARY

The partially acetylated acidic mucilage isolated from the cactus Opuntia ficus-indica consists of a highly branched molecule containing D-galactose, L-arabinose, D-xylose, D-galacturonic acid and L-rhamnose in the ratio 2.9:6.6:3.8:1.3:1.0. A partial hydrolysis study led to the isolation of fourteen oligosaccharides and a degraded polysaccharide. Periodate oxidation of the degraded polysaccharide and methylation analysis of the degraded and reduced degraded polysaccharides led to the proposal of a structure for the degraded polysaccharide consisting of a chain of alternating α-1,4-D-galactopyranosyluronic acid and β-1,2-L-rhamnopyranosyl units with branches consisting of short chains of β-1,6-D-galactopyranosyl units linked to C-3 of the rhamnose residues. An insight into the nature of the peripheral side-chains was obtained by methylation studies of the oligosaccharides and the native polysaccharide. The majority of the side-chains are terminated by D-xylopyranosyl and L-arabinofuranosyl units while there is a small proportion of D-galactopyranosyl end-groups. The remaining units of the peripheral chains consists of mainly 1,3- and 1,5-linked L-arabinofuranosyl units.

The acidic polysaccharide isolated from the jointed cactus Opuntia aurantiaca contains D-galactose, L-arabinose, D-xylose, D-galacturonic acid and L-rhamnose in the ratio 5.9:5.5:3.4:1.2:1.0. A partial hydrolysis study revealed a similar series of galactose containing oligosaccharides as was identified from the mucilage of Opuntia ficus-indica. The degraded polysaccharide isolated from the partial hydrolysate was methylated and a possible structure

proposed for the repeating unit. Methylation studies of the native polysaccharide indicated a polysaccharide with a more complex structure than that for the mucilage of Opuntia ficus-indica.

Polysaccharides with a galacturonorhamnan core

1. Introduction

Gum exudates are produced by a surprisingly large number of plants and various parts of the plant may secrete gum. Exposed to the air and allowed to dry the gum exudates form clear, glassy masses which are usually coloured from dark brown to pale yellow. The most likely function of gum formation is that the tree produces the gum in order to seal off the injured parts, not so much to prevent infection, but to prevent loss of moisture.

The so-called vegetable mucilages which occur in the bark, roots, leaves, seeds, and in some cases, the flowers of plants are products of normal plant metabolism and may serve as food reserves in much the same manner as starch in many plants and glycogen in animals. The mucilages may arise from starch and, in certain plants at least, they seem to act, as do the polysaccharides in succulent plants, as agents for holding water.

The gums and mucilages which possess a galacturonorhamnan core have recently been reviewed by Aspinall[1] and prior to that by Smith and Montgomery[2].

The important feature of this group of polysaccharides is that their core (or backbone) consists of D-galacturonic acid and L-rhamnose. However, there are a few polysaccharides which, in addition, contain D-galactose in the core and these have been included in this group. The sugars making up the side-chains in these polysaccharides vary from polysaccharide to polysaccharide.

This group of polysaccharides may be broadly classified into three main groups:-

a) those in which the core consists of alternating rhamnose and galacturonic acid residues

b) those in which rhamnose is randomly dispersed in the galacturonan core

c) those in which galactose and rhamnose occur in the galacturonan core.

1.1 Polysaccharides with an alternating rhamnose and galacturonic acid core

1.1.1 The mucilage from the bark of Ulmus fulva (Slippery Elm Mucilage)

Slippery elm mucilage contains D-galacturonic acid, D-galactose, L-rhamnose and 3-O-methyl-D-galactose[3,4,5]. The existence of an acid resistant residue, composed of D-galacturonic acid and L-rhamnose and shown by methylation analysis[6] to be 2-O-(α-D-galactopyranosyluronic-acid)-L-rhamnose, was also demonstrated.

Methylation of the mucilage followed by hydrolysis gave a complex mixture of methylated monosaccharides (Table 1)[7].

Table 1

Hydrolysis products of methylated mucilage	
3,4-Me_2-L-rhamnose	(2 parts)
4-Me-L-rhamnose	(2 parts)
2,3,4,6-Me_4-D-galactose	(2 parts)
2,3,6-Me_3-D-galactose	(1 part)
2,4,6-Me_3-D-galactose	(1 part)
2,3,4-Me_3-D-galacturonic acid	(trace)
2,3-Me_2-D-galacturonic acid	(4 parts)

A re-examination of the methylated polysaccharide by Beveridge et al[8] indicated the presence of the methylated sugars shown in Table 2.

Table 2

3-Me-L-rhamnose	(1 part)
4-Me-L-rhamnose	(4 parts)
2,3,4,6-Me_4-D-galactose	(6 parts)
2,3,6-Me_3-D-galactose	(6 parts)
2,3,4-Me_3-D-galacturonic acid	(1 part)
2,3-Me_2-D-galacturonic acid	(4 parts)
2,3,4-Me_3-L-rhamnose	(trace)
3,4-Me_2-L-rhamnose	(trace)
2,4,6-Me_3-D-galactose	(trace)

The two methylation studies are almost identical. However, the earlier sample contained less 4-O-methyl-L-rhamnose and more 3,4-di-O-methyl-L-rhamnose and 2,4,6-tri-O-methyl-D-galactose in addition to small amounts of rhamnose. The D-galactose and 3-O-methyl-D-galactose residues are thus either non-reducing end-groups and/or 4-O-substituted (assuming pyranose forms are present in the polysaccharide). Very occasionally some D-galactose residues are 3-O-substituted. If the backbone of the polysaccharide consists of alternating units of 2-O-substituted L-rhamnopyranose and 4-O-substituted D-galactopyranosyluronic acid then D-galactose and 3-O-methyl-D-galactose residues, or chains containing these residues, are attached to C-3 and occasionally to C-4 of the L-rhamnopyranose residues.

An idea of the distribution of D-galactose and 3-O-methyl-D-galactose residues in the side-chains was obtained by the isolation of an oligosaccharide, containing 3-O-methyl-D-galactose and L-rhamnose, from the partial acid hydrolysate of the polyalcohol obtained on borohydride reduction of the periodate-oxidized polysaccharide. The N.M.R. spectrum of the oligosaccharide in D_2O showed a doublet at $\tau 8.53$ which was assigned

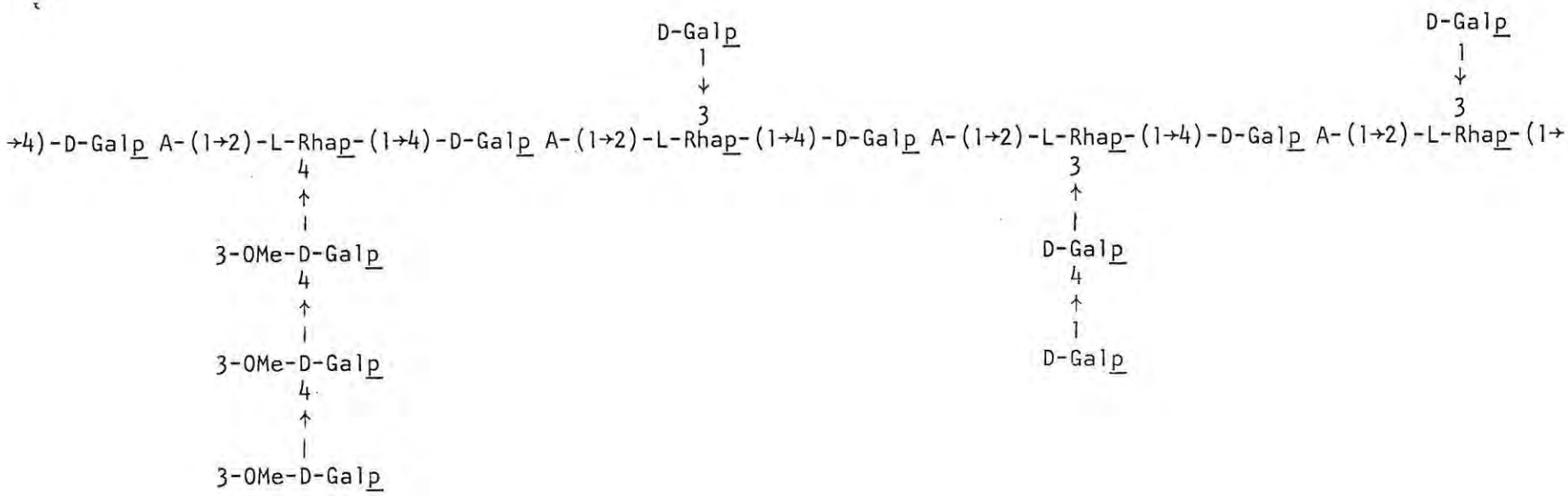
to the methyl group of the rhamnose residue and the signals at τ6.37 and 6.40 were assigned to the methoxyl groups of the 3-O-methyl-D-galactose residues. The molar proportion of 3-O-methyl-D-galactose to L-rhamnose was 2.34:1 suggesting that the oligosaccharide is a trisaccharide. Borohydride reduction showed that the rhamnose occupied the reducing end of the trisaccharide. Methylation analysis of the trisaccharide showed the presence of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose and 2,3-di-O-methyl-L-rhamnose. The trisaccharide is thus O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-O-(3-O-methyl-β-D- galactopyranosyl)-(1→4)-L-rhamnose, the β linkage being inferred from the low positive specific rotation.

Two further oligosaccharides [9,10] were isolated from the borohydride reduced periodate oxidized polysaccharide viz. O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-L-rhamnose and O-(3-O-methyl-β-D-galactopyranosyl)-(1→4)-(3-O-methyl-D-galactose).

The polyalcohol, derived from the reduced periodate oxidized polymer, was shown to contain D-galacturonic acid, 3-O-methyl-D-galactose and L-rhamnose but only traces of D-galactose residues. Thus, nearly all of the D-galactose residues are vulnerable to periodate and therefore occur either as non-reducing end-groups or as 4-O-substituted residues. These conclusions are consistent with the methylation evidence for the polyalcohol (Table 3).

Mild hydrolysis of the polyalcohol[11] yielded a small amount of non-dialysable material which contained D-galacturonic acid, 3-O-methyl-D-galactose and L-rhamnose . Methylation analysis showed the presence of 2,3-di-O-methyl-L-rhamnose in addition to those O-methyl sugars already mentioned in the methylated polyalcohol, except that the proportion of 4-O-methyl-L-rhamnose was greatly diminished and 2,4,6-tri-O-methyl-D-galactose was absent. These observations provide

Figure 1



Tentative structure for the mucilage of Ulmus fulva.

further evidence that chains of 3-O-methyl-D-galactose residues are attached to the C-4 position of certain L-rhamnose residues whilst some D-galactose or 4-O-substituted D-galactose residues are attached to the C-3 positions. Trace amounts of 3,4-di-O-methyl-L-rhamnose in the methylated polysaccharide indicate that very few L-rhamnose residues are unbranched. A structure for the mucilage which is consistent with the above data is shown in Fig. 1.

Table 3

Hydrolysis products of methylated polyalcohol	
3,4-Me_2-L-rhamnose	(trace)
3-Me-L-rhamnose	(1 part)
4-Me-L-rhamnose	(2 parts)
2,3,4,6-Me_4-D-galactose	(1 part)
2,3,6-Me_3-D-galactose	(3 parts)
2,4,6-Me_3-D-galactose	(trace)
2,3-Me_2-D-galacturonic acid	(trace)

1.1.2 The gum from Rhizophora mangle Linn. (Mangle gum)

This homogeneous gum $[\alpha]_D$ + 140°, has been shown[12,13,14] to contain D-galactose (15.2%), L-arabinose (20%), L-rhamnose (26.8%), D-galacturonic acid (37%) and 4-O-methyl-D-glucuronic acid (5%). Autohydrolysis yielded a degraded gum $[\alpha]_D$ + 30°, which was shown by electrophoresis to be homogeneous and which contained residues of L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid and 4-O-methyl-D-glucuronic acid[13] in the molar proportions 15:13.5:26.7:36:4.1.

Methylation analysis of the degraded gum followed by separation of the acid and neutral fractions on an anion exchange resin afforded the sugars shown in Table 4.

Table 4

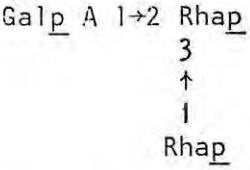
Neutral sugars		Acidic mono and oligosaccharides	
2,3,5-Me_3-L-arabinose	(4 moles)	2,3,4-Me_3-D-galacturonic acid	(3 moles)
2,3,4,6-Me_4-D-galactose	(1 mole)	2,3-Me_2-D-galacturonic acid	(1 mole)
3,4-Me_2-L-rhamnose	(1 mole)	4-O-Me-2-O-(2-O-Me-D-Gal*p* A)-L-Rha	(4 moles)
2,5-Me_2-L-arabinose	(2 moles)	3,4-Me_2-2-O-(2-O-Me-D-Gal*p* A)-L-Rha	(2 moles)
2,4,6-Me_3-D-galactose	(1 mole)	3-O-Me-2-O-(2,3,4-Me_3-D-Gal*p* A)-L-Rha	(2 moles)
3-Me-L-rhamnose	(1 mole)	2,4,6-Me_3-3-O-(2,3,4-Me_3-D-Gal*p* A)-D-Gal	(2 moles)
		2,3,6-Me_3-4-O-(2,3,4-Me_3-D-Glc*p* A)-D-Gal	(2 moles)

The core of the degraded gum is made up of aldobiouronic acid residues, the bulk of which are 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose. All of the D-galacturonic acid residues and most of the L-rhamnose residues in the main chain are also glycosidically linked through C-3. Of the units linked to acid sugar residues in the main chain, some are D-galacturonic acid residues which either form the non-reducing ends or are, in turn, joined through C-4 to other sugar units.

Fig. 2a shows a possible structure of degraded mangle gum. Periodate oxidation and Smith degradation studies confirm this as a possible structure. The percentages of galactose, rhamnose and galacturonic acid residues resistant to periodate are in reasonable agreement with the theoretical values, but, for arabinose, the expected value is less than that found experimentally.

Barry degradation of the degraded gum yielded galacturonic acid, galactose, rhamnose, and aldotriouronic acid and high molecular weight material. The aldotriouronic acid contained rhamnose and galacturonic acid in the ratio 2:1. Periodate oxidation studies on this material suggest the structure shown in Fig. 3

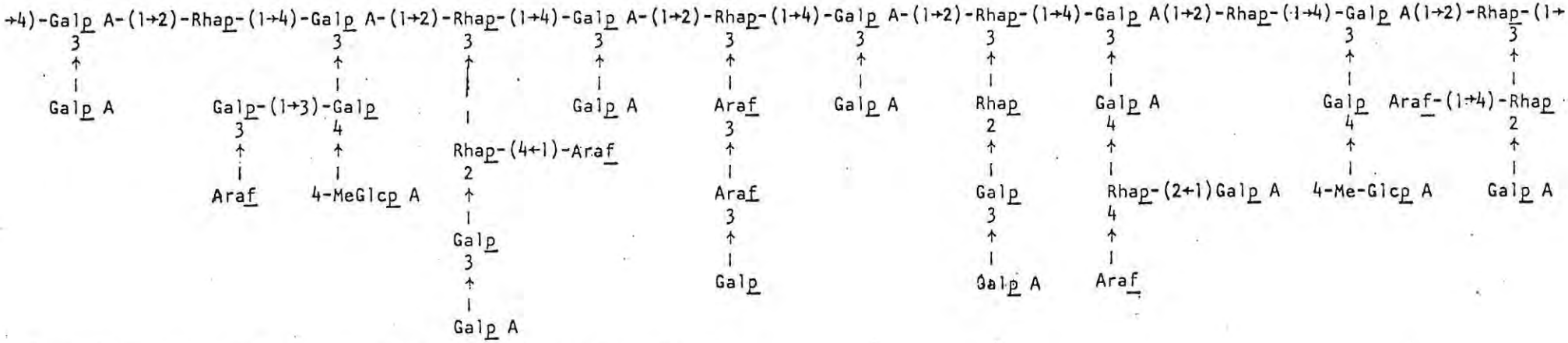
Fig. 3



The high molecular weight material was electrophoretically homogeneous and contained galactose (22.0%), arabinose (12.5%), rhamnose (32.3%) and galacturonic acid (30.4%). Based on the structure proposed for the degraded gum, the high molecular weight fragment shown in Fig. 2b should be obtained on Barry degradation of the degraded gum. This fragment contains galactose (23.0%), arabinose (14.0%), rhamnose (30.0%) and galacturonic acid (30.0%).

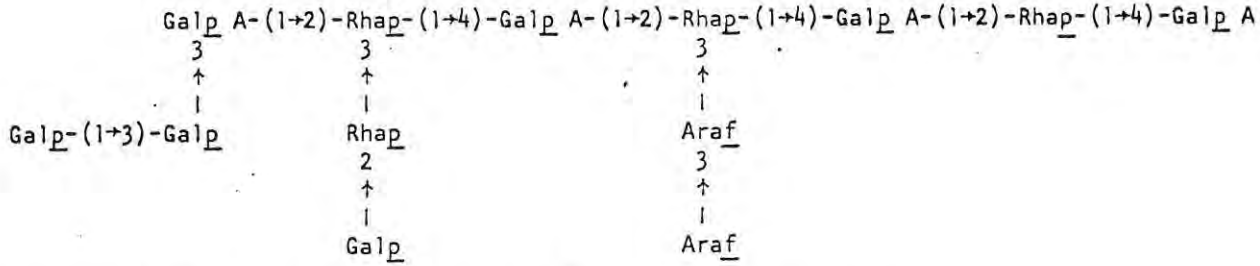
Figure 2

(a)



Tentative structure of the repeating unit of degraded mangle gum.

(b)



Structure of fraction isolated from the Barry-degraded product.

The supernatant liquid obtained during autohydrolysis of mangle gum contained arabinose, rhamnose and two oligosaccharides[14] which were identified as 3-O-β-L-arabinofuranosyl-L-arabinose and 5-O-β-D-galactopyranosyl-L-arabinose.

Methylation of the native gum[14] followed by hydrolysis gave a series of neutral (Table 5) and acidic sugars. The seven methylated acidic sugars were found to be identical with those obtained in the acidic fraction of the degraded gum[13] and were in the same molar proportions. Thus the positions of all aldobiouronic acid residues in the two polysaccharides are considered to be the same.

Table 5

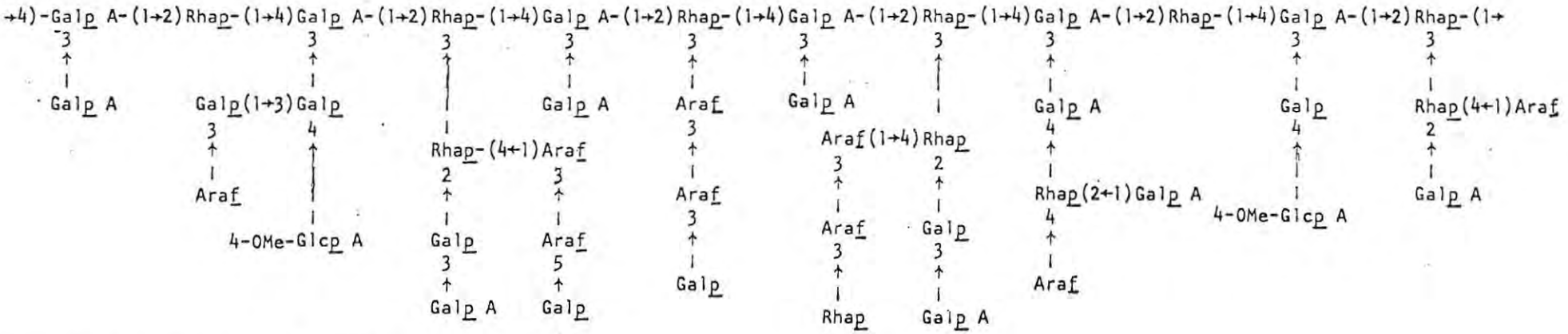
Neutral sugar	Mole ratio*
2,3,4-Me_3-L-rhamnose	1.0
2,3,5-Me_3-L-arabinose	2.8 (4)
2,3,4,6-Me_4-D-galactose	2.0 (1)
2,5-Me_2-L-arabinose	4.0 (2)
2,4,6-Me_3-D-galactose	1.2 (1)
2,3-Me_2-L-arabinose	1.0
3-Me-L-rhamnose	2.1 (1)

* Mole ratios in degraded gum are given in parentheses

Furthermore, removal of the acid-labile sugar units from the gum, followed by methylation, did not introduce further methoxyl groups into the uronic acid residues of the macromolecule, indicating that the acid-labile sugar units are not linked directly to the uronic acid units of the gum.

A comparison of the mole ratios of the neutral methylated sugars of the whole and degraded gum indicates that the acid labile sugars are linked to C-3 of arabinofuranose residues and to C-4 of 1,2-linked rhamnopyranose residues in the side chain.

Figure 4



Tentative structure of the repeating unit of mangle gum.

Methylation studies suggest that there are four mole proportions of 1,3-linked arabinofuranose residues in the repeating unit of the whole gum. However, on autohydrolysis a disaccharide having 1→3 linked arabinofuranose units was obtained. At some positions in the whole gum this disaccharide is present, but it cannot be stated with certainty that every repeating unit contains this sugar grouping. The presence of arabinose in the autohydrolysate of the polysaccharide is due to further hydrolysis of the disaccharide. On the basis of these considerations and from the deduced structure of the degraded gum Fig. 4 gives a tentative structure for the whole gum. Periodate oxidation studies seem to confirm this structure.

1.1.3 Structure of the mucilage of Hibiscus ficulneus Linn.

Initial studies[15] showed that the mucilage contained D-galacturonic acid, D-galactose, L-rhamnose and two minor sugars, one of which is D-glucose and the other probably xylose. Partial hydrolysis of the mucilage[16] afforded 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose and neutral monosaccharides.

A degraded mucilage $[\alpha]_D$ + 75°[17] obtained by autohydrolysis of the mucilage contained D-galactose, L-rhamnose and D-galacturonic acid in the ratio 1:5:6. Hydrolysis of the degraded methylated polymer gave 2,3,4-tri-O-methyl-D-galactose (1 mole), 3,4-di-O-methyl-L-rhamnose (5 moles) and 2,3-di-O-methyl-D-galacturonic acid (6 moles).

No uronic acid or aldobiouronic acid is released during the formation of the degraded polymer and, since the rhamnose content of the degraded mucilage corresponds to the amount in the aldobiouronic acid obtained from the parent mucilage, it follows that all the L-rhamnose residues are linked through C-2 to C-1 of the D-galacturonic acid residues. The remaining galacturonic acid residue must be linked to

position 6 of a D-galactose residue.

The structure for the degraded mucilage (Fig 5) accounts for the experimental data.

Figure 5

$[-4\text{-D-Gal}\underline{p}\ A1\rightarrow2\ L\text{-Rha}\underline{p}\ 1(\rightarrow4\ D\text{-Gal}\underline{p}\ A\ 1\rightarrow2\ L\text{-Rha}\underline{p})_4\rightarrow4D\text{-Gal}\underline{p}\ A\ 1\rightarrow6D\text{-Gal}\underline{p}\ 1-]_n$

The above structure is supported by periodate oxidation studies.

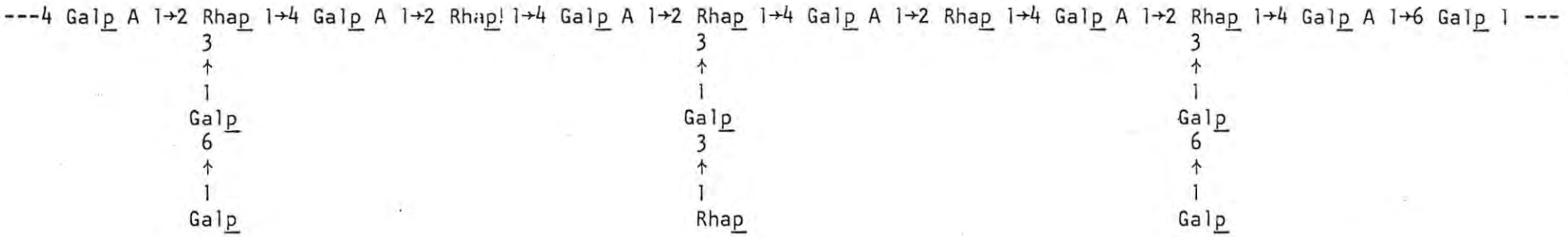
Methylation studies of the whole mucilage[17a] led to the characterization of a series of neutral and acidic sugars (Table 5a).

Table 5a

Neutral Sugars	Acidic Sugars
2,3,4-Me_3-L-rhamnose (1 mole)	2,3-Me_2-D-galacturonic acid (6 moles)
2,3,4,6-Me_4-D-galactose (2 moles)	D-galacturonic acid (trace)
3,4-Me_2-L-rhamnose (2 moles)	
2,4,6-Me_3-D-galactose (1 mole)	
2,3,4-Me_2-D-galactose (3 moles)	
4-Me-L-rhamnose (3 moles)	
rhamnose (trace)	

In the degraded polysaccharide all the rhamnose was recovered as 3,4-di-O-methyl-L-rhamnose whereas in the native mucilage only two moles were recovered as 3,4-di-O-methyl-L-rhamnose and the rest (3 moles) was recovered as 4-O-methyl-L-rhamnose. Thus in the repeating unit of eighteen residues in the native polymer three of the rhamnose units in the main galacturonorhamnan chain must carry branches at C-3, two of which are terminated by galactopyranose units and the

Figure 5a



Tentative structure for the mucilage of Hibiscus ficulneus.

third by a rhamnopyranose unit. A possible structure of the repeating unit of the native mucilage is given in Fig 5a. This structure is supported by periodate oxidation studies.

1.2 Polysaccharides with a galacturonan core in which the rhamnose residues are randomly dispersed

1.2.1 The Sterculia Gums

1.2.1.1 Sterculia urens gum

The exudate from the bark of Sterculia urens, karaya gum, consists of a partially acetylated polysaccharide[18] (8% acetyl groups, 37% uronic acid residues). Deacetylation of the gum affords a homogeneous polysaccharide which contains D-galactose, L-rhamnose, D-galacturonic acid and D-glucuronic acid. Partial acid hydrolysis of the gum affords the oligosaccharides (I-VI) shown in Table 6 while acetolysis also showed the presence of oligosaccharide (VII)[18,19].

Table 6

I	α-D-Gal*p* A 1→4 D-Gal*p*
II	α-D-Gal*p* A 1→2 L-Rha
III	D-Gal*p* A 1→2 L-Rha*p* 1→4 D-Gal*p*
IV	D-Gal*p* A 1→2 L-Rha*p* 1→4 D-Gal*p* A
V	β-D-Glc*p* A 1→3 D-Gal*p* A 1→2 L-Rha
VI	β-D-Glc*p* A 1→3 D-Gal A
VII	D-Gal*p* 1→2 D-Gal*p* A 1→4 D-Gal*p*

A degraded polysaccharide[20] was obtained from the periodate oxidized carboxy-reduced gum which contained essentially linear chains

of alternating 4-O-substituted D-galactopyranose and 2-O-substituted rhamnopyranose residues. Acetolysis of the degraded polysaccharide gave oligosaccharides (VIII), (IX), (XIII), (XVI) and (XVII) (Table 7), while acetolysis of the carboxy-reduced gum afforded oligosaccharides (VIII), (IX), (XIII), (X), (XI), (XII), (XIV) and (XV) (Table 7).

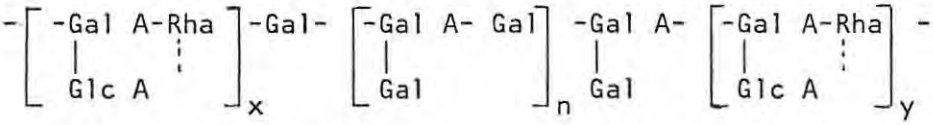
Table 7

L-Rhap 1→4 D-Galp	VIII
D-Galp 1→2 L-Rha	IX
D-Galp 1→4 D-Galp	X
D-Galp 1→2 D-Gal	XI
D-Galp 1→2 D-Galp 1→4 D-Galp	XII
D-Galp 1→2 L-Rhap 1→4 D-Galp	XIII
L-Rhap 1→4 D-Galp 1→2 L-Rha	XIV
L-Rhap 1→4 D-Galp 1→2	
L-Rhap 1→4 D-Galp	XV
D-Galp 1→2 L-Rhap 1→4	
D-Galp 1→2 L-Rha	XVI
D-Galp 1→2 L-Rhap 1→4	
D-Galp 1→2 L-Rhap 1→4 D-Galp	XVII

By the nature of its formation, the degraded polymer must arise from interior chains in the gum in which the corresponding D-galacturonic acid and L-rhamnose residues carry side-chains.

It is difficult to interpret the results from the acetolysis of the carboxy-reduced gum as the origin of the galactose is not known. However, the results point to two main structural regions in the gum. Fig 6 indicates one way in which these blocks may be linked.

Figure 6



Raymond and Nagel[21] degraded S.urens gum using a Cephalosporium fungus. Their studies indicate at least three different types of chains. One chain is postulated to contain repeating units of four galacturonic acid residues containing β-D-galactose branches and having an L-rhamnose residue at the reducing end of the unit. A second chain is postulated to contain an oligorhamnan chain containing D-galacturonic acid branching residues and interrupted occasionally by a D-galactose residue. D-Glucuronic acid is apparently confined to a third type of chain. However, as no oligosaccharides were isolated and identified, their results must be viewed with a certain degree of caution.

1.2.1.2 Sterculia caudata gum

The gum which contains D-galacturonic acid, D-glucuronic acid, D-galactose and L-rhamnose residues,[22,23] is a highly branched, partially acetylated homogeneous polysaccharide. Six acidic oligosaccharides[23] (Table 8) have been characterized as partial hydrolysis products.

Table 8

I	α-D-Gal*p* A 1→2 L-Rha
II	β-D-Gal*p* A 1→4 D-Gal
III	β-D-Gal*p* A 1→4 (2-O-Ac)-D-Gal
IV	β-D-Glc*p* A 1→3 D-Gal A
V	β-D-Glc*p* A 1→3 α-D-Gal*p* A 1→2 L-Rha
VI	D-Gal*p* A 1→4 D-Gal*p* A 1→2 L-Rha

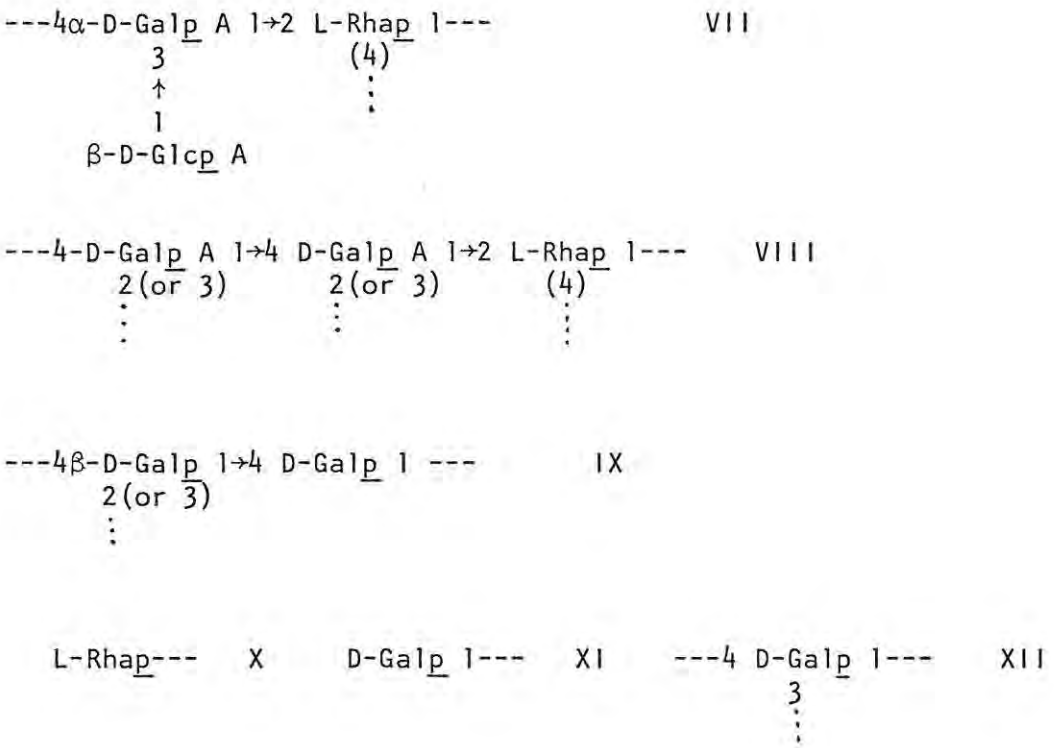
Methylation studies[23] showed the presence, in the methylated gum, of the sugar residues listed in Table 9.

Table 9

2,3,4,6-Me_4-D-galactose	3-Me-L-rhamnose
2,3,6-Me_3-D-galactose	2,3,4-Me_3-D-glucuronic acid
2,6-Me_2-D-galactose(s)	2,3,4-Me_3-D-galacturonic acid
2,3,4-Me_3-L-rhamnose	2,3-Me_2-D-galacturonic acid
3,4-Me_2-L-rhamnose	2- and 3-Me-D-galacturonic acid

(s) = small amount only

In the light of the methylation results it is possible to indicate the location, in the polysaccharide structure, of some of the fragments which were isolated as partial hydrolysis products. D-Glucuronic acid residues are present only as non-reducing end-groups linked 1→3 to D-galacturonic acid and since 3-O-substituted D-galacturonic acid residues in the polysaccharide are also 4-O-substituted, partial structure (VII) defines the mode of attachment of D-glucuronic acid residues as single unit side chains. With the exception of a small proportion of end groups, D-galacturonic acid residues are all 4-O-substituted and the majority are also 2- or 3-O-substituted and are mainly present in interior chains, frequently as branching points, and may be accommodated in the partial structures (VII-IX). L-Rhamnopyranose residues are also present in the sequences (VII) and (VIII), and may be branched at C-4 or may, to a small extent, be present as non-reducing end groups (X). D-Galactopyranose residues are located mainly as end groups (XI) and in non-terminal positions in the units (IX), although a small proportion may be present as branching points (XII).



1.2.1.3 Sterculia setigera gum

The gum is a partially acetylated, highly branched, homogeneous polysaccharide containing D-galacturonic acid, D-galactose, L-rhamnose and D-glucuronic acid[24,25,26]. Partial hydrolysis of the gum[24,25] furnished a mixture of acidic oligosaccharides, identified as oligosaccharides I and II in Table 11. The sugar residues obtained from the methylated gum are shown in Table 10.

Table 10

2,3,4,6-Me_4-D-galactose	(6 parts)
2,3,6-Me_3-D-galactose	(3 parts)
2,6-Me_2-D-galactose	(trace)
3,4 (?)-Me_2-L-rhamnose	(4 parts)
2 and/or 3-Me-L-rhamnose	(3 parts)
L-rhamnose	(trace)
2-Me-D-galacturonic acid	(16 parts)

Aspinall, Fraser and Sanderson[26] isolated and characterized the oligosaccharides listed in Table 11 from a partial hydrolysate.

Table 11

I	α-D-Galp A 1→2 L-Rha
II	β-D-Galp A 1→4 D-Gal
III	β-D-Glcp A 1→3 D-Gal A
IV	β-D-Glcp A 1→3 α-D-Galp A 1→2 L-Rha
V	β-D-Galp A 1→4 (2-O-Ac)-D-Gal

It is evident that the isolation procedure of the polysaccharide, which involves deacetylation of the native gum with aqueous ammonia, does not result in complete deacylation. An additional trisaccharide, probably O-(D-galactopyranosyluronic acid)-(1→4)-O-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnose was also isolated.

Results indicate that the gum from the three Sterculia species contain not only the same structural units but also that many of the same sequences of sugar residues are present in the three polysaccharides.

1.2.1.4 Cochlospermum gossypium gum

The gum is a partially acetylated, acidic polysaccharide containing L-rhamnose, D-galactose, D-galacturonic acid and D-glucuronic acid[27,28]. The modes of linkage of the neutral sugar residues were established by methylation. Reduction of the methylated acidic fraction[28] followed by hydrolysis led to the isolation of the neutral sugars listed in Table 12.

With the exception of 2,3,6-tri-O-methyl-D-galactose and 3-O-methyl-L-rhamnose none of the methylated sugars listed in Table 12 was present amongst the neutral products formed on direct hydrolysis of the methylated gum and it may be assumed that they arise from the reduction of the corresponding methyl ethers of hexuronic acids.

Table 12

2,3,4-Me_3-D-glucose (m)
3,4-Me_2-D-glucose
3-Me-D-galactose
2,3,6-Me_3-D-galactose (m)
2,3-Me_2-D-galactose
3,4-Me_2-D-galactose
2-Me-D-galactose
3-Me-L-rhamnose

(m) = major

Thus D-glucuronic acid is present in the gum mainly as non-reducing end-group while D-galacturonic acid is found in the inner chains of the polysaccharide, in part as branching points.

Aspinall, Fraser and Sanderson[26] separated and identified the acidic oligosaccharides, shown in Table 13, from a partial hydrolysate of the gum.

Table 13

α-D-Gal<u>p</u> A 1→2 L-Rha
β-D-Gal<u>p</u> A 1→4 D-Gal
β-D-Glc<u>p</u> A 1→3 D-Gal A
β-D-Glc<u>p</u> A 1→3 α-D-Gal<u>p</u> A 1→2 L-Rha
β-D-Gal<u>p</u> A 1→4 (2-O-Ac)-D-Gal

It is apparent that the structure of the polysaccharide from <u>Cochlospermum gossypium</u> gum is very similar to that of the <u>Sterculia</u> gums already discussed.

1.2.2 <u>Soy-bean polysaccharides</u>

1.2.2.1 <u>The acidic polysaccharide from Soy-bean Cotyledon Meal</u>

Soy-bean cotyledon meal has been shown to contain an

arabinogalactan[29,30], at least one other neutral polysaccharide (probably an araban) and an acidic polysaccharide complex. Fractionation[31] of the acidic complex gave two main polysaccharide components, both of which, on partial hydrolysis, furnished the same oligosaccharides but in different proportions. Partial hydrolysis studies were therefore performed on the unfractionated polysaccharide.

Mild acid hydrolysis of the polysaccharide resulted in the rapid release of arabinose followed by fucose, while under more forcing hydrolytic conditions both neutral and acidic oligosaccharides were released. Table 14 shows the acidic and neutral oligosaccharides isolated from a partial hydrolysate and an acetolysate of the polysaccharide.

Table 14

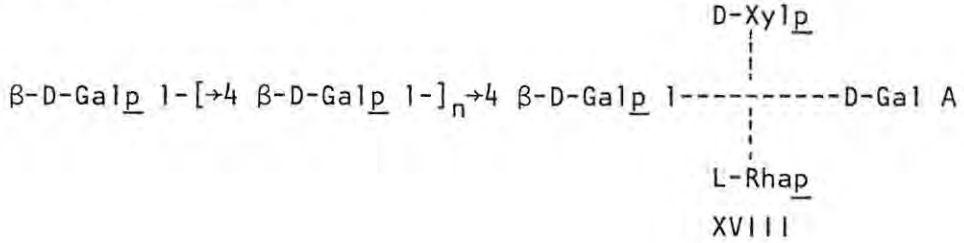
		Partial Acid Hydrolysis	Acetolysis
I	D-Gal*p* 1→4 D-Gal*p*	+	
II	D-Gal*p* 1-[→4 D-Gal*p* 1-]$_n$→4 D-Gal*p* n = 1	+	+
III	n = 2	+	+
IV	n = 3	+	+
V	n = 4	+	
VI	L-Fuc*p* 1→2 D-Xyl		+
VII	D-Gal*p* 1→2 D-Xyl		+
VIII	D-Gal*p* A 1→4 D-Gal*p* A	+	
IX	D-Gal*p* A 1→4 D-Gal*p* A 1→4 D-Gal*p* A	+	
X	D-Gal*p* A 1→2 L-Rha	+	+
XI	Gal*p* A 1→2 Rha*p* 1→4 Gal*p* A 1→2 Rha	+	+
XII	D-Gal*p* A 1→2 L-Rha*p* 1→2 L-Rha		+
XIII	D-Gal*p* A 1→4 D-Gal*p* A 1→2 L-Rha*p* 1→2 L-Rha		+
XIV	D-Glc*p* A 1→6 D-Gal	+	
XV	D-Glc*p* A 1→4 D-Gal*p*	+	
XVI	D-Glc*p* A 1→4 L-Fuc*p*	+	

The main components of the neutral fractions were 4-O-β-D-galactopyranosyl-D-galactose and its polymer homologues. Two additional oligosaccharides were characterized in the acetolysate viz. 2-O-α-L-fucopyranosyl-D-xylose (VI) and 2-O-β-D-galactopyranosyl-D-xylose (VII).

The acidic oligosaccharides isolated in greatest yield were 4-O-(α-D-galactopyranosyluronic acid)-D-galactose (VIII) and the polymer homologous trisaccharide (IX) and 2-O-(α-D-galactopyranosyluronic-acid)-L-rhamnose (X). Higher acidic oligosaccharides were also characterized. In addition, three glucuronic acid containing aldobiouronic acids were isolated as minor products of the partial hydrolysis. No evidence exists for their location in the polymer.

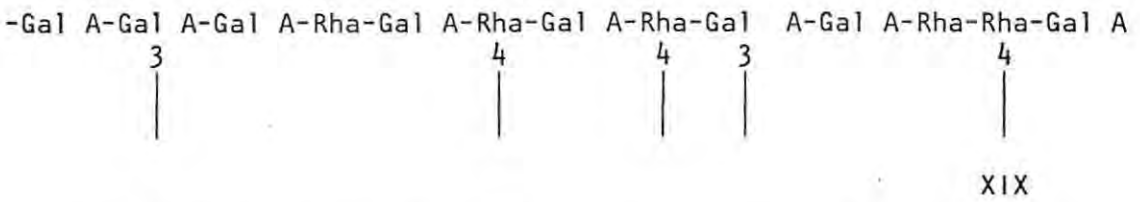
When the acidic polysaccharide was partially degraded by dilute acid, with cleavage of most of the arabinofuranose and fucopyranose residues, and then digested with a commercial enzyme preparation, three oligosaccharides were isolated. One was characterized as 3-O-β-D-xylopyranosyl-D-galacturonic acid (XVII). Methylation and partial hydrolysis studies indicated the presence in the other two of linear chains of 1→4 linked β-D-galactopyranose residues. One fraction was shown to have a single galacturonic acid residue as the reducing end-group. Since no clear evidence was obtained for the location of xylose and rhamnose residues, only a partial structure (XVIII) was advanced for this oligosaccharide.

β-D-Xyl<u>p</u> 1→3 D-Gal A XVII



XVIII

These results indicate a partial structure for the polysaccharide in which interior chains comprise units of D-galacturonic acid and L-rhamnose arranged somewhat randomly as shown in structure (XIX).



XIX

Clearly oligosaccharides (IX), (XI), (XII) and (XIII) may be derived from (XIX).

The results also indicate that some of the L-rhamnose units are branched at C-4 and some of the D-galacturonic acid units are branched at C-3.

The structural units in the side chains may be considered in three groups:-

a) linear chains of 1→4 linked β-D-galactopyranose residues (XX). Results do not however show whether the galactan chains are linked directly to galacturonic acid residues in the main chain or indirectly through xylose or rhamnose residues.

b) D-xylopyranose residues which may occur as end-groups or in units of the disaccharide 2-O-β-D-galactopyranosyl-D-xylopyranose and 2-O-α-L-fucopyranosyl-D-xylopyranose. Since 3-O-β-D-xylopyranosyl-D-galacturonic acid (XVII) was characterized it is clear that single unit side chains of D-xylopyranose residues are linked to C-3 of D-galacturonic acid residues.

c) side chain units containing L-arabinofuranose residues and probably include those of the disaccharide 5-O-L-arabinofuranosyl-L-arabinofuranose. There is no evidence for the location of the three glucuronic acid containing aldobiouronic acids. It is possible that the acidic unit (XVI) arises from further

substitution of a few of the 2-O-α-L-fucopyranosyl-D-xylopyranose (VI) side chains. Likewise the acidic disaccharides (XIV) and (XV) may arise from further substitution of D-galactopyranose units in the side chains (II) and (VII).

The soy-bean cotyledon meal acidic polysaccharide complex contains structural features in common with several pectinic acids and with tragacanthic acid. It may be regarded as a member of the pectinic acid group of polysaccharides, but one which contains an unusually high proportion of neutral sugar units, these being both in the interior structure where chains of 1→4 linked α-D-galacturonic acid residues are interrupted frequently by L-rhamnose residues and in a variety of side-chains.

1.2.2.2 Acidic polysaccharides from Soy-bean hulls

Successive extractions of soy-bean hulls with aqueous reagents furnished five acidic polysaccharides [32,33,34] (A-E), with uronic acid contents 40%, 40%, 76%, 45% and 45% respectively. Polysaccharide C, extracted with hot aqueous ammonium oxalate, was the main component.

The quantities of the various polysaccharide fractions did not permit a full structural examination of each, and selected series of experiments only were carried out on the various fractions. Polysaccharides A and B, slightly contaminated with galactomannans, were partially hydrolysed to furnish acidic oligosaccharides (I), (II), (III), (IV), (V), (VII), (VIII), (IX) and (X). Polysaccharide C was partially hydrolysed with mineral acid, under conditions which would form acidic oligosaccharides only. Polysaccharides D and E, which contained high proportions of neutral sugar residues, were depolymerized by acetolysis. Table 15 lists the neutral and acidic oligosaccharides isolated and characterized.

Table 15

Polysaccharide		$H_{I\ \&\ II}$	H_{III}	H_{IV}	H_{V}	M	
Mode of Formation		a	a	b	b	a	b
I	α-D-Galp A 1→4 D-Galp A	+	+			+	
II	α-D-Galp A 1→4 α-D-Galp A 1→4 D-Galp	+				+	
III	α-D-Galp A 1→2 L-Rha	+	+	+	+	+	+
IV	Galp 1→2 Rhap 1→4 Galp A 1→2 Rha	+		+		+	+
V	Galp A 1→4 Galp A 1→2 Rha	+					
VI	Galp A 1→2 Rhap 1→2 Rha				+		+
VII	β-D-Glcp A 1→4 L-Fucp	+	+			+	
VIII	β-D-Glcp A 1→6 D-Gal	+	+			+	
IX	β-D-Glcp A 1→4 D-Galp	+				+	
X	β-D-Glcp A 1→2 D-Man	+					
XI	β-D-Galp 1→4 D-Galp			+	+	+	+
XII	β-D-Galp 1→4 β-D-Galp 1→4 D-Galp			+		+	+
XIII	β-D-Galp 1→4 β-D-Galp 1→4 β-D-Galp 1→4 D-Galp			+		+	+
XIV	α-D-Fucp 1→2 D-Xyl			+	+		+
XV	β-D-Galp 1→2 D-xyl			+	+		+

H = Acid polysaccharide from soy-bean hulls

a = Partial acid hydrolysis

M = Acid polysaccharide from soy-bean cotyledon-meal

b = Acetolysis

The acidic and neutral oligosaccharides, which have been characterized from the various acidic polysaccharide fractions from soy-bean hulls, together with those isolated from the acidic polysaccharides from cotyledon-meal are shown in Table 15. It is evident that these polysaccharides contain similar sequences of structural units.

The results clearly indicate that the various acidic polysaccharide fractions from soy-bean hulls are of the same general structural type i.e. they contain interior chains of 4-O-substituted D-galacturonic acid and 2-O-substituted L-rhamnopyranose residues with side-chains composed mainly of neutral sugar residues, in which sequences including L-arabinose residues, chains of 1→4 linked β-D-galactopyranose residues, 2-O-α-L-fucopyranosyl-D-xylopyranose and 2-O-β-D-galactopyranosyl-D-xylopyranose units are present. However, only the L-rhamnose residues have been precisely located. The hull polysaccharide, like the cotyledon-meal polysaccharide[24], also contains small proportions of glucuronic acid residues.

1.2.3 Lemon-peel pectin

Two homogeneous pectins have been isolated from dried lemon-peel[35, 36,37]. The two pectins, C and L, show slight differences in uronic acid content (75% and 84% respectively) and in degrees of esterification (10.1% and 8.8% respectively). Both pectins contain D-galacturonic acid, L-arabinose, D-galactose, L-rhamnose, D-xylose and traces of D-glucuronic acid and L-fucose. Most of the structural determination has been carried out on the unfractionated pectin.

Partial acid hydrolysis studies of the whole pectin resulted in the identification of acidic oligosaccharides 1-6, shown in Table 16, while oligosaccharides 1, 4 and 7 were identified from the acetolysate of pectin C.

Table 16

1.	α-D-Galp A 1→2 L-Rha
2.	Glcp A 1→6 Gal
3.	Glcp A 1→4 Fuc
4.	Galp A 1→2 Rhap 1→4 Galp A 1→2 Rha
5.	α-D-Galp A 1→4 D-Galp A
6.	α-D-Galp A 1→4 D-Galp A 1→4 D-Galp A
7.	Galp A 1→2 Rhap 1→2 Rha
8.	Xylp 1→3 Gal A
9.	4-deoxy-β-L threo-hex-4-enopyranosyluronic acid 1→2 L-Rha
10.	Galp A 1→2 Rhap 1→4 Galp A

Degradation by a commercial enzyme preparation gave the three acidic oligosaccharides 8, 9 and 10 (Table 16). The formation of disaccharide 9 shows that the enzyme preparation used contains some pectin and/or pectic acid hydro-lyase activity in addition to hydrolytic activity.

Hydrolysis of the carboxyl-reduced polysaccharide gave galactose as the major product, with only traces of other sugars. The presence and nature of the other sugar residues were established by g.l.c. analysis of the methylated polysaccharide. The major products were methyl glycosides of 2,3,6-tri-O-methyl-galactose with smaller proportions of 2,3,4,6-tetra-O-methyl-galactose, 2,6-di-O-methyl-galactose, 2,3,5-tri-O-methyl-arabinose , 2,3-di-O-methyl-arabinose, 3,4-di-O-methyl-rhamnose and 3-O-methyl-rhamnose.

The only neutral sugar located with any degree of accuracy is L-rhamnose. The isolation of oligosaccharides 1, 4, 7 and 10 suggests that residues of L-rhamnose are present in the main chains of the pectin and are probably concentrated in certain regions where

they either alternate with D-galacturonic acid residues, as in oligosaccharide 4 or they occur as adjacent units, as in oligosaccharide 7.

The other neutral-sugar constituents of lemon-peel pectin probably occur in the outer chains. Methylation of the carboxyl-reduced pectin showed that arabinofuranose residues were present together with some non-terminal units. The only evidence for the location of galactose is supplied by oligosaccharide 2, but since no galactose containing oligosaccharides were obtained as acetolysis products, under conditions in which they were formed in soy-beans, it appears that chains of contiguous galactose residues are absent in lemon-peel pectin. The isolation of oligosaccharide 8 shows that residues of xylose are attached to the galacturonan chain as in soy-bean cotyledon pectin and tragacanthic acid.

The presence of glucuronic acid and fucose is shown by the isolation of oligosaccharides 2 and 3, but there is no evidence for their structural location.

1.2.4 Rapeseed polysaccharides

1.2.4.1 Rapeseed Hull Pectin[38]

The pectin was extracted with aqueous ammonium oxalate and then purified on DEAE sephadex. It contains uronic acid (76%), D-galactose (2-3%), L-arabinose (8-9%), D-xylose (2%) L-rhamnose (2-3%) and L-fucose (1%). The degree of esterification is 83%.

Table 17 lists the oligosaccharides identified as partial hydrolysis products.

Methylation studies were carried out in parallel on rapeseed-hull and lemon-peel pectic acids and on the carboxyl reduced pectic acids. The results are shown in Table 18.

Table 17

1. α-D-Gal*p* A 1→2 L-Rha
2. Glc*p* A 1→4 L-Fuc
3. α-D-Gal*p* 1→4 D-Gal*p* A
4. α-D-Gal*p* A 1→4 α-D-Gal*p* A 1→4 D-Gal*p* A
5. α-D-Gal*p* A 1→4 α-D-Gal*p* A 1→4 α-D-Gal*p* A 1→4 D-Gal*p* A
6. L-Ara*f* 1→5 L-Ara*f*
7. D-Gal*p* 1→4 D-Gal*p*

Table 18

	Sugars formed from methylated pectins	Proportions (%) in methylated reduced	
		Rapeseed pectin	Lemon-peel pectin
2,3,4-Me_3-Xyl	+	1.7	0.6
2,3,5-Me_3-Ara	++	4.6	5.9
2,3-Me_2-Ara	+	2.0	1.9
2-Me-Ara	tr		
3,4-Me_2-Rha	+	2.1	2.6
3-Me-Rha	tr	0.4	0.6
2,3,4,6-Me_4-Gal	+	2.0	3.7
2,3,6-Me_3-Gal	+*	82.5	81.1
2,6-Me_2-Gal		3.7	3.6
2,3,4-Me_3-Gal A	++		
2,3-Me_2Gal A	+++++		

* Not detected from methylated lemon-peel pectin

The methylation results and the partial hydrolysis studies on the rapeseed pectic acid, taken together with those previously reported for lemon-peel pectic acid indicates a close similarity between the two polysaccharides. The neutral sugars occupy similar structural roles to those in other pectins. L-Rhamnose residues are present in interior chains interrupting the galacturonan chain at intervals. As acidic oligosaccharide 1 was the only rhamnose-containing oligosaccharide isolated from the rapeseed pectic acid, it is not known whether residues of this sugar are concentrated in certain regions of the polysaccharide chain as in lemon-peel pectin[35]. Other neutral sugars occur in side-chains as single L-arabinofuranose, D-xylopyranose and D-galactopyranosyluronic acid residues and less frequently in longer chains such as oligosaccharides 6 and 7. The proportions of non-terminal arabinose and galactose residues preclude the existence of a significant proportion of chains longer than two residues. The major branch points are through C-3 of D-galacturonic acid residues.

1.2.4.2 Rapeseed cotyledon-meal pectic polysaccharide[39]

The pectin isolated from rapeseed cotyledon-meal yielded two closely related pectic polysaccharides A and H, which were homogeneous in the ultra centrifuge. Each on hydrolysis gave L-arabinose, D-xylose, D-galactose, glucose, L-rhamnose, fucose (trace) galacturonic acid and a trace of an unidentified component.

The minor fraction A had general features similar to those of H but showed an extremely poor correspondence between the ratios of the sugar components determined on the unmethylated polysaccharide and those calculated after methylation and hydrolysis. Hence the results must be regarded as qualitative.

Since glucose is not a known constituent of pectic substances and fucose and the unidentified component were present in amounts too small to allow unambiguous identification, the significance of these

components is doubtful.

Fraction H had $[\alpha]_D$ -69° and was homogeneous. The major constituents were shown to be arabinose, xylose, galactose and rhamnose in the molar ratios 5.3: 3.2: 1.0: 0.57 and a uronic acid content of 33%. The methyl ester content (2.41%) indicated a degree of estification of uronate residues of 36%.

The major acidic component, on hydrolysis, was tentatively identified as 2-O-(D-galactosyluronic acid) 1→2 L-rhamnose.

The results of methylation studies (Table 19) show H to be a highly branched structure.

The polysaccharide contains twenty eight uronic acid residues in an average unit of one hundred and twelve residues.

Table 19

Neutral sugars	Mole proportions
2,3,5-Me_3-arabinose	21
2,3,4-Me_3-xylose	20
3,4-Me_2-rhamnose	5
2,3-Me_2-arabinose	10
2,3,4,6-Me_4-galactose	4
2-Me-arabinose	14
3-Me-arabinose	3
2,3,6-Me_3-galactose	3
arabinose	3
2,4-Me_2-galactose	1

In order to achieve a balance between the end-groups and the branch points, twenty one (i.e. 75%) of the galacturonic acid residues ought to be branched.

Repeated attempts at reduction, by the procedure of Taylor and

Conrad[40], resulted in a polysaccharide which had 40% of the uronic acid residues still intact. Depolymerization of the methylated, carboxyl-reduced polysaccharide showed that 2,3,6-tri-O-methyl- and 2,6-di-O-methyl-D-galactose, derived from 2,3-di-O-methyl-D-galacturonic acid and 2-O-methyl-D-galacturonic acid were present in the ratio 1.4:1. Hence, major branch-points are through C-3 of D-galacturonic acid, and in order to provide a reasonable balance between end-groups and branch points it was assumed that all the remaining, unreduced uronic acid residues were also branched at C-3.

The above results are consistent with a 1→4 linked galacturonan chain containing 2-linked rhamnose residues. About 75% of the galacturonic acid residues and a negligible amount of rhamnose residues carry side-chains made up mainly of arabinose residues. Most of the xylose is attached as single units to the galacturonan chain.

The rapeseed meal pectin polysaccharides have structural features in common with the pectinic acids from soy-bean and mustard cotyledon-meal and constitute a group which is characterized by the presence of an unusually high proportion of neutral sugars.

1.2.5 Pectic substances from Lucerne (Medicago sativa)

The pectic acids extracted from the leaves and stems[41] of lucerne contained a low proportion (ca. 1 in 6) of esterified galacturonic acid residues and estimates of uronic acid contents of both leaf and stem were about 73%. The polysaccharide samples were chromatographed on DEAE cellulose and in each case more than 95% of the sample was eluted in a single band, indicating that the neutral sugars are integral constituents of the pectic acid rather than of contaminating neutral polysaccharide.

Partial hydrolysis[42] of leaf and stem pectic acids yielded, in both cases, degraded galacturonans which were substantially devoid of

neutral sugar residues and which separated from solution or were readily precipitated with acetone. The soluble sugars were separated and each polysaccharide gave oligosaccharides I-IV and VI-VII (Table 20).

Table 20

Source of pectic acid		Stem		Leaf	
Mode of fragmentation		a	b	a	b
I	α-D-Galp A 1→2 L-Rha	+	+	+	+*
II	β-D-Glcp A 1→4 L-Fucp	+		+	
III	β-D-Glcp A 1→6 D-Gal	+		+	
IV	Galp A 1→2 Rhap 1→4 Galp A 1→2 Rha	+	+	+	+*
V	Galp A 1→4 Galp A 1→2 Rha		+	+	
VI	α-D-Galp A 1→4 D-Galp A	+		+	
VII	α-D-Galp A 1→4 α-D-Galp A 1→4 D-Galp A	+		+	
VIII	Galp A 1→2 Rhap 1→2 Rha		+		+*

a = Partial acid hydrolysis b = Acetolysis

* = Detection by paper-chromatography only

Oligosaccharide (V) was only isolated from the leaf pectic acid. Acetolysis of stem pectic acid led to the isolation of oligosaccharides (I), (IV), (V) and (VIII). In the case of leaf pectic acid the amount of material was insufficient to permit isolation of individual compounds from acetolysis, but paper chromatography indicated the formation of acidic oligosaccharides (I), (IV) and (VIII).

The results clearly show that there is no discernible structural difference between the pectic acids isolated from lucerne leaves and stems. The characterization of (I) as a partial hydrolysis product shows unambiguously that L-rhamnose is a constituent sugar. The

further characterization of higher oligosaccharides (IV), (V) and (VIII) containing galacturonic acid and rhamnose residues points to the presence of 2-O-substituted rhamnose units in the main chains of 4-O-substituted galacturonic acid units. Since a degraded galacturonan substantially devoid of neutral sugar units was also isolated during partial hydrolysis, rhamnose units appear to be unevenly distributed in the galacturonorhamnan chains, so that there are a) regions of uninterrupted galacturonic acid residues and b) regions in which the rhamnose residues are concentrated. In the latter regions there are both alternating sequences of galacturonic acid and rhamnose residues and sequences of at least two adjacent rhamnose residues. Lucerne pectic acid is similar in this respect to the acid polysaccharide from soy-bean cotyledons and hulls, lemon-peel pectin and rape-seed pectin.

There is no direct evidence yet for the location of units of the two disaccharides (II) and (III) in the pectic acid. The same two oligosaccharides were also isolated from soy-bean cotyledon and hull pectic acids and from citrus pectin.

Neutral sugars, other than rhamnose, probably occur in outer chains[41]. Evidence in support of this was obtained by examination of the acidic oligosaccharides formed when the polysaccharide was treated with a pectinase preparation. Partial separation showed the presence of a number of O-glycosyl-galacturonic acid containing residues of galactose, arabinose and xylose. Two further oligosaccharides, 3-O-xylopyranosyl-galacturonic acid and 3-O-arabinofuranosyl-galacturonic acid were isolated in pure form.

Although present in only trace quantities xylose is clearly an integral part of lucerne pectic acid and is linked to the

galacturonan chain in the same manner as in the pectic acids from soy-bean cotyledon and lemon-peel.

The characterization of 3-O-arabinofuranosyl-galacturonic acid is the first conclusive evidence that arabinose is necessarily a constituent of pectin. Evidence for the nature of the linkage of galactose to galacturonic acid residues in this polysaccharide has not yet been obtained.

1.2.6 Gum tragacanth

The gum is grossly heterogeneous, containing at least two components, tragacanthic acid and an arabinogalactan[43,44].

The homogeneous tragacanthic acid, $[\alpha]_D$, + 105° gave, on hydrolysis, D-xylose (40%), L-fucose (10%), D-galactose (4%), L-arabinose (trace), D-galacturonic acid (43%) and L-rhamnose (trace).

Mild hydrolysis of 2-hydroxyethyl tragacanthate[45] released most of the fucose, a small amount of xylose and a trace of galacturonic acid. A degraded tragacanthic acid I[46] and two disaccharides (1) and (2) (Table 21) were also isolated. The degraded polysaccharide contained xylose and galacturonic acid residues in approximately equimolecular proportions and only traces of fucose, galactose and arabinose residues. Stronger hydrolysis released further xylose residues and tragacanthic acid II was isolated. This degraded polysaccharide had a high optical rotation ($[\alpha]_D$ + 228°) approximating to those of polysaccharides of the pectic acid group.

Depolymerization of I and II yielded xylose, galacturonic acid and a similar series of acidic oligosaccharides ((1)-(5) Table 21).

The main acidic oligosaccharide from degraded tragacanthic acid I was (3) while degraded tragacanthic acid II gave relatively large proportions of di- and tri- galacturonic acids (4) and (5) (Table 21).

Table 21

(1)	α-L-Fucp 1→2 D-Xyl
(2)	β-D-Galp A 1→2 D-Xyl
(3)	β-D-Xylp 1→3 D-Gal A
(4)	α-D-Galp A 1→4 D-Galp A
(5)	α-D-Galp A 1→4 α-D-Galp A 1→4 D-Galp A
(6)	α-D-Galp A 1→2 L-Rha
(7)	β-D-Glcp A 1→4 L-Fuc
(8)	α-D-Glcp A 1→4 D-Gal

Table 22 lists the sugars isolated from methylated carboxyl-reduced tragacanthic acid.

Table 22

2,3,4-Me_3-D-glucose	2,3,6-Me_3-D-galactose
2,3,4-Me_3-L-fucose	2,3-Me_2-D-galactose
2,3,4-Me_3-D-xylose	2,4-Me_2-D-galactose
3,4-Me_2-D-xylose	2-Me-D-galactose
4-Me-D-xylose	D-xylose
2,3,4,6-Me_4-D-galactose	D-galactose
2,3,4-Me_3-D-galactose	

The following sugars were also present but only in small amounts:- 2,3-di-O-methyl-L-fucose, mono-O-methyl-L-fucose, di-O-methyl- and 3-O-methyl-D-xylose, 2,4,6-tri-O-methyl-D-galactose and 3,5-di-O-methyl-L-arabinose. With the exception of tetra- and the small proportion of 2,4,6-tri-O-methyl-D-galactose the methyl ethers of D-galactose could have arisen either from D-galactose residues originally present in the polysaccharide or from D-galacturonic acid residues after reduction. Results showed that 2,3,4-tri-O-methyl-, 2,3-(and probably 2,4)-di-O-methyl and 2-O-methyl-D-galactose were derived largely, if not

exclusively, from the reduction of D-galacturonic acid residues.

The methylation results indicate that D-xylopyranose, L-fucopyranose and D-galactopyranose residues occur mainly as end-groups. However, some D-xylopyranose residues are 2-O-substituted. The very small proportion of L-arabinose residues, which gives rise to 3,5-di-O-methyl-L-arabinose, represents a genuine minor structural feature since contamination of the polysaccharide with arabinogalactan would produce the 2,3,5-tri-methyl ether as the main derivative of L-arabinose.

The polysaccharide is clearly based on essentially linear chains of 1,4-linked α-D-galacturonic acid residues (Fig 7), the majority of which carry xylose containing side-chains through C-3. Three types of side-chains have been recognized viz. single β-D-xylose residues and disaccharide units of 2-O-α-L-fucopyranosyl-D-xylopyranose and 2-O-β-D-galactopyranosyl-D-xylopyranose and these must account for the majority of the sugar residues in the outer chain. The location of the small proportion of 2-O-substituted L-arabinofuranose residues in the polymer is not known.

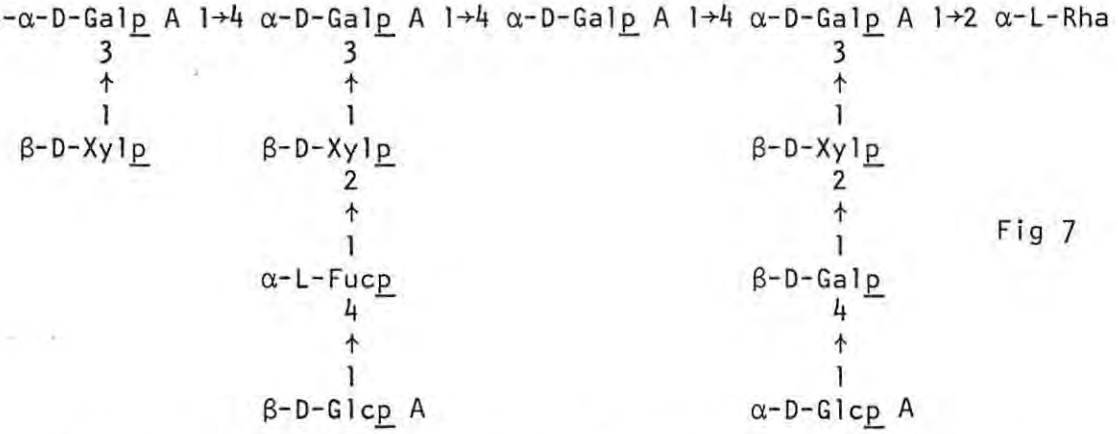


Fig 7

Although units of disaccharide (6)[47] were not precisely located, in respect of its core structure the polysaccharide may be regarded as a galacturonorhamnan with very low proportion of L-rhamnose residues.

The identification of small amounts of 2,3,4-tri-O-methyl-D-glucose,

2,3,6-tri-O-methyl-D-galactose and 2,3-di-O-methyl-L-fucose together with the characterization of disaccharides (7) and (8)[47] shows that glucuronic acid residues occur as non-reducing end-groups and is consistent with the presence in tragacanthic acid of small proportions of the trisaccharide units [A] and [B] arising from further substitution of some 2-O-β-D-galactopyranosyl-D-xylopyranose and 2-O-α-L-fucopyranosyl-D-xylopyranose side-chains.

β-D-Glc*p* A 1→4 α-L-Fuc*p* 1→2 β-D-Xyl*p* 1--- [A]

α-D-Glc*p* A 1→4 β-D-Gal*p* 1→2 β-D-Xyl*p* 1--- [B]

Tragacanthic acid is similar to polysaccharides of the pectic acid group in giving rise to glucuronic acid containing aldobiouronic acids on partial hydrolysis. Lucerne pectic acid, citrus pectin and the acidic polysaccharides from soy-beans afford the same disaccharide (7) as tragacanthic acid but the various polysaccharides yield O-(D-glucopyranosyluronic acid)-D-galactose with different modes of linkage and probably with different glycosidic configuration.

1.2.7 Pectic polysaccharides from suspension-cultured sycamore cells

1.2.7.1 Extracellular polysaccharides[48]

Aspinall *et al* isolated three extracellular polysaccharides a) an arabinogalactan, b) a fucoxylan and c) a pectinic acid containing galacturonic acid (67%), arabinose, galactose, fucose, glucuronic acid, xylose and rhamnose, from the culture medium of suspension-cultured sycamore cells (*Acer pseudoplatanus* L.).

Evidence for the location of some of the sugar residues in the pectinic acid was obtained by the detection, as products of partial acid hydrolysis, of a series of acidic oligosaccharides (Table 23).

As material was limited a more detailed structural examination was not possible, but the polysaccharide showed no essential differences from other pectins.

Table 23

I	α-D-Galp A 1→2 L-Rha
II	β-D-Glcp A 1→4 L-Fuc
III	β-D-Glcp A 1→6 D-Gal
IV	α-D-Galp A 1→4 D-Gal A
V	α-D-Galp A 1→4 α-D-Galp A 1→4 D-Gal A

1.2.7.2 The structure of cell-walls isolated from suspension-cultured sycamore cells (Acer pseudoplatanus L.)[49]

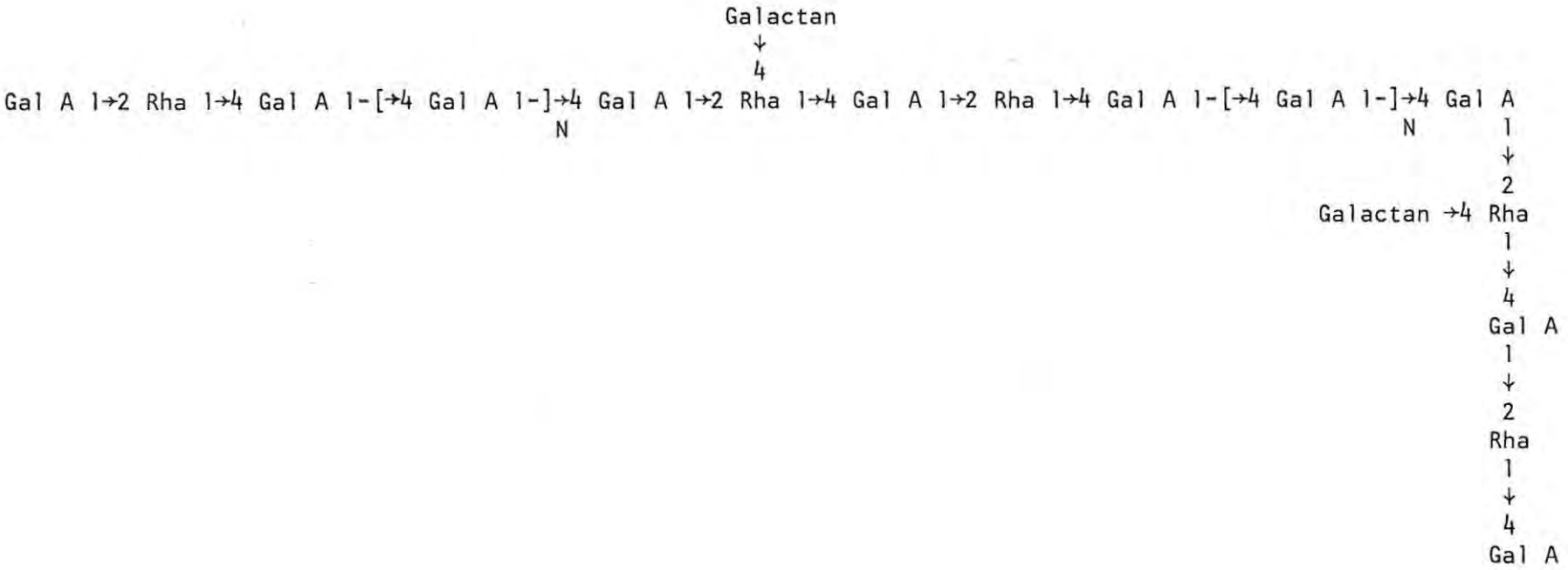
The sycamore cell walls are composed of an arabinan (10%), a 3,6-linked arabinogalactan (2%), cellulose (23%), oligo-arabinosides (9%), a 4-linked galactan (8%), a hydroxyproline-rich protein (10%), a rhamnogalacturonan (16%) and a xyloglucan (21%).

The structure of the rhamnogalacturonan was obtained, in part, by methylation analysis of fragments of the polymer which were released by the action of a highly purified endopolygalacturonase. The data suggests a rhamnogalacturonan consisting of an α-(1→4)-linked galacturonan chain which is interspersed with 2-linked rhamnose residues Fig 8. The rhamnose residues are not randomly distributed in the chain but are thought to occur as rhamnosyl-(1→4)-galacturonosyl-(1→2)-rhamnosyl units. This sequence alternates with a homogalacturonan sequence containing approximately eight residues of 4-linked galacturonic acid. About half of the rhamnose residues are branched, having a substituent attached to C-4.

The results also suggest a branched arabinan and a linear 4-linked galactan, which is linked to C-4 of the rhamnose.

The anomeric configuration of the sugar residues was examined by chromic acid oxidation of the acetylated polysaccharide. The results

Figure 8



Tentative structure for the rhamnogalacturonan from suspension-cultured sycamore cells.

suggest that each of the neutral sugar residues are linked in the β-configuration except for the fucosyl residues which appear to have α-linkages. The anomeric configuration of the arabinose residues was not determined since the above method degrades both α- and β-linked furanosides.

The results point to an overall structure in which the galactan serves as a bridge between the xyloglucan and rhamnogalacturonan.

1.2.8 Cotyledon of White Mustard

The mucilage from the seed of white mustard contains galacturonic acid, arabinose, galactose, rhamnose and xylose[50,51].

The results of a methylation study on the polymer are shown in Table 24 [51]. The relative yields of all the methylated sugars, apart from arabinose and rhamnose, were in reasonable agreement with the proportions of sugars before methylation. The two discrepancies can be explained by

i) overestimation of the yields of 2,3,5-tri-O-methyl-arabinose and 2,3,4-tri-O-methyl-xylose.

ii) probable underestimation of the rhamnose in the native polysaccharide due to it being incompletely released on acid hydrolysis.

The methylation evidence is consistent with a (1→4) linked galacturonan chain with frequent interruption by insertion of 2-linked rhamnose. The various rhamnose ethers, obtained from the neutral fraction of the hydrolysed methylated acidic polymer, were in different proportions from the 'reduced fraction' (i.e. polymer in which uronic acid has been reduced). This implies that contiguous rhamnose residues occur in the chain. About 60% of the galacturonic acid and rhamnose residues, in the part of the polysaccharide that was recovered as the methyl ether, would seem to carry side chains. The main types

Table 24

	Relative molar ratio of ether	Relative molar ratio of parent sugar calculated from ether ratio	Relative molar ratio of parent sugar by direct analysis
2,3-Me_2-galacturonic acid	1.00	1.0	1.0
2-Me-galacturonic acid	1.14		
3-Me-galacturonic acid	1.16		
galacturonic acid	0.03		
2,3,5-Me_3-arabinose	2.86	3.5	1.6
2,3-Me_2-arabinose	2.50		
2,5-Me_2-arabinose	0.04		
2-Me-arabinose	2.24		
3-Me-arabinose	0.42		
arabinose	0.15		
2,3,4-Me_3-xylose	1.88	0.8	0.6
2,3,4-Me_3-rhamnose	0.03	0.6	0.1
3,4-Me_2-rhamnose	0.54		
3-Me-rhamnose	0.74		
4-Me-rhamnose	0.11		
rhamnose	0.03		
2,3,4,6-Me_4-galactose	0.32	0.3	0.3
2,4,6-Me_3-galactose	0.15		
2,3,4-Me_3-galactose	0.09		
2,3,6-Me_3-galactose	0.05		
2,4-Me_2-galactose	0.04		
2,3,6-Me_3-glucose	0.15	0.2	0.2
2,3-Me_2-glucose	0.25		

of branching are through C-3 of galacturonic acid and C-4 of rhamnose. A little branching probably occurs through other positions as well.

The arabinose ethers suggest that a large part of the molecule is made up of arabinan side-chains. The high yield of 2,3,4-tri-O-methyl-xylose suggests that the backbone is densely substituted by single unit xylose side-chains linked to C-3 of galacturonic acid. The modes of linkage of the galactose residues were established by the isolation of several methyl ethers.

The results indicate that the pectin is a highly branched galacturonan chain which is frequently interrupted by rhamnose.

1.2.9 Pectic substances in cured and uncured tobacco[52]

Pectin was obtained from the cured-leaf stems and fresh leaves by ammonium oxalate extraction. The cured pectin had an anhydrogalacturonic acid content of more than 90% and on partial hydrolysis, with a pectinase preparation, yielded di-, tri- and galacturonic acids with α-1,4-linkages. The neutral sugars present were D-galactose, L-arabinose, L-rhamnose and smaller amounts of glucose, xylose, fucose and 2-O-methyl-D-xylose. The polysaccharide failed to fractionate on DEAE cellulose.

Methylation of the carboxyl-reduced polysaccharide gave 2,3,6-tri-O-methyl-D-galactose as the major product and 3,4-di-, 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-galactose as minor products.

Partial enzymic hydrolysis of the pectin from fresh leaves yielded a neutral and two acidic (I and II) fractions. The neutral fraction contained only monosaccharides while the acidic fraction (I) consisted of three galacturonic acid containing oligosaccharides, one of which was tentatively identified as 2-O-(D-galactopyranosyluronic acid)-L-rhamnose.

Fraction II consisted of galacturonic acid and traces of three

oligosaccharides which yielded only galacturonic acid on acid hydrolysis. These were thought to be of the same homologous series as that isolated from cured pectin.

Cured leaf pectin contains over 90% galacturonic acid whereas pectin from fresh leaves has a surprisingly low (ca. 20%) uronic acid content. The acidic component of fresh leaf appears to have the same basic structure as that of the cured pectin.

The general inference from the above is that during curing the polysaccharide is considerably degraded and most of the neutral sugar components attached to the acidic chain are lost.

Analysis of both pectins revealed that the L-rhamnose content relative to other neutral sugars is higher. In the case of pectin from fresh leaves the absolute percentage is extremely high (ca. 30%). This would suggest that rhamnose is either incorporated in the uronic acid chain and/or exists as single branch points off the main acidic chain. This is supported by the isolation of the galacturonic acid-rhamnose containing oligosaccharide and by the apparent increase in rhamnose content when cured pectin is reduced. If many L-rhamnose residues are incorporated into the uronic acid chain then it is unlikely that fresh-leaf polysaccharide will contain many regions where relatively large numbers of uronic acid residues are adjacent. This being so, the apparent fall in rhamnose content (ca 30 → 5%) during curing suggests a complete breakdown of the acidic chain should have occurred unless, as Northcote[53] has shown in apple pectin, part of the acidic component exists free from neutral sugar.

1.2.10 Pectin in the bark of white-willow (Salix alba L.)

The acidic polysaccharide contained D-galacturonic acid, D-galactose and L-arabinose, together with small amounts of L-rhamnose,

D-xylose and L-fucose. Partial acid hydrolysis yielded the acidic and neutral oligosaccharides[54,55] listed in Table 25.

Table 25

(1)	β-D-Gal<u>p</u> 1→4 D-Gal
(2)	β-D-Gal<u>p</u> 1→4 β-D-Gal<u>p</u> 1→4 D-Gal
(3)	β-D-Gal<u>p</u> 1→4 β-D-Gal<u>p</u> 1→4 β-D-Gal<u>p</u> 1→4 D-Gal
(4)	β-D-Gal<u>p</u> 1→6 D-Gal
(5)	β-D-Gal<u>p</u> 1→6 β-D-Gal<u>p</u> 1→6 D-Gal
(6)	L-Ara<u>f</u> 1→6 D-Gal<u>p</u>
(7)	β-D-Glc<u>p</u> A 1→6 D-Gal
(8)	β-D-Glc<u>p</u> A 1→4 L-Fuc
(9)	α-D-Gal<u>p</u> A 1→2 L-Rha
(10)	α-D-Gal<u>p</u> A 1→2 β-L-Rha<u>p</u> 1→2 L-Rha<u>p</u>
(11)	α-D-Gal<u>p</u> A 1→2 β-L-Rha<u>p</u> 1→4 D-Gal<u>p</u> A
(12)	α-D-Gal<u>p</u> A 1→4 D-Gal A
(13)	α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> A 1→4 D-Gal A
(14)	α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> A 1→4 D-Gal A
(15)	α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> A 1→4 α-D-Gal<u>p</u> 1→2 L-Rha<u>p</u>
(16)	β-D-Gal<u>p</u> A 1→6 D-Gal
(17)	D-Gal<u>p</u> 1→3 D-Gal<u>p</u>
(18)	α-D-Gal<u>p</u> A 1→4 D-Gal
(19)	α-D-Gal<u>p</u> A 1→4 D-Xyl

The most abundant aldobiouronic acid residue is 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose which derives from the rhamnogalacturonan chain of the pectinic acid. The position of the other aldobiouronic acid residues is unknown. They are probably present as terminal residues or in the outer chains of the pectinic acid. The

isolation of 6-O-(β-D-galactopyranosyluronic acid)-D-galactose apparently provides the first evidence for the existence of this structural analogue of the frequently occurring unit 6-O-(β-D-glucopyranosyluronic acid)-D-galactose.

The neutral oligosaccharides probably originate from the polymeric chains attached to a rhamnogalacturonan core. The β-(1→4)- and β-(1→6)-linked D-galactose residues, together with L-arabinose residues may be structural units of the polysaccharide chains.

In general these results further confirm that the L-rhamnose residues are unevenly distributed in the rhamnogalacturonan chain and are probably concentrated in blocks as adjacent units as in (10) or alternate with D-galacturonic acid as in (11) and (15). Such L-rhamnose rich areas may interpose relatively long chains of uninterrupted α-(1→4) linked D-galacturonic acid residues. The neutral oligosaccharide (6) and (17) originate probably from the 3,6-linked L-arabinose-D-galactan chains.

1.2.11 Pectin present in the bark of Amabilis Fir[56]

The ammonium pectate was isolated and chromatographed on DEAE cellulose giving three fractions a) a galactoglucomannan (52%) b) an acidic arabinoxylan (7%) and c) a fraction tentatively called a pectic acid (28%).

The pectic acid fraction was separated into two fractions 1) a galacturonan $[\alpha]_D$ +246° and 2) a pectic acid $[\alpha]_D$ +225° containing rhamnose (trace) and D-galacturonic acid, D-galactose and L- arabinose in the ratio 74:7:19 respectively.

Mild partial acid hydrolysis of the pectic acid yielded first arabinose and then galactose. On prolonged hydrolysis the di- to penta-oligomers of D-galacturonic acid were obtained.

. Purified but unfractionated ammonium pectate was partially hydrolysed and gave four oligosaccharides (Table 26).

Table 26

I	α-D-Gal*p* A 1→4 D-Gal A
II	α-D-Gal*p* A 1→4 α-D-Gal*p* A 1→4 D-Gal A
III	α-D-Gal*p* A 1→2 L-Rha
IV	a galacturonosyl-rhamnosyl-rhamnose

The isolation of oligosaccharides (III) and (IV) indicates that the rhamnose is probably an integral part of the pectic acid.

The sugar constituents of the methylated pectic acid are given in Table 27.

Table 27

Component	Mole %	Component	Mole %
Unknown sugars	1.8	D-galacturonic acid	4.3
3-Me-L-rhamnose	2.6	2-Me-D-galacturonic acid	10.5
Me_2-L-arabinose	2.4	3-Me-D-galacturonic acid	9.7
2,3,5-Me_3-L-arabinose	13.3	Me_2-D-galacturonic acid	0.3
2,3,4-Me_3-D-galactose	1.2	2,3-di-O-Me-D-galacturonic acid	45.3
2,3,4,6-Me_4-D-galactose	7.2	2,3,4-Me_3-D-galacturonic acid	1.4

The large amount of 2,3-di-O-methyl-D-galacturonic acid shows that the pectic acid contains a framework of α-(1→4) linked D-galacturonic acid residues. The uronic acid chain is frequently branched and contains terminal non-reducing D-galacturonic acid residues. The majority of the D-galactose and L-arabinose residues are present as non-reducing end groups. A small amount of inner arabinose and (1→6)

linked galactose residues are also present. The nature of the galacturonorhamnose obtained on partial hydrolysis, in conjunction with the presence of 3-O-methyl-L-rhamnose amongst the O-methyl sugars, strongly indicates that rhamnose residues are an integral part of the galacturonan framework, with at least some of them serving as branching points through C-4.

1.2.12 Cress-seed mucilage

The mucilage contains D-galactose, L-arabinose, D-xylose and L-rhamnose in the ratio 10:12:8:8 and D-galacturonic acid (30.9%). 4-O-Methyl-D-glucuronic acid was also a major component[57,58].

A degraded mucilage[59], V, was obtained by autohydrolysis of the cellulose-rich mucilage, R. The uronic acid fraction was methylated and had $[\alpha]_D$ +67°, OMe, 38.3%. The neutral methylated sugars identified are given in Table 28.

Table 28

2,3,4-Me_3-D-xylose (n)	
2,3,4,6-Me_4-D-galactose (n)	
2,3,6-Me_3-D-galactose (m)	m = major
2,6-Me_2-D-galactose (n)	n = minor
3-Me-L-rhamnose (m)	tr. = trace
L-rhamnose (m)	
2,3,4-Me_3-L-rhamnose (tr.)	
3,4-Me_2-L-rhamnose (tr.)	

Methylated barium uronates were converted into the free acids which were separated into three fractions A, B and C. Portions of each fraction were examined chromatographically after prolonged hydrolysis and the remainder converted into the methyl ester methyl

glycoside, which were reduced, hydrolysed and the methylated neutral sugars examined.

Free 2,3,4-tri-O-methyl-D-glucuronic acid was present in the mixture of acids from A. Prolonged acid hydrolysis gave 2,3,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-glucuronic acid and a di-O-methyl-hexuronic acid as major components and 2,6-di-O-methyl-D-galactose and 3-O-methyl-L-rhamnose as minor ones.

The neutral sugars from the reduction were identified and are given in Table 29.

2,3,4-Tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-galactose are derived from 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3-di-O-methyl-D-galacturonic acid respectively. The proportions of neutral sugars

Table 29

2,3,4-Me_3-D-glucose
2,3,6-Me_3-D-galactose
2,3-Me_2-D-galactose
2,6-Me_2-D-galactose
3-Me-rhamnose
2,3-Me_2-glucose (trace)
6-Me-galactose (trace)

and the high mobility of the original fraction A indicates that in addition to much free 2,3,4-tri-O-methyl-D-glucuronic acid the aldobiouronic acid 2,3,6-tri-O-methyl-4-O-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-D-galactose is a major component of the mixture. Lack of information concerning aldobiouronic acids precludes the assignment of aldobiouronic acid structures to the minor components.

3-O-Methyl-L-rhamnose and 2,3-di-O-methyl-D-galacturonic acid were the major components of fraction B and as the galactose and rhamnose methyl ethers were isolated in the approximate ratio 2:1 it seems likely that methylated reduced B consists mainly of O-2,3-di-O-methyl-galactopyranosyl-(1→4)-O-2,3-di-O-methyl-D-galactopyranosyl-(1→2(4))-3-O-methyl-L-rhamnose.

Fraction C gives approximately equal amounts of rhamnose and 3-O-methyl-rhamnose and a larger proportion of 2,3-di-O-methyl-D-galacturonic acid; the low mobility of the fraction suggests it is an oligouronide containing adjacent 1,4-linked galacturonic acid residues.

Fully methylated degraded polymer, V, $[\alpha]_D$ +82°, OMe, 39.1%, was hydrolysed and separated into neutral and acidic fractions. The neutral sugars isolated are given in Table 30.

Table 30

2,3,4,6-Me_4-D-galactose
2,3,6-Me_3-D-galactose
2,6-Me_2-D-galactose
3,4-Me_2-L-rhamnose
4-Me-L-rhamnose
3-Me-L-rhamnose
L-rhamnose
2,3,4-Me_3-xylose (minor)
2,3,4-Me_3-rhamnose (minor)

The mixture of methylated uronic acids were separated into fractions A', B' and C'. B' and C' proved to be identical to B and C in the native polymer. A' yielded the neutral sugars in Table 31 on reduction.

Table 31

2,3,4-Me_3-D-glucose
3,4-Me_2-L-rhamnose
2,3,4-Me_3-D-galactose
2,3-Me_2-D-galactose
2-Me-D-galactose
3-Me-L-rhamnose
2,3,6-Me_3-D-galactose
2,6-Me_2-D-galactose

The 2,3,4-tri-O-methyl-D-glucose, 2,3,4-tri-O-methyl- and 2,3-di-O-methyl-D-galactose must be derived from the related uronic acid residues.

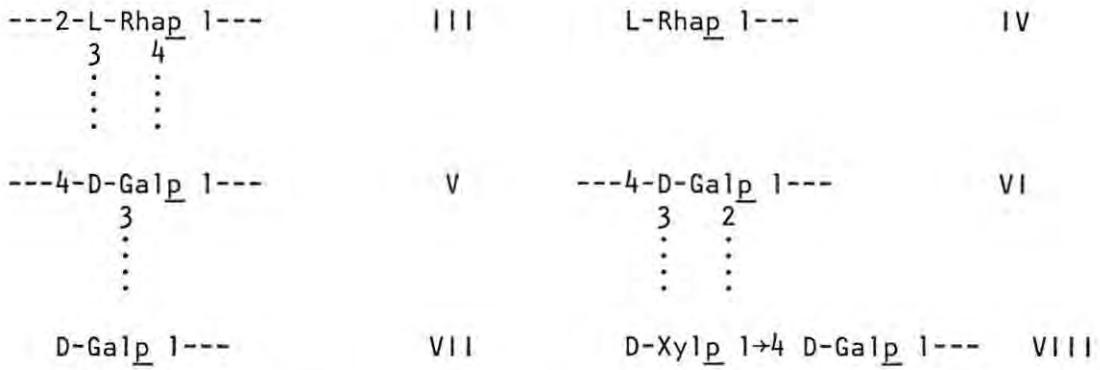
The major differences between the degraded and native polymers lie in the isolation of 4-O-methyl-rhamnose and the production of increased proportions of 3,4-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-galactose and the decreased proportions of 2,3,4-tri-O-methyl-D-xylose, rhamnose, 3-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-, 2,6-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid. 2,3,4-Tri-O-methyl-D-galacturonic acid residues are present only in the methylated degraded mucilage.

The two most important features of the whole mucilage which emerges from the above results are represented in structures (I) and (II) below. (I) constitutes, in part, the backbone of the mucilage and (II) represents the major side chain

---4-D-Gal$\underline{p}$ A 1→4 D-Gal$\underline{p}$ A 1→2 L-Rha$\underline{p}$ 1---- I

4
↑
R

4-Me-D-Glc$\underline{p}$ A 1→4 D-Gal$\underline{p}$ 1---- II



The absence of 2,3,4-tri-O-methyl-D-galacturonic acid in the products from methylated native mucilage shows that all the galacturonic acid is 1,4-linked. Fractions B and B' indicate that the uronic acid residues are linked to the 2- or 4- position of rhamnose. Linkage is assigned to the 2 position on the basis of the degraded polymer. About one in three rhamnose residues is fully substituted (III). Occasional non-reducing terminal residues (IV) are present in the native mucilage.

4-O-Methyl-D-glucuronic acid occurs only as terminal residues and constitutes about half the acidic residues of the whole polysaccharide. It is possibly attached to the 4-position of a galactose residue as in the aldobiouronic acid unit (II). On degradation the proportions of D-galactose and 4-O-methyl-D-glucuronic acid residues are decreased relative to galacturonic acid and rhamnose thus emphasising the close association of the first two residues and their possible situation in the outer chains of the polysaccharide. (II) is possibly attached to position 4 of the rhamnose residues. Some 1,3,4-linked galactose residues (V) occur in the whole mucilage, but whether these are present as additional units in the side-chain or in the core of the polysaccharide is uncertain. The 1,2,3,4-linked galactose residues (VI) occur infrequently and only a small proportion of the terminal residues of the whole mucilage are galactose units (VII).

4-O-α-D-Xylopyranosyl-D-galactose was isolated from the autohydrolysis products of the whole mucilage[58] and therefore (VIII) constitutes a minor side-chain.

1.3. Polysaccharides with a galacturonan core containing both galactose and rhamnose

1.3.1 Combretum leonense gum

The neutral sugars arabinose, galactose and rhamnose were shown to be present in the ratio 9:10:1[60], but the uronic anhydride content varied (ca. 15-20%) when the polysaccharide was isolated from different nodules of the gum. A stepwise degradation released monosaccharides and various neutral and acidic olisaccharides[61,62] (Table 32) while autohydrolysis resulted in the preferential release of arabinose residues accompanied by a small proportion of galactose residues, with the formation of a degraded gum almost devoid of arabinose residues.

Table 32

(a) an arabinobiose of unknown structure
(b) β-L-Araf 1→3 L-Ara
(c) an arabinosylgalactose
(d) β-D-Galp 1→4 D-Gal
(e) β-D-Galp 1→6 D-Gal
(f) β-D-Galp 1→3 L-Ara
(g) β-D-Galp 1→6 β-D-Galp 1→6 D-Gal
(h) β-D-Galp 1→6 β-D-Galp 1→6 β-D-Galp 1→6 D-Gal
(i) β-D-Galp 1→6 β-D-Galp 1→6 β-D-Galp 1→6 β-D-Galp 1→6 D-Gal
(j) α-D-Galp A 1→2 L-Rha
(k) β-D-Glcp A 1→6 D-Gal
(l) D-Galp A 1→2 L-Rhap 1→4 D-Gal
(m) D-Galp 1→4 (or 3) D-Galp A 1→2 L-Rha

The degraded gum was methylated[62] and the hydrolysis products separated into neutral and acidic sugars. The acidic sugars were reduced to give the corresponding neutral sugars (Table 33). Trace quantities of other sugars were also present.

Table 33

Neutral sugars	Reduced acidic sugars
2,3,4,6-Me_4-D-galactose	2,3,4-Me_3-D-glucose
2,3,4-Me_3-D-galactose	3,4-Me_2-L-rhamnose
2,3-Me_2-D-galactose	3-Me-L-rhamnose
3,4-Me_2-L-rhamnose	2,3,4-Me_3-D-galactose
3-Me-L-rhamnose	2,3-Me_2-D-galactose

Most of the neutral sugars from the methylated degraded gum have their origin in the 1,6-linked chains of β-D-galactopyranose residues but the isolation of 2,3-di-O-methyl-D-galactose shows that some residues carry side-chains at C-4 as in (I). It is probable that the acidic fraction, obtained on hydrolysis of the methylated degraded gum, consisted largely of fully or partially methylated derivatives of the aldobiouronic acids (j) and (k). 2,3-Di-O-methyl-D-galactose and the mixture of 3,4-di-O-methyl- and 3-O-methyl-L-rhamnose were formed in approximately equimolecular proportions as the major components of the mixture of sugars derived from the acidic fraction and it is probable that the former sugar arises from 4-O-substituted D-galacturonic acid as in (II), in which the majority of L-rhamnose residues are present as branching points.

D-Gal*p* 1→6 D-Gal*p* 1→6 D-Gal*p* 1→6 D-Gal*p* 1--- I

4

⋮

---4 D-Gal*p* A 1→2 L-Rha*p* 1---- II

D-Glc*p* A 1→6 D-Gal*p*--- III

The D-glucuronic acid residues in the degraded gum are clearly present as end groups, while 2,3,4-tri-O-methyl-D-galactose can arise from the neutral and acidic fractions so it is not possible to ascribe unique structural features. If it is assumed to arise from the neutral fraction it follows that D-glucuronic acid residues terminate side-chain units as in (III)

The neutral and acidic sugars isolated from methylated *Combretum leonense* gum are given in Table 34.

Table 34

Neutral sugars	Acidic sugars (after reduction)
2,3,5-Me_3-L-arabinose	2,3,4-Me_3-D-galactose
2,3-Me_2-L-arabinose	2,3-Me_2-D-galactose
2,5-Me_2-L-arabinose	2,3-Me_2-D-glucose
2,3,4-Me_3-D-galactose	3-Me-L-rhamnose
2,3,6-Me_3-D-galactose	2,4-Me_2-D-galactose (tr.)
2,3-Me_2-D-galactose	
2,4-Me_2-D-galactose	
2,6-Me_2-D-galactose	
2-Me-D-galactose	
3-Me-L-rhamnose	
L-rhamnose	

Traces of 2,3,4-tri-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-, 3-O-methyl-D-galactose and 3,4-di-O-methyl-L-rhamnose were identified in the neutral sugar fractions.

The majority of the peripheral arabinose units occur as L-arabinofuranose end groups (IV). The isolation of disaccharides (b) and (f) and 2,5-di-O-methyl-L-arabinose indicates that the disaccharides arise from (V) and (VI) which are probably present in the outer chains. The isolation of 2,3-di-O-methyl-L-arabinose points to the presence of other non-terminal L-arabinose residues (VII) in the gum.

L-Araf 1--- IV

β-D-Galp 1→3 L-Araf 1--- V

β-L-Arap 1→3 L-Araf 1--- VI

---5-L-Araf 1--- (or ---4-L-Arap 1---) VII

As 2,3,4-tri-O-methyl-, 2,4-di-O-methyl- and 2-O-methyl-D-galactose were isolated in approximately equimolecular proportions it would appear that the majority of acid labile groups (R = IV - VII) are attached to C-3 of 1,6-linked β-D-galactopyranose residues as in (VIII)

```
                        R          R
                        ↓          ↓
                        3          3
---6-β-D-Galp 1→6 β-D-Galp 1→6 β-D-Galp 1---        VIII
                        4
                        :
```

The presence of 2,3-di-O-methyl-D-glucuronic acid residues shows that the D-glucuronic acid residues in the gum are 4-O-substituted and are probably present in the partial structure (IX). The evidence for

R→4 β-D-Glcp A 1→6 D-Galp 1--- IX

---D-Galp 1→4 (or 3) D-Galp A 1→2 L-Rhap 1→4 D-Galp 1--- X

```
                                  3
                                  :
                                  :
                                  :
```

the attachment of acid-labile substituents to other aldobiouronic acid units (II) is less certain, although some such substituents may be linked to L-rhamnose residues. However, the isolation of two isomeric trisaccharides (l) and (m) for the partial hydrolysis of the gum[61]

suggests that these aldobiouronic acid units are flanked on either side by D-galactose residues and partial structure (X) may be proposed for this part of the molecule. The gum also contains a small proportion of contiguous D-galactopyranose residues joined by 1→4 linkage, as shown by the isolation of (d) as a minor product of partial hydrolysis, but the location of these units in the molecular structure is not yet known.

The major structural features of the gum may be summarized in partial structures (VIII), (IX) and (X) to which the various acid-labile groups R are attached at sites indicated.

The possibility that the gum might contain a mixture of polysaccharides of different structural types was investigated[62]. The results provided strong evidence against the presence in the gum of polysaccharides of structurally different types and suggests that the gum contains a mixture of polysaccharides composed of the same structural units which are linked in a similar manner but are present in slightly differing proportions. This type of microheterogeneity may be contrasted with the gross heterogeneity of gum tragacanth and Khaya senegalensis gum where each gum contains a mixture of structurally unrelated polysaccharides.

1.3.2 The Khaya gums

1.3.2.1 Khaya grandifoliola gum

The gum occurs naturally as a partially acetylated polysaccharide containing galactose, rhamnose, galacturonic acid and 4-O-methyl-D-glucuronic acid[63,64] and is very resistant to hydrolysis, even when methylated.

Table 35 lists the sugars isolated from the neutral and acidic

fractions of the methylated gum[64].

Table 35

Neutral fraction	Acidic fraction (after reduction)
2,3,4,6-Me_4-D-galactose	2,3,6-Me_3-D-galactose
2,3,6-Me_3-D-galactose	3-Me-L-rhamnose
3-Me-L-rhamnose	2,3,4-Me_3-D-glucose
Me_2-D-galactose	2,3-Me_2-D-galactose

2,3,4-Tri-O-methyl-D-glucose and 2,3-di-O-methyl-D-galactose obviously arise from reduction of 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3-di-O-methyl-D-galacturonic acid respectively.

Fractionation of the mixture of acidic substances obtained on partial hydrolysis of the gum gave galacturonic acid (I), 2-O-D-galacturonosyl-L-rhamnose (II), 4-O-(4-O-methyl-D-glucuronosyl)-D-galactose (III) and O-D-galacturonosyl-(1→2)-O-L-rhamnosyl-(1→4)-D-galactose (IV).

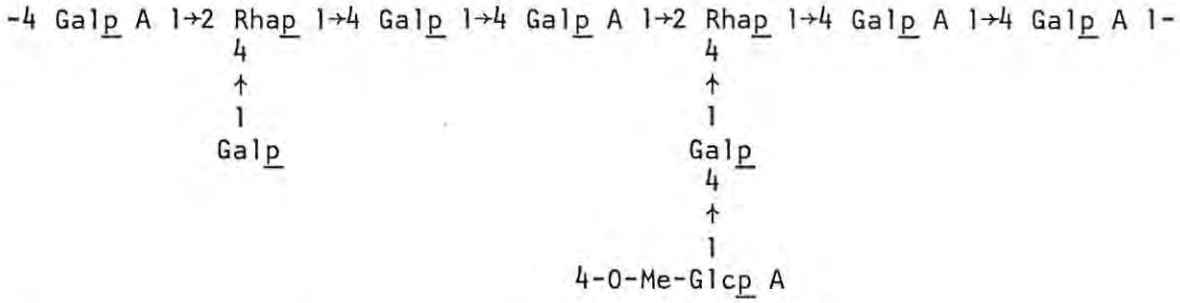
Other acidic oligosaccharides were obtained in low yield, but although these were all shown to be composed of galactose, rhamnose and galacturonic acid residues, insufficient quantities were isolated for detailed structural studies.

The above results indicate that a) D-galactose and 4-O-methyl-D-glucuronic acid occur as end-groups, b) L-rhamnose residues are linked 1,2 in the main chain and are branched at C-4 and c) D-galacturonic acid residues are 1,4-linked in the main chain. On mild acid hydrolysis galactose (and a trace of arabinose) is released which suggests that galactose is linked directly to the rhamnose. The isolation of (III) on partial acid hydrolysis shows that 4-O-methyl-D-

glucuronic acid end groups are linked to the main chain through 1,4-linked D-galactose residues in two-residue side chains. The isolation of (IV) shows that some D-galactose residues are present in the main chain. In addition, the main chain probably contains adjacent D-galacturonic acid residues as indicated by the oligosaccharides of high uronic acid content isolated on partial hydrolysis; indeed the high uronic acid content of the gum itself can only be explained on this basis.

Although no unique structure can be put forward for the gum the repeating unit (Fig 9) includes all the known types of linkage present in the gum and the ratio of constituent sugars, viz. D-galactose (3 parts), L-rhamnose (2 parts), D-galacturonic acid (4 parts) and 4-O-methyl-D-glucuronic acid (1 part) is consistent with the quantitative measurements made, when allowance is made for the decomposition of sugars on hydrolysis.

Figure 9



1.3.2.2 Khaya senegalensis gum

The gum is partially acetylated in its natural state and contains residues of D-galactose, L-rhamnose, D-galacturonic acid and 4-O-methyl-D-glucuronic acid. The gum exudate is made up of two polysaccharide components, the major component falling into the galacturonorhamnan class. This has $[\alpha]_D$ +140° and contains 55% uronic acid residues and has a methoxyl content of 1.8%.

4-O-Methyl-D-glucuronic acid and the two aldobiouronic acids 4-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-galactose (I) and 2-O-(D-galactopyranosyluronic acid)-L-rhamnose (II) were identified[65] as partial hydrolysis products.

Examination of the hydrolysis products of the methylated polymer afforded a mixture of neutral sugars (Table 36) and six fractions containing oligosaccharides, three of which were identified as disaccharides (I) and (II) and an aldobiouronic acid consisting of 1,4-linked D-galacturonic acid residues.

Table 36

2,3,4,6-Me_4-D-galactose
2,3,6-Me_3-D-galactose
2,3,4-Me_3-D-galactose
2,4-Me_2-D-galactose
2,3,4-Me_3-L-rhamnose
3,4-Me_2-L-rhamnose
3-Me-L-rhamnose
2,3,5-Me_3-L-arabinose (s)
2,6-Me_2-D-galactose (s)
2,3-Me_2-D-galactose (s)
L-rhamnose (s)

(s) Present in small amounts only

Reduction of the combined methylated acids followed by hydrolysis resulted in the characterization of the methylated sugars shown in Table 37.

2,3,4-Tri-O-methyl- and 3,4 -di-O-methyl-D-glucose obviously arise from the hexuronic acid but 2,3,4-tri-O-methyl-D-galactose could arise from either galactose or galacturonic acid residues. However, since

Table 37

2,3,4-Me_3-D-glucose	
3,4-Me_2-D-glucose	
2,3,4-Me_3-D-galactose	
2,3,6-Me_3-D-galactose	
2,3-Me_2-D-galactose	
2,4-Me_2-D-galactose	
3-Me-L-rhamnose	
2,3,4-Me_3-L-rhamnose	(s)
rhamnose	(s)

this sugar is also formed on direct hydrolysis of the methylated polysaccharide and hence from galactose residues, it is probable that some, if not all, of this sugar arises similarly from 1,6-linked galactose units.

From the quantities of sugars isolated 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose, 3-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3-di-O-methyl-D-galacturonic acid are of major importance.

Information on the sequence of sugar residues in the interior chains was sought from acetolysis of the carboxy-reduced polysaccharide[66]. Deacetylation of the reaction products and chromatographic separation of the resulting sugars gave the six oligosaccharides in Table 38.

(VIIa) is more likely than (VIIb) as no other oligosaccharides were isolated containing contiguous rhamnose residues.

Since the polysaccharide contains 4-O-substituted-D-galacturonic acid residues and D-galactose residues, no unambiguous assignment of the structural significance of the D-galactose containing

Table 38

I	L-Rha*p* 1→4 D-Gal*p*
II	α-D-Gal*p* 1→2 L-Rha
III	α-D-Gal*p* 1→4 D-Gal*p*
IV	α-D-Gal*p* 1→4 α-D-Gal*p* 1→4 D-Gal*p*
V	4-O-Me-α-D-Glc*p* 1→4 D-Gal*p*
VI	D-Gal*p* 1→2 L-Rha*p* 1→4 D-Gal*p*
VIIa	D-Gal*p* 1→2 L-Rha*p* 1→4 D-Gal*p* 1→2 L-Rha
or VIIb	D-Gal*p* 1→4 D-Gal*p* 1→2 L-Rha*p* 1→2 L-Rha

oligosaccharides (I-IV), (VI) and (VII) from the carboxy-reduced polysaccharide is possible. However, in view of the high uronic acid content (55%) of the polysaccharide and of the methylation results[65], the majority of the D-galactose residues in these oligosaccharides probably arise from the D-galacturonic acid in the parent polysaccharide. Disaccharide (V) on the other hand must arise from units of the corresponding aldobiouronic acid isolated on partial hydrolysis.

The major structural units (VIII-XI) are inferred from the above results. Results also show that structural units (XII-XIV) are of minor but definite structural significance in the polysaccharide

---4 α-D-Gal*p* 1→4 α-D-Gal*p* A 1→4 α-D-Gal*p* A 1--- VIII

---4 α-D-Gal*p* A 1→2 L-Rha*p* 1→4
4 :
: :
: (3)
α-D-Gal*p* A 1→2 L-Rha*p* 1--- IX
4
:
:

D-Gal*p* 1--- X

4-O-Me-α-D-Glc*p* A 1→4 D-Gal*p* 1--- XI

L- Araf 1--- XII ---6 D-Galp 1--- XIII

-6 D-Galp 1--- XIV
3
⋮

1.3.2.3 Khaya ivorensis gum

The gum is partially acetylated and contains galactose, arabinose, rhamnose, galacturonic acid and 4-O-methyl-glucuronic acid[67]. Isolation followed by graded precipitation resulted in a deacetylated homogeneous polysaccharide (38% uronic acid). Partial hydrolysis gave an insoluble galacturonan and the acidic oligosaccharides in Table 39.

Table 39

I	α-D-Galp A 1→2 L-Rha
II	4-O-Me-Glcp A 1→4 Gal
III	α-D-Galp A 1→4 D-Galp A
IV	Galp A 1→2 Rhap 1→4 Galp A 1→2 Rha

Hydrolysis of the methylated reduced polysaccharide gave the sugars listed in Table 40. In addition, the following sugars were isolated in trace quantities:- 2,6-di-O-methyl-, 2-O-methyl- and 3-O-methyl-D-galactose and 3,4-di-O-methyl-D-glucose. The methylation results indicate that the 2,3-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucose arise from the reduction of the corresponding uronic acids.

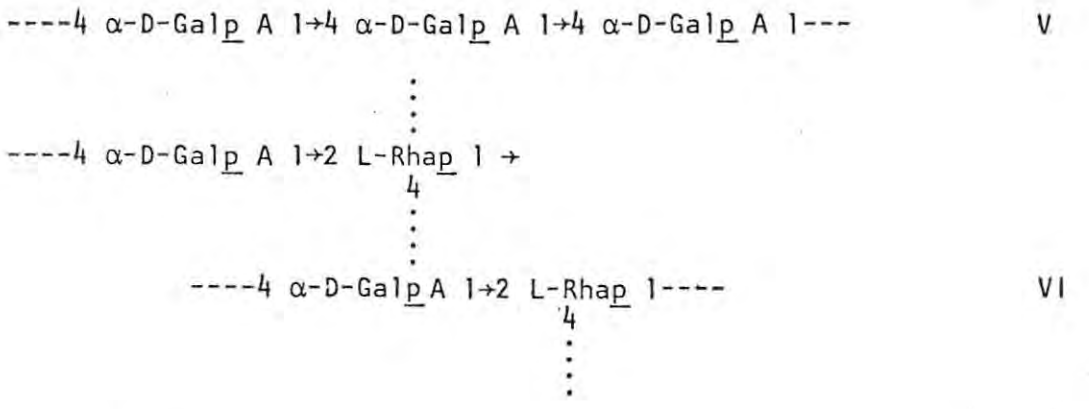
The partial hydrolysis and methylation results lead to the following conclusion concerning the structure. The interior chains consist of 4-O-substituted α-D-galacturonic acid and 2-O-substituted L-rhamnopyranose residues . The chains contain regions of contiguous

Table 40

	Relative proportions
2,3,4,6-Me_4-D-galactose	+++
2,3,4-Me_3-D-galactose	+
2,3,6-Me_3-D-galactose	+++
2,3-Me_2-D-galactose	+++
2,4-Me_2-D-galactose	+
2,3,4-Me_3-D-glucose	++
2,3,5-Me_3-L-arabinose	+
3,4-Me_2-L-rhamnose	+
3-Me-L-rhamnose	+++
L-rhamnose	++

+++ = 10-15% ++ = 6-8% + = 2.5%

galacturonic acid residues (V) and regions in which residues of the two sugars alternate (VI). In this respect there is a qualitative resemblance to pectins of lemon-peel, soy-beans and lucerne.



D-Galp 1--- VII

4-O-Me-D-Glcp A 1→4 D-Galp 1--- VIII

L-Araf 1---- IX

----6 D-Galp 1--- X

----6 D-Galp 1--- XI
3

Rhamnose residues provide the main branching points in the polysaccharide and although no direct proof is yet available, the semi-quantitative results leave little doubt that single D-galactopyranose residues (VII) and units of the disaccharide (VIII) are the more important side-chains and that the majority of these are joined by 1→4' linkages to L-rhamnopyranose residues in the interior chains. In these respects the polysaccharide from Khaya ivorensis gum, although containing a lower proportion of uronic acid residues, closely resembles the main polysaccharide components from Khaya grandifoliola and Khaya senegalensis gums. The structural units (VIII-XI) in Khaya senegalensis are essentially the same as structural units (V-VIII) in Khaya ivorensis. The minor structural units (IX-XI) cannot yet be placed in the overall molecular structure but, in the absence of evidence for heterogeneity in the polysaccharide preparation, they are regarded as integral constituents.

1.3.3 Apple Fruit Pectic Substances[53]

The pectic substances of apple have been separated into a pure pectinic acid and a neutral arabinogalactan complex.

The pectinic acid, shown by zone and free-boundary electrophoresis to be homogeneous, contains galacturonic acid (87%), arabinose (9.3%), galactose (1.4%), rhamnose (1.2%), xylose (0.85%), fucose (trace), 2-O-methyl-xylose (trace) and 2-O-methyl-fucose (trace).

That some of the neutral sugar residues were directly combined with the galacturonic acid residues was shown by the presence of three aldobiouronic acids in the partial hydrolysate of the pectinic acid. The two aldobiouronic acids present in largest amounts were tentatively identified as a galacturonosyl-galactose and galacturonosyl-(1→2)-rhamnose. The third aldobiouronic acid had a

chromatographic mobility consistent with its being a uronosylxylose.

A large proportion of the total neutral sugar residues of the pectinic acid was found in a high molecular-weight component, A, obtained by degradation of the polymer in hot neutral solution. A low molecular-weight component, B, also isolated, contained a very high percentage of galacturonic acid. Detailed analysis of the fractions showed that, whereas arabinose was over one hundred times more abundant in A than in B the distribution of galactose, rhamnose and xylose was rather less unequal. This implies that some galactose, rhamnose and xylose residues are linked directly to galacturonic acid. The very slow release of rhamnose during hydrolysis and the presence of this sugar in the most abundant aldobiouronic acid detected, provides an explanation for the fact that rhamnose is the neutral sugar present in the greatest amount in B.

1.4 Miscellaneous

Many other polysaccharides have been studied which contain both galacturonic acid and rhamnose residues. In some cases the aldobiouronic acid, 2-O-α-(D-galactopyranosyluronic acid)-L-rhamnose[68-75], has been identified as a partial hydrolysis product, but usually insufficient evidence is available in the study to decide whether the oligosaccharide is part of a galacturonorhamnan core or present in side-chains. These polysaccharides have therefore not been included in the survey and their classification into the present study must await further experimental evidence.

2. Experimental

Unless otherwise stated solutions were concentrated at 40°/20 m.m. and specific rotations were determined in water in a Perkin-Elmer 141 Polarimeter. Paper chromatography was carried out on Whatman No.1 filter paper using the following solvent systems:- (1) ethyl acetate-acetic acid-formic acid-water (18:3:1:4), (2) ethyl acetate-pyridine-water (8:2:1) and (3) ethyl acetate-pyridine-water (10:4:3). 2% p-Anisidine hydrochloride[76], triphenyltetrazolium chloride[77] and 10% sulphuric acid in ethanol are sprays a, b and c respectively. R_{Gal} and $R_{T.M.G}$ values refer to rates of movement of sugars on paper chromatograms relative to galactose and 2,3,4,6-tetra-O-methyl-D-galactose respectively. Thin layer chromatography (t.l.c.) was carried out on glass plates coated with Silica Gel G, containing calcium sulphate as binder, using chloroform-methanol (9:1) as the solvent system. Gas liquid chromatography (g.l.c.) was carried out using a Beckman GC-4 chromatograph equipped with dual flame-ionization detectors and using nitrogen as a carrier gas. Separation of methyl glycosides was made on columns containing 15% w/w butane-1,4-diol succinate polyester on Chromsorb W (acid washed; 80-100 mesh) at 175° and a flow-rate of 70 ml./min. (Column I) and 3% w/w neopentylglycol adipate on Chromsorb W (acid washed; 80-100 mesh) at 150° and a flow-rate of 70 ml./min. (column II). Separation of alditol acetates was made on columns containing 15% w/w Apiezon T on Gas Chrom Q (100-120 mesh) at 180° and a flow-rate of 60-80 ml./min. (Column III), 3% w/w ECNSS M on Gas Chrom Q (100-120 mesh) at 175° and a flow-rate of 60-80 ml./min. (Column IV) and 3% w/w OV 225 on Gas Chrom Q (100-120 mesh) at 190° and a flow-rate of 60-80 ml./min. (Column V).

Methyl acetate was determined on 20% w/w Carbowax 1000 on Chromsorb W (80-100 mesh) at 50°. G.l.c. - mass spectrometry (m.s.) analyses were carried out using a Pye Unicam 104 Gas Chromatograph linked to an AEI MS 30 Double Beam Mass Spectrometer. The alditol acetates were separated on Column V at 173° and a flow-rate of 30 ml./min. Mass spectra were determined at 70 eV and a source temperature of 230°.

Table 1 lists the retention times of the methyl glycosides and alditol acetates relative to 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-galactitol respectively.

Table 1

	Methyl glycosides		Alditol acetates[78]		
	15% Butane-1,4-diol succinate (i)	3% Neopentyl-glycol adipate (ii)	15% Apiezon T (iii)	3% ECNSS M (iv)	3% OV 225 (v)
2,3,5-Me_3-arabinose	0.52, 0.77		0.46	0.47	0.46
2,3,4-Me_3-arabinose	1.01	1.0	0.57	0.59	0.55
2,4-Me_2-arabinose	2.03	1.89			
2,3-Me_2-arabinose	1.45, 1.61, 1.75	1.22, 1.44	0.74	1.15	0.93
2,5-Me_2-arabinose	1.75		0.61	0.93	0.85
3-Me -arabinose					1.59
arabinose			1.09	2.60	2.00
2,3,4-Me_3-xylose	0.46, 0.58	0.61	0.56	0.61	0.58
2,3-Me_2-xylose	1.31, 1.37, 1.51	1.12, 1.34	0.74	1.28	1.03
xylose			1.09	2.91	2.55
2,3,4,6-Me_4-galactose	1.64	1.72	1.00	1.00	1.00
2,3,4-Me_3-galactose	5.92		1.61	2.47	2.10
2,3,6-Me_3-galactose	2.80, 3.42, 3.66, 3.95		1.17	1.80	1.66
2,4,6-Me_3-galactose	3.55, 3.97		1.35	1.72	1.55
2,4-Me_2-galactose			2.19	4.47	3.43
2,6-Me_2-galactose			1.44	2.67	2.32
2-Me-galactose			2.22	5.60	4.26
3,4-Me_2-rhamnose	0.99		0.72	0.76	0.79
3-Me-rhamnose			0.96	1.35	1.37
rhamnose			1.02	1.61	1.52

2.1 The Mucilage of Opuntia ficus-indica

2.1.1 Isolation and purification of the polysaccharide

Six cladodes (6606 g), collected in July 1974, were macerated in a Waring blender to afford a mucilaginous pulp which was centrifuged, filtered through sintered glass and then precipitated in acetone (5 vols.). The polysaccharide was allowed to settle and was separated from the supernatant by decantation and centrifugation. The polysaccharide was then soaked in ether, filtered, and dried in vacuo at 50°. The crude polysaccharide (110 g) was dissolved in distilled water (550 ml.) and dialysed against distilled water for 7 days. The contents of the dialysis bag was filtered through a celite pad and the polysaccharide (A) was isolated by freeze-drying (8.34 g). It had $[\alpha]_D$ -62.2° (c 0.34), N, 0.87% and OAc, 5.06%.

Further samples of the polysaccharide were isolated from the same plant in March 1976 (Polysaccharide B), June 1976 (Polysaccharide C) and October 1976 (Polysaccharide D). See Table 3.

2.1.2 Separation and characterization of the components of polysaccharide A

Polysaccharide A (433 mg) in sulphuric acid (0.25 M; 5 ml) was heated on a boiling water bath for 8 h. The solution was cooled, neutralized ($BaCO_3$), filtered and evaporated to a syrup (366 mg). Paper chromatography (solvent 2; spray a) revealed the presence of four sugars with R_{Gal} values 1.00, 1.76, 2.26 and 3.28 (trace) corresponding to galactose, arabinose, xylose and rhamnose respectively. The syrup was separated by paper chromatography (Whatman No.1, solvent 2, 18 h) to afford three fractions.

Fraction 1, a syrup (39 mg) had R_{Gal} 1.0 (solvent 2), was crystallized from methanol-ethanol and had $[\alpha]_D$ +77° (c 0.39) m.p. 163-164° and

mixed m.p. with authentic D-galactose 164-165°.

Fraction 2, a syrup (40 mg), had R_{Gal} 1.76 (solvent 2) and crystallized from methanol-ethanol after standing at room-temperature for some time. The sugar had $[\alpha]_D$ +85.4° (c 0,80) and m.p. 152-153° and mixed m.p. with authentic L-arabinose 153-154°.

Fraction 3, a syrup (28 mg), had R_{Gal} 2.26 (solvent 2) and crystallized from methanol-ethanol and had $[\alpha]_D$ +22° (c 0,36) m.p. 143-144° and mixed m.p. with authentic D-xylose m.p. 142-143°.

2.1.3 Fractionation of Polysaccharide A

a) On DEAE cellulose

Polysaccharide (250 mg) in the minimum quantity of water, was applied onto a DEAE cellulose column (340 x 30 mm, acetate form) and the column was eluted sequentially with distilled water (2 ℓ), 5% v/v acetic acid (2 ℓ) and 5% v/v formic acid (2 ℓ). Fractions (20 mℓ) were collected and tested for carbohydrate. Only the formic acid fractions contained carbohydrate material. These were combined into three fractions, dialysed against running tap water (16 h) and the polysaccharides isolated by freeze-drying. The properties of the three fractions A_1 (114 mg), A_2 (43 mg) and A_3 (46 mg) are given in Table 2.

b) With cetyltrimethylammonium bromide (CTAB)

A solution of CTAB (10%; 15 mℓ) was added with stirring to a solution of polysaccharide A (2%; 50 mℓ). No precipitate formed, even after standing at room-temperature for 48 h. The solution was therefore dialysed against frequently changed distilled water for about seven days, during which time the CTAB - polysaccharide complex precipitated from solution.

The precipitate was removed by centrifugation, dissolved in potassium chloride solution (2 M; 125 mℓ) and precipitated into ethanol (700 mℓ). The mixture was centrifuged, the precipitate was dissolved

in distilled water, dialysed against running deionized water (16 h) and the polysaccharide (A_4) was isolated by freeze-drying [735 mg; $[\alpha]_D$ - 43.9° (c 1.2)].

The supernatant liquid remaining after removal of the CTAB - polysaccharide complex was freeze-dried to afford solid material (109 mg).

2.1.4 Quantitative determination of the sugar residues in the polysaccharide

To polysaccharide (ca 7 mg) was added a known quantity of erythritol (ca 1 mg) and sulphuric acid (0.5 M; 2 mℓ). The solution was heated at 100°, on a glycerine bath, for 16 h, cooled, neutralized ($BaCO_3$), centrifuged, filtered and allowed to stand at room-temperature for 20 min. Sodium borohydride (16 mg) was added and the solution allowed to stand at room-temperature for 4 h, after which the solution was treated with Amberlite I R 120 [H^+] ion exchange resin, evaporated to dryness and distilled with methanol to remove borate. The sample was then treated with acetic anhydride-pyridine (1:1) in a sealed tube at 100° for 1 h. Samples were then directly analysed by gas chromatography (column V).

Since the response of the flame-ionization detector may vary from one glycitol acetate to another, the molar responses of the glycitol acetate of each sugar present in the polysaccharide was determined relative to erythritol acetate. This was done by reducing a solution of a known weight of the sugar to be determined with sodium borohydride and then acetylating as outlined above. The concentration of each sugar was estimated from its peak area.

The uronic acid content of the polysaccharide was determined titrimetrically.

2.1.5 Partial hydrolysis of Polysaccharide A

a) Preliminary hydrolysis

In order to determine the optimum conditions for the formation of oligosaccharides, polysaccharide (99.4 mg) in sulphuric acid (0.05 M; 2 mℓ) was heated at 80° (glycerine bath) and aliquots were withdrawn and spotted onto Whatman No.1 filter paper at hourly intervals over a period of 12 h. The temperature was then raised to 100° and the solution heated for a further 18 h, aliquots once again being removed hourly and applied to Whatman No.1 filter paper. The paper chromatograms were run in solvent 2 (16 h, spray a).

b) Large scale partial hydrolysis

The polysaccharide (4.0 g) was dissolved in sulphuric acid (0.05 M; 80 mℓ) and heated at 80° for 10 h on a glycerine bath. The hydrolysate was then dialysed against two changes of a saturated solution of barium carbonate. The combined diffusates were filtered and freeze-dried (930 mg). A partially degraded polysaccharide, AD_1, was isolated from the dialysate by freeze-drying [2.6 g; $[\alpha]_D$ - 52.0° (c 0.96)]. A small amount of each of the diffusate and the degraded polysaccharide, AD_1, was hydrolysed with 0.25 M sulphuric acid for 4.5 h on a boiling water-bath. A paper chromatogram of the diffusate, hydrolysed diffusate, degraded polysaccharide and hydrolysed degraded polysaccharide was run in solvent 2 (16 h; spray a). The hydrolysed diffusate contained arabinose, xylose and a small amount of galactose. The diffusate contained xylose, arabinose, galactose and two oligosaccharides, one of which had R_{Gal} 0.99 (solvent 1). The hydrolysed degraded polysaccharide contained galactose, arabinose, xylose and a small amount of rhamnose.

The degraded polysaccharide, AD_1, (2.4 g) was heated with sulphuric

acid (0,05 M; 48 mℓ) at 80° for 10 h. The hydrolysate was dialysed and treated as above to give a freeze-dried diffusate (880 mg) and a degraded polysaccharide, AD_2 [1.16 g; $[\alpha]_D$ - 0.8° (c 2.43)].

Hydrolysis of the second diffusate followed by paper chromatography (solvent 2; spray a) showed the presence of arabinose, xylose and a small amount of galactose. Hydrolysis of the degraded polysaccharide, AD_2, followed by paper chromatography (solvent 2; spray a) showed the presence of galactose and smaller amounts of xylose and arabinose. Paper chromatography of the diffusate (solvent 2; spray a) revealed the presence of xylose, arabinose and several oligosaccharides.

The degraded polysaccharide, AD_2, was heated with sulphuric acid (0.05 M; 14 mℓ) under reflux at 100° for 10 h. The hydrolysate was treated as before to yield a diffusate (257 mg) and degraded polysaccharide AD_3 [169 mg; $[\alpha]_D$ + 45.5° (c 1.23)].

The polysaccharides A, AD_2 and AD_3 were hydrolysed, and the derived alditol acetates were examined by g.l.c. The uronic acid contents of each was determined titrimetrically. Results and physical properties are shown in Table 4.

The combined diffusates were dissolved in a minimum amount of water and applied to a Darco G 60 Charcoal-Johns-Manville Celite 535 column (1:1; 450 x 30 mm). Monosaccharides were eluted with water and oligosaccharides by applying an alcohol gradient from 0-40%. Fractions (ca. 16 mℓ) were collected and examined by paper chromatography. Like fractions were combined, evaporated to dryness, weighed and, where necessary, separated further by paper chromatography.

Fraction 1. The syrup (190 mg) was shown by paper chromatography (solvents 1 and 2; spray a) to contain arabinose.

Fraction 2. The syrup (409 mg) was shown by paper chromatography (solvents 1 and 2; spray a) to contain arabinose, xylose and galactose.

Fraction 3. The syrup (6 mg) was shown by paper chromatography (solvents 1 and 2; spray a) to contain only galactose.

Fraction 4. The syrup (8.1 mg) was shown by paper chromatography to contain only one component, oligosaccharide I, R_{Gal} 0.82, 0.56, 0.80 in solvents 1, 2 and 3 respectively, $[\alpha]_D$ +76° (c 0.63). The oligosaccharide gave a red colour with triphenyltetrazolium chloride spray and on hydrolysis with sulphuric acid (0.05 M; 0.5 mℓ) for 2 h on a boiling water-bath followed by neutralization ($BaCO_3$) and paper chromatography (solvents 1 and 2; spray a) the presence of arabinose was revealed.

The oligosaccharide (2 mg) in distilled dimethyl formamide (0.2 mℓ), methyl iodide (0.2 mℓ) and silver oxide (200 mg)was stirred in the dark at 5° [79]. The progress of the reaction was followed by t.l.c. A small amount of chloroform was added, the solution was centrifuged, filtered through cotton wool, and the filtrate was then evaporated to dryness. The methylated oligosaccharide was then refluxed with 2% methanolic hydrogen chloride (0.3 mℓ) for 6 h and the solution was injected directly into the gas chromatograph (columns I and II). Peaks with retention times of 2,3,4-tri-O-methyl-, 2,5-di-O-methyl- and 2,4-di-O-methyl-arabinosides were observed. Alditol acetates, prepared from the methyl glycosides by hydrolysis, reduction and acetylation were examined on column IV. Peaks corresponding to the alditol acetates of 2,3,4-tri-O-methyl- and 2,5-di-O-methyl-arabinose were observed. These results indicate that this is the disaccharide 3-O-α-L-arabinopyranosyl-L-arabinose, the α-configuration being assigned on the basis of the optical rotation.

Fraction 5. The syrup (22.4 mg) was shown by paper chromatography (solvents 1 and 2; spray a) to contain three components which were

separated by paper chromatography (solvent 1; 16 h).

Oligosaccharide II. The sugar (5.7 mg), R_{Gal} 0.28 and 0.36 in solvents 1 and 3 respectively, had $[\alpha]_D$ +18° (c 0.50). Hydrolysis of the oligosaccharide followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of galactose only. The oligosaccharide gave a red colour when treated with triphenyltetrazolium chloride spray. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) revealed the presence of 2,3,4,6-tetra-O-methyl- and 2,3,4-tri-O-methyl-galactose in the methylated oligosaccharide. These results indicate that oligosaccharide II is 6-O-β-D-galactopyranosyl-D-galactose. The optical rotation and R_{Gal} values are in reasonable agreement with those reported for the disaccharide by Parikh and Jones[80].

Oligosaccharide III. The sugar (5.74 mg), R_{Gal} 0.55 and 0.53 in solvents 1 and 2 respectively, had $[\alpha]_D$ +25° (c 0.48). The oligosaccharide gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the oligosaccharide followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of equal proportions of galactose and arabinose. The sugar (1 mg) was dissolved in water (2 mℓ) and sodium borohydride (2 mg) was added and the mixture allowed to stand for 2 h. The solution was then treated with Amberlite IR 120 [H^+] ion exchange resin, evaporated to dryness and repeatedly distilled with methanol to remove borate. Hydrolysis of the reduced oligosaccharide, followed by paper chromatography (solvent 2; spray a), revealed the presence of galactose only. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) revealed the presence of 2,3,4,6-tetra-O-methyl-galactose and 2,4-di- and 2,5-di-O-methyl-

arabinose in the methylated oligosaccharide. These results indicate that oligosaccharide III is 3-O-β-D-galactopyranosyl-L-arabinose, the β-linkage being assigned on the basis of the optical rotation. The R_{Gal} value in solvent 1 is in reasonable agreement with that reported by Parikh and Jones[80].

Oligosaccharide IV. The sugar (15.28 mg), R_{Gal} 0.91, 0.87 and 1.15 in solvents 1, 2 and 3 respectively, had $[\alpha]_D$ +15° (c 0.76). The oligosaccharide gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the oligosaccharide followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of equal proportions of xylose and arabinose. Reduction of the oligosaccharide, followed by paper chromatography (solvents 1 and 2; spray a) of the hydrolysate of the non-reducing syrup, revealed the presence of xylose. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides and alditol acetates (columns I, II, III and IV) revealed the presence of 2,3,4-tri-O-methyl-xylose and 2,4-di- and 2,5-di-O-methyl-arabinose in the methylated oligosaccharide. These results indicate that oligosaccharide IV is 3-O-β-D-xylopyranosyl-L-arabinose, the β-linkage being assigned on the basis of the optical rotation.

Fraction 6. The syrup (23.42 mg), shown by paper chromatography (solvents 1 and 2; spray a) to consist of eight components, was separated by paper chromatography (solvent 2; 48 h) to give oligosaccharide III (2.43 mg), oligosaccharide IV (5.7 mg), oligosaccharides V and VI (2.7 mg) and oligosaccharide VII (see Fraction 7).

Oligosaccharides V and VI. The syrup (2.7 mg), R_{Gal} 1.44 and 1.77 in solvents 1 and 2 respectively, had $[\alpha]_D$ -11.0° (c 0.45). Hydrolysis of the sugar followed by paper chromatography (solvents 1 and 2; spray a)

revealed the presence of arabinose only. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived alditol acetates (columns III and V) revealed the presence of 2,3,5-tri-, 2,3-di- and 2,5-di-O-methyl-arabinose in the methylated oligosaccharide. 2,3-Di-O-methyl-arabinose was present in the largest amount. These results indicate the syrup to be an approximately 1:4 mixture of 5-O-α-L-arabinofuranosyl-L-arabinose (oligosaccharide V) and 3-O-α-L-arabinofuranosyl-L-arabinose (oligosaccharide VI). Smith and Stephen[81] isolated both these oligosaccharides from Virgilia oroboides gum. The former had R_{Gal} 1.40 (solvent 1) and $[\alpha]_D$ -87° and the latter had R_{Gal} 1.28 (solvent 1) and $[\alpha]_D$ 0°.

Fraction 7. The syrup (215.5 mg), R_{Gal} 1.06, 1.40 and 1.42 in solvents 1, 2 and 3 respectively and $[\alpha]_D$ -52.6° (c 1.2), was shown to be the same as oligosaccharide VII. The oligosaccharide gave a red colour when treated with triphenyltetrazollum chloride spray. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose in approximately equal proportions. Reduction of the oligosaccharide, followed by paper chromatography of the hydrolysate of the non-reducing syrup, revealed the presence of xylose. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) and alditol acetates (column IV) revealed the presence of 2,3,4-tri-O-methyl-xylose and 2,3-di-O-methyl-arabinose in the methylated oligosaccharide. These results indicate that oligosaccharide VII is 5-O-β-D-xylopyranosyl-L-arabinose. The R_{Gal} values (solvents 1 and 3) and the optical rotation are in reasonable agreement with those reported by Parikh and Jones[80] and Smith and Stephen[81].

Fraction 8. The syrup (13.98 mg), shown by paper chromatography (solvents 1 and 2; spray a) to consist of two components, was separated by paper chromatography (solvent 1; 16 h) to give oligosaccharides VII and VIII.

Oligosaccharide VIII. The syrup (4.8 mg), R_{Gal} 0.09 (solvent 1), had $[\alpha]_D$ +13.2° (c 0.48) and m.p. 152-155°. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of galactose only. The oligosaccharide was reduced and the non-reducing syrup hydrolysed on a boiling water-bath for 25 minutes with 0.05 M sulphuric acid. The solution was neutralized ($BaCO_3$), evaporated to dryness and on paper chromatographic examination the presence of galactose and oligosaccharide II was revealed. The results indicate oligosaccharide VIII to be O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose. Smith and Stephen[81] reported $[\alpha]_D$ +22° for the trisaccharide.

Fraction 9. The syrup (9.6 mg), R_{Gal} 0.61 and 0.58 in solvents 1 and 2 respectively, had $[\alpha]_D$ -21.2° (c 0.57). The oligosaccharide gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose in the approximate ratio 1:2. Partial hydrolysis followed by paper chromatography revealed the presence of xylose, arabinose and oligosaccharide IV. Methylation followed by g.l.c. analysis of the derived methyl glycosides (column I) revealed the presence of 2,3,4-tri-O-methyl-xyloside and 2,4-di- and 2,5-di-O-methyl-arabinoside. These results indicate oligisaccharide IX to be the trisaccharide O-β-D-xylopyranosyl-(1→3)-O-α-L-arabinofuranosyl-(1→3)-L-arabinose, the α-linkage being assigned on the basis of the optical rotation.

Fraction 10. The syrup (17 mg), shown by paper chromatography (solvents 1 and 2; spray a) to contain two components, was separated by paper chromatography (solvent 2; 72 h) to yield oligosaccharides X and XI.

Oligosaccharide X. The sugar (3.16 mg), R_{Gal} 0.54, 0.53 and 1.01 in solvents 1, 2 and 3 respectively, had $[\alpha]_D$ -58.8° (c 0.53). The oligosaccharide gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose in the approximate ratio 1:2. Repeated attempts at partial hydrolysis failed to afford any identifiable disaccharides.

Oligosaccharide XI. The sugar (2.82 mg), R_{Gal} 1.08, 0.61 and 0.60 in solvents 1, 2 and 3 respectively, had $[\alpha]_D$ -32° (c 0.47) and gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose in the approximate ratio 1:2. Partial hydrolysis followed by paper chromatography revealed the presence of arabinose, xylose and oligosaccharide VII. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) and alditol acetates (column IV) revealed the presence of 2,3,4-tri-O-methyl-xylose, 2,3-di-, 2,4-di- and 2,5-di-O-methyl-arabinose in the methylated oligosaccharide. These results indicate oligosaccharide XI to be a trisaccharide with the structure O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→3)-L-arabinose, the α-linkage being assigned on the basis of the optical rotation. Parikh and Jones[80] reported $[\alpha]_D$ -74.2° for the trisaccharide.

Fraction 11. The syrup (72 mg), R_{Gal} 0.75, 0.70 and 1.35 in solvents

1, 2 and 3 respectively, had $[\alpha]_D$ -56.5° (c 0.52) and gave a red colour when treated with triphenyltetrazolium chloride spray. Hydrolysis of the oligosaccharide followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of arabinose and xylose in the approximate ratio 2:1. Partial hydrolysis followed by paper chromatography revealed the presence of xylose, arabinose and oligosaccharide VII. Reduction and partial hydrolysis revealed the same products. Methylation followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) and alditol acetates (column IV) revealed the presence of 2,3,4-tri-O-methyl-xylose and 2,3-di-O-methyl-arabinose in the methylated oligosaccharide. These results indicate that oligosaccharide XII is a trisaccharide with the structure O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→5)-L-arabinose, the α-linkage being assigned on the basis of the optical rotation.

Fraction 12. The syrup (35.54 mg) was shown by paper chromatography (solvents 1 and 2; spray a) to consist of two components which were separated by paper chromatography (solvent2; 5 days) to give oligosaccharides XIII and XIV.

Oligosaccharide XIII. The sugar (13.26 mg), R_{Gal} 0.41, 0.34 and 0.83 in solvents 1, 2 and 3 respectively, had $[\alpha]_D$ -26.0° (c 0.54) and gave a red colour with triphenyltetrazolium chloride spray. Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose. Partial hydrolysis followed by paper chromatography revealed the presence of xylose, arabinose and oligosaccharides VII and XII. The ratio of xylose to arabinose was shown (alditol acetates; column V) to be 1:1 suggesting a tetrasaccharide. Reduction followed by hydrolysis and paper chromatography revealed a decrease in the arabinose concentration

suggesting an arabinose end-group. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides (columns I and II) and alditol acetates (columns III and IV) revealed the presence of 2,3,4-tri- and 2,3-di-O-methyl-xylose and 2,3-di-O-methyl-arabinose. These results indicate oligosaccharide XIII to be O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→5)-L-arabinose.

Oligosaccharide XIV. The syrup (6.78 mg), R_{Gal} 0.40, 0.47 and 1.16 in solvents 1, 2 and 3 respectively, had $[\alpha]_D$ - 22.6° (c 0.88). Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of xylose and arabinose. The ratio of xylose to arabinose was shown (alditol acetates; column V) to be 1:1, suggesting a tetrasaccharide. Repeated partial hydrolysis experiments failed to reveal any recognizable di- or trisaccharides.

Fraction 13. The syrup (65.46 mg) was shown by paper chromatography to contain several oligosaccharides which did not move any appreciable distance from the origin (solvent 2; 8 days). Hydrolysis of the syrup followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of arabinose, xylose and galactose. This fraction was not further investigated.

2.1.6 Methylation of polysaccharide A.

Polysaccharide (1.0 g) in water (10 mℓ) was treated slowly and simultaneously with dimethyl sulphate (10 mℓ) and sodium hydroxide solution (40% w/v; 20 mℓ) with vigorous stirring during 7 h after which the mixture was stirred for a further 17 h. The solution was then dialysed against running deionized water (72 h) and the polysaccharide isolated by freeze-drying (0.8 g).

The partially methylated polymer (0.8 g) in dimethyl formamide (30 mℓ) and methyl iodide (20 mℓ) was treated with silver oxide (5 g)

in portions while stirring under reflux at 40° for 24 h. Methyl iodide (20 mℓ) and silver oxide (10 g) were added as above and the solution allowed to stir under reflux for a further 24 h. The mixture was then centrifuged, filtered and evaporated to dryness (0.74 g). The infra-red spectrum of the polysaccharide ($CHCl_3$ solution) showed a large hydroxyl peak. The methylation procedure using dimethyl formamide and Purdie's reagents[82] were repeated several times on a portion of the partially methylated polysaccharide to afford fully methylated polysaccharide [176 mg; $[\alpha]_D$ -68.2° (c 5.4, chloroform)].

A portion of the methylated polysaccharide (5 mg) was hydrolysed with sulphuric acid (0.5 M; 3 mℓ) at 100° for 16 h. The solution was neutralized ($BaCO_3$), filtered and evaporated to a small volume. Paper chromatography of the hydrolysate (solvent 2; spray a) revealed the presence of a mono-O-methyl-galactose (R_{TMG} 0.26), a mono-O-methyl-arabinose (R_{TMG} 0.39), 2,4-di-O-methyl-galactose (R_{TMG} 0.44), a mono-O-methyl-rhamnose (R_{TMG} 0.63), 2,3,4-tri-O-methyl-galactose (R_{TMG} 0.69), 2,3-di-O-methyl-arabinose (R_{TMG} 0.79), 2,3,4,6-tetra-O-methyl-galactose (R_{TMG} 1.00), 2,3,4-tri-O-methyl-xylose (R_{TMG} 1.12) and 2,3,5-tri-O-methyl-arabinose (R_{TMG} 1.12).

A small proportion of the methylated polysaccharide (15 mg) was methanolysed and samples were directly analysed by gas chromatography (column I).

The methyl glycosides were converted to the alditol acetates which were examined by gas chromatography (columns III, IV and V). The sugars identified are shown in Table 11.

2.1.7 Reduction of methylated polysaccharide A.

The methylated polysaccharide (76 mg) in dry tetrahydrofuran (3 mℓ) was heated under reflux with lithium aluminium hydride (LAH) for 18 h.

Excess LAH was destroyed with ethyl acetate and the mixture was then dialysed against running deionized water (18 h). The polysaccharide was isolated by freeze-drying (69 mg).

2.1.8 Methylation of the carboxyl-reduced methylated polysaccharide

The reduced, methylated polysaccharide in dimethyl formamide (1 mℓ) and methyl iodide (4 mℓ) was treated with silver oxide (100 mg) and stirred under reflux for 48 h. This procedure was repeated and the mixture was then centrifuged, filtered and the filtrate evaporated to dryness. The syrup was dissolved in chloroform, the solution washed with potassium cyanide solution, dried over sodium sulphate, filtered and evaporated to dryness (39 mg).

The polysaccharide was hydrolysed with sulphuric acid (0.5 M; 3 mℓ) at 100° for 16 h, the solution was neutralized ($BaCO_3$) centrifuged, filtered and sodium borohydride (80 mg) added. After 4 h the solution was treated with Amberlite IR 120 [H^+] ion exchange resin, the solution evaporated to dryness, distilled with methanol to remove borate and the alditol acetates prepared as described previously. The methylated alditol acetates were analysed by g.l.c. (columns III-V) and g.l.c. - m.s. The results are shown in Table 11.

2.1.9 Methylation of polysaccharide B

The polysaccharide (200 mg) in dry dimethyl sulphoxide (distilled over CaH_2; 3 mℓ) was stirred, under an atmosphere of dry nitrogen, with methyl-sulphinyl anion[83,84] at room-temperature for 6 h with intermittent ultrasonication. Methyl iodide (1 mℓ) was added over a period of 15 min. care being taken to maintain the temperature below 20°. The mixture was allowed to stir for a further 20 min., was dialysed against running deionized water (16 h) and the partially methylated polysaccharide was isolated by freeze-drying. The

polysaccharide was further methylated with methyl iodide (7 mℓ), dimethyl formamide (0.5 mℓ) and silver oxide (200 mg), under reflux, with stirring for 48 h. The fully methylated polysaccharide (145 mg) was isolated as previously described and the derived methylated alditol acetates were examined by g.l.c. (columns III-V). A small amount of the methylated polysaccharide was methanolysed and examined by g.l.c. on column I.

The polysaccharide was reduced, methylated, hydrolysed, converted to the methylated alditol acetates as previously described and examined by g.l.c. (columns III-V) and g.l.c.-m.s. The results are shown in Table 12.

2.1.10 Periodate oxidation of polysaccharide B

Polysaccharide (104.2 mg) was dissolved in deionized distilled water (50 mℓ) and 0.0299 M sodium metaperiodate (50 mℓ) was added. The solution was set aside at room-temperature in the dark, and at intervals aliquots (0.1 mℓ) were removed and the reduction of periodate measured[85]. Ethylene glycol was added to the solution to destroy excess periodate and sodium borohydride (200 mg) added. The solution was then dialysed against running deionized water and the polyalcohol isolated by freeze-drying (36 mg). Further treatment of the polyalcohol with periodate showed oxidation to be essentially complete. A small portion of the polyalcohol (10 mg) was hydrolysed with sulphuric acid (0.5 M; 3 mℓ) and the hydrolysate examined by paper chromatography (solvents 1 and 2; spray a). The remainder was converted to alditol acetates and examined by g.l.c.

2.1.11 Chromic acid oxidation of polysaccharide A[86]

To polysaccharide A (46.04 mg) in formamide (5 mℓ) was added acetic anhydride (4 mℓ) and pyridine (3 mℓ) and the solution was

allowed to stand in the dark at room temperature. After 24 h the mixture was poured into water, dialysed against running deionized water (16 h), centrifuged and evaporated to dryness (70 mg). The above procedure was repeated to give acetylated polysaccharide A (66.64 mg).

To acetylated polysaccharide A in glacial acetic acid (5 mℓ) was added chromium trioxide (157.35 mg) and 2-deoxygalactitol acetate (36 mg) as internal standard. The solution was allowed to stir at room temperature and aliquots (0.6 mℓ) were withdrawn at intervals, poured into water (2 mℓ) and the polysaccharide extracted with chloroform. The chloroform layer was evaporated to dryness and the polysaccharide was hydrolysed, reduced, acetylated and examined by g.l.c. (column V). The results are given in Table 6.

2.1.12 Determination of the degree of acetylation of the natural polysaccharides

Polysaccharides A and B (15.71 mg and 3.41 mg) were heated separately in a sealed tube with M methanolic HCl (0.25 mℓ) on a steam bath for 1.5 h. The methanolic hydrogen chloride contained toluene (3.57mg/mℓ) as an internal standard. Penta-acetyl glucose (1.45 mg) in methanolic hydrogen chloride (0.25 mℓ) was similarly treated. All samples were injected directly into the gas-chromatograph (column VI).

2.1.13 Preparation of degraded polysaccharide BD_1

The polysaccharide (4 g) was hydrolysed with sulphuric acid (0.05 M; 80 mℓ) on a glycerine bath at 100° for 10 h. The solution was dialysed against running deionized water (16 h) and the degraded polysaccharide isolated by freeze-drying [1.0 g; $[\alpha]_D$ +30.1° (c 2.73)]. The partially degraded polysaccharide (1 g) was further hydrolysed with sulphuric acid (0.05 M; 50 mℓ) at 100° for 1 h. The solution was

dialysed against running deionized water (16 h) and the degraded polysaccharide BD_1 was isolated by freeze-drying [800 mg; $[\alpha]_D$ +31.4° (c 3.79)] and the component neutral sugars estimated by g.l.c. of the derived alditol acetates and the uronic acid content by titration.

2.1.14 Periodate oxidation of degraded polysaccharide BD_1

The polysaccharide (48.32 mg) was dissolved in deionized distilled water (25 mℓ) and sodium metaperiodate (0.0307 M; 25 mℓ) was added. The same procedure was then followed as described previously.

2.1.15 Isolation and characterization of L-rhamnose and D-galacturonic acid

Degraded polysaccharide BD_1 (150 mg) was hydrolysed with sulphuric acid (0.5 M; 20 mℓ) at 100° for 16 h, the solution neutralized ($BaCO_3$), centrifuged, filtered and passed through Amberlite IRA 400 [CH_3COO^-] ion exchange resin. The resin was washed with deionized distilled water (20 mℓ), the eluate concentrated and the mixture separated by paper chromatography (solvent 2; 16 h) to afford L-rhamnose [14.1 mg; $[\alpha]_D$ +7.1° (c 1.41)] and D-galactose [23 mg; $[\alpha]_D$ +71.4° (c 2.30)].

The resin was then eluted with zinc acetate (0.05 M; 200 mℓ) and three fractions were collected. Fraction i was passed through Amberlite IR 120 [H^+] ion exchange resin and evaporated to dryness to give a syrup [25.4 mg; $[\alpha]_D$ +41.3° (c 2.54)]. Paper chromatography (solvents 1 and 2; spray a) showed that this fraction was pure galacturonic acid. The syrup was oxidized with nitric acid-water (1:1) at 80° for 2 h to afford mucic acid, m.p. 217-218°.

Fraction ii was passed through Amberlite IR 120 [H^+] ion exchange resin and evaporated to dryness [3.15 mg; $[\alpha]_D$ +25.4° (c 0.32); R_{Gal} 0.84 (solvent 1)]. Hydrolysis followed by paper chromatography (solvents 1 and 2; spray a) revealed the presence of rhamnose and

galacturonic acid. The oligosaccharide is probably 2-O-α-(D-galactopyranosyluronic acid)-L-rhamnose, which has been reported by Aspinall and Sanderson[19] to have R_{Gal} 0.75 (solvent 1) and $[\alpha]_D$ +88°. The oligosaccharide was also isolated from Okra mucilage and had R_{Gal} 0.97 (solvent 1) and $[\alpha]_D$ +15.1° [70].

Fraction iii contained no polysaccharide material.

2.1.16 Reduction of degraded polysaccharide BD_1

The degraded polysaccharide (100 mg), moistened with dry methanol was vigorously stirred at 0° and to this was added an ether-diazomethane solution[87] (10 mℓ). The mixture was allowed to stir at 0° for 2 h, evaporated to dryness and the polysaccharide dissolved in distilled water. Sodium borohydride (50 mg) was added to the solution and after 4 h the solution was treated with Amberlite IR 120 [H^+] ion exchange resin[88]. The solution was evaporated to dryness and then distilled with methanol to remove borate. The polysaccharide was dissolved in distilled water, the solution treated with Amberlite IR 120 [H^+] ion exchange resin and then freeze-dried. The above procedure was repeated twice to give reduced degraded polysaccharide (98mg). Estimation of the uronic acid content of the polysaccharide by titration showed that reduction was essentially complete.

2.1.17 Methylation of polysaccharide BD_1 and reduced polysaccharide BD_1

The degraded polysaccharide (100 mg) and reduced degraded polysaccharide (85 mg) were dissolved separately in dimethyl sulphoxide (3 mℓ) and methyl-sulphinyl anion[83,84] (3 mℓ) was added and the polysaccharides methylated as previously described to afford partially methylated BD_1 and partially methylated reduced BD_1 (45 and 64 mg respectively). The partially methylated polysaccharides in dimethyl

formamide-methyl iodide (1 mℓ: 8 mℓ) were treated with silver oxide (250 mg) in portions while stirring under reflux for 48 h. The mixtures were centrifuged, filtered and evaporated to dryness. They were then redissolved in chloroform, washed with potassium cyanide solution, dried over sodium sulphate and evaporated to dryness to yield yellow syrups (22.8 mg and 67.6 mg respectively).

2.1.18 Hydrolysis of methylated BD_1 and methylated reduced BD_1

The methylated degraded polysaccharide (22.8 mg) and the methylated reduced degraded polysaccharide (30 mg) were hydrolysed separately with sulphuric acid (0.5 M; 3 mℓ) at 100° for 16 h. The solutions were neutralized ($BaCO_3$) centrifuged and filtered. Paper chromatography of the hydrolysates (solvent 2; spray a) showed the presence in each of a mono-O-methyl-rhamnose (R_{TMG} 0.70), 2,3,4-tri-O-methyl-galactose (R_{TMG} 0.76) and 2,3,4,6-tetra-O-methyl-galactose (R_{TMG} 1.00) respectively. In addition 2,3,6-tri-O-methyl-galactose (R_{TMG} 0.87) was observed in the hydrolysate of methylated reduced BD_1. The hydrolysates were then reduced, acetylated and the methylated alditol acetates examined by g.l.c. (column III and V) and g.l.c.-m.s. (Table 7).

2.2. The mucilage of Opuntia aurantiaca

2.2.1 Isolation and purification of the polysaccharide

The cladodes (581 g) in water (1 ℓ) were macerated in a Waring blender. The mixture was centrifuged, filtered through cotton wool and the solution was poured slowly into ethanol (5 vols). The fibrous polysaccharide which precipitated was collected by decantation and centrifugation. The polysaccharide was dissolved in water (200 mℓ), dialysed against running tap-water for 16 h and then poured into 95% ethanol (1.6 ℓ). The polysaccharide was allowed to settle and the supernatant liquid decanted off. The polysaccharide was then washed with methanol-ether (3:1) and dried in vacuo at 50°. Yield 6.38 g, $[\alpha]_D$ -35.4° (c 1.67), N, 0.46%.

2.2.2 Identification and determination of the ratios of the neutral sugars in the polysaccharide

To polysaccharide (15 mg) was added a known quantity of erythritol (ca 2 mg) and sulphuric acid (0.5 M; 3 mℓ). The solution was heated at 100° (glycerine bath) for 16 h. The solution was neutralized ($BaCO_3$), centrifuged, filtered and evaporated to dryness. Paper chromatography of the hydrolysate (solvents 1 and 2; spray a) revealed the presence of galactose, arabinose, xylose and rhamnose (trace). The hydrolysate was reduced, acetylated and analysed by g.l.c. (column V). The neutral sugar ratios were calculated and the uronic acid content determined titrimetrically (Table 14).

2.2.3 Methylation of the polysaccharide

The polysaccharide (190 mg) in dimethyl sulphoxide (3 mℓ) was stirred,with intermittent ultrasonication, with methyl sulphinyl anion[83,84] (3 mℓ) at room-temperature for 6 h under an atmosphere of nitrogen. Methyl iodide (1 mℓ) was added and the partially

methylated polysaccharide isolated as previously described. The partially methylated polysaccharide in dimethyl formamide (0.5 mℓ) and methyl iodide (7 mℓ) was treated with silver oxide (200 mg) under reflux for 48 h and the methylated polysaccharide (130 mg) isolated as before.

2.2.4 Methylation of carboxyl-reduced methylated polysaccharide

Methylated polysaccharide in tetrahydrofuran (3 mℓ) was refluxed with LAH at 75° for 16 h. Excess LAH was destroyed (ethyl acetate) and the mixture dialysed against running tap-water (24 h) and the reduced methylated polysaccharide was isolated by freeze-drying. Methylation of the polysaccharide[82] with methyl iodide (6 mℓ) and silver oxide (200 mg) afforded methylated reduced polysaccharide (95 mg).

The methylated reduced polysaccharide was hydrolysed with sulphuric acid (0.5 M; 3 mℓ) at 100° (glycerine bath) for 16 h and the methylated sugars converted to the alditol acetates as previously described and examined by g.l.c. (columns III and V). The results are shown in Table 16.

2.2.5 Preparation and analysis of degraded polysaccharide

Polysaccharide (4 g) was hydrolysed with sulphuric acid (0.05 M; 80 mℓ) under conditions essentially similar to those described for O. ficus-indica mucilage to afford degraded polysaccharide [1.17 g; $[\alpha]_D$ +24.4° (c 3.45)] and dialysable material (2.66 g).

To polysaccharide (20 mg) was added a known quantity of erythritol (ca 2 mg) and sulphuric acid (0.5 M; 3 mℓ) and the solution was heated at 100° (glycerine bath) for 16 h. Paper chromatography of the neutralized ($BaCO_3$) solution (solvents 1 and 2; spray a) revealed the presence of galactose, rhamnose and smaller amounts of arabinose and xylose. The hydrolysate was reduced, acetylated and

the alditol acetates examined by g.l.c. (column V). The sugar ratios are shown in Table 14, the uronic acid content having been determined by titration.

The diffusates were examined by paper chromatography (solvents 1 and 2; spray a).

2.2.6 Reduction of the degraded polysaccharide

The acid form of the degraded polysaccharide (101 mg) in dry methanol was methylated with diazomethane[87] and reduced with sodium borohydride as previously described for degraded O. ficus-indica polysaccharide, to afford reduced polysaccharide[88] (90 mg). Reduction was shown to be essentially complete by titration of the polysaccharide after being passed through a column of Amberlite IR 120 [H^+] ion exchange resin.

2.2.7 Methylation of degraded and reduced degraded polysaccharide

Degraded polysaccharide (54.5 mg) and reduced degraded polysaccharide (62 mg) dissolved separately in dimethyl sulphoxide (2 mℓ) were methylated with methyl-sulphinyl anion and methyl iodide[83,84] (1 mℓ) as previously described. The partially methylated polysaccharides in dimethyl formamide (0.5 mℓ) were further methylated with methyl iodide (7 mℓ) and silver oxide (200 mg) under reflux for 48 h to afford methylated degraded (61 mg) and methylated reduced degraded (69 mg) polysaccharides.

A portion (20 mg) of each methylated polysaccharide was hydrolysed with 0.5 M sulphuric acid and converted to the methylated alditol acetates which were examined by g.l.c. (columns III and V) and g.l.c.-m.s. The results are shown in Table 15.

3. Discussion

3.1 Opuntia ficus-indica

Opuntia ficus-indica (L) Mill. is the most common and widespread species of cactus in South Africa. It has reached noxious weed proportions in the Eastern Cape and is extremely costly to control. The specimen used in the present investigation was found about 8 km north of Grahamstown in the Cape Province. The plant can grow to a height of 5 m, forming a sturdy trunk with age. Leaves, if present, are minute thorns[89].

Extraction of the cladodes (modified stems) followed by precipitation in acetone, decantation and centrifugation afforded crude polysaccharide material in 1.67% yield. Further purification was achieved by dissolving the polysaccharide in water, dialysing, filtering and freeze-drying. Polysaccharide A, isolated in approximately 0.13% yield, had $[\alpha]_D$ -62.2°, N 0.87, and OAc 5.06% and on hydrolysis and paper chromatography was shown to contain galactose, arabinose, xylose and a trace of rhamnose. When the time of hydrolysis was increased uronic acid was detected and tentatively identified as galacturonic acid by paper chromatography. The ratios of the neutral sugars were determined by g.l.c. analysis of the derived alditol acetates and the uronic acid content determined titrimetrically (Table 2).

Two methods of fractionation were applied to determine whether the polysaccharide was homogeneous. Treatment of a solution of the polysaccharide with cetyltrimethylammonium bromide (CTAB) failed to produce a precipitate even after 48 h. The solution was therefore dialysed against frequently changed distilled water over a period of 7 days, during which time a precipitate formed. The precipitate was dissolved in potassium chloride solution and precipitated into ethanol.

Table 2. Properties of polysaccharides A, A_1, A_2, A_3 and A_4

Polysaccharide	A	A_1	A_2	A_3	A_4
$[\alpha]_D$	-62.2°	-55.5°	-54.0°	-57.7°	-48.9°
% N	0.87	0.90	-	-	0.34
Constituent sugar units (Mole %)					
Galactose	18.42	23.82	20.59	21.57	17.81
Arabinose	42.38	42.78	44.91	44.15	45.98
Xylose	24.48	22.32	20.68	19.92	23.31
Rhamnose	6.38	5.06	8.39	9.17	5.97
Galacturonic acid	8.35	6.02	5.44	5.19	6.93

Further purification of the polysaccharide was effected by dissolution in water and dialysis and the polysaccharide, A_4, was isolated by freeze-drying in 75% yield. The properties of polysaccharides A_4 and A are compared in Table 2. The supernatant liquid remaining after removal of the CTAB - polysaccharide complex was dialysed and freeze-dried and the material isolated (11% yield) was shown to contain the same sugar residues and in similar proportions as those present in polysaccharide A_4. This material probably consists of low molecular weight material whose CTAB complex failed to precipitate.

Polysaccharide A was applied to a DEAE cellulose column (acetate form) and the column eluted sequentially with water, 5% acetic acid and 5% formic acid. No carbohydrate material was eluted with either water or acetic acid, indicating the absence of neutral polysaccharide. Three polysaccharide fractions were obtained from the formic acid eluate. A comparison of the sugar residues of the three fractions,

A_1, A_2 and A_3, and the whole mucilage, A, is given in Table 2. These fractionation results suggest that polysaccharide A consists of a single polydisperse acidic heteropolysaccharide. Structural work was therefore performed on the unfractionated polysaccharide.

In an earlier preliminary investigation[90] the mucilage of O. ficus-indica was reported to be devoid of uronic acid and to contain arabinose, xylose, galactose and rhamnose in the molar ratio 3.6:1.5:2.8:1.0. In view of the presence of uronic acid in polysaccharide A it was decided to investigate the seasonal variation in the sugar composition of the mucilage. Samples of polysaccharide (B, C and D) were accordingly isolated from the same plant in March, June and October 1976 and analysed. The properties of A, B, C and D are compared in Table 3.

Table 3. Properties of polysaccharides A, B, C and D

Polysaccharide	A	B	C	D
$[\alpha]_D$	-62.2°	-67.0°	-50.4°	-53.9°
% N	0.87	1.10	1.56	1.12
Constituent sugar units (Mole %)				
Galactose	18.42	22.83	25.16	21.65
Arabinose	42.38	41.30	39.95	42.75
Xylose	24.48	22.08	20.01	20.45
Rhamnose	6.38	6.97	6.86	7.34
Galacturonic acid	8.35	6.82	8.02	7.81

The results show very slight variation in the composition of the polysaccharides. It is interesting to note the approximately 2:1 ratio of arabinose to xylose and 1:1 ratio of rhamnose to galacturonic acid in each of the polysaccharide samples.

The neutral sugar residues in polysaccharide A were isolated from the hydrolysate by paper chromatography and characterized as D-galactose, L-arabinose and D-xylose. Only traces of rhamnose and uronic acid were detected in the hydrolysate.

A partially degraded polysaccharide, AD_1, was prepared by heating polysaccharide A in 0.05 M sulphuric acid at 80° for 10 h. The release of sugars during the hydrolysis was followed by paper chromatography. After 0.5 h an oligosaccharide corresponding to 5-O-β-D-xylopyranosyl-L-arabinose appeared in the hydrolysate. Arabinose appeared after 1 h and was followed by a second oligosaccharide. Xylose was only detected after 8 h hydrolysis. The partially degraded polysaccharide was isolated by dialysis and freeze-drying. The diffusate was neutralized, freeze-dried and shown by paper chromatography to contain xylose, arabinose, galactose and the two oligosaccharides mentioned above. The diffusate was hydrolysed and shown by paper chromatography to contain arabinose, xylose and a small amount of galactose. Partially degraded polysaccharide AD_1 was heated with sulphuric acid at 80° for a further 10 h and a second degraded polysaccharide, AD_2, isolated from the hydrolysate as before. Paper chromatography of the second diffusate, isolated as above, revealed the presence of xylose, arabinose and several oligosaccharides. A paper chromatogram of the hydrolysed diffusate revealed the presence of the same sugars and in similar concentrations as before. Hydrolysis of polysaccharide AD_2 with sulphuric acid for a further 10 h at 100° followed by dialysis and freeze-drying led to the isolation of degraded polysaccharide AD_3. The properties of the degraded (AD_3), partially degraded (AD_2) and whole mucilages are shown in Table 4. The results indicate that after 20 h hydrolysis a large proportion of the arabinose and xylose has been removed. However,

the uronic acid : rhamnose : galactose ratio is very nearly the same.

Table 4. Properties of polysaccharides A, AD_2 and AD_3.

Polysaccharide	A	AD_2	AD_3
$[\alpha]_D$	-62.2°	-0.8°	+45.5°
Constituent sugar units (mol. ratios)			
Galactose	22.1	26.9	15.0
Arabinose	50.8	14.7	0.9
Xylose	29.3	8.8	0.6
Rhamnose	7.6	7.4	9.9
Galacturonic acid	10.0	10.0	10.0

After 30 h hydrolysis nearly all the arabinose and xylose has been removed as well as a large proportion of the galactose. However, because of the resistance to hydrolysis of the uronosyl linkages compared with other glycosidic linkages the results cannot strictly be regarded as quantitative. The ease with which the arabinose units are released on hydrolysis suggests that they are present in the furanose form while the more resistant nature of the xylose units suggests that they are in the pyranose form. The change of optical rotation of the polysaccharide from negative to positive with the removal of the arabinofuranose and xylose suggests that most of these are α- and β-linked respectively.

A degraded polysaccharide, BD_1, $[\alpha]_D$ +31.4°, was similarly prepared from polysaccharide B. The sugar composition, as determined by g.l.c. of the alditol acetates, is shown in Table 5. Polysaccharide BD_1 was converted to the carboxyl-reduced polysaccharide and the sugar rations determined (Table 5).

Table 5. Properties of degraded polysaccharide BD_1

Polysaccharide	Acid form (BD_1)	Reduced form (BD_1)
Constituent sugar ratios (Mole %)		
Galactose	41.34	68.49
Rhamnose	34.14	31.51
Galacturonic acid	24.52	-
Arabinose	trace	trace
Xylose	trace	trace

Degraded polysaccharide BD_1 was hydrolysed with 0.5 M sulphuric acid for 16 h at 100°, neutralized, filtered and passed through a column containing Amberlite IRA 400[CH_3COO^-] ion exchange resin. After washing with distilled water the eluate was concentrated and the mixture of neutral sugars separated by paper chromatography. L-Rhamnose and D-galactose were isolated and characterized. Elution of the resin with 0.05 M zinc acetate led to the isolation of two carbohydrate-containing fractions. The first fraction contained D-galacturonic acid and was characterized by its optical rotation and conversion to mucic acid. The second fraction contained a biouronic acid which gave rhamnose and galacturonic acid on hydrolysis and was tentatively identified by paper chromatography [19,70] as 2-O-α-(D-galactopyranosyluronic acid)-L-rhamnose.

Treatment of fully acetylated glycosides with chromic acid affords information regarding the anomeric configuration of the sugar. The chromic acid oxidizes fully acetylated pyranosidic pentose and hexose residues in which the aglycone occupies an equatorial position[86]. When acetylated polysaccharide A was treated with chromic acid the results shown in Table 6 were obtained.

Table 6. Chromic acid oxidation of polysaccharide A

% Sugar Consumed	2h	4h	8h	12h	24h
D-Galactose	65.58	75.18	78.42	82.65	88.86
L-Arabinose	19.53	41.64	49.83	70.76	82.57
D-Xylose	52.02	72.42	80.27	89.24	94.40
L-Rhamnose	15.87	59.52	66.67	76.19	92.06

The above results indicate that L-rhamnose, D-galactose and D-xylose are β-linked. However, no information is forthcoming regarding the anomeric configuration of arabinose as all acetylated furanosidic pentose residues are oxidized.

Both degraded polysaccharides BD_1 and reduced BD_1 were methylated by the method of Hakomori[83,84] followed by the method of Haq and Percival[79], to yield fully methylated derivatives which were then subjected to hydrolysis. Paper chromatography of the hydrolysates of methylated BD_1 and reduced BD_1 revealed the presence in both of 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-galactose, di-O-methyl- and mono-O-methyl-rhamnose. In addition 2,3,6-tri-O-methyl-galactose was observed in the hydrolysate of methylated reduced BD_1. Conversion of the hydrolysates to the alditol acetates followed by g.l.c. analysis revealed the presence of the methylated sugar residues shown in Table 7.

It is clear from the methylation results that the 2,3,6-tri-O-methyl-galactose must be derived from the galacturonic acid residues present in polysaccharide BD_1. The identities of the methylated sugars were confirmed by g.l.c. - m.s. analysis. The significant m.s. peaks[91] are shown in Table 7. From the results (Table 7) it is possible to propose a tentative repeating structure for the degraded polysaccharide BD_1. (Fig 1)

Table 7.

Methylation analysis of degraded polysaccharide BD_1 and reduced degraded polysaccharide BD_1

	Significant m.s. peaks[91]	methylated BD_1	methylated reduced BD_1 Mole %	Sugars (Mole %) (i) From reduced methylated BD_1	Sugars (Mole %) (ii) From reduced BD_1
2,3,5-Me_3-arabinose		+	+		
2,3,4-Me_3-xylose		+	+		
2,3,4,6-Me_4-galactose	45, 117, 161, 205	+++	23.14	galactose	
2,3,4-Me_3-galactose	117, 161, 189 233	+++	17.82	68.84	68.49
2,3,6-Me_3-galactose	45, 117, 233	+	27.88	rhamnose	
3,4-Me_2-rhamnose	131, 189	++	7.94	31.16	31.55
3-Me-rhamnose	189, 203	+++	23.22		

+++ = major ++ = minor + = trace

Fig 1

→4 Gal A 1→2 Rha 1→4 Gal A 1→2 Rha 1→4 Gal A 1→2 Rha 1→4 Gal A 1→2 Rha 1→
4 4 4
↑ ↑ ↑
1 1 1
Gal Gal Gal
6 6
↑ ↑
1 1
Gal Gal

To verify the results of the methylation studies, polysaccharide BD_1 was subjected to periodate oxidation and the progress of the reaction was monitored spectrophotometrically[85] (Table 8). The results of periodate oxidation of polysaccharide B are also shown in Table 8.

Table 8. Periodate reduced per "average anhydro unit" *

Time (h)	4	8	24	36	48	72
Deg. polysaccharide BD_1	0.85		1.09		1.21	1.21
Polysaccharide B	0.25	0.43	0.76	0.79	0.86	0.86

* Average anhydro unit of degraded polysaccharide = 161.4

Average anhydro unit of native polysaccharide = 142.8

The value of the average anhydro unit was calculated from the mole ratios of the sugars in the polysaccharides. The structure proposed for the degraded polysaccharide would be expected to consume 1.15 moles per average anhydro unit. This result is within reasonable agreement with the experimental value of 1.21 moles per average anhydro unit. Chromic acid oxidation studies of the whole polymer showed both rhamnose and galactose to be β-linked. Both of these linkages have a low positive contribution to the optical rotation of the degraded polysaccharide. Thus to achieve a specific rotation of +31.4° the galacturonic acid must be α-linked. Hydrolysis of the periodate oxidized material followed by paper chromatographic and g.l.c. analysis of the hydrolysate revealed that only rhamnose survives periodate oxidation. This is consistent with the structure proposed for the degraded polysaccharide.

The combined diffusates derived from the partial hydrolysis of polysaccharide A were applied to a charcoal-celite column and the sugars eluted first with water and then with an ethanol gradient into thirteen fractions. Some of the fractions contained only single components, while others were obtained as mixtures and were separated by paper chromatography. The monosaccharides identified were galactose, arabinose and xylose while fourteen oligosaccharides were isolated, of which twelve were characterized (Table 9).

Oligosaccharide I was an arabinobiose and showed a high positive rotation. Treatment with triphenyltetrazolium chloride gave a red colour, indicating the absence of a 1,2-linked terminal reducing unit. G.l.c. analysis of the derived methyl glycosides and alditol acetates revealed the presence of 2,3,4-tri-, 2,4-di- and 2,5-di-O-methyl-arabinose in the methylated oligosaccharide. The oligosaccharide

is thus 3-O-α-L-arabinopyranosyl-L-arabinose, the α-linkage being assigned as a result of the high positive rotation. The β anomer of this disaccharide has been reported in the hydrolysates of several other polysaccharides[61,92-96]. Oligosaccharide II, a

Table 9

I	α-L-Ara p 1→3 L-Ara
II	β-D-Galp 1→6 D-Gal
III	β-D-Galp 1→3 L-Ara
IV	β-D-Xylp 1→3 L-Ara
V	α-L-Araf 1→5 L-Araf
VI	α-L-Araf 1→3 L-Ara
VII	β-D-Xylp 1→5 L-Araf
VIII	β-D-Galp 1→6 β-D-Galp 1→6 D-Gal
IX	β-D-Xylp 1→3 α-L-Araf 1→3 L-Ara
X	D-Xyl: L-Ara :: 1:2
XI	β-D-Xylp 1→5 α-L-Araf 1→3 L-Ara
XII	β-D-Xylp 1→5 α-L-Araf 1→5 L-Araf
XIII	β-D-Xylp 1→4 β-D-Xylp 1→5 α-L-Araf 1→5 L-Araf
XIV	D-Xyl: L-Ara :: 1:1

galactobiose, gave a red colour when treated with triphenyltetrazolium chloride and the methanolysis products of the methylated disaccharide were shown by g.l.c. to have the retention times of 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-galactoside. The oligosaccharide is thus 6-O-β-D-galactopyranosyl-D-galactose, previously identified in white willow[54], Combretum leonenses[61] and several other polysaccharides[80,81,97,98]. Paper chromatography of oligosaccharide VIII and its partial hydrolysis products indicated this oligosaccharide to be O-β-D-galactopyranosyl-

(1→6)-O-β-D-galactopyranosyl-(1→6)-D-galactose. Oligosaccharide III gave approximately equimolecular proportions of galactose and arabinose on hydrolysis while reduction and hydrolysis revealed arabinose to be the reducing end-group. The oligosaccharide gave a red colour with triphenyltetrazolium chloride and the methanolysis products of the methylated oligosaccharide were shown by gas chromatography to be 2,3,4,6-tetra-O-methyl-galactoside and 2,4-di- and 2,5-di-O-methyl-arabinoside. The oligosaccharide is thus 3-O-β-D-galactopyranosyl-L-arabinose, the β-linkage being assigned as a result of the fairly low positive optical rotation. This disaccharide has been reported in the polysaccharides of Combretum leonenses[61], O. fulgida[80], gum ghatti[98], Abizzia zygia[99] and Anogeissus schimperi[100]. Oligosaccharide IV gave approximately equimolecular proportions of xylose and arabinose on hydrolysis and a pink colour when treated with triphenyltetrazolium chloride spray. Xylose was shown by reduction and hydrolysis of the oligosaccharide to be the non-reducing end-group while g.l.c. analysis of the derived methyl glycosides and alditol acetates of the methylated oligosaccharide indicated a 1,3-linkage. The oligosaccharide is thus 3-O-β-D-xylopyranosyl-L-arabinose, the β-linkage being inferred from the low positive optical rotation. The α anomer of this disaccharide has been reported in golden apple gum[93], corn-hull[101] and corn-fibre[102] cellulose and had $[\alpha]_D$ +173° to 182°. Oligosaccharides V and VI were isolated as a mixture by paper chromatography and had very similar R_{Gal} values. Paper chromatographic analysis of the hydrolysate of the syrup revealed the presence of arabinose only. G.l.c. analysis of the derived methyl alditol acetates revealed the presence of a mixture of sugars identified as 2,3,5-tri-O-methyl-arabinose and 2,3-di- and 2,5-di-O-methyl-arabinose. This fraction is a mixture of

5-O-α-L-arabinofuranosyl-L-arabinose and 3-O-α-L-arabinofuranosyl-L-arabinose in the approximate ratio 4:1 (inferred from the specific rotation and g.l.c. analysis). Both these oligosaccharides have been reported previously, the former in sugar-beet araban[103] and V. oroboides[81] and the latter in O. fulgida[80] and V. oroboides[81]. The β anomer of disaccharide VI has been isolated from a number of sources[100,103,104] and is reported to have $[\alpha]_D$ +94°. Oligosaccharide VII, isolated in greatest quantity, gave approximately equimolecular proportions of xylose and arabinose on hydrolysis and gave a red colour when treated with triphenyltetrazolium chloride. Reduction and hydrolysis revealed arabinose to be the reducing end-group. G.l.c. analysis of the derived methyl glycosides and alditol acetates revealed the presence of 2,3,4-tri-O-methyl-xylose and 2,3-di-O-methyl-arabinose in the methylated oligosaccharide. The oligosaccharide is thus 5-O-β-D-xylopyranosyl-L-arabinose which has been reported in peach[95], cholla[80] and V. oroboides[81] gums.

Oligosaccharide IX was shown by hydrolysis to contain xylose and arabinose in the ratio 1:2 and gave a red colour when treated with triphenyltetrazolium chloride spray. Paper chromatography of the partial hydrolysis products revealed the presence of xylose, arabinose and oligosaccharide IV. The methanolysis products from the methylated oligosaccharide were shown by gas chromatography to have the retention times of the methyl glycosides of 2,3,4-tri-O-methyl-xylose, 2,4-di- and 2,5-di-O-methyl-arabinose. The oligosaccharide is thus O-β-D-xylopyranosyl-(1→3)-O-α-L-arabinofuranosyl-(1→3)-L-arabinose, the α-linkage and furanose configuration being assigned as a result of the negative optical rotation and the ease of release of arabinose on hydrolysis. All other possible structures viz. β-furanosyl and

α-and β-pyranosyl would result in a positive optical rotation for the oligosaccharide. As in the case of oligosaccharide IX, oligosaccharide X contained arabinose and xylose in a 2:1 ratio and gave a red colour when treated with triphenyltetrazolium chloride. Repeated attempts at partial hydrolysis failed to produce any recognizable disaccharides and due to the small amount of material available no further investigation was possible. However, it is clear that the oligosaccharide is a trisaccharide containing two residues of arabinose and one of xylose. Oligosaccharide XI gave a red colour when treated with triphenyltetrazolium chloride and on hydrolysis was shown to contain arabinose and xylose in a 2:1 ratio. Xylose, arabinose and oligosaccharide VII were identified in the partial hydrolysate while methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides and alditol acetates established the presence of 2,3,4-tri-O-methyl-xylose, 2,3-di-, 2,4-di- and 2,5-di-O-methyl-arabinose in the oligosaccharide. The results indicate that the sugar is the trisaccharide O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→3)-L-arabinose, the α-linkage being inferred from the negative optical rotation. This trisaccharide has previously been reported in cholla gum[80] and had $[\alpha]_D$ - 74.2°. Oligosaccharide XII contained arabinose and xylose (2:1) and gave a red colour with triphenyltetrazolium chloride. Partial hydrolysis produced xylose, arabinose and oligosaccharide VII. G.l.c. analysis of the derived methyl glycosides and alditol acetates of the methylated oligosaccharide revealed the presence of 2,3,4-tri-O-methyl-xylose and 2,3-di-O-methyl-arabinose. The oligosaccharide is thus 5-O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→5)-L-arabinose, the α-linkage being assigned as result of the optical rotation.

Oligosaccharide XIII was shown by paper chromatography to contain arabinose and xylose in approximately a 1:1 ratio. This was confirmed by g.l.c. of the derived alditol acetates. The sugar gave a red colour when treated with triphenyltetrazolium chloride spray while paper chromatographic analysis of the partial hydrolysate revealed the presence of xylose, arabinose and oligosaccharides VII and XII. Paper chromatographic analysis of the hydrolysate of the non-reducing syrup indicated arabinose to be the reducing end-group. Methylation of the oligosaccharide followed by g.l.c. analysis of the derived methyl glycosides and alditol acetates revealed the presence of 2,3,4-tri- and 2,3-di-O-methyl-xylose and 2,3-di-O-methyl-arabinose. All the results point to oligosaccharide XIII having the structure O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→5)-O-α-L-arabinofuranosyl-(1→5)-L-arabinose. However, such a structure would have been expected to yield xylobiose as the major partial hydrolysis product. The structure must therefore be regarded as tentative. Oligosaccharide XIV was shown by paper chromatography and g.l.c. analysis of the alditol acetates to contain xylose and arabinose in a 1:1 ratio. Repeated partial hydrolysis experiments failed to reveal any recognizable di- or trisaccharides and due to the limited amount of material no further investigation was possible.

The final fraction eluted from the column by 40% ethanol was shown by paper chromatography to contain several oligosaccharides which did not move any appreciable distance from the origin even after prolonged elution. A portion of the syrup was hydrolysed and paper chromatographic analysis revealed the presence of xylose, arabinose and galactose. This material was not further investigated.

Polysaccharide A was exhaustively methylated by the Haworth and Haq and Percival[79] procedures. A small amount of the methylated polysaccharide was hydrolysed and the hydrolysate examined by paper chromatography. Nine methylated sugars were observed (Table 10).

Table 10. Sugars present in hydrolysate of methylated polysaccharide A

mono-Me-galactose
mono-Me-arabinose
mono-Me-rhamnose
2,4-Me_2-galactose
2,3-Me_2-arabinose
2,3,4-Me_3-galactose
2,3,5-Me_3-arabinose
2,3,4-Me_3-xylose
2,3,4,6-Me_4-galactose

Methanolysis of the methylated polysaccharide followed by g.l.c. analysis of the derived methyl glycosides clearly indicated the absence of 2,3,4-tri-O-methyl-arabinose.* The methyl glycosides were converted to the alditol acetates and examined by g.l.c. The sugars identified are shown in Table 11. Methylated polysaccharide A was reduced by refluxing with LAH, the carboxyl-reduced polysaccharide methylated, hydrolysed, reduced, acetylated and examined by g.l.c. and g.l.c. - m.s. (Table 11).

It is clear from the methylation data that 2,3,6-tri-O-methyl-galactose arises as a result of the reduction and methylation of 1,4-linked galacturonic acid residues in the polymer.

*FOOTNOTE. Since arabinopyranose end groups have not been detected as constituents of the methylated polysaccharide, the isolation of oligosaccharide I implies that it is an artifact, possibly of acid-reversion or indicates that there are non-terminal arabinopyranose residues. This latter possibility would imply that some of the 2,3-O-methyl arabinose represents 4-O-substituted arabinopyranose in addition to 5-O-substituted arabinofuranose residues.

Table 11. Methylation analysis of polysaccharide A

	Native methylated	Carboxyl-reduced native methylated Mole %	Significant m.s. peaks[91]
2,3,5-Me_3-arabinose	+++	13.83	45, 117, 161
2,3-Me_2-arabinose	+++	18.70	117, 189
2,5-Me_2-arabinose	++	3.98	45, 117, 233
3-Me-arabinose	++	4.50	189
arabinose	++	2.81	-
2,3,4-Me_3-xylose	+++	24.63	117, 161
2,3-Me_2-xylose	++	1.23	-
2,3,4,6-Me_4-galactose	++	3.73	45, 117, 161, 205
2,3,4-Me_3-galactose	++	2.40	117, 161, 189, 233
2,3,6-Me_3-galactose	+	4.52	45, 117, 233
2,4-Me_2-galactose	++	3.57	117, 189
2,6-Me_2-galactose	++	3.99	45, 117
2-Me-galactose	++	6.22	117
3-Me-rhamnose	++	5.90	189, 203

+ = trace ++ = minor +++ = major

The mole ratios of the methylated sugars were calculated from the results obtained on the OV 225 column. All samples were also analysed on both ECNSS M and Apiezon T columns. The Apiezon T column was found to be most suitable for the separation and identification of the alditol acetates derived from methylated galactoses as all other methylated alditol acetates present in the sample have shorter retention times than the first eluted

methylated galactitol acetate. The alditol acetates of 2,4-di- and 2-O-methyl-galactose are not separated on Apiezon T but are well resolved on OV 225. The ECNSS M column gives very similar results to the OV 225 column with the exception that it fails to resolve the alditol acetates of 2,6-di-O-methyl-galactose and arabinose sufficiently to quantitatively determine each sugar. The OV 225 column does resolve these sugars sufficiently.

Polysaccharide B was methylated by the method of Hakomori[83,84] followed by treatment with dimethyl formamide and Purdie's reagents. A small amount of methylated polysaccharide B was methanolysed, analysed by g.l.c. and, as in the case of methylated polysaccharide A, the methyl glycosides were hydrolysed, reduced, acetylated and examined by g.l.c. and g.l.c. - m.s. The results are shown in Table 12. As in the case of methylated polysaccharide A no 2,3,4-tri-O-methyl-arabinose was present in the methylated polysaccharide. Methylated polysaccharide B was converted to the carboxyl-reduced polysaccharide methylated and analysed as described above (Table 12).

A comparision of the methylation results of methylated polysaccharides A and B shows similar amounts of most of the methylated sugars with the exception of a) 1,5-linked arabinofuranose which occurs to a far greater amount in polysaccharide B b) 2,3,4-tri-O-methyl-galactose which appears in methylated polysaccharide A but not in methylated polysaccharide B, indicating 1,6-linked galactose residues which are not further branched and c) the amount of 2,3,6-tri-O-methyl-galactose in methylated reduced polysaccharide A is less than in methylated reduced polysaccharide B. The methylation results indicate a very similar type of structure in both polysaccharides, the main difference would appear to be a slight

variation in the structure of the side-chains. From the mole ratios of the methylated sugars it is possible to calculate a theoretical value for periodate-uptake/mole for polysaccharide B.

Table 12. Methylation analysis of polysaccharide B

	Native methylated	Carboxyl-reduced native methylated Mole %	Significant m.s. peaks[91]
2,3,5-Me_3-arabinose	+++	12.79	45, 117, 161
2,3-Me_2-arabinose	+++	26.07	117, 189
2,5-Me_2-arabinose	++	2.77	45, 117, 233
3-Me-arabinose	++	3.76	189
arabinose	++	3.13	-
2,3,4-Me_3-xylose	+++	21.59	117, 161
2,3-Me_2-xylose	++	1.20	-
2,3,4,6-Me_4-galactose	++	3.64	45, 117, 161, 205
2,3,6-Me_3-galactose	+	6.00	45, 117, 233
2,4-Me_2-galactose	++	2.40	117, 189
2,6-Me_2-galactose	++	3.68	45, 117
2-Me-galactose	++	7.87	117
3-Me-rhamnose	++	5.10	189, 203

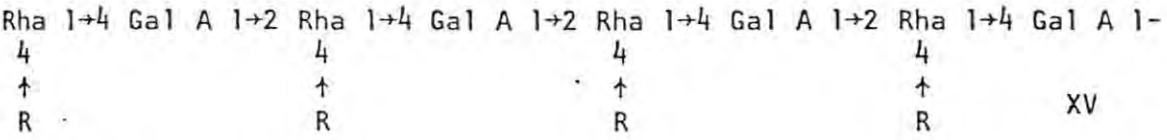
+ = trace ++ = minor +++ = major

Assuming a repeating structural unit of one hundred and thirty eight residues the periodate uptake would be 0.95 moles/mole of sugar. This is within reasonable agreement with the experimental value of 0.86/anhydro unit.

Paper chromatographic analysis of the hydrolysate of the

periodate oxidized material revealed that no xylose or galacturonic acid survive periodate oxidation. G.l.c. analysis of the alditol acetates of the hydrolysate revealed that approximately 68% of the galactose, 20% of the arabinose and all the rhamnose residues survive periodate oxidation. This is in reasonable agreement with the results of the methylation analysis of polysaccharide B. Those units which give rise to 2,3,5-tri-, 2,3—di-O-methyl-arabinose, 2,3,4-tri-, 2,3-di-O-methyl-xylose and 2,3,4,6-tetra-O-methyl-galactose would be oxidized by periodate.

Although no unique structure can be advanced for the mucilage several partial structures can be proposed. The backbone of the mucilage probably consists of alternating galacturonic acid and rhamnose residues (XV). The methylation results indicate that the



rhamnose is branched at C-4. The galacturonic acid must be unbranched as no 2,6-di-, 3,6-di- or 6-O-methyl-galactose was identified in reduced methylated polysaccharide BD_1. The nature of the side chains (R) cannot be defined with certainty, however, it is clear from the methylation analysis of the native polysaccharides that they are of a highly branched nature. The structure proposed for the degraded polysaccharide (Fig 1) suggests that most of the side-chains are made up partly of 1,6—linked galactose units (XVI) which are linked to C-4 of the rhamnose units in the core. The β-1,6-linked D-galactose residues present in the pectin of white-willow[54] may be structural units of side-chains. The length of the galactose chain appears to be no longer than three units, this assumption being based on two factors a) only

1,6-linked galactobiose and triose oligosaccharides were identified (II and VIII) and b) the average ratio of 1,6-linked galactose units and end-group galactose units to rhamnose branch points in the core is 2:1, this fact being derived from the methylation results. If the average length of the 1,6-linked galactose chain is two units then, as a galactotriose was identified, some of the galactose side chains must consist of single units. From the structure proposed for degraded polysaccharide BD_1, one in four rhamnose units is unbranched. The possibility therefore exists that residues other than galactose are directly linked to rhamnose. The 2,6-di-O-methyl-galactose identified in the hydrolysis products of the methylated polysaccharides is not derived from reduced methylated galacturonic acid as it was also present as a hydrolysis product of the methylated polysaccharides. There is thus some 1,3,4-linked galactose (XVII) in the polysaccharides.

Oligosaccharides VII and XII were the oligosaccharides isolated in greatest amounts from the partial hydrolysate and partial structures XVIII and XIX must therefore be of major structural importance. These two structures form the majority of side-chains attached to the 1,6-linked galactose chains.

The methylation results of both polysaccharides indicate a large proportion of arabinofuranose end-group. If we assume that most of the 1,5-linked arabinose is attached to end-group xylose, an assumption based on the fact that oligosaccharides VII and XII were isolated in greatest proportion, then most of the end-group arabinofuranose probably occur as single unit side-chains. A large part of the molecule of white mustard[51] is made up of 1,5-linked arabinose side-chains while structure XX has also been identified in rapeseed-hull pectin[38]. The structural unit XXI appears in the side chains of mangle gum[14] and Combretum leonense gum[60-62], however, the majority

of peripheral L-arabinofuranose units in Combretum leonense gum appear as arabinofuranose end-groups. There is also a small amount of end-group arabinofuranose in the Khaya gums[63-67]. As in the case of the mucilage of O. ficus-indica the majority of acid labile groups in Combretum leonense gum[60-62] are attached to the 1,6-linked β-D-galactopyranose residues. In the Khaya gums[63-67] single galactose residues are attached to C-4 of rhamnose. In the present study this is a possibility but several factors indicate otherwise. Firstly, a small amount of galactose is released after only a few hours of partial hydrolysis suggesting that some galactose is near the periphery. Secondly, a galactosyl-arabinose disaccharide (III) was identified as one of the partial hydrolysis products, indicating some, if not all, of the end group galactose is linked to arabinose.

The identification of a small amount of 2,3-di-O-methyl-xylose in the hydrolysis products of the methylated mucilages coupled with the fact that two oligosaccharides (XIII and XIV) contain arabinose and xylose in a 1:1 ratio indicates that structure XXII is of minor but definite structural significance. The identification of small amounts of oligosaccharides IV, IX and XI indicates that structures XXIII, XXIV and XXV make a minor contribution to the overall structure of the polysaccharide.

-6 Gal 1→6 Gal 1→6 Gal 1→4 Rha- (XVI)

.....4 Gal 1..... (XVII)

3

⋮

Xyl 1→5 Araf 1..... (XVIII)

Xyl 1→5 Araf 1→5 Araf 1..... (XIX)

Araf 1→5 Araf ..... (XX)

Araf 1→3 Ara ..... (XXI)

.....4 Xyl 1..... (XXII)

Xyl 1→3 Ara..... (XXIII)

Xyl 1→3 Araf 1→3 Ara ..... (XXIV)

Xyl 1→5 Araf 1→3 Ara ..... (XXV)

The polysaccharide from the mucilage of O. ficus-indica thus falls into the group of polysaccharides containing a galacturonorhamnan backbone in which the galacturonic acid and rhamnose residues alternate. The overall structure appears to be far more complex than that for the mucilage of Ulmus fulva[3-11] although both polysaccharides have galactose side-chains, in Ulmus fulva through both C-3 and C-4 of rhamnose. Mangle gum, which also has an alternating galacturonic acid and rhamnose core, has a highly branched structure with both galacturonic acid and rhamnose carrying branch points. However, there is no evidence for galactose being attached directly to rhamnose. Hibiscus ficulneus[15-17a] mucilage like that of O. ficus-indica mucilage has an alternating galacturonic acid - rhamnose core with 1,6 linked-galactose side chains attached to the rhamnose, in this case attached to C-3. The structure of the mucilage of O. ficus-indica is far more complex than that of Hibiscus ficulneus as the galactose chains in Hibiscus ficulneus are not further branched.

The properties and composition of the mucilage of O. ficus-indica are compared (Table 13) with those reported for gums and mucilages obtained from other Opuntia species.

In all the polysaccharides except O. dillenii[106] the same neutral sugar residues are present but in different proportions. Amin et al[90]

isolated a mucilage from O. ficus-indica but paper chromatographic

Table 13. Comparison of properties from different Opuntia species

	O. fulgida gum	O. megacantha gum	O. ficus-indica mucilage[a]	O. ficus-indica mucilage[b]	O. dillenii mucilage
$[\alpha]_D$	-86	+28	-170	-62	+268
% Composition					
Galactose	45	46	36	19	75
Arabinose	29	30	37	42	25
Rhamnose	2	11	12	6	-
Xylose	13	tr.	15	25	-
Glucuronic acid	-	13	-	-	-
Galacturonic acid	11	-	-	8	-

a. Isolated by Amin et al[90] b. Present study

analysis of the hydrolysate of the mucilage revealed the presence of the same neutral sugars as in the present study but failed to reveal the presence of uronic acid (Table 13). Methylation of the mucilage followed by paper chromatographic analysis of the hydrolysate revealed the presence of 2,3,5-tri-O-methyl-arabinose, 2,3,6-tri-, 2,4-di-O-methyl-galactose and 3,4-di-O-methyl-rhamnose. No methylated xyloses were reported in spite of the large amount of xylose in the polysaccharide. The results must be regarded as suspect as all information is based on results obtained only from paper chromatographic analysis. The gum from O. megacantha examined by Churms et al[105] was an exudate caused by insect attack of the cladodes. It is worthy of note that plants previously classified in the Eastern Cape as O. megacantha have now been recognised as O. ficus-indica. This gum has the same neutral sugar constituents as the mucilage in the present study but differs in the uronic acid present (Table 13).

Methylation results indicate that arabinofuranose and galactopyranose end-groups are the major constituents of the gum, 1,4-, 1,6-and 1,3-linked galactopyranose chains being present. Rhamnopyranose residues occur either as end groups or part of a chain while there is some evidence that glucopyranosyluronic acid also occurs either as end groups or as part of a chain. Arabinose residues also occur within the polysaccharide chain. A major difference between O. megacantha gum and O. ficus-indica mucilage is therefore the lack of xylose end-groups in the former. There is no information regarding an inner core in the gum of O. megacantha. O. fulgida gum would appear to resemble O. ficus-indica mucilage in the nature of the side chains i.e. xylose and arabinofuranose end-groups occur; all the 1,6 linked galactose units carry side-chains. The same oligosaccharides (II, III, VI, VII and XI) were isolated from O. fulgida gum[80] as from O.ficus-indica mucilage. The mucilage from Opuntia dillenii[106] was obtained from the "pods", assumed to mean the fruit, which is a berry. The structure of this mucilage is postulated to be composed of interior chains of 1,6-linked galactopyranose residues to which the arabinofuranose residues are attached at C-3. This is therefore a far simpler structure than that of the mucilage of O. ficus-indica, the only similarity being the presence of the arabinofuranose residues. It is evident that considerable variation occurs among gums and mucilages from different species of Opuntia.

3.2 A preliminary investigation of the mucilage of Opuntia aurantiaca.

Opuntia aurantiaca Lindl. is found in the eastern area of the Cape Province and occassionally in the other three provinces of South Africa. The infestation in the eastern Cape has become uncontrollable and eradication by biological and chemical poisons has so far met with little success. The object of this prelimminary investigation was to determine whether there is any similarity between the mucilages of O. aurantiaca and O. ficus-indica. The cladodes used in the study were obtained from "THURSFORD FARM" which is 20 km north of Grahamstown. The plant is a low spreading, creeping shrublet up to 1 m high. A feature of the cladodes is the 2-7 sharp spines which are up to 3 cm long.

Extraction of the cladodes followed by precipitation into ethanol, decantation, centrifugation, dissolution in water, dialysis and reprecipitation in ethanol afforded a fibrous polysaccharide in 1.10% yield. The polysaccharide had $[\alpha]_D$ -35.4°, N 0.46% and on hydrolysis of the polysaccharide followed by paper chromatographic and g.l.c. analysis revealed the presence of the same constituent sugars as found in the mucilage of O. ficus-indica. The properties of the polysaccharide are shown in Table 14.

The polysaccharide was partially hydrolysed under conditions essentially similar to those described for O. ficus-indica mucilage. The degraded polysaccharide isolated was hydrolysed and the hydrolysate examined by paper chromatography and g.l.c. (alditol acetates). The same sugars were shown to be present as in the native polysaccharide but in different proportions (Table 14).

Table 14. Properties of the mucilages of native and degraded O. aurantiaca.

Polysaccharide	Native polysaccharide	Degraded polysaccharide
$[\alpha]_D$	-35.4°	+24.4°
% N.	0.46	—
Constituent sugars (Mole %)		
Galactose	34.87	22.63
Arabinose	32.25	18.35
Xylose	19.80	17.14
Rhamnose	5.90	29.16
Galacturonic acid	7.18	12.72

The polysaccharide appears to be far more resistant to acid hydrolysis than the polysaccharide from O. ficus-indica as under almost identical conditions of partial hydrolysis a degraded polysaccharide was obtained from the mucilage of O. ficus-indica which was almost devoid of xylose and arabinose residues whereas the degraded polysaccharide from O. aurantiaca contains a large proportion of both arabinose and xylose. This suggests that not all the arabinose is in the furanose form as is mainly the case in the mucilage of O. ficus-indica. A comparison of the ratio galacturonic acid : rhamnose in the native and degraded polysaccharides indicates a decrease of galacturonic acid relative to rhamnose in the degraded polysaccharide. This implies that galacturonic acid is removed during the partial hydrolysis. This is in contrast to the situation found in the mucilage of O. ficus-indica where uronic acid appears to reside solely in the core of the polysaccharide. Examination of the diffusates from the partial

hydrolysis of the native polysaccharide revealed the presence of two oligosaccharides which were chromatographically indistinguishable from 6-O-β-D-galactopyranosyl-D-galactose and the homologous trisaccharide. No other oligosaccharides were observed but arabinose and xylose were present in all the diffusates.

The degraded polysaccharide was reduced and the degraded and degraded reduced polysaccharides were methylated by the method of Hakomori[83,84] followed by treatment with Purdie's reagents[82]. The methylated polysaccharides were hydrolysed, reduced, acetylated and examined by g.l.c. and g.l.c. - m.s. (Table 15). The methylation results clearly indicate that the degraded polysaccharide has a more complex structure than that found in the degraded polysaccharide from the mucilage of O. ficus-indica. The ratio 2,4,6-tri-O-methyl-galactose : 2,3,4,6-tetra-O-methyl-galactose : 2,3,4-tri-O-methyl-galactose is approximately the same in both the degraded reduced and degraded methylated polysaccharides indicating the absence of 1,3 and non-reducing end-group galacturonic acid in the degraded polymer. On the other hand the amount of 2,3,6-tri-O-methyl-galactose in the reduced methylated degraded polysaccharide is more than double that in the methylated degraded polysaccharide. This indicates that approximately 65% of the 2,3,6-tri-O-methyl-galactose in the reduced degraded methylated polysaccharide is derived from 1,4-linked galacturonic acid. From the mole ratios of the methylated sugars it is possible to propose a structure for the partially degraded polysaccharide. Fig. 2 shows a possible structure for a repeating unit of the degraded mucilage consisting of ninety eight sugar residues. However, this is only one of many possible structures which can be proposed from the available evidence.

Table 15. Methylation analysis of the degraded and reduced degraded polysaccharide.

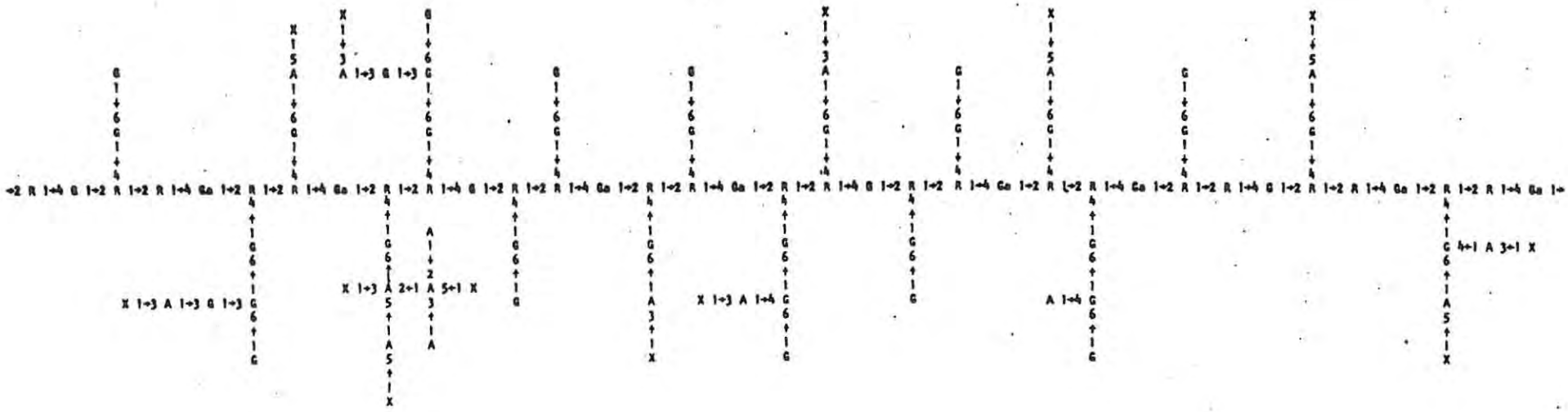
	Degraded polysaccharide	Reduced degraded polysaccharide Mole %	Significant m.s. peaks[91]
2-Me-galactose	+	+	
2,4-Me_2-galactose	++	2.03	
2,3-Me_2-galactose	++	3.06	
2,3,4-Me_3-galactose	+++	17.23	117, 161,189 233
2,3,6-Me_3-galactose	+++	12.38	45, 117, 233
2,4,6-Me_3-galactose	++	2.08	
2,3,4,6-Me_4-galactose	+++	11.20	45, 117, 161 205
arabinose	++	2.25	
2,5-Me_2-arabinose	++	5.76	
2,3-Me_2-arabinose	++	4.76	
2,3,5-Me_3-arabinose	++	3.23	
2,3,4-Me_3-xylose	+++	12.44	117, 161
3-Me-rhamnose	+++	18.56	189, 203
3,4-Me_2-rhamnose	++	5.02	

+ = trace ++ = minor +++ = major

The whole mucilage was partially methylated by the method of Hakomori[83,84], reduced with LAH and then methylated with Purdie's reagents several times to afford a fully methylated reduced polysaccharide. The methylated polysaccharide was hydrolysed, reduced, acetylated and examined by g.l.c. (Table 16).

Most of the methylated sugars identified in the hydrolysate of the methylated mucilage of O. aurantiaca were also observed in the

Fig. 2



A = arabinose G = galactose Ga = galacturonic acid R = rhamnose X = xylose

Possible structure for a repeating unit of degraded O. aurantiaca mucilage

methylated mucilage of *O. ficus-indica.* However, 2,3-di-O-methyl-galactose and an unidentified methylated sugar were observed in the methylated mucilage of *O. aurantiaca*. Neither of these was present

Table 16. Methylation analysis of reduced methylated native polysaccharide.

2,3,5-Me_3-arabinose	+++
2,3-Me_2-arabinose	+++
2,5-Me_2-arabinose	++
3-Me-arabinose	++
arabinose	+
2,3,4-Me_3-xylose	+++
2,3,4,6-Me_4-galactose	++
2,3,6-Me_3-galactose	++
2,3,4-Me_3-galactose	+
2,6-Me_2-galactose	+
2,3-Me_2-galactose	++
2,4-Me_2-galactose	++
2-Me-galactose	++
3-Me-rhamnose	++
unidentified	++

+++ = major ++ = minor + = trace

in the methylated mucilage of *O. ficus-indica*. The methylated mucilage of *O. ficus-indica* contained a small amount of 2,3-di-O-methyl-xylose which was not observed in the methylated mucilage of *O. aurantiaca*.

This preliminary study clearly indicates that there are marked

differences in the structures of the mucilages of O. ficus-indica and O. aurantiaca. A more detailed study of the mucilage of O. aurantiaca and preliminary studies on the mucilages of other Opuntia species is therefore warranted.

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