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STRUCTURAL FEATURES OF THE HEMICELLULOSE A FROM THE STEM OF *Mimosa bracatinga*

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ABSTRACT

The hemicellulose A from the stem of *Mimosa bracatinga* contains residues of D-xylose and 4-*O*-methyl-D-glucuronic acid. Smith-degradation and methylation data indicate it to consist of a backbone of (1 → 4)-linked β-D-xylopyranosyl residues with side chains consisting of single units of 4-*O*-methyl-α-D-glucosyluronic acid attached to position 3. The branched structure was also indicated by Smith-degradation and methylation analysis of the oligosaccharides obtained by enzymic hydrolysis of the hemicellulose.

INTRODUCTION

The hemicellulose A, which has been isolated from various species of woody angiosperms (hardwoods), is known to be a 4-*O*-methylglucuronoxylan. These polysaccharides usually consist[1,2] of a linear framework of at least 70 (1 → 4)-linked β-D-xylopyranosyl residues with an average of every tenth residue substituted at position 2 with a 4-*O*-methyl-α-D-glucopyranosyluronic acid unit. We now report that the hemicellulose A isolated from *Mimosa bracatinga*, an arborescent angiosperm (hardwood) that grows in south Brazil, has a backbone of (1 → 4)-linked β-D-xylopyranosyl residues to which are attached 4-*O*-methyl-α-D-glucopyranosyluronic acid units at position 3.

MATERIALS AND METHODS

General. — Ascending paper chromatography (p.c.) was carried out on Whatman Nos. 1 and 3MM papers with *A*, benzene–1-butanol–pyridine–water (1:5:3:3, upper layer); *B*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); *C*, ethyl acetate–acetic acid–water (18:7:8); and detection with alkaline silver nitrate[3] and *p*-anisidine hydrochloride[4]. Total carbohydrate was determined by the phenol–sulphuric acid method[5], reducing sugar by the Nelson[6] and Somogyi[7] method, and uronic acid by the carbazole method[8] and by titration with standard base[9]. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter at 25°. Acid

hydrolysis was performed with 0.5M trifluoroacetic acid or 0.5M sulphuric acid for 5 h at 100°. Electrophoresis of the dyed polysaccharide on cellulose acetate was carried out according to the procedure of Dudman and Bishop[10]. I.r. spectra were determined for KBr discs using a Beckman IR-18 spectrophotometer. G.l.c. was performed on an F & M chromatograph Model 810R-12 (flame ionization), using helium as carrier gas, with *A*, a column (100 × 0.4 cm) of 14% of LAC-4R-886 on Chromosorb W (80–100 mesh) at 160° (flow rate, 50 ml/min); and *B*, a column (120 × 0.15 cm) of 3% of ECNSS-M on Gas Chrom (100–120 mesh) at 130–180° (flow rate, 10 ml/min). Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside. Column *A* was used for quantitative analysis of methylated sugars. Column *B* was used with temperature programming (130 → 180°, 10°/min) for the determination of molar ratios of Smith-degradation products after conversion into the acetylated alditols. The *T* values of partially methylated sugars [except that of methyl (methyl 2,3,4-tri-*O*-methyl-D-glucosid)uronate] were also compared with those of pure samples, and they agree well with those obtained by Stephen *et al.*[11] (Table I). Peak areas were measured by a triangulation procedure. Acetylation of Smith-degradation products, after conversion into the alditols, was performed with acetic anhydride–70% perchloric acid (14:0.1) for 5 h at 28°.

Extraction of the hemicellulose. — The stem of *Mimosa bracatinga* (obtained from Departamento de Engenharia e Technologia da Madeira do Setor de Ciencias Agrárias da Universidade Federal do Paraná) was ground in a Wiley mill (60–80 mesh). The powdered material was exhaustively extracted (Soxhlet) with hot 2:1 benzene–ethanol for 24 h and with hot water for 24 h. The residue was collected, and washed with ethanol and then acetone. The residue from the previous extraction treatment (500 g) was extracted with 6% aqueous potassium hydroxide by the procedure of Whistler and Feather[12]. The alkaline slurry was vacuum-filtered through cloth, and the residue was washed with water.

The combined extract and washings were immediately cooled in an ice-bath, and hemicellulose A was precipitated by acidification to pH 5 with 50% acetic acid. The precipitate was collected by centrifugation, washed with water (at pH 5), ethanol, and acetone, and then dried to yield crude polysaccharide (50 g).

Hemicellulose B was isolated from the supernatant solution by precipitation with ethanol.

A solution of hemicellulose A (20 g) in M sodium hydroxide (500 ml) was centrifuged, and the supernatant solution was dialysed against running tap water for 48 h, and then poured into ethanol (3 vol.). The precipitate was washed with ethanol (2×) and acetone (2×), and dried *in vacuo* to give the polysaccharide (15 g), $[\alpha]_D^{25}$ −60° (*c* 1, 2M sodium hydroxide) (Found: 4-*O*-methyl-D-glucuronic acid, 5.5; D-xylose, 94.5%). Electrophoresis of the dyed polysaccharide on cellulose acetate indicated the presence of one component.

Periodate oxidation and Smith degradation of the Hemicellulose A. — A sample of the polysaccharide (150 mg) was oxidized in 0.05M sodium metaperiodate (100 ml) in the dark for 168 h at 28–30°. Aliquots (1 ml) were analysed for periodate uptake.

After 168 h, the polysaccharide had consumed 1.07 mol of periodate, and liberated 0.27 mol of formic acid, per mol of "anhydro sugar". The reaction was stopped by the addition of ethylene glycol (1 ml), and the solution was dialysed against running tap water for 24 h. Sodium borohydride (250 mg) was then added and the mixture was kept for 24 h at room temperature. The excess of sodium borohydride was destroyed with acetic acid, and the solution was dialysed against running tap water for 48 h. The resulting polyalcohol was hydrolysed with M sulphuric acid for 5 h at 100°, and the hydrolysate was neutralized with barium carbonate and then treated with sodium borohydride (150 mg) for 12 h. After removal of cations with Dowex 50W-X8 (H^+) resin (200–400 mesh), borate ions were eliminated by successive treatments with methanol and concentration. Analysis of the products by g.l.c. (alditol acetates, column *B*) revealed glycerol (79%), xylitol (12%), and an unidentified compound (8%) which was believed to be a derivative of 4-*O*-methyl-glucuronic acid.

Methylation of the hemicellulose A. — The polysaccharide (1 g) was methylated (5×) by the Haworth procedure[13]. The reaction mixture was neutralized (3M sulphuric acid), salts were removed by dialysis against tap water, and the solution was lyophilized. The dry product was methylated (4×) with tetrahydrofuran–methyl sulphate–sodium hydroxide[14], and then by the Purdie method. A solution of the methylated polysaccharide in chloroform was treated with light petroleum (b.p. 30–60°) and centrifuged. The dry polysaccharide, $[\alpha]_D^{25}$ −50° (*c* 0.8, chloroform), showed no i.r. absorption for hydroxyl. A sample (50 mg) was treated in a sealed tube with 3% methanolic hydrogen chloride (5 ml) for 6 h at 100°. The cooled solution was neutralized with silver carbonate, and the mixture of methyl glycosides was analysed by g.l.c. (column *A*). The *T* values of the products are given in Table I.

Isolation of the aldobiouronic acid after partial hydrolysis of the hemicellulose A with acid. — The polysaccharide (2 g) was treated with 0.5M sulphuric acid (50 ml) for 8 h at 100°. After neutralization with barium carbonate, the hydrolysate was passed through a column of Dowex 50W-X8 (H^+) resin (200–400 mesh), and concentrated. The syrupy residue was applied to a column of Dowex 1-X8 (AcO^-) resin (200–400 mesh), which was washed with water to remove neutral sugar. Acidic components were then eluted with 30% acetic acid, and the eluate was concentrated.

TABLE I

METHANOLYSIS PRODUCTS FROM METHYLATED HEMICELLULOSE A

Product	T[a]		*Relative molar proportion*
Methyl 2,3,4-tri-*O*-methyl-D-xyloside	0.40m	0.59s	4.20
Methyl 2,3-di-*O*-methyl-D-xyloside	1.73m	2.16s	78.30
Methyl (methyl 2,3,4-tri-*O*-methyl-D-glucosid)uronate[b]	2.95m	3.92s	5.00
Methyl 2-*O*-methyl-D-xyloside	6.38	9.23	12.50

[a]Relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside. Key: s, strong; m, moderate. [b]As methyl ester methyl glycoside.

P.c. (solvent *B*) of the syrupy residue revealed components having R_{XYL} 1.35, 0.68 (major), and 0.20. The major component was purified by chromatography on Whatman No. 3MM paper (solvent *B*) to give an aldobiouronic acid, $[\alpha]_D^{25}$ +78° (*c* 0.8, water). The disaccharide (10 mg) was converted into the methyl ester methyl glycoside by treatment with boiling 3% methanolic hydrogen chloride (5 ml) for 8 h. A sample (2 mg) of this product was reduced with sodium borohydride. After deionisation, the product was hydrolysed with 0.5M trifluoroacetic acid for 5 h at 100°. P.c. (solvents *A* and *B*) of the hydrolysate revealed equimolar amounts of 4-*O*-methyl-D-glucose and xylose.

Smith degradation of the aldobiouronic acid. — A sample (5 mg) of the foregoing methyl ester methyl glycoside of the aldobiouronic acid was oxidized with 0.05M sodium metaperiodate (10 mg) in the dark for 120 h at 0–2°. Excess of oxidant was reduced with ethylene glycol (0.1 ml). Sodium borohydride (10 mg) was added, and after 24 h the excess of reductant was decomposed with 2M acetic acid. After deionisation, the product was hydrolysed with 0.5M sulphuric acid for 5 h at 100°, and the hydrolysate was neutralized with barium carbonate. Sodium borohydride (10 mg) was added to the stirred solution during 12 h and the excess of sodium borohydride was decomposed by addition of 2M acetic acid. After deionization, borate was removed by successive treatments with methanol and concentration, and the residue was then subjected to g.l.c. (column *B*) which revealed 2-*O*-methylerythritol and xylitol.

Digestion of hemicellulose A with xylanase of Polyporus circinatus. — A solution of hemicellulose A (1 g) in M sodium hydroxide (200 ml) was acidified to pH 5 with acetic acid, which gave a colloidal suspension to which was added a solution (40 ml) of active fraction α of xylanase (1.4 mg/ml) of *Polyporus circinatus*[16]. The mixture was incubated at 37° with toluene as preservative for 8.5 h, ethanol (3 vol.) was then added, and the residual hemicellulose A was removed by centrifugation. The supernatant solution was concentrated to ~5 ml, and then neutral and acidic sugar fractions were separated by using first a column (10 × 2 cm) of Dowex 50W-X8 (H^+)

TABLE II

NEUTRAL OLIGOSACCHARIDES PRODUCED FROM DIGESTION OF HEMICELLULOSE A WITH XYLANASE OF *Polyporus circinatus*

Fraction[a]	R_{XYL}	*Total sugar*[b] *(μg/ml)*	*Reducing sugar*[c] *(μg/ml)*	*D.p.*	*Glycerol and xylose after Smith degradation*
1	0.89	26.58	22.04	1.20	–
2	0.58	195.00	110.00	1.77	–
3	0.30	245.00	81.00	3.02	+
4	0.16	119.00	29.00	4.10	+
5	0.05	121.00	22.00	5.50	+

[a]Neutral sugars were separated from acidic sugars by treatment with Dowex 1-X8 (AcO^-) resin. Fractions were resolved by preparative paper chromatography in solvent *C*. [b]Phenol–sulphuric acid method.[c] Nelson–Somogyi method.

resin (200–400 mesh) and then a column (20 × 2 cm) of Dowex 1-X8 (AcO^-) resin. The eluate and water washings were concentrated to small volume to give the neutral sugar (oligosaccharide) fraction. The acidic sugars were eluted with 30% acetic acid, and the eluate was concentrated to dryness. The neutral sugar fraction was subjected to preparative p.c. (solvent *C*). The d.p. of each neutral oligosaccharide was measured by the ratio of total carbohydrate[4] to reducing sugar[5]. Each oligosaccharide was determined, after acid hydrolysis, by p.c. (solvent *A*). The results are recorded in Table II.

Smith degradation of the neutral oligosaccharides. — Samples of fractions 3, 4, and 5 of the neutral oligosaccharides (Table II) were oxidized in 0.1M sodium metaperiodate in the dark for 120 h at room temperature. After reduction with sodium borohydride, the solutions were hydrolysed with 0.5M trifluoroacetic acid for 5 h at 100°, and the hydrolysates were again reduced with sodium borohydride. After deionization, the products were analysed as the alditol acetates by g.l.c. (column *B*), which revealed glycerol and xylitol (Table II). Combined fractions 3–5, after oxidation with sodium metaperiodate, followed by reduction with sodium borohydride, were methylated twice with methyl iodide and silver oxide in *N*,*N*-dimethylformamide[17]. G.l.c. (column *A*) of the methanolysis products suggested the presence of methyl 2-*O*- or 3-*O*-methyl-D-xyloside.

RESULTS AND DISCUSSION

The stem of *Mimosa bracatinga* was milled, and extracted sequentially with benzene–ethanol and hot water. The residue was treated with aqueous potassium hydroxide under nitrogen, and hemicellulose A was precipitated from the extract by acidification to pH 5 with acetic acid. Purification was effected by centrifugation of an alkaline solution, dialysis, and precipitations with ethanol which gave a polysaccharide that was homogeneous by electrophoresis.

The polysaccharide contained D-xylose (94.5%) and 4-*O*-methyl-D-glucuronic acid (5.5%), and the $[\alpha]_D^{25}$ values (−60° and −50°, respectively) of the polysaccharide and the methylated derivative are indicative of β-linkages.

The hemicellulose was methylated, and the product was fractionally precipitated from solution in chloroform by the addition of light petroleum to give one main fraction which showed no i.r. absorption for hydroxyl. Methanolysis of this fraction and g.l.c. of the products (Table I) revealed the methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylose (4.20%), 2,3-di-*O*-methyl-D-xylose (78.30%), 2-*O*-methyl-D-xylose (12.50%), and methyl 2,3,4-tri-*O*-methyl-D-glucuronate (5.00%). The methylation data allow the proposal of a partial structure for this hemicellulose, involving (1 → 4)-linked β-D-xylopyranosyl residues with 4-*O*-methyl-D-glucuronic acid attached as single, terminal side-chains to the xylose residues at position 3. However, in contrast to linear glucuronoxylans from other angiosperms (hardwoods)[1,2], the present hemicellulose is branched.

Periodate-oxidation and Smith-degradation data corroborated the structure

proposed from the methylation data. The polysaccharide consumed 1.07 mol of periodate, and released 0.27 mol of formic acid, per mol of "anhydropentose" units. The relatively large amount of formic acid produced indicated a considerable proportion of end groups. The results of Smith degradation (glycerol, 79.20%; xylose, 12.30%; unidentified components, 8.5%) are in good agreement with those from methylation analysis. The linkage between the 4-*O*-methyl-D-glucuronic acid and the D-xylosyl residue was established by examination of the aldobiouronic acid obtained by partial hydrolysis of the hemicellulose with acid. The $[\alpha]_D^{25}$ value (+78°) of the aldobiouronic acid indicated an α-configuration for the glycosidic bond, and the constituent sugars were determined by reduction of the methyl ester methyl glycoside with sodium borohydride followed by acid hydrolysis, which gave equimolar proportions of D-xylose and 4-*O*-methyl-D-glucose.

Smith degradation of the methyl ester methyl glycoside of the aldobiouronic acid gave 2-*O*-methylerythritol and xylitol (g.l.c. of alditol acetates). Therefore, the aldobiouronic acid was 3-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

Further evidence for a branched chain in this hemicellulose was provided on examination of the neutral oligosaccharides obtained by enzymic hydrolysis with a purified xylanase from *Polyporus circinatus*[16]. The xylanase effected random hydrolysis, producing neutral and acidic oligosaccharides and a resistant polysaccharide. The neutral sugars were fractionated by paper chromatography to give the products in Table II. On the basis of Smith-degradation data and g.l.c. of alditol acetates, the neutral oligosaccharides 3–5 were probably formed by branched molecules, as they contained xylose residues that were resistant to periodate oxidation. Detection (g.l.c.) of 2-*O*-methyl-D-xylose following methanolysis of the methylated, Smith-degraded oligosaccharides confirmed the presence of branching points at C-3 of the D-xylosyl residues.

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