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Note

Analysis of water-soluble polysaccharides from unretted jute plant *(Corchorus capsularis)*

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Alkali-soluble hemicelluloses from both retted[1–5] and unretted[6,7] jute bark and stem have been studied. However, there has been no report of water-soluble hemicellulose from jute samples. We now report the isolation, fractionation, and structural studies of different polysaccharide fractions obtained by extraction of unretted jute bark and stem (*Corchorus capsularis*) with water.

The dried and powdered bark and stem from unretted jute (*C. capsularis*) were first extracted with refluxing aqueous 80% ethanol followed by extraction with hot chloroform (Scheme 1) in order to remove all low-molecular-weight material[8], waxes, lipids, *etc.* Extraction with water at room temperature followed by dialysis gave the dialysable and non-dialysable components. The bark contained ~5% and the stem ~2% of water-soluble material (Table I), of which the non-dialysable part was the highest in amount.

P.c. revealed no free sugar (or lower oligosaccharides) in the dialysable fractions (*A* from bark and *R* from stem in Table I). The dialysable fractions contained small proportions of uronic acid residues and hence were not pectins. G.l.c. of the alditol acetates derived from the products of acid hydrolysis showed that the dialysable fractions from both the bark and the stem were rich in glucose, galactose, and mannose. There was also a small proportion of xylose and higher proportions of arabinose and rhamnose.

The non-dialysable fractions (*B* and *S* from bark and stem, respectively) were rich in galactose, glucose, and arabinose (Table I) and also contained significant proportions of mannose and rhamnose. Fraction *S* contained a small proportion of rhamnose and a rather high proportion of xylose, which, together with ~10% of uronic acid, indicated that some xylan was extracted from the stem.

The uronic acid content of fraction *B* was quite high. Carboxyl-reduction[9] of

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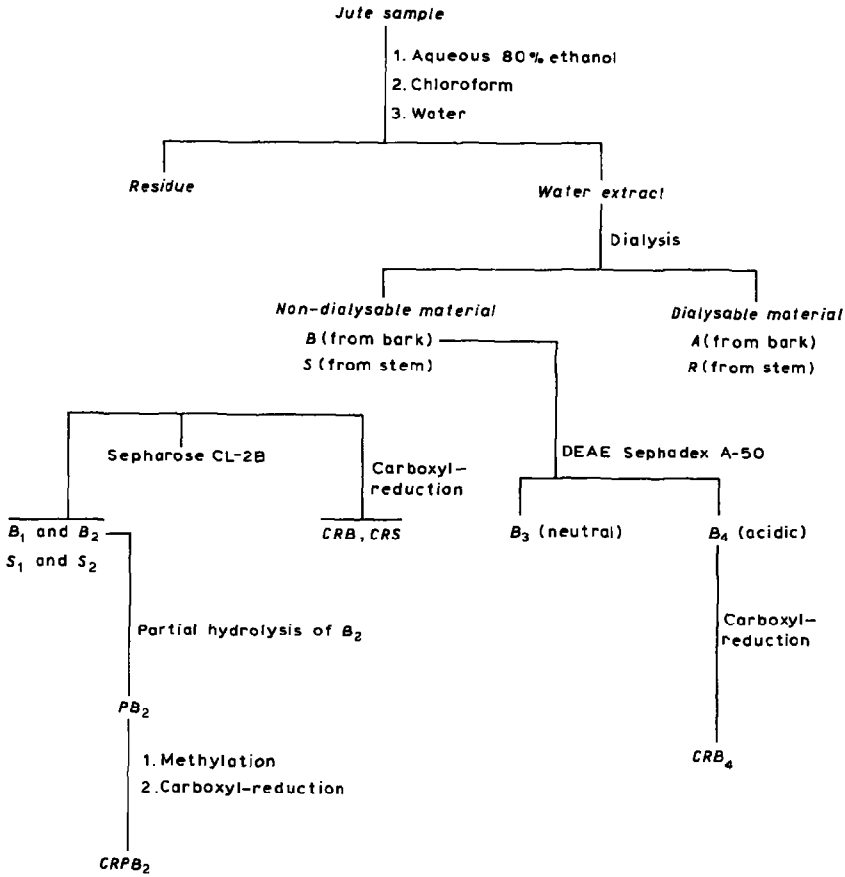

Scheme 1. Extraction and fractionation steps (see Experimental for details).

fractions *B* and *S* followed by sugar analysis of the products (*CRB* and *CRS*, respectively) indicated that the bark extract was rich in galacturonic acid and that the stem extract was rich in glucuronic acid. Paper electrophoresis of the hydrolysates of the original fractions also confirmed this finding.

Chromatography of fraction *B* on DEAE-Sephadex A-50 gave an apparently neutral fraction B_3 which contained >50% of glucose. The acidic fraction B_4 was enriched in rhamnose (Table I). Carboxyl-reduction of fraction B_4 enriched the glucose content, indicating the presence, originally, of glucuronic acid, although fraction *B* contained only a small proportion of glucuronic acid (paper electrophoresis). The recovery from the ion-exchange column was <50%, and it is possible that the material rich in galacturonic acids remained in the fractions that were not collected.

Fractionation of *B* on Sepharose CL-2B gave two main fractions, namely, B_1,

TABLE I

RELATIVE COMPOSITION[a] OF THE POLYSACCHARIDE CONSTITUENTS IN THE WATER-SOLUBLE POLYSACCHARIDE FRACTIONS (%)

Water-soluble polysaccharide fraction	*Yield*[b]	*Composition of neutral sugars in the hydrolysates*						*Uronic acid*
		Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	
A[c]	1.9	7.5	10.2	5.5	19.6	27.0	30.2	3.2
B	3.1	8.5	21.7	6.6	5.8	34.2	23.2	23.6
CRB	—	7.0	15.1	4.1	3.2	46.7	23.9	—
R	0.6	7.9	12.3	1.2	10.9	31.3	36.4	2.0
S	1.4	2.6	13.6	11.9	8.5	46.6	16.8	10.0
CRS	—	3.2	10.9	6.3	9.0	28.5	42.1	—
B_1	0.5	31.1	4.2	trace	6.7	12.6	45.2	—
B_2	1.7	trace	27.7	12.3	5.2	44.4	10.2	5.0
PB_2	—	2.7	trace	4.3	8.3	63.4	21.3	—
B_3	0.6	10.2	7.0	12.0	5.0	10.6	53.1	—
B_4	0.9	24.9	4.9	3.0	5.2	31.3	30.9	—
CRB_4	—	10.8	3.9	2.5	4.3	28.1	50.3	—
S_1	0.3	8.7	4.6	2.6	12.1	14.9	56.9	—
S_2	0.6	5.6	12.6	25.6	5.8	42.1	8.3	—

[a]Average of two replicate analyses. [b]Percent of dry jute sample. [c]See Scheme 1 for the origin of the samples.

which was rich in rhamnose and glucose, and B_2, which was rich in arabinose and galactose. The two fractions combined constituted ~70% of *B*. As the proportion of fraction B_2 was by much the highest (Table I), this fraction was used for further study.

The ^{1}H-n.m.r. spectrum of fraction B_2 in D_2O showed three peaks for anomeric protons, which could be assigned to α-arabinose, β-galactose, and β-arabinose and or α-galactose. There were also weak signals for acetyl groups. The ^{13}C-n.m.r. spectrum contained signals for C-6 of uronic acid and for four anomeric carbons (see Experimental), of which one could be assigned to C-1 of 3-linked glucose. The main anomeric peak was assigned to 4-linked galactose.

The molecular weight of fraction B_2 was estimated to be ~70,000 by passing it through a Sepharose CL-2B column (calibrated with dextran T-series). The negative optical rotation indicated a preponderance of β linkages in the polymer.

As the methylation analysis of *CRB* and *CRS* indicated the presence of substantial proportions of terminal arabinose (Table II), fraction *B* was partially hydrolysed with 25mM H_2SO_4 at 96° for 2 h, which cleaved arabinose and left a fraction (PB_2) that was enriched in galactose and glucose. Methylation analysis of PB_2 (Table II) revealed 4-linked galactose, 3- and 6-linked glucose, and terminal

TABLE II

METHYLATION ANALYSIS OF DIFFERENT WATER-SOLUBLE POLYSACCHARIDE FRACTIONS (RELATIVE MOL %)

Partially methylated sugar	*Polysaccharide fraction*			
	CRB	CRS	B_2	$CRPB_2$
2,3,5-Tri-*O*-methylarabinose	21.5	14.5	12.1	—
2,3-Di-*O*-methylarabinose	4.9	2.5	1.4	—
2,3,4-Tri-*O*-methylxylose	3.4	4.8	0.7	2.2
2,3-Di-*O*-methylxylose	10.4	27.4	9.0	22.6
3-*O*-Methylxylose	3.7	6.3	1.2	7.6
2,3,4,6-Tetra-*O*-methylglucose	2.2	6.7	trace	10.9
2,3,4-Tri-*O*-methylglucose	1.9	6.7	—	7.4
2,4,6-Tri-*O*-methylglucose	4.6	6.6	20.0	9.6
2,3,4,6-Tetra-*O*-methylgalactose	11.5	5.6	3.6	16.7
2,3,6-Tri-*O*-methylgalactose	30.0	15.4	44.6	18.6
2,6-Di-*O*-methylgalactose	4.1	2.1	5.4	—
2,3-Di-*O*-methylgalactose	2.0	1.5	1.6	4.4

glucose and galactose residues, much of the last two probably coming from uronic acid residues. The formation of 2,3-di-*O*-methylgalactose indicated C-6 branches through uronic acid residues, because these sugars were not cleaved by partial hydrolysis. The high negative optical rotation of PB_2 indicated the main-chain linkages to be mainly β.

The foregoing results indicate the presence of a complex mixture of polymers in the water-soluble polysaccharides isolated from the jute bark and stem. Substantial amounts of xylans (usually extracted with alkali[7]) were also extracted with water (most from the stem). The major polymer extracted from bark was a branched arabinogalactan containing mainly a (1→4)-linked β-galactan backbone with a terminal arabinofuranosyl group and mono- and oligo-meric branches of arabinofuranose residues. Glucuronic acid and xylose residues may be present also as further branches.

EXPERIMENTAL

Plant material. — Jute plants were collected from fields in the Jessore area of Bangladesh in the season of 1983. Bark and stem (top part, root, and leaf rejected) were separated manually by peeling, cut into small pieces, and sun-dried. The dried materials were powdered (jute sample) in a Wiley mill with 1-mm screen.

General methods. — All evaporations were carried out under reduced pressure at $\gtrless 40°$ (bath). G.l.c. was performed on a Packard 427 instrument equipped with a flame-ionisation detector and a quartz capillary column (12.5 m × 0.2 mm i.d.). Separations were performed on OV-275 (alditol acetates), OV-225

(partially methylated alditol acetates), and CP sil 88 (alditol and partially methylated alditol acetates) WCOT columns. Peak areas were determined with a Hewlett–Packard 3390A integrator. G.l.c.–m.s. was performed on a Finnigan 4021 instrument. N.m.r. spectra (^1H, 90 MHz; ^{13}C, 15.14 MHz) were recorded at 35° with a JEOL FX 90Q instrument on solutions in D_2O (^{13}C, internal Me_4Si; ^1H, internal sodium 3-trimethylsilyl[$^2H_4$]propionate). Optical rotations were determined with a Perkin–Elmer 141 instrument. Unless otherwise stated, uronic acids were determined by a decarboxylation method[10]. Paper electrophoresis was performed on Whatman No. 1 paper, using 0.1M sodium acetate buffer (pH 4.5) at 1500 V for 2 h with detection by *p*-anisidine hydrochloride. P.c. was performed with ethyl acetate–acetic acid–water (3:1:1).

Extraction. — Unretted jute bark (150 g) was extracted by refluxing with aqueous 80% ethanol (3 × 1.5 L, 30 min each time) followed by chloroform (3 × 750 mL, 30 min each time). The residue was then stirred with water (2 × 1 L, 10 h each time) at room temperature. The extract was filtered, concentrated (to ~200 mL), and dialysed (4 × 4 L, 12 h each time). All the diffusates were combined, concentrated, and freeze-dried, to give *A* (2.85 g, 1.9%). The aqueous solution in the dialysis tubing was also concentrated and freeze-dried, to give *B* (4.65 g, 3.1%). The extraction and fractionation procedure is outlined in Scheme 1.

Unretted jute stem (150 g) was also extracted by the above procedure, to give the dialysable (*R*, 0.9 g, 0.6%) and non-dialysable part (*S*, 2.11 g, 1.4%).

P.c. of *A* and *R* revealed no mono-, di-, or lower oligosaccharides.

Analysis of fractions A, B, R, *and* S. — Each fraction (5 mg) was hydrolysed with 0.5M trifluoroacetic acid (2 mL) at 96° for 16 h with *myo*-inositol (1 mg) as internal standard. The resulting sugars were converted into their alditol acetates[11] and analysed[12] by g.l.c. (Table I). The uronic acid content of each sample was also determined (Table I). The hydrolysates from fractions *B* and *S* were concentrated separately to small volume with added water, and analysed by paper electrophoresis.

Carboxyl-reduction and methylation analysis of fractions B *and* S. — Fraction *B* (30 mg) was de-esterified by treatment[13] with aqueous sodium hydroxide (pH 12.0) at 0° for 2 h. The solution was neutralised (pH 7.0), dialysed, and freeze-dried. The de-esterified material (25 mg) was reduced[9] (→*CRB*) and hydrolysed (0.5M trifluoroacetic acid), and the resulting neutral sugars were analysed as their alditol acetates by g.l.c. (Table I).

Fraction *CRB* (5 mg) was methylated first by the Haworth method and then by the Hakomori method. The product was hydrolysed and the resulting partially methylated sugars were analysed[14,15] as their alditol acetates by g.l.c. and g.l.c.–m.s. (Table II).

Fraction *S* (30 mg) was also de-esterified and reduced (→*CRS*). Sugar and methylation analysis results of fraction *CRS* are given in Tables I and II, respectively.

Ion-exchange chromatography. — A solution of fraction *B* (500 mg) in 0.1M

phosphate buffer (20 mL, pH 6.0) was applied to a column (100 × 2 cm) of DEAE-Sephadex A-50. The column was eluted at room temperature with the same buffer (1 L) and in sequence with the buffer containing 0.1 (200 mL), 0.5 (200 mL), and 1M (600 mL) NaCl. The fractionation was monitored by the phenol–sulphuric acid method[16]. A neutral fraction (B_3, 100 mg) and an acidic fraction (B_4, 150 mg) were collected by dialysis and freeze-drying. Fraction B_4 was reduced[9] ($\rightarrow CRB_4$). Sugar analyses of the fractions B_3, B_4, and CRB_4 are given in Table I.

Gel-filtration. — A solution of fraction *B* (200 mg) in 0.1M phosphate buffer (10 mL, pH 7.0) containing 0.02% of NaN_3 was applied to a column (100 × 2 cm) of Sepharose CL-2B and eluted with the same buffer solution. The fractionation was monitored by the phenol–sulphuric acid method. Two main fractions (B_1, 30 mg; and B_2, 100 mg) were collected by dialysis of the appropriate combined fractions and freeze-drying.

Fraction *S* (200 mg) was fractionated by following the same procedure, to give mainly fractions S_1 (33 mg) and S_2 (67 mg). Sugar analyses of B_1, B_2, S_1, and S_2 are given in Table I.

Characterisation of fraction B_2. — The ^{1}H-n.m.r. spectrum (D_2O) of B_2 gave three peaks for anomeric protons at δ 4.51 (β-gal), 5.23 (α-ara, β-gal or β-ara, α-gal), and 5.35 (α-ara) with $J_{1,2}$ values of 1, 4, and 3 Hz, respectively. The ^{13}C-n.m.r. spectrum contained peaks at δ 176.83, 110.67, 109.08, 108.37, 103.77, 100.52, 86.57, 85.48, 82.72, 73.49, 69.64, 64.01, and 62.63. Fraction B_2 contained 5% of uronic acid (carbazole method) and had $[\alpha]_{578}^{20}$ −20° (*c* 0.4, water).

The mol. wt. of B_2, estimated by passage (in 0.1M phosphate buffer) through a column of Sepharose CL-2B calibrated with dextran T-series (Pharmacia), was ~70,000.

Fraction B_2 (5 mg) was methylated by the Hakomori method, the product was hydrolysed, and the resulting partially methylated sugars were analysed as their alditol acetates by g.l.c. and g.l.c.–m.s.

Partial hydrolysis of fraction B_2. — A solution of B_2 (20 mg) in 25mM H_2SO_4 (10 mL, pH 2.3) was kept at 96° for 2 h. Neutralisation (pH 7.0) with 0.1M NaOH, dialysis, and freeze-drying then gave the partially hydrolysed product (PB_2, 12 mg), $[\alpha]_{578}^{20}$ −120° (*c* 0.4, water).

PB_2 (1 mg) was hydrolysed with 0.5M trifluoroacetic acid (1 mL) at 96° for 16 h with *myo*-inositol (0.2 mg) as internal standard. The resulting sugars were analysed as their alditol acetates by g.l.c. (Table I).

Fraction PB_2 (5 mg) was methylated by the Hakomori method. The methylated product was reduced with $LiBH_4$ in tetrahydrofuran ($CRPB_2$) and then hydrolysed, and the resulting partially methylated sugars were reduced with $NaBD_4$, acetylated, and analysed by g.l.c. and g.l.c.–m.s. (Table II).

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