Carbohydrate Research, 88 (1981) 305–314
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

METHYLATION ANALYSIS OF THE MUCILAGE OF *Opuntia ficus-indica**

DONALD MCGARVIE AND HARALAMBOS PAROLIS†
Department of Chemistry, Rhodes University, P.O. Box 94, Grahamstown 6140 (South Africa)
(Received March 31st, 1980; accepted for publication, May 7th, 1980)

ABSTRACT

The mucilage of *Opuntia ficus-indica* and a partially degraded mucilage have been investigated by methylation analysis and periodate oxidation. The results show that the mucilage is composed of 1,4-α-D-galactopyranosyluronic acid and 1,2-β-L-rhamnopyranosyl residues to which are attached short chains of 1,6-β-D-galactopyranosyl residues at position 4 of all of the rhamnopyranosyl residues. Most of the galactosyl residues in the side chains carry branches at O-3, while some are also branched at O-4. The branches are mainly composed of 1,5-linked arabinofuranosyl, end-group arabinofuranosyl, and end-group xylopyranosyl residues.

INTRODUCTION

The mucilage extracted from the modified stems of *Opuntia ficus-indica*[2] contains β-D-xylopyranosyl, β-L-rhamnopyranosyl, β-D-galactopyranosyl, α-D-galactopyranosyluronic acid, and α-L-arabinosyl residues mainly in the furanoid form. Mild hydrolysis[2] of the mucilage releases virtually all of the xylose and arabinose residues together with some of the galactose residues. The resulting, degraded polysaccharide has been shown by methylation analysis[1] to be composed of chains of alternating 1,4-α-D-galactopyranosyluronic acid and 1,2-β-L-rhamnopyranosyl residues to which are attached chains of 1,6-β-D-galactopyranosyl residues at position 4 of most of the rhamnopyranosyl residues. We now report on the periodate-oxidation and methylation analysis of the mucilage.

RESULTS AND DISCUSSION

Polysaccharide A[2] and polysaccharide B[2], extracted from the same plant in June 1974 and March 1976, respectively, and a partially degraded polysaccharide A (AD_2)[2] were used in the present investigation. The properties of these polysaccharides are shown in Table I.

---

*The Mucilage of *Opuntia ficus-indica*, Part III. For Part II, see ref. 1.
†Present address: School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140, South Africa.

TABLE I

COMPOSITION OF POLYSACCHARIDES *A*, AD_2, AND *B*

Polysaccharide	A	AD_2	B
$[\alpha]_D$ (degrees)	−62	−0.8	−67
N (%)	0.87		1.10
Constituent sugar residues (mol. ratios)			
Galactose	18.4	22.6	22.8
Arabinose	42.4	12.4	41.3
Xylose	24.5	7.4	22.1
Rhamnose	6.4	6.2	7.0
Galacturonic acid	8.4	8.4	6.8

Periodate oxidation of polysaccharide *B* was complete after 48 h, when 0.86 mole of periodate had been consumed per "average anhydro-unit" (mol. wt. 142.8, calculated from the molar ratios of the sugars in the mucilage). Periodate-oxidised polysaccharide *B* was reduced with borohydride and hydrolysed. Paper chromatography of the hydrolysate and g.l.c. of the derived alditol acetates revealed that no xylose or galacturonic acid survived oxidation, whereas ~68% of the galactose, ~20% of the arabinose, and all of the rhamnose residues did survive.

Polysaccharide *A* was exhaustively methylated by the Haworth procedure[3] followed by the method of Percival and Haq[4]; polysaccharide *B* was first methylated by the Hakomori method[5] and then with silver oxide and methyl iodide in *N*,*N*-dimethylformamide. The alditol acetates derived from methylated polysaccharides *A* and *B* were examined by g.l.c. and g.l.c.–m.s. (see Table II). The molar ratios of the sugars in the methylated polysaccharides were not determined accurately, because of the resistance of the glycosiduronic acid linkages to complete hydrolysis by acid, which results in an underestimation of those neutral sugars to which the uronic acids are glycosidically attached. Methylated polysaccharides *A* and *B* were therefore reduced with lithium aluminium hydride, and each carboxyl-reduced, methylated polysaccharide was re-methylated. The resulting, methylated polysaccharides were hydrolysed and the derived, methylated alditol acetates were quantitatively analysed by g.l.c. and the identities of the components were confirmed by g.l.c.–m.s. (Table II).

None of the stationary phases[6] (Apiezon T, ECNSS-M, OV-225) used to examine the methylated alditol acetates is capable of resolving the alditol acetates derived from 2,3,4-tri-*O*-methylarabinose and 2,3,4-tri-*O*-methylxylose. Fortunately, these sugars are readily resolved as their methyl glycosides on either neopentyl glycol adipate or butane-1,4-diol succinate. Examination of the methyl glycosides derived from methylated polysaccharides *A* and *B* showed the absence of 2,3,4-tri-*O*-methylarabinosyl residues in both methylated polysaccharides. The molar ratios of the sugars were determined on OV-225. This phase does not resolve satisfactorily the alditol acetates derived from 2,3-di-*O*-methylxylose and 2,3,4,6-tetra-*O*-methyl-

TABLE II

NEUTRAL SUGARS FROM METHYLATED POLYSACCHARIDES *A* AND *B*, AND THEIR CARBOXYL-REDUCED, REMETHYLATED DERIVATIVES

	Methylated polysaccharide A	*Carboxyl-reduced, methylated polysaccharide* A *(mole %)*	*Methylated polysaccharide* B	*Carboxyl-reduced, methylated polysaccharide* B *(mole %)*	*Significant m.s. peaks* (m/z)	*Retention times of alditol acetates on Column 3*
2,3,5-Me_3-Ara	+++[a]	13.8	+++	12.8	45, 117, 161	0.46
2,3-Me_2-Ara	+++	18.7	+++	26.1	117, 189	0.93
2,5-Me_2-Ara	++	4.0	++	2.8	45, 117, 233	0.85
3-Me-Ara	++	4.5	++	3.8	189	1.59
Ara	++	2.8	++	3.1	—	2.00
2,3,4-Me_3-Xyl	+++	24.6	+++	21.6	117, 161	0.58
2,3-Me_2-Xyl	++	1.2	++	1.2	—	1.03
2,3,4,6-Me_4-Gal	++	3.7	++	3.6	45, 117, 161, 205	1.00
2,3,4-Me_3-Gal	++	2.4				2.10
2,3,6-Me_3-Gal	+	4.5	+	6.0	45, 117, 233	1.66
2,4-Me_2-Gal	++	3.6	++	2.4	117, 189	3.43
2,6-Me_2-Gal	++	4.0	++	3.7	45, 117	2.32
2-Me-Gal	++	6.2	++	7.9	117	4.26
3-Me-Rha	++	5.9	++	5.1	189, 203	1.37

[a]Key: +, trace; ++, minor; +++, major.

galactose, but these are well-separated on ECNSS-M. Apiezon T[6] was the most suitable phase for the separation and identification of methylated galactitol acetates and is particularly useful in distinguishing between 2,6- and 4,6-di-*O*-methylgalactitol acetates and 2,4,6- and 2,3,6-tri-*O*-methylgalactitol acetates.

The methylation results show that the 2,3,6-tri-*O*-methylgalactose, which is present in the hydrolysate of each carboxyl-reduced, methylated polysaccharide and only in traces in those of methylated polysaccharides *A* and *B*, is derived from 1,4-linked galacturonic acid residues in the mucilages. All of the galacturonic acid residues are unbranched and reside in the backbone of the macromolecule, since (*a*) all of the galacturonic acid residues in the degraded[1] and undegraded mucilages are cleaved by periodate; (*b*) no 2,6-di-,3,6-di-, or 6-*O*-methylgalactose was detected in the hydrolysate of the degraded, reduced, methylated polysaccharide *BD*[1]; (*c*) no acidic saccharides were released from the mucilages[2] during the preparation of the degraded polysaccharides.

The presence of 3-*O*-methylrhamnose as the only methylated rhamnose in the hydrolysates of both methylated polysaccharides, coupled with the methylation results of the degraded polysaccharide[1], indicates that the rhamnose residues are 1,2-linked and branched through O-4. Since no rhamnose or rhamnose-containing saccharides were released during partial hydrolysis of the mucilage[2], it follows that all of the rhamnose residues reside in the core of the macromolecule and are linked to galacturonic acid residues.

The methylation results of the degraded mucilage[1] established that the side chains attached to the galacturonorhamnan core are composed of 1,6-linked β-D-galactopyranosyl residues. The presence of 2,4-di-*O*-methyl- and 2-*O*-methylgalactose in the methylated polysaccharides indicates that some of the 1,6-linked galactopyranosyl residues are branched through O-3, whereas others are branched through both O-3 and O-4. Polysaccharide *B* has 30% fewer units branched through O-3 and 30% more units branched through both O-3 and O-4 than polysaccharide *A*. Polysaccharide *B*, in contrast to polysaccharide *A*, is devoid of unbranched, 1,6-linked β-D-galactopyranosyl residues. In addition, polysaccharides *A* and *B* both contain galactose residues linked through positions 1, 3, and 4. The precise location of these units in the mucilage is not known.

Virtually all of the xylose residues in both polysaccharides *A* and *B* are present as non-reducing end-groups. The small proportion of 2,3-di-*O*-methylxylose probably indicates the presence of some 1,4-linked xylopyranosyl residues, although it could be an undermethylation product.

Arabinose is present in both polysaccharides, mainly as 1,4- and/or 1,5-linked residues and arabinofuranosyl end-groups. A small proportion is also present as 1,3-linked arabinofuranosyl, 1,2,5(4)-linked arabinosyl, and 1,2,3,5(4)-linked arabinosyl residues. The ease with which the arabinosyl residues were released during graded hydrolysis of the mucilage strongly suggested[2] that the majority of these residues are present in the furanoid form. The methylation results of polysaccharides *A* and *B* revealed that ~40 and ~30%, respectively, of the arabinosyl residues are present

in the furanoid form; the remaining units could be present in either furanoid or pyranoid forms, or both. A consideration of the specific rotation of polysaccharide *A* and the partially degraded polysaccharide AD_2, together with the sugars released[2] during the production of polysaccharide AD_2 (Table I), provides additional information on the ring size and linkage configuration of the arabinose residues. It follows that, since β-D-xylopyranosyl and arabinosyl residues and only small proportions of β-D-galactopyranosyl residues are cleaved, in order to account for the difference in specific rotation of polysaccharides *A* and AD_2, the majority (if not all) the arabinosyl residues must be present in the α-L-furanoid form.

Comparison of the methylation results for polysaccharides *A* and *B* with those of the degraded polysaccharide[1] does not provide any information concerning the location of the 1,3,4-linked galactosyl residues in the mucilage. Methylation analysis of polysaccharide AD_2 should provide this information since, unlike the production of the degraded polysaccharide[2] in which ~50% of the galactosyl residues were cleaved, only a small proportion of galactose was lost during the production of polysaccharide AD_2. The results of the methylation analysis of polysaccharides AD_2 and carboxyl-reduced, methylated AD_2 are shown in Table III.

Comparison of the methylation results of polysaccharides *A* and AD_2 shows that most of the end-group arabinofuranose and end-group xylopyranose and a large

TABLE III

NEUTRAL SUGARS FROM METHYLATED POLYSACCHARIDE AD_2 AND CARBOXYL-REDUCED, METHYLATED AD_2

	Methylated polysaccharide AD_2	*Carboxyl-reduced, methylated polysaccharide* AD_2 *(mol. ratios)*	*Significant m.s. peaks* (m/z)	*Retention times of alditol acetates on Column* 3
2,3,5-Me_3-Ara	+++[a]	4.4	45, 117, 161	0.46
2,3-Me_2-Ara	+++	7.1	117, 189	0.93
2,5-Me_2-Ara	++	1.3	45, 117, 233	0.85
3-Me-Ara	++	1.0	189	1.59
2,3,4-Me_3-Xyl	+++	5.4	117, 161	0.58
2,3-Me_2-Xyl	++	0.6	—	1.03
2,3,4,6-Me_4-Gal	+++	4.7	45, 117, 161, 205	1.00
2,3,4-Me_3-Gal	+++	5.1	117, 161, 189, 233	2.10
2,3,6-Me_3-Gal	+++	8.6	45, 117, 233	1.67
2,4,6-Me_3-Gal	++	0.6	45, 117, 161	1.55
2,3-Me_2-Gal	++	1.4	117, 261	3.18
2,4-Me_2-Gal	++	2.0	117, 189	3.43
2,6-Me_2-Gal	+	0.3	45, 117	2.32
2-Me-Gal	++	0.9	117	4.26
3,4-Me_2-Rha	++	0.9	131, 189	0.79
3-Me-Rha	+++	5.0	189, 203	1.36

[a]Key: +, trace; ++, minor; +++, major.

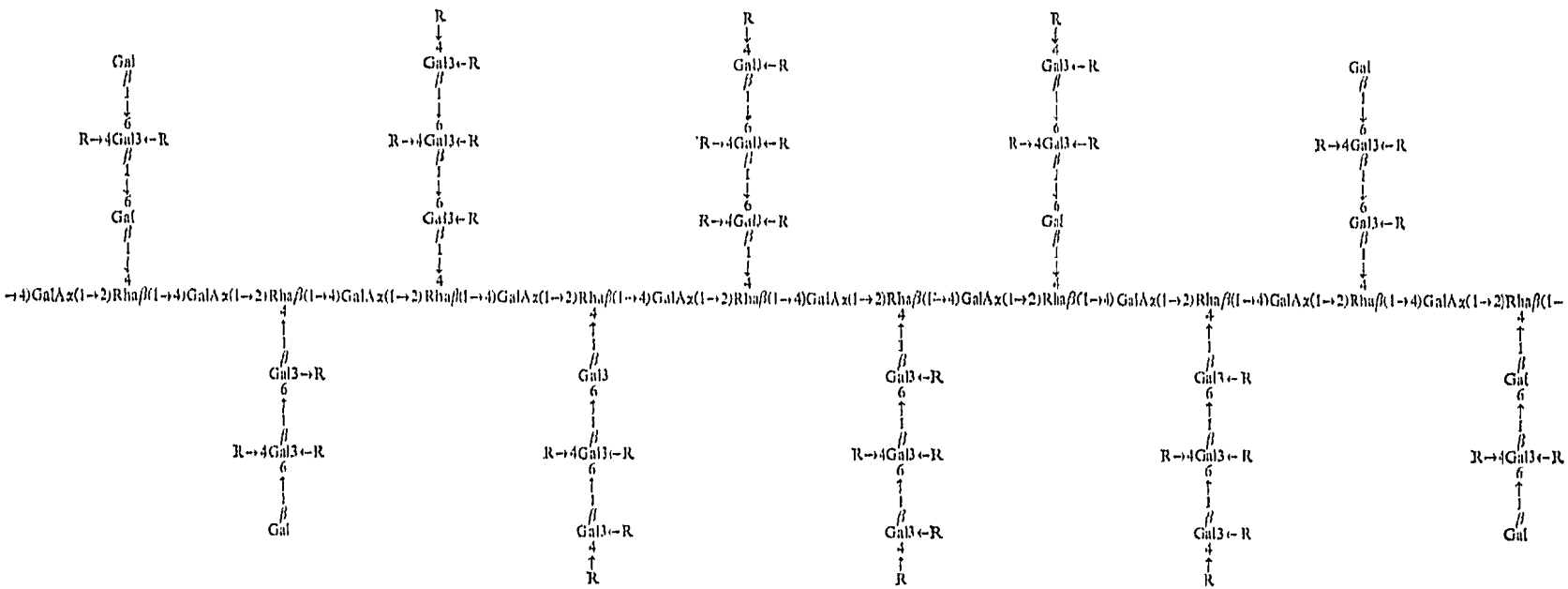

Fig. 1. Proposed partial structure for *O. ficus-indica* mucilage.

proportion of the 1,3- and 1,5-linked arabinofuranosyl residues are cleaved during mild hydrolysis of polysaccharide *A*. The release of these labile sugars was accompanied by a decrease in the amount of 2-*O*-methyl- and 2,4- and 2,6-di-*O*-methyl-galactose, an increase in the amount of 2,3,4,6-tetra-*O*-methyl- and 2,3,4- and 2,3,6-tri-*O*-methyl-galactose, and the appearance of 2,3-di-*O*-methyl- and 2,4,6-tri-*O*-methyl-galactose amongst the methylation products. On the basis of the present results plus the structure assigned to the degraded polymer[1], it is proposed that the structure shown in Fig. 1 represents a possible, partial repeating-structure for the mucilage of *Opuntia ficus-indica*. The R chains are composed of xylopyranosyl and arabinofuranosyl end-groups and also 1,3-, 1,5-, 1,2,5-, and 1,2,3,5-arabinofuranosyl residues. The exact sequence of the sugars in the R groups is not known. However, previous results[2] have suggested that at least some of the end-group xylopyranose is linked to arabinofuranosyl residues. It is also possible that, since some galactose was cleaved during the production of the partially degraded polymers[1], these units may be present in the R chains linked to arabinofuranosyl residues. Further information concerning the nature of the peripheral acid-labile chains must await the results of the analysis of the oligosaccharides produced during the graded hydrolysis of the mucilage.

An earlier methylation study[7] by Egyptian workers on the mucilage from the stems of *O. ficus-indica* revealed (p.c.) 2,3,5-tri-*O*-methylarabinose and 2,3,6-tri-*O*-methylgalactose as major structural components and 3,4-di-*O*-methylrhamnose and 2,4-di-*O*-methylgalactose as minor components. No methylated xyloses were reported, although 15.5% of this sugar is present.

Comparison of the methylation results presented here with those for the mucilages isolated from the stems of *O. monacantha*[8], *O. nopalea-coccinillifera*[8], *O. aurantiaca*[9], and *O. brasiliensis*[9] shows that arabinofuranosyl, xylopyranosyl, and galactopyranosyl end-groups and 1,5-linked arabinofuranosyl residues are features common to all these mucilages. Whereas the present results have established the positions of the branch-points in the mucilage of *O. ficus-indica*, this has not been the case for the above-mentioned *Opuntia* mucilages nor has the composition of the backbone in these mucilages been established unequivocally.

In addition to the above mucilages, an arabinogalactan isolated from the fruit of *O. dilleni*[10] was shown by methylation analysis to be composed of chains of 1,4-linked galactopyranosyl residues, approximately half of which carry arabinofuranosyl end-groups at O-3. A small number of galactopyranosyl residues are also attached to the main chain through O-3.

Cholla gum[11–13], an exudate from *O. fulgida*, differs considerably from *Opuntia ficus-indica* mucilage. It has been shown by partial hydrolysis and methylation analysis to have a complex structure with 1,6-linked β-D-galactopyranosyl residues as the main chain with branching at O-3. The branches consist of arabinofuranosyl, arabinopyranosyl, rhamnopyranosyl, and galactopyranosyluronic acid residues and terminate in xylopyranosyl or arabinofuranosyl groups.

The green, jelly-like material (frass) produced by *Cactoblastis* species feeding

on *O. megacantha*[14] (now recognised[15] as *O. ficus-indica*) contains polysaccharide material that has been shown to have a complex structure. Arabinofuranosyl and galactopyranosyl end-groups and 1,4-, 1,6-, and 1,3-linked galactopyranosyl residues are the most important structural features of the polysaccharide. A major difference between "*O. megacantha*" polysaccharide and the mucilages obtained from the stems of other *Opuntia* species and cholla gum is the absence of end-group xylopyranose in the former. In addition, "*O. megacantha*" polysaccharide is reported to contain glucuronic acid, whereas other acidic *Opuntia* mucilages contain galacturonic acid. The differences between *O. ficus-indica* mucilage and "*O. megacantha*" gum could be attributed to enzyme attack on the mucilage during its passage through the larva's gut or to subsequent bacterial action on the frass[15].

EXPERIMENTAL

General and analytical methods are described in Parts I[2] and II[1]. Separation of methyl glycosides was effected on a column containing 15% of butane-1,4-diol succinate polyester on Chromosorb W (acid-washed; 80–100 mesh) at 175° (Column *4*) at a flow rate of 70 ml/min. Retention times of methyl glycosides and alditol acetates are relative to methyl 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.

Periodate oxidation of polysaccharide B. — The polysaccharide (104.2 mg) was dissolved in deionised, distilled water (50 ml), and sodium metaperiodate (29.9mM, 50 ml) was added. The solution was kept at room temperature in the dark, and, at intervals, aliquots (0.1 ml) were removed and the reduction of periodate was measured[17]. After 72 h, ethylene glycol was added and the oxopolysaccharide reduced with sodium borohydride. The solution was then dialysed against running, deionised water and the polyalcohol isolated by freeze-drying. A portion of the polyalcohol was hydrolysed and the hydrolysate examined by paper chromatography (solvents *1* and *2*). The remainder was converted into alditol acetates and examined by g.l.c.

Methylation of polysaccharide A. — Polysaccharide *A* (1.0 g) in water (10 ml) was treated slowly and simultaneously with dimethyl sulphate (10 ml) and aqueous sodium hydroxide (40% w/v; 20 ml) with vigorous stirring during 7 h. The mixture was stirred for a further 17 h and then dialysed against running, deionised water (72 h), and the polysaccharide was isolated by freeze-drying (0.8 g). The partially methylated polysaccharide in *N,N*-dimethylformamide (30 ml) and methyl iodide (20 ml) was treated with silver oxide (5 g) in portions with stirring under reflux for 24 h. More methyl iodide (20 ml) and silver oxide (10 g) were added and the solution was stirred under reflux for a further 24 h, after which the polysaccharide (0.74 g) was isolated. The polysaccharide showed a large i.r. absorption for hydroxyl. The methylation procedure, using *N,N*-dimethylformamide and Purdie's reagent, was repeated several times on a portion of the partially methylated polysaccharide, to afford fully methylated material (176 mg), $[\alpha]_D$ −68.2° (*c* 5.4, chloroform). A portion (5 mg) of the methylated polysaccharide was hydrolysed with sulphuric acid

(0.5M; 3 ml) at 100° for 16 h. Examination (p.c.) of the neutralised ($BaCO_3$) hydrolysate (solvent *2*) revealed a mono-*O*-methylarabinose (R_{TMG} 0.39), 2,4-di-*O*-methylgalactose (R_{TMG} 0.44), a mono-*O*-methylrhamnose (R_{TMG} 0.63), 2,3,4-tri-*O*-methylgalactose (R_{TMG} 0.69), 2,3-di-*O*-methylarabinose (R_{TMG} 0.79), 2,3,4,6-tetra-*O*-methylgalactose (R_{TMG} 1.00), 2,3,4-tri-*O*-methylxylose (R_{TMG} 1.12), and 2,3,5-tri-*O*-methylarabinose (R_{TMG} 1.12). A portion of the methylated polysaccharide (15 mg) was methanolysed, and analysed by g.l.c. (column *4*). The methyl glycosides were converted into the alditol acetates and examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s. (Table II).

Reduction and re-methylation of methylated polysaccharide A. — Methylated polysaccharide *A* (76 mg) in dry tetrahydrofuran (3 ml) was heated under reflux with lithium aluminium hydride for 18 h. Excess of reductant was decomposed with ethyl acetate, the mixture was then dialysed against running, deionised water (18 h), and the carboxyl-reduced polysaccharide was isolated by freeze-drying (69 mg). The product was further methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide, to give methylated, carboxyl-reduced polysaccharide *A* (39 mg). The polysaccharide was hydrolysed, reduced, and acetylated, and the methylated alditol acetates were examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s. (Table II).

Methylated polysaccharide B *and derivatives.* — Polysaccharide *B* (200 mg) in dry methyl sulphoxide (3 ml) was stirred, under an atmosphere of dry nitrogen, with methylsulphinyl anion at room temperature for 6 h with intermittent ultrasonication. Methyl iodide (1 ml) was added during 15 min, the mixture was stirred for a further 20 min, and the partially methylated polysaccharide was isolated by freeze-drying after dialysis against running, deionised water. The polysaccharide was further methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide, to give fully methylated polysaccharide *B* (145 mg). The derived alditol acetates were examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s., and the derived methyl glycosides were examined by g.l.c. (column *4*). Methylated polysaccharide *B* was reduced with lithium aluminium hydride, and the carboxyl-reduced, methylated polysaccharide was re-methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide. Methylated, carboxyl-reduced polysaccharide *B* was converted into methylated alditol acetates that were examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s.

Methylated polysaccharide AD_2 *and derivatives.* — Polysaccharide AD_2 (100 mg) was methylated, as described for polysaccharide *B*, to afford a fully methylated polysaccharide AD_2 (80 mg). The derived alditol acetates were examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s. Methylated polysaccharide AD_2 was reduced with lithium aluminium hydride, and the carboxyl-reduced, methylated polysaccharide was re-methylated and the derived alditol acetates were examined by g.l.c. (columns *1*, *2*, and *3*) and g.l.c.–m.s.

ACKNOWLEDGMENTS

The authors thank Mr. A. W. Sonemann for recording the mass spectra,

Rhodes University and the South African Council for Scientific and Industrial Research for financial support, and Professor G. O. Aspinall and (the late) Professor J. K. N. Jones for gifts of methylated arabinoses.

REFERENCES

1 Part II: D. McGarvie and H. Parolis, *J. Chem. Soc., Perkin Trans. 1*, (1979) 1464–1466.
2 D. McGarvie and H. Parolis, *Carbohydr. Res.*, 69 (1979) 171–179.
3 W. N. Haworth, *J. Chem. Soc.*, 107 (1915) 8–16.
4 Q. N. Haq and E. Percival, in H. Barnes (Ed.), *Some Contemporary Studies in Marine Science*, Allen and Unwin, London, 1966, p. 355.
5 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208; J. Kärkkainen, *Carbohydr. Res.*, 14 (1970) 27–33.
6 H. Parolis and D. McGarvie, *Carbohydr. Res.*, 62 (1978) 363–367.
7 El S. Amin, O. M. Awad, and M. M. El-Sayed, *Carbohydr. Res.*, 15 (1970) 159–161.
8 L. Mindt, K. Saag, and G. R. Sanderson, *J. Sci. Food. Agric.*, 26 (1975) 993–1000.
9 P. Moyna and J. L. DiFabio, *Planta Med.*, 34 (1978) 207–210.
10 B. K. Srivastava and C S. Pande, *Planta Med.*, 24 (1974) 92–97.
11 F. Brown, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, (1949) 1761–1766.
12 V. M. Parikh and J. K. N. Jones, *Can. J. Chem.*, 44 (1966) 327–333.
13 V. M. Parikh and J. K. N. Jones, *Can. J. Chem.*, 44 (1966) 1531–1539.
14 S. C. Churms, A. M. Stephen, and P. van der Bijl, *J. S. Afr. Chem. Inst.*, 26 (1973) 45–52.
15 A. A. Obermeyer, *Flora of Southern Africa*, 22 (1976) 144–156.
16 J. H. Hoffman, M.Sc. Thesis, Rhodes University.
17 G. O. Aspinall and R. J. Ferrier, *Chem. Ind. (London)*, (1957) 1216.