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STRUCTURAL FEATURES OF ALKALI-SOLUBLE ACIDIC XYLANS ISOLATED FROM THE BARK OF *Ceiba pentandra* var. indica

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ABSTRACT

The insoluble residue of *C. pentandra* var. indica, obtained by delignification of the bark, gave an alkali-soluble polysaccharide fraction containing L-fucose, D-xylose, L-arabinose, D-glucose, D-galactose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid. Fractional precipitation with Cetavlon and barium hydroxide afforded three hemicellulose-type acidic xylans that differed in the contents of uronic acid and mol. wts., and a fucose-containing acidic polysaccharide. Methylation analysis of the carboxyl-reduced acidic xylans suggested a (1→2)- or (1→4)-linked backbone and different patterns of substitution. Partial hydrolysis of one acidic xylan gave 4-*O*-β-D-xylopyranosyl-D-xylose and 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose. The oxidation of this polysaccharide with chromium trioxide and periodate was studied.

INTRODUCTION

The bark of the Kapok tree (*C. pentandra* var. indica) contains a mucilaginous substance that gave an aqueous extract and an insoluble residue on delignification[1]. The water-soluble polysaccharide comprised mainly a mixture of two similar acidic polysaccharides. Treatment of the insoluble residue with alkali gave an alkali-soluble polysaccharide fraction which is the subject of this report.

RESULTS AND DISCUSSION

The alkali-soluble polysaccharide fraction, isolated in 18% yield, contained ~87.5% of carbohydrate[2], 7.21% of protein[3], L-fucose, D-xylose, L-arabinose, D-glucose, D-galactose, 4-*O*-methyl-D-glucuronic acid, and D-glucuronic acid, and gave 3.11% of ash[4]; the contents of sulfate[5] (0.018%), phosphate[6] (0.016%), and lignin[7] were negligible.

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With Cetavlon[8], the polysaccharide fraction gave insoluble (ACP) and soluble (ACS) fractions in yields of 44% and 47%, respectively. ACP contained D-xylose, L-arabinose, D-glucose, D-galactose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid, whereas ACS contained L-fucose in addition to these components.

Treatment of ACP with barium hydroxide[9] afforded insoluble (ACPBP) and soluble (ACPBS) fractions in yields of 55% and 30%, respectively. ACPBP was composed of D-xylose and D-glucuronic acid together with a minor proportion of D-glucose, whereas ACPBS contained D-xylose and 4-*O*-methyl-D-glucuronic acid together with minor proportions of L-arabinose and D-galactose. Similar treatment of ACS with barium hydroxide also gave insoluble (ACSBP) and soluble (ACSBS) fractions in yields of 45% and 50%, respectively. ACSBP contained D-xylose and D-glucuronic acid together with minor proportions of L-arabinose and D-galactose, whereas ACSBS was composed of L-fucose, D-xylose, L-arabinose, D-glucose, D-galactose, and 4-*O*-methyl-D-glucuronic acid.

Thus, each polysaccharide fraction contained D-xylose and either D-glucuronic acid or 4-*O*-methyl-D-glucuronic acid, suggesting them to be hemicellulosic in nature[10].

Ion-exchange chromatography[11] of ACPBP on DEAE-cellulose gave a minor neutral fraction (eluted with water) and major and minor acidic fractions eluted with a linear gradient (0.1→M) of sodium chloride. The major acidic fraction (acidic xylan ACPBP) contained D-xylose and D-glucuronic acid. Likewise, ACPBS gave a minor neutral fraction and a major acidic fraction (acidic xylan ACPBS, eluted with 0.5M sodium chloride) composed of D-xylose and 4-*O*-methyl-D-glucuronic acid. Chromatography of ACSBP on DEAE-cellulose also gave a minor neutral fraction and a major acidic fraction (acidic xylan ACSBP, eluted with 0.5M sodium chloride) which contained D-xylose and D-glucuronic acid.

The acidic xylans ACPBS and ACSBP were eluted as single symmetrical peaks from Sephadex G-200 at slightly included volumes, whereas the acidic xylan ACPBP was eluted at the void volume as a single peak. In gel-permeation chromatography[12] on Bio-GelA-15m, the acidic xylan ACPBP gave a single symmetrical peak with an elution volume corresponding to a mol. wt. of $\sim 5 \times 10^5$. Likewise, the acidic xylans ACPBS and ACSBP were eluted as single symmetrical peaks corresponding to mol. wts. of $\sim 1.905 \times 10^5$ and $\sim 1.445 \times 10^5$, respectively. The chemical compositions of these polysaccharides were unaltered after elution from Sephadex G-200 and Bio-GelA-15m, suggesting homogeneity. The acidic xylans ACPBP, ACPBS, and ACSBP were readily soluble in alkali and were precipitated readily with barium hydroxide, Cetavlon, and Fehling's solution. The sugar compositions of these polysaccharides remained unaltered after several precipitations with these reagents, and the acidic xylan ACPBP gave a single symmetrical peak on sedimentation analysis[13], further indicating the homogeneity of the acidic xylans ACPBP, ACPBS, and ACSBP.

Structure of the acidic xylan ACPBP. — The carboxyl-reduced polysaccharide

contained D-xylose and D-glucose in the molar ratio ~8:1. Methylation analysis of this carboxyl-reduced polysaccharide (Table I) suggested that all of the D-glucosyl residues and 1 in 16 of the D-xylosyl residues were present as non-reducing end-groups. The molar ratios of 1.1:6.8:1.0 computed for the derivatives of 3-*O*-methyl-D-xylose, 2,3- or 3,4-di-*O*-methyl-D-xylose, and 2,3,4,6-tetra-*O*-methyl-D-glucose suggested that at least 1 in 8 of the D-xylosyl residues carried D-glucopyranosyl residues at O-2 or O-4. The formation of the derivatives of 2,3- or 3,4-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose suggested that at least 1 in 8 of the D-xylosyl residues carried non-reducing D-glucopyranosyl residues at O-2 or O-4. The formation of the derivatives of 2,3- or 3,4-di-*O*-methyl-D-xylose (6.8 mol) and 3-*O*-methyl-D-xylose (1.1 mol) implied that the backbone contained (1→4)- or (1→2)-linked D-xylopyranosyl residues.

Partial hydrolysis of the acidic xylan ACPBP with trifluoroacetic acid afforded a neutral and acidic disaccharide which were isolated by preparative p.c. The neutral disaccharide (R_{Glc} 0.72, solvent *A*) contained only D-xylose, and acid hydrolysis of its alditol derivative gave D-xylose and D-xylitol in equimolar proportions indicative of a disaccharide. Methylation analysis of the disaccharide-alditol indicated a (1→4) linkage. The disaccharide-alditol obtained by reduction with sodium borodeuteride, on methylation followed by e.i.-m.s.[14], gave ions at *m/z* 111 (aA_3), 143 (aA_2), 175 (aA_1), 192 ($aldJ_2$) and 252 ($aldJ_1$) which confirmed the disaccharide structure. These results and the $[\alpha]_D$ value of −28° (lit.[15] −20 to −30°) suggested the disaccharide to be 4-*O*-β-D-xylopyranosyl-D-xylose.

The acidic disaccharide (R_{GlcA} 0.68, solvent *E*) contained D-glucuronic acid and D-xylose, and acid hydrolysis of its carboxyl-reduced methyl glycoside gave D-glucose and D-xylose in the molar ratio 1:1 indicative of a disaccharide. Acid hydrolysis of the carboxyl-reduced disaccharide-alditol gave D-glucose and xylitol in equimolar proportions. Methylation analysis of the carboxyl-reduced methyl glycoside indicated a (1→2) linkage. The e.i.-m.s.[14] of the methylated disaccharide-

TABLE I

METHYLATION ANALYSIS DATA OF CARBOXYL-REDUCED ACPBP

Alditol acetate of	T[a] *on*		*Average molar proportion*	*Characteristic mass fragments* (m/z)	*Mode of linkage*
	OV-225	*SE-54*			
2,3,4-Me_3-Xyl[b]	0.67	0.61	0.57	58, 71, 87, 101, 117, 161	D-Xyl*p*-(1→
2,3,4,6-Me_4-Glc	1.00	1.00	1.00	87, 101, 113, 117, 129, 145, 161, 173, 205	D-Glc*p*-(1→
2,3- or 3,4-Me_2-Xyl	1.19	0.89	6.48	101, 117, 129, 161	→2 or 4)-D-Xyl*p*-(1→
3-Me-Xyl	1.68	1.22	1.06	87, 129, 189	→2,4)-D-Xyl*p*-(1→

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]2,3,4-Me_3-Xyl = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-xylitol, *etc.*

alditol, obtained by reduction with sodium borodeuteride followed by methylation, contained ions at *m/z* 169 (aA_3), 192 ($aldJ_2$), 201 (aA_2), 233 (aA_1), and 251 ($aldJ_1$) confirming the disaccharide structure. These results together with the $[\alpha]_D$ value of +92° (lit.[15] +88–98°) suggested the structure 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylopyranose.

The formation of the aldobiouronic acid and the xylobiose confirmed that, in the polysaccharide, all the D-glucopyranosyluronic acid residues were (1→2)-linked to β-D-xylopyranosyl residues and that the β-D-xylopyranosyl residues of the backbone were (1→4)-linked.

Oxidation[16,17] of the carboxyl-reduced polysaccharide with chromium trioxide suggested the D-glucopyranosyl residues to be α and the D-xylopyranosyl residues to be β. Further, the $[\alpha]_D$ value of −68° (*c* 1, 0.1M NaOH) of the acidic xylan ACPBP suggested the D-xylopyranosyl residues to be mainly β.

On periodate oxidation[18], the acidic xylan ACPBP consumed 0.98 mol of oxidant per "anhydropentose" residue. Likewise, the carboxyl-reduced polysaccharide consumed 1.01 mol of periodate and released 0.33 mol of formic acid. Smith-degradation[19] experiments showed that all the D-glucopyranosyluronic acid or D-glucopyranosyl residues of the native or caboxyl-reduced polysaccharide, respectively, were oxidised. These results accorded with the methylation analysis data.

Thus, it can be inferred that the acidic xylan ACPBP most probably contained a (1→4)-linked β-D-xylan backbone in which 1 in 8 of the residues was substituted with D-glucopyranosyl residues at O-2. Also, 1 in 16 of the D-xylopyranosyl residues carried non-reducing D-xylopyranosyl residues at O-2. Further, the acidic xylan ACPBP was precipitated from aqueous solution by adjusting the pH to 4.5 with acetic acid at ~5°, suggesting it to be a hemicellulose-A type polysaccharide[10].

Structure of the acidic xylan ACPBS. — The carboxyl-reduced polysaccharide

TABLE II

METHYLATION ANALYSIS DATA OF THE CARBOXYL-REDUCED ACPBS

Alditol acetate of	T[a] *on DB-1*	*Average molar proportion*	*Characteristic mass fragments* (m/z)	*Mode of linkage*
2,3,4-Me_3-Xyl[b]	0.74	0.56	101, 117, 127, 147 161, 173, 205, 247[d]	D-Xyl*p*-(1→
2,3,4,6-Me_4-Glc	1.00	1.0	101, 117, 129, 139, 145, 161, 171, 205, 263[c]	D-Glc*p*-(1→
2,3- or 3,4-Me_2-Xyl	0.9	2.19	101, 117, 129, 189, 205, 247[c]	→2 or 4)-D-Xyl*p*-(1→
3-Me-Xyl	1.1	1.42	117, 129, 145, 173, 189, 275[c]	→2,4)-D-Xyl*p*-(1→

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]2,3,4-Me_3-Xyl = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-xylitol, *etc.* [c]The ions are $(M^+ + H) - 60$, obtained by the "Finnigan ion trap" method of ionisation. [d]The ion is $(M^+ + H) - 60$.

of the acidic xylan ACPBS contained D-xylose and 4-*O*-methyl-D-glucose in the molar ratio ~5:1, and methylation analysis (see Table II) suggested that all the 4-*O*-methyl-D-glucose and 1 in 8 of the D-xylose residues were present as non-reducing end-groups. The formation of the derivatives of 2,3- or 3,4-di-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose indicated that the backbone was a (1→2)- or (1→4)-linked D-xylan. The molar ratios 0.5:1.0:2.2:1.4 computed for the derivatives of 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3- or 3,4-di-*O*-methyl-D-xylose, and 3-*O*-methyl-D-xylose suggested that at least 1 in 4 of the D-xylosyl residues carried non-reducing 4-*O*-methyl-D-glucuronic acid residues at O-2 or O-4. Thus, the structure of the acidic xylan ACPBS closely resembled that of the acidic xylan ACPBP.

Structure of the acidic xylan ACSBP. — Carboxyl-reduced acidic xylan ACSBP contained D-xylose and D-glucose in the molar ratio 12:1. The molar ratios 1.09:8.7:1.0:1.6:0.5, calculated for the derivatives of 2,3,4-tri-*O*-methyl-D-xylose, 2,3- or 3,4-di-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3-*O*-methyl-D-xylose, and D-xylose, respectively, on the basis of methylation analysis data (see Table III), indicated that at least 1 in 11 of the xylosyl residues of the polysaccharide carried non-reducing D-glucopyranosyl residues at O-2 or O-4. The data also suggested that all the D-glucopyranosyl residues and 1 in 10 of the D-xylopyranosyl residues were present as non-reducing end-groups. The identification of the derivatives of 2,3- or 3,4-di-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose in the molar ratio 8.8:1.6 suggested that the backbone was a (1→4)- or (1→2)-linked D-xylan. The methylation analysis data also suggested that at least 1 in 22 of the D-xylopyranosyl residues carried substituents at O-2,3,4. Thus, the acidic xylan ACSBP

TABLE III

METHYLATION ANALYSIS DATA OF THE CARBOXYL-REDUCED ACSBP

Alditol acetate of	T[a] *on DB-1*	*Average molar proportion*	*Characteristic mass fragments* (m/z)	*Mode of linkage*
2,3,4-Me_3-Xyl[b]	0.73	1.1	101, 117, 127, 147 161, 173, 205, 247[d]	D-Xyl*p*-(1→
2,3,4,6-Me_4-Glc	1.0	1.0	101, 117, 129, 139, 145, 161, 171, 205, 263[c]	D-Glc*p*-(1→
2,3- or 3,4-Me_2-Xyl	0.9	0.9	101, 117, 129, 189, 205, 247[c]	→2 or 4)-D-Xyl*p*-(1→
3-Me-Xyl	1.12	1.6	117, 129, 145, 173, 189, 275[c]	→2,4)-D-Xyl*p*-(1→
Xyl	1.27	0.5	103, 115, 129, 145, 158, 171, 187, 217, 227, 291, 303[c]	→2,3,4)-D-Xyl*p*-(1→

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]2,3,4-Me_3-Xyl = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-xylitol, *etc.* [c]The ions are $(M^+ + H) - 60$, obtained by the "Finnigan ion trap" method of ionisation. [d]The ion is $(M^+ + H) - 60$.

seems to have similar types of glycosidic bonds to those in the acidic xylans ACPBP and ACPBS.

It is evident that the acidic xylans ACPBP, ACPBS, and ACSBP each has a (1→2)- or (1→4)-linked D-xylan backbone. However, they differ in their composition with respect to the uronic acid and the degree of substitution of the D-xylosyl residues of the backbone. It is interesting to note that these polysaccharides, which have similar structures, can be precipitated selectively using Cetavlon[8] and barium hydroxide[9].

EXPERIMENTAL

The materials, general methods, and analytical procedures employed have been reported[1].

Isolation of the polysaccharides. — The insoluble residue (5 g), obtained by delignification of the bark of *C. pentandra* var. indica, was extracted with cold water (3 × 100 mL). The residue was extracted at room temperature with aqueous 5% sodium hydroxide (3 × 100 mL) in an inert atmosphere. The extract was neutralised with cold acetic acid at 0°, concentrated, dialysed, and diluted with ethanol (4 vol.), to give the alkali-soluble polysaccharide as a white powder (900 mg).

A solution of the polysaccharide (500 mg) in 0.1M sodium hydroxide (50 mL) was dialysed against water, then diluted to 500 mL with water, and warmed to 40°, and aqueous 5% Cetavlon[8] (25 mL) was added with stirring. The precipitate was collected by centrifugation and dissolved in 4M sodium chloride (50 mL), and the polysaccharide (ACP, 220 mg) was recovered by precipitation with ethanol. The centrifugate was extracted with 1-pentanol, concentrated, and dialysed, and ethanol (4 vol.) was added. The polysaccharide (ACS, 235 mg) was recovered by centrifugation.

An aqueous solution of the ACP (200 mg in 100 mL) was stirred with saturated aqueous barium hydroxide[9] (20 mL). The precipitate was collected by centrifugation and washed with dilute aqueous barium hydroxide, and a suspension in water was acidified with cold dilute acetic acid at ~0°. Ethanol (4 vol.) was added, and the precipitate was collected, suspended in water, dialysed against water, and diluted with ethanol to give the polysaccharide ACPBP (110 mg). The centrifugate and the washings of the precipitate were combined, concentrated, acidified, dialysed, and diluted with ethanol to give the polysaccharide ACPBS (60 mg). Similar fractionation of ACS (200 mg) with saturated aqueous barium hydroxide[9] gave a precipitable fraction (ACSBP, 90 mg) and a non-precipitable fraction (ACSBS, 100 mg).

The sugar compositions of the alkali-soluble polysaccharide fraction and the polysaccharides, ACS, ACP, ACPBP, ACPBS, ACSBP, and ACSBS were determined.

DEAE-Cellulose chromatography[11]. — A solution of ACPBP (100 mg) in

0.1M sodium hydroxide (5 mL) was dialysed and then applied to a column (20 × 2.5 cm) of DEAE-cellulose. The column was eluted first with water (200 mL), then with a linear gradient of 0.1→M sodium chloride (250 + 250 mL). Fractions (10 mL) were collected and monitored by the phenol–sulfuric acid method[2]. The polysaccharide-containing fractions were combined, concentrated, and dialysed, and the polysaccharides were recovered by precipitation with ethanol. A major portion of the polysaccharide (acidic xylan ACPBP) was eluted as a single peak at ~0.2M sodium chloride. A minor acidic fraction (8 mg) was eluted at ~0.9M sodium chloride, and a neutral fraction (7 mg) was eluted with water.

Likewise, ACPBS (100 mg) was chromatographed on a column (20 × 2.5 cm) of DEAE-cellulose. The column was washed with water to give the neutral fraction (5 mg). Elution with 0.5M sodium chloride gave a major acidic fraction (86 mg, acidic xylan ACPBS). Likewise, ACSBP (100 mg) gave a neutral fraction (6 mg) on elution with water, and a major acidic fraction (90 mg, acidic xylan ACSBP) eluted with 0.5M sodium chloride. The sugar compositions of the fractions obtained by DEAE-cellulose chromatography were determined.

Gel-permeation chromatography[12]. — (*a*) *On Sephadex G-200*. Separate solutions of ACPBP, ACPBS, and ACSBP (10 mg of each) in 0.1M sodium hydroxide were dialysed, treated with solid sodium chloride to 0.1M, applied to a column (82 × 2 cm) of Sephadex G-200, and eluted with 0.1M sodium chloride. Fractions (10 mL) were collected, and aliquots were analysed for carbohydrate by the phenol–sulfuric acid method[2]. ACPBP was eluted at the void volume as a single peak, whereas ACPBS and ACSBP were eluted at slightly included volumes (at 130 and 150 mL, respectively; V_0 120 mL).

(*b*) *On Bio-GelA-15m*. ACPBP, ACPBS, and ACSBP (10 mg of each) were chromatographed separately on a column (82.5 × 2.0 cm) of Bio-GelA-15m which was calibrated with dextrans T-2000, T-500, T-40, and T-10 (Pharmacia). The column was eluted with 50mM sodium acetate buffer (pH 5.6) at 15 mL/h. Fractions (10 mL) were collected and monitored by the phenol–sulfuric acid method[2].

Sedimentation analysis[13]. — A 1% solution of ACPBP in 0.1M sodium hydroxide was centrifuged at 25° in a Beckman analytical ultracentrifuge Model E at 59,780 r.p.m. The movement of the boundary was followed using Schlieren optics.

Carboxyl-reduction and methylation analysis. — ACPBP, ACPBS, and ACSBP (100 mg of each) were separately carboxyl-reduced[20] with 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride and sodium borohydride. The sugar compositions of the products were determined after hydrolysis by p.c. and by g.l.c. of the alditol acetates.

Each carboxyl-reduced polysaccharide (10 mg of each) was methylated (Hakomori procedure[21]), the product was hydrolysed, and the resulting partially methylated sugars were converted into their alditol acetates and analysed by g.l.c. and g.l.c.–m.s.[22]. The results are given in Tables I–III.

Partial hydrolysis of ACPBP. — A solution of ACPBP (500 mg) in 0.1M

sodium hydroxide (25 mL) was dialysed, mixed with M trifluoroacetic acid (1 vol.), and heated at 100° for 30 min. The acid was removed under vacuum and a solution of the syrupy residue was passed through columns of Amberlite IR-120(H^+) and IRA-400($HCOO^-$) resins. The eluate and washings were combined and concentrated. P.c. (solvent *A*) of the residue revealed D-xylose and a neutral saccharide which was isolated (15.0 mg) by preparative p.c. (solvent *A*). The Amberlite IRA-400($HCOO^-$) resin was eluted with 2M formic acid and the eluate was concentrated under vacuum. P.c. (solvent *G*) of the residue revealed D-glucuronic acid and a higher acidic saccharide which was isolated (23 mg) by preparative p.c. (solvent *E*). The homogeneity of the neutral and acidic higher saccharides was ascertained by p.c. (solvents *C* and *G*, respectively). The higher saccharides were (*a*) hydrolysed with acid before and after reduction with sodium borohydride followed by p.c.; (*b*) reduced with sodium borohydride and then methylated by the Hakomori procedure[21] (the acidic oligosaccharide was first treated with methanolic 2% hydrogen chloride for 24 h at room temperature, reduced with sodium borohydride, then methylated), and the methylated product was isolated by reversed phase chromatography using Sep-pak C_{18} cartridge[23], hydrolysed with acid, and the products were analysed as their alditol acetates by g.l.c. and g.l.c.–m.s.[21]; and (*c*) reduced with sodium borodeuteride, methylated, and isolated by reversed phase chromatography using Sep-pak C_{18} cartridges[23], and the alditol derivative was subjected to e.i.-m.s.[14]. The higher acidic saccharide was treated with methanolic 2% hydrogen chloride, then carboxyl-reduced with sodium borohydride. The product was hydrolysed with 0.5M sulfuric acid for 4–6 h at 100°, and the products were analysed by p.c. and by g.l.c. of their alditol acetates. Further, the higher acidic saccharide was reduced with sodium borohydride, then treated with methanolic 2% hydrogen chloride, reduced with sodium borohydride, and hydrolysed with acid, and the products were analysed by p.c. and g.l.c. as above.

Oxidations. — (*a*) *With chromium trioxide*[16,17]. Carboxyl-reduced ACPBP (15 mg) was acetylated and the product was oxidised with chromium trioxide (350 mg) in glacial acetic acid (3 mL). The oxidation was followed as described[1].

(*b*) *With periodate*[18]. The native polysaccharide and carboxyl-reduced ACPBP (50 mg of each) were oxidised with sodium periodate as described[1].

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