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STRUCTURAL STUDIES OF AN ACIDIC POLYSACCHARIDE ISOLATED FROM THE LEAF FIBRE OF PINEAPPLE (*Ananas comosus* MERR.)

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ABSTRACT

Pineapple-fibre holocellulose was extracted with alkaline solutions of increasing concentration to give hemicellulose fractions I–III. Purified fraction I (major component) contained D-xylose, L-arabinose, and 4-*O*-methyl-D-glucuronic acid in the molar proportions 10:0.6:1, and an average of ~116 sugar residues, whereas its methylated product contained ~94 sugar residues. Structural studies of the hemicellulose and its arabinose-free portion showed it to be a (1→4)-linked β-D-xylan with approximately every tenth residue carrying a 4-*O*-methyl-α-D-glucopyranosyluronic acid group at O-2 and every sixteenth residue carrying an L-arabinofuranosyl group at O-3.

INTRODUCTION

Pineapple fibre[1,2] is extracted from the leaves of the pineapple plant (*Ananas comosus* Merr., Family: Bromeliaceae), which is widely cultivated in the tropical and subtropical regions of the world. The fibre is strong and white, with a silky lustre, and is used for making ropes, twines, and threads. The chemical composition of the fibre extracted from the leaves of three different varieties of the plant, *viz.* Giant Kew, Queen, and Mauritius, has been reported[3]. We now report on the carbohydrate constituents of the fibre.

RESULTS AND DISCUSSION

The extractive-free fibre containing 4.4% of lignin and 1.2% of pectin was delignified by treatment with 0.5% sodium chlorite in an acid medium[4]. The resulting holocellulose was depectinised by treatment with aqueous 0.5% ammonium oxalate and then successively extracted with aqueous 5%, 10%, and 24% sodium hydroxide, to give hemicellulose fractions I–III (see Table I for yields and analyti-

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TABLE I

ANALYTICAL DATA FOR HEMICELLULOSE FRACTIONS

Hemicellulose fraction	*Yield*[a] *(%)*	*Pentosan (%)*	*Uronic anhydride (%)*	*Methoxyl (%)*	*Equivalent weight*	$[\alpha]_D^{30}$ *(MNaOH) (degrees)*
I	9.6	77.5	11.5	2.6	1540	−50
IA		81.6	11.9	2.1	1500	−46.5
II	4.6	73.7	12.8	2.5	1420	−52
III	1.2	72.5	12.5	1.8	1467	−53

[a]Based on the dry weight of the defatted fibre; the rest of data are expressed on the weight of the dry fraction

TABLE II

MOLAR PROPORTION OF NEUTRAL SUGARS IN HEMICELLULOSE FRACTIONS AND CELLULOSE RESIDUE

Hemicellulose fraction	*Gal (%)*	*Glc (%)*	*Man (%)*	*Ara (%)*	*Xyl (%)*	*Rha (%)*
I	8.5	3.1	—	6.5	81.6	0.3
IA	1.7	1.7	—	5.6	91.0	—
II	0.3	12.9	0.5	2.9	83.4	—
III	2.5	11.9	2.6	2.9	80.0	—
Cellulose residue	—	99.1	—	0.2	0.6	0.1

cal data). Fraction I (major component) was purified by two precipitations from its solution in aqueous 5% sodium hydroxide by neutralisation and then addition of ethanol. The analytical data of the purified hemicellulose IA are also given in Table I. Samples of the hemicellulose fraction and the cellulose residue left after extraction were hydrolysed[5], and the neutral sugars in the hydrolysates were quantified as their alditol acetates[6] by g.l.c. The results are summarised in Table II.

Hemicellulose IA contained small proportions of galactose and glucose which could not be removed and could be associated with impurities. The homogeneity of the polysaccharide was confirmed by electrophoresis in borate buffer (pH 9.5). Carboxyl-reduced hemicellulose IA, obtained by the method of Taylor and Conrad[7], yielded, on hydrolysis, xylose (81.1%), arabinose (5.2%), 4-*O*-methylglucose (9.6%), and galactose and glucose (3.9%). The presence of 4-*O*-methylglucose indicated the presence of 4-*O*-methylglucuronic acid in the hemicellulose.

Hemicellulose IA was soluble in water and dilute alkali. Its uronic acid content and equivalent weight suggested the presence of 10 xylose residues per uronic acid residue. The arabinose content was consistent with one arabinose unit per 16 xylose residues. The hemicellulose IA was, therefore, an arabino-4-*O*-methylglucuronoxylan.

Treatment of hemicellulose IA with 0.01M sulphuric acid (~100°, 3 h) caused

maximal removal of arabinose. The degraded hemicellulose IA had $[\alpha]_D^{27}$ −42° (M sodium hydroxide) and an equivalent weight of 1310, and contained pentosan, 80.2%; uronic anhydride, 12.4%; methoxyl, 2.2%; xylose, 96.7%; arabinose, 0.5%; and galactose and glucose, 2.8%.

Graded hydrolysis of hemicellulose IA with 0.1M sulphuric acid (~100°, 6 h) yielded a mixture of mono- and oligo-saccharides from which 4-*O*-β-D-xylopyranosyl-D-xylopyranose (xylobiose) and *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (xylotriose) were isolated and identified (see Experimental), indicating that the hemicellulose contained a backbone of (1→4)-linked β-D-xylopyranosyl residues. Two acidic oligomers were also isolated, and identified (see Experimental) as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose and *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose.

Hemicellulose IA and the degraded hemicellulose IA were methylated by the Hakomori procedure[8,9] and then by the Purdie method[10]. The methylated hemicellulose IA had $[\alpha]_D^{27}$ −59.5° (chloroform) and was fractionated with boiling chloroform–light petroleum. The main fraction was soluble in a 3:7 mixture, and had $[\alpha]_D^{31}$ −57.5° (chloroform) and a d.p. of 94 as determined by osmometry. Hydrolysis gave (p.c.) an acidic product together with 2-*O*-methylxylose, 2,3-di-*O*-methylxylose, 2,3,4-tri-*O*-methylxylose, and 2,3,5-tri-*O*-methylarabinose. 2-*O*-Methylxylose and 2,3-di-*O*-methylxylose were isolated and characterised. Paper electrophoresis of 2-*O*-methylxylose revealed a trace of 3-*O*-methylxylose. The acidic component was characterised as 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-D-xylose.

The methylated, degraded hemicellulose IA had $[\alpha]_D^{27}$ −47.5° (chloroform), and a d.p. of 50 as determined by osmometry. Methylated hemicellulose IA and methylated, degraded hemicellulose IA were hydrolysed, and the resulting partially methylated sugars were converted into alditol acetates[11] and analysed by g.l.c. The results are summarised in Table III.

TABLE III

METHYLATED NEUTRAL SUGARS IN THE HYDROLYSATES OF METHYLATED HEMICELLULOSE FRACTION IA (C) AND METHYLATED, DEGRADED HEMICELLULOSE FRACTION IA (D)

Sugars	T[a]		*Approximate molar proportion*	
	A	B	C	D
2,3,5-Tri-*O*-methylarabinose	0.47	0.40	2.1	—
2,3,4-Tri-*O*-methylxylose	0.67	0.53	1.0	1.0
2,3-Di-*O*-methylxylose	1.53	1.19	38.5	19.2
2-*O*-Methylxylose	1.90	2.14	4.5	1.5

[a]Retention times at 165° of the corresponding alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on *A*, 3% of ECNSS-M; and *B*, 3% of OV-225.

The large proportion of the 2,3-di-*O*-methylxylose derivative obtained, together with the high negative $[\alpha]_D$ value of the original and methylated hemicellulose IA, indicated that the polysaccharide contained a backbone of (1→4)-linked β-D-xylopyranosyl residues. The molar proportions of the neutral sugars in the methylated hemicellulose IA indicated the presence of 44 xylose residues for each non-reducing end-group producing 2,3,4-tri-*O*-methylxylose (see Table III). The uronic acid content of hemicellulose IA indicated that 44 units of xylose sugars would be associated with 4.8 units of aldobiouronic acid. The approximate molar proportions of the sugars, *viz.*, 2,3,4-tri-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylxylose, 2-*O*-methylxylose, 3-*O*-methylxylose, and 2,3,4-tri-*O*-methylglucuronic acid would therefore be 1:2.1:38.5:4.5:4.8:4.8. The total number of sugar residues was 55.7. Thus, for a molecule of d.p. 94, there would be 1.7 (94/55.7) non-reducing xylosyl end-groups or 0.7 branch-point.

The formation of 2,3,5-tri-*O*-methylarabinose confirmed the presence of terminal L-arabinofuranosyl groups, indicated by the loss of arabinose on mild, acid hydrolysis of hemicellulose IA. The amount (2.1 mol) of tri-*O*-methylarabinose per mol of tri-*O*-methylxylose was lower than the value expected (3 mol) from the ratio of sugars in hemicellulose IA. This was probably due to loss of 2,3,5-tri-*O*-methylarabinose by volatilisation, as has been observed elsewhere[12,13]. If 3 mol were the correct value, 3 mol of 2-*O*-methylxylose would be expected if all the arabinofuranosyl groups were 3-linked to the xylose residues. The excess (1.5 mol) of 2-*O*-methylxylose was probably due to branching, incomplete methylation, and demethylation during hydrolysis of the methylated hemicellulose[14,15]. The presence of traces of 3-*O*-methylxylose in the 2-*O*-methylxylose might be due to slight hydrolysis of the methylated aldobiouronic acid.

That all the arabinose residues were attached directly to the xylan backbone and that there were no interposed xylose residues was indicated by the number of non-reducing xylosyl end-groups per molecule before and after selective removal of the arabinosyl groups. The molar proportions of neutral sugars in the methylated, degraded hemicellulose IA indicated 21.7 xylosyl residues for each non-reducing xylosyl end-group (see Table III). Taking 2.4 units of partially methylated aldobiouronic acid into account for the 21.7 units of xylose residues in the molecule, the ratio of 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, 2-*O*-methylxylose, 3-*O*-methylxylose, and 2,3,4-tri-*O*-methylglucuronic acid would be 1:19.2:1.5:2.4:2.4. Thus, one non-reducing xylosyl end-group (producing 2,3,4-tri-*O*-methylxylose) was present per 26.5 sugar residues of the methylated polysaccharide. The d.p. of the methylated polysaccharide was 50. Therefore, the molecule would contain 1.9 (50/26.5) non-reducing end-groups or 0.9 branch-point; *i.e.*, there was no significant change in the number of branch points on removal of arabinosyl groups. Consequently, the arabinose residues were directly linked to the xylan framework. The increase in non-reducing xylosyl groups after acid hydrolysis of methylated, degraded hemicellulose IA might be due to some depolymerisation of the xylan framework. The d.p. values of hemicellulose IA and its

degraded portion, as determined by osmometry of solutions in dimethyl sulphoxide, were 116 and 60, respectively.

From the above evidence, a simplified structure can be proposed for hemicellulose IA. The polysaccharide contains an average of at least 100 (1→4)-linked β-D-xylopyranosyl residues with slightly less than one branch point; side chains of 4-*O*-methyl-α-D-glucopyranosyluronic acid groups are attached, on average, to every tenth xylosyl residue through O-2, and L-arabinofuranosyl groups are attached, on average, to every sixteenth xylosyl residue through O-3.

The results of periodate oxidation and Smith degradation accord with this structure. The hemicellulose IA and the degraded hemicellulose IA consumed 0.85 and 0.89 mol of periodate, respectively, liberating 0.013 and 0.02 mol of formic acid, respectively, per pentosyl residue. Acid hydrolysis of each reduced oxopolysaccharide released mainly glycerol and xylose. Thus, pineapple-leaf fibre contains an arabino-4-*O*-methylglucuronoxylan as the main hemicellulose component, in contrast to such bast and leaf fibres as jute[16], mesta[17], roselle[18], sisal[19], flax[20], and sansevieria[21], which contain 4-*O*-methylglucuronoxylans, or sunn hemp[22], which contains a glucomannan as the major hemicellulose component.

EXPERIMENTAL

Materials. — Pineapple fibre was obtained by decortication of the leaves of the pineapple plant, variety Giant Kew. The fibre was cleaned by combing before analysis.

General methods. — Optical rotations were equilibrium values measured at 589.6 nm. Evaporations were performed under diminished pressure at 40–45°. Melting points are uncorrected. Electrophoresis was performed on Whatman No. 1 paper with a Shandon High Voltage Electrophoresis Apparatus, Model L-24. Benzidine–periodate was used as spray reagent. I.r. spectra were recorded with a Beckman IR-20A instrument. P.c. was performed on Whatman Nos. 1 and 3 MM papers with *A*, butyl acetate–pyridine–ethanol–water (8:2:2:1); *B*, 1-butanol–pyridine–water (6:4:3); *C*, 1-butanol–ethanol–water (40:11:19); *D*, ethyl acetate–acetic acid–water (9:2:2); *E*, 1-butanol–ethanol–water (4:1:5, upper layer); and *F*, benzene–ethanol–water (169:47:15, upper layer); and detection with aniline hydrogenoxalate or alkaline silver nitrate. Polysaccharides were hydrolysed according to the method of Jeffery *et al.*[5]. The hydrolysates were neutralised with barium carbonate, centrifuged, and treated successively with Amberlite IR-120 (H^+) and IR-402 (HO^-) resins. The neutral sugars were analysed by p.c. and by g.l.c. of their alditol acetates[6]. For g.l.c., a Hewlett-Packard 5830A gas chromatograph equipped with f.i.d. was used, with stainless-steel columns (6 ft × 0.125 in.) containing *A*, 3% of ECNSS-M on Supelcoport (80–100 mesh) at 190° for alditol acetates of sugars and at 165° for alditol acetates of partially methylated sugars; and *B*, 3% of OV-225 on Supelcoport (80–100 mesh) at 165° for alditol acetates of partially

methylated sugars. Molar proportions of sugars were determined conventionally from peak areas.

Composition of the fibre. — Analysis by standard methods revealed α-cellulose, 69.5; pentosan, 17.8; uronic anhydride, 5.3; lignin, 4.4; pectin, 1.2; acetyl, 2.7; ash, 0.9; fat and wax, 3.3; N, 0.3; galactose, 2.9; glucose, 75.2; mannose, 1.0; arabinose, 2.0; xylose, 17.9; and rhamnose, 1.1%.

Isolation of hemicellulose fractions. — The pineapple fibre was cut into small pieces, and extracted exhaustively with ethanol–benzene (1:2). The defatted fibre was delignified by treatment with 0.5% sodium chlorite in an acid medium[4], to give holocellulose (yield, 90.4%; pectin, 1.2%) which was depectinised by treatment with aqueous 0.5% ammonium oxalate at 75–80° for 4 h. The depectinised holocellulose (160.4 g) was extracted successively with 10 parts of aqueous 5%, 10%, and 24% sodium hydroxide for 2, 2, and 24 h, respectively, under nitrogen. Each extract was neutralised with acetic acid, and ethanol was added to precipitate hemicellulose fractions I–III, respectively. The cellulose residue was washed and dried. Samples of each hemicellulose fraction and the cellulose residue were hydrolysed[5], and the neutral sugars in the hydrolysates were analysed as their alditol acetates[6] by g.l.c. on column *A*.

Purification of fraction I. — A solution of fraction I (16 g) in aqueous 5% sodium hydroxide (160 mL) was centrifuged, cooled, and neutralised with acetic acid, and the hemicellulose was precipitated with ethanol (2 vol.). The process was repeated, and the hemicellulose was made ash-free by treatment with ethanolic hydrochloric acid (M). The purified hemicellulose (fraction IA) was washed free from acid with ethanol and dried (yield, 13.6 g). The proportions of the sugar components of the hemicellulose were almost constant after two purifications. The homogeneity of the polysaccharide was confirmed by high-voltage electrophoresis in borate buffer (0.02M, pH 9.5), when a single spot with slight tailing was observed. A part of the sample was hydrolysed, and the resulting neutral sugars were analysed as the alditol acetates by g.l.c. on column *A*. The hydrolysate was fractionated on Whatman No. 3 MM paper with solvent *A*. The zones containing xylose and arabinose were each extracted with water (20 mL), each extract was concentrated, and the syrupy residue was crystallised from aqueous ethanol, to give D-xylose, m.p. 144°, $[\alpha]_D^{27}$ +18° (*c* 1, water); lit.[23a] m.p. 143–144°, $[\alpha]_D^{20}$ +19° (water); and L-arabinose, m.p. 156°, $[\alpha]_D^{27}$ +105° (*c* 1, water); lit.[23b] m.p. 156°, $[\alpha]_D^{20}$ +108° (water).

Carboxyl-reduction of fraction IA[7]. — 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulphonate (200 mg) was added to a stirred solution of fraction IA (20 mg) in water (20 mL), and the pH of the solution was kept at 4.75 by dropwise addition of 0.01M hydrochloric acid. After 2 h, 2M sodium borohydride (35 mL) was added dropwise during 45 min, and the pH was kept at 7 by simultaneous addition of 4M hydrochloric acid. After being stirred for 1 h, the solution was dialysed against distilled water for 24 h and then concentrated to dryness. The whole process was repeated, the reduced sample was hydrolysed with M

sulphuric acid for 18 h at 100°, and the resulting sugars were analysed as their alditol acetates by g.l.c. on column *A*.

Graded hydrolysis studies. — A solution of fraction IA (4 g) in 0.1M sulphuric acid (200 mL) was heated on a boiling water-bath for 6 h; these conditions gave a maximum yield of lower oligosaccharides. The solution was neutralised with barium carbonate, centrifuged, treated with Amberlite IR-120 (H^+) resin, concentrated to ~25 mL, and passed through a column of Amberlite IR-402 (HCO_3^-) resin on which the acidic sugars were absorbed. The column was washed with distilled water to give the neutral saccharides, and then eluted with 0.5M sulphuric acid and water. The eluate was neutralised with barium carbonate, centrifuged, treated with Amberlite IR-120 (H^+) resin, and concentrated (~1 mL), to give the acidic saccharides. The neutral and acidic saccharides were subjected to preparative p.c. (solvents *B* and *D*, respectively) to give the following compounds.

4-*O*-β-D-Xylopyranosyl-D-xylopyranose (xylobiose, 158 mg), m.p. 184° (from methanol), $[\alpha]_D^{27}$ −24° (*c* 1, water); lit.[24] m.p. 185–186°, $[\alpha]_D^{25}$ −25.5° (water). Hydrolysis with 0.5M sulphuric acid (100°, 18 h) gave only xylose, identified by p.c. (solvent *A*) and by g.l.c. (column *A*) of the alditol acetate. Methylation by the Kuhn method[25] and then hydrolysis with M sulphuric acid (100°, 18 h) gave 2,3-di-*O*-methylxylose and 2,3,4-tri-*O*-methylxylose (in approximately equal proportions) identified by g.l.c. (column *A*) of the alditol acetates.

O-β-D-Xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (xylotriose, 112 mg), m.p. 204° (from aqueous ethanol), $[\alpha]_D^{27}$ −46° (*c* 1.1, water); lit.[24] m.p. 205–206°, $[\alpha]_D^{25}$ −47° (water). Hydrolysis with 0.5M sulphuric acid (100°, 18 h) gave xylose (p.c. and g.l.c. of the alditol acetate). Methylation[25] and then hydrolysis yielded 2,3-di-*O*-methylxylose and 2,3,4-tri-*O*-methylxylose in the ratio 2:1 (g.l.c. of alditol acetates).

2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose (215 mg), $[\alpha]_D^{27}$ +85° (*c* 0.5, water); equivalent wt., 338. It was chromatographically identical with the authentic compound obtained from jute[16]. Hydrolysis with 0.5M sulphuric acid in a sealed tube (100°, 12 h) yielded xylose and 4-*O*-methylglucuronic acid (p.c., solvent *D*). The methyl ester methyl glycoside was reduced with lithium aluminium hydride in dry tetrahydrofuran[26] and then hydrolysed with 0.5M sulphuric acid (100°, 18 h), to give xylose and 4-*O*-methylglucose in nearly equal proportions (identified by g.l.c. of the alditol acetates on column *A*). The aldobiouronic acid (100 mg) was methylated by the Kuhn method[25], and the product was reduced with lithium aluminium hydride in ether, and then methylated with methyl iodide and silver oxide. The fully methylated disaccharide (60 mg) was hydrolysed with 0.5M sulphuric acid (100°, 18 h) to yield 3,4-di-*O*-methylxylose and 2,3,4,6-tetra-*O*-methylglucose (p.c., solvent *C*).

O-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (52 mg), $[\alpha]_D^{27}$ +57° (*c* 0.5, water). Hydrolysis with 0.5M sulphuric acid (100°, 18 h) gave (p.c., solvent *D*) xylose, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose, and a trace of 4-*O*-methylglucuronic

acid. Methylation[25] and then hydrolysis with 0.5M sulphuric acid (100°, 18 h) yielded 2,3-di-*O*-methylxylose and 3,4-di-*O*-methylxylose in nearly equal amounts (p.c., solvent *C*).

Degraded fraction IA. — A solution of fraction IA (4 g) in 0.01M sulphuric acid (40 mL) was heated on a boiling water-bath for 3 h, conditions under which the maximum amount of arabinose was released. The degraded hemicellulose was precipitated from the solution by adding ethanol, collected by centrifugation, washed with ethanol, and dried (yield, 2.5 g). A part of the sample was hydrolysed with M sulphuric acid (100°, 18 h), and the resulting neutral sugars were analysed by g.l.c. (column *A*) of the alditol acetates.

Methylation analysis. — Fraction IA (1 g) and degraded fraction IA (100 mg) were separately methylated, first by the Hakomori method[8,9] and then by the Purdie method[10], until free from i.r. absorption for hydroxyl. Methylated fraction IA (OMe, 39.2%) was purified by fractionation with a mixture of boiling chloroform–light petroleum (b.p. 60–65°); the main fraction (OMe, 38.9%) was obtained with a solvent ratio of 3:7. The methylated, degraded hemicellulose IA had OMe, 38.4%. Each methylated product was hydrolysed first with 85% formic acid (100°, 2 h) and then, after removal of formic acid, with 0.5M sulphuric acid (100°, 18 h). The resulting partially methylated sugars were converted into their alditol acetates[11], and analysed by g.l.c. (columns *A* and *B*). P.c. (solvents *C*, *E*, and *F*) of the hydrolysate of methylated fraction IA revealed 2-*O*-methylxylose, 2,3-di-*O*-methylxylose, 2,3,4-tri-*O*-methylxylose, and 2,3,5-tri-*O*-methylarabinose, together with an acidic component. The mixture was subjected to preparative p.c. (solvent *C*). The following compounds were isolated.

2-*O*-Methyl-D-xylose, m.p. 132°, $[\alpha]_D^{27}$ +35° (*c* 1, methanol); lit.[27] m.p. 132–133°, $[\alpha]_D$ +35° (methanol). The sugar had the same mobility in paper electrophoresis as an authentic sample, and contained a trace of 3-*O*-methylxylose. The anilide derivative had m.p. 130°; lit.[27] m.p. 130–131°.

2,3-Di-*O*-methyl-D-xylose, m.p. 91°, $[\alpha]_D^{27}$ +26° (*c* 1, water); lit.[27] m.p. 91–92°, $[\alpha]_D$ +26° (water). The anilide derivative had m.p. 124°, $[\alpha]_D^{27}$ +192° (*c* 0.5, ethyl acetate); lit.[27] m.p. 124–125°, $[\alpha]_D$ +192° (ethyl acetate).

3-*O*-Methyl-2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-D-xylose was converted into the methyl ester methyl glycoside, reduced with lithium aluminium hydride in ether, and hydrolysed with 0.5M sulphuric acid (100°, 18 h), to yield 3-*O*-methylxylose and 2,3,4-tri-*O*-methylglucose (p.c., solvent *C*), which were isolated by preparative p.c. 3-*O*-Methylxylose, $[\alpha]_D^{27}$ +17° (*c* 0.5, water) {lit.[19] $[\alpha]_D^{31}$ +17.4° (water)}, had the same mobility as an authentic sample in paper electrophoresis, and gave an anilide, m.p. 137° (from ethyl acetate), $[\alpha]_D^{30}$ +80° (*c* 0.4, ethyl acetate); lit.[19] m.p. 137°, $[\alpha]_D$ +80° (ethyl acetate). 2,3,4-Tri-*O*-methylglucose had $[\alpha]_D^{30}$ +67° (*c* 1, water) {lit.[19] $[\alpha]_D^{31}$ +66.9° (water)}, and the anilide derivative had m.p. 144°; lit.[19] m.p. 143–145°.

Periodate oxidation. — Fraction IA and degraded fraction IA (800 mg of each) were oxidised with sodium metaperiodate (0.1M, 75 mL) in the dark at 10°.

Uptake of periodate was measured by the excess arsenite method[28]. The sodium salts of the two hemicellulose samples (850 mg of each) were oxidised with sodium metaperiodate (0.1M, 75 mL) in the dark at 10°, according to the method of Halsall *et al.*[29]. The formic acid liberated was titrated with 0.01M sodium hydroxide.

Smith degradation[30]. — Fraction IA and degraded fraction IA (200 mg of each) were oxidised with sodium metaperiodate (0.1M, 75 mL) in the dark at 10° for 250 h. Excess of periodate was reduced with ethylene glycol, each solution was dialysed and concentrated, and the residue was reduced with sodium borohydride[30]. Hydrolysis of the reduced products with 0.5M sulphuric acid (100°, 8 h) yielded glycerol and xylose, as shown by p.c. (solvent *E*), and g.l.c. (column *A*) of the alditol acetates.

Molecular weight determination. — The number-average molecular weights of fraction IA and degraded fraction IA were determined by osmometry of solutions in dimethyl sulphoxide. Butyl acetate was used for the methylated hemicelluloses. Measurements were made by the static method at 35 ±0.02° with a Hellfritz[31] osmometer and a regenerated cellulose membrane SM 11536 (Sartorius). The membrane was conditioned to solvent according to the Wagner procedure[32]. The molecular weights of fraction IA and degraded fraction IA were estimated to be 15,963 and 8,210, respectively, and the values for their methylated products were 15,583 and 7,595, respectively.

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