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LIGNIN–XYLAN ESTER LINKAGE IN JUTE FIBER (*Corchorus capsularis*)

NARENDRA NATH DAS*, SANKAR CHANDRA DAS, ADITYA SEKHAR DUTT, AND ASHIMANANDA ROY
Applied Chemistry Division, Indian Jute Industries' Research Association, 17 Taratola Road, Calcutta-700088 (India)

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ABSTRACT

Treatment of jute fiber (I) with aqueous potassium borohydride afforded an off-white fiber (II) (containing 77% of the original lignin) and a water-soluble, lignin–carbohydrate complex (III). On hydrolysis, both II and III furnished a new sugar, *viz.*, 4-*O*-methyl-D-glucose, in admixture with the same acidic and neutral sugar components as those of I. Alkaline extraction of holocelluloses prepared from I and II afforded crude D-xylans which, on fractionation with aqueous barium hydroxide, yielded two enriched D-xylans, containing respectively, 15.9 and 10.5 units of uronic acid side-chains per 100 xylosyl residues. These results indicate that ~34% of the acidic side-chains of the D-xylan in I are linked to lignin by an ester linkage.

INTRODUCTION

The hemicelluloses in jute fiber comprise an intricate mixture of polysaccharides, of which D-xylan is the major constituent[1]. The backbone of the D-xylan is made up of (1→4)-linked D-xylopyranosyl residues, every seventh unit of which carries a side chain of 4-*O*-methyl-α-D-glucopyranosyluronic acid attached glycosidically to the main chain through[2–7] O-2. On the basis of circumstantial evidence[8–10], it was suggested that some of these uronic acid residues are linked to lignin *via* ester linkages. This finding was based on the observation[9] that the acid value of jute fiber, arising from the acidic side-chains on the D-xylan, increases linearly on progressive delignification of the fiber with sodium chlorite. The supposition is, however, questionable, because it is unlikely that the delignifying reagent would cleave the ester bonds. In fact, polysaccharide preparations containing ester linkages in the form of *O*-acetyl groups may be extracted from chlorite-treated, jute fiber[11], or wood[12].

Additional conflicting evidence came from studies on lignin model-compounds[13], which showed that these compounds produce various carboxylic acids on oxidation with chlorine dioxide, one of the oxidants[13] used in the chlorite-delignification reaction. The behavior[10] of jute fiber towards dilute alkali, however, points to the presence of an ester linkage in it, but the evidence is inconclusive for establishing an

*To whom enquiries should be directed.

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ester linkage between the lignin and the xylan. It may be relevant that, in some plants, the ester linkages occur in the lignin portion itself[14-16]. Considering all of these aspects, it was deemed necessary to reinvestigate the exact nature of the ester linkages present in jute fiber. Direct evidence indicating the existence of an ester linkage between the lignin and the D-xylan is now reported.

EXPERIMENTAL

Materials and methods. — The reagents and solvents employed in the experiments were all of A.R. grade, and the latter were distilled before use. Unless mentioned otherwise, the evaporation of water or organic solvents was conducted at 35–40°, under diminished pressure. Paper, partition chromatography (hereafter, chromatography) was performed by the descending technique, using Whatman No. 1 papers for qualitative tests, and Whatman No. 3MM papers for the separation of sugar mixtures on a preparative scale. The papers were irrigated by using the following solvent mixtures (v/v): (*A*) 8:2:1 ethyl acetate–pyridine–water; and (*B*) 10:1:2 1-butanol–ethanol–water. The chromatograms were developed by staining with (*a*) alkaline silver nitrate[17], or (*b*) aniline hydrogenoxalate[18].

The fiber strands (~2.5 m long) were collected from medium-quality, white jute (*Corchorus capsularis*; grade W_5 as specified[19] by ISI). The root and top ends (~60 cm) of these strands were rejected, and the middle portion of each was cut into smaller pieces (2–3 cm long), pulverized in a Wiley mill, and then dewaxed by extraction with 1:2 (v/v) ethanol–benzene in a Soxhlet apparatus for 12 h. The resulting fiber (termed as control fiber, CJ) was air-dried, and used as the starting material in the present investigation.

Borohydride-reduced, and untreated, jute fibers were delignified[20] by treatment of them with aqueous sodium chlorite (0.7%) in sodium acetate–acetic acid buffer (0.1M, pH 4.0, fiber to liquor ratio, 1:50) for 5 h at 75 ±1°.

Alditol acetate derivatives of neutral monosaccharides were prepared according to Lindahl's method[21].

Hydrolyzates of the fibers or polysaccharides were prepared by the following methods. (*A*) The fiber, or oligo- or poly-saccharide, sample was heated with 0.5M sulfuric acid on a boiling-water bath, and the hydrolyzate, obtained by filtration of the reaction mixture through a Gooch crucible (porosity G-4), was treated in the usual way for performing chromatographic tests. (*B*) A polysaccharide sample (~5 mg) was partially hydrolyzed by treatment overnight at 5–10° with 72% sulfuric acid (0.5 mL). The resulting solution was diluted ten-fold, the acid neutralized with ice-cold, aqueous sodium hydroxide (4M), and the final volume made up to 25.0 mL. This solution was used for the estimation of the constituent monosaccharides. (*C*) The polysaccharide sample was treated with sulfuric acid (72%) as in method *B*, and the solution was heated on a boiling-water bath after 24-fold dilution with water.

Total hexoses and pentoses were determined by L-cysteine–H_2SO_4 methods[22,23]. The apparent content of uronic acid was estimated by the Bitter–Muir method[24],

using D-glucuronic acid as the standard. The amount of D-glucuronic acid that would produce the same degree of color as that given by the proportion of hexoses and pentoses present in a hydrolyzate was determined in a separate experiment by preparing calibration curves, using D-glucose, D-xylose, and D-glucuronic acid. This amount was deducted from the apparent uronic acid content, to obtain the exact proportion of uronic acid in the hydrolyzate.

The *O*-acetyl content of the fibers was determined according to the method of Macmillan and Sen Gupta[25]. Lignin (Klason lignin) was estimated gravimetrically[26] by dissolving the carbohydrate constituents of the fiber with sulfuric acid (72%) at 2–4°.

The specific rotations of the sugars at equilibrium were determined with a Perkin–Elmer Spectropolarimeter, model 241. Spectrophotometric measurements in the u.v. and visible regions were conducted with a Hitachi Spectrophotometer, model 200-20. G.l.c. of neutral sugars (as their alditol acetate derivatives) was performed, isothermally at 195°, in a glass column (1.83 m × 3.2 mm) containing 1% of OV-225, supported on SIL.RUB. (80–100 mesh), using a Hewlett–Packard Gas Chromatograph, model 5730 A, equipped with f.i.d. and a recorder, model 7127, nitrogen being used as the carrier gas.

Reduction of jute fiber with aqueous potassium borohydride. — The control fiber (CJ; ~10 g), containing 3.57% of *O*-acetyl groups, was soaked in aqueous potassium borohydride (M; fiber to liquor ratio, 1:15) for 24 h at room temperature. The initial pH of the solution was found to be 8.0, and this increased to 10.0 after 24 h. The suspended fibers were filtered through a Nylon cloth, squeezed well, and the residue washed with distilled water (2 × 200 mL). The filtrate and washings were pooled, to afford solution S_1, which was set apart for further treatments. The reduced fiber was treated with 1% oxalic acid (200 mL) for 1 h, filtered, and then rewashed with water until the washings were neutral to Methyl Red. The resulting, creamy white fiber (RJ-1), after air-drying, was found to contain 0.84% of *O*-acetyl. Two successive treatments of RJ-1 with aqueous borohydride afforded an off-white fiber (RJ-3). The fibers obtained after the second and third borohydride treatments of CJ contained 0.33 and 0.00% of *O*-acetyl, respectively. The weight loss encountered during these treatments was determined in a separate experiment, using 1 g of CJ (in duplicate). Reduced fiber (RJ-3) was prepared in two more batches, exactly as already described, using 10 g of CJ in each experiment.

The solution S_1 (obtained earlier) was decationized with Dowex-50 X-8 (H^+) resin, the suspension filtered (glass wool), and the filtrate centrifuged at 4° (12,000 r.p.m., 40 min). The clear, supernatant liquor was dialyzed, concentrated to ~40 mL, and then lyophilized, to obtain a lignin–carbohydrate complex (LCC) as a pinkish-white powder; yield 297.3 mg. A portion (1.0 mL) of an aqueous solution of LCC (~5 mg/mL) was tested for lignin with a drop of phloroglucinol–hydrochloric acid reagent[27], which responded negatively. Another solution S_2 (~0.02%) of LCC was diluted ten-fold, to afford solution S_3, which responded positively to the characteristic test for carbohydrates with 1-naphthol–H_2SO_4 reagent[28]. The u.v. spectrum of S_3

against water as the blank showed a peak at 274 nm ($E^{1\,cm}_{1\%}$ 29.5). This peak disappeared, with the appearance of new ones having absorption maxima at 248 and 300 nm, when the spectrum of solution S_4 [containing S_2 (2.5 mL), aqueous sodium hydroxide (M; 2.5 mL) and water (20.0 mL)] was recorded against S_3 as the reference solution.

Preparation of 4-O-methyl-D-glucose. — This compound was prepared by conversion of 4-*O*-methyl-D-glucuronic acid (obtained as oligouronic acids by hydrolyzing CJ for 10 h by method *A*) to the corresponding, neutral sugar. Fractionation of the hydrolyzate containing the uronic acid on a column of Dowex-1 X-8 (formate; 100–200 mesh) afforded a neutral (f_1) and an acidic (f_2) fraction by using water and formic acid (0.5M) as the respective eluant. Fraction f_2 was esterified by boiling its solution (0.5%) in absolute methanol under reflux in the presence of Dowex-50 X-8 (H^+) resin (100–200 mesh) for 20 h. The mixture was cooled, and filtered, and the filtrate evaporated to a syrup. The residue (containing the esters) was reduced with aqueous potassium borohydride[29], to afford, after the usual treatment, a mixture of neutral (f_3) and acidic (f_4) sugar "appendages" as their

TABLE I

CHEMICAL COMPOSITION OF CONTROL (CJ) AND REDUCED (RJ-3) FIBERS, AND LIGNIN–CARBOHYDRATE COMPLEX (LCC)

Sample	*Sugars detected*[a]	*Composition (%)*[b]		
		Ash	*Lignin*	*O-Acetyl*
Control fiber (CJ)	Galactose Glucose Mannose Arabinose Xylose Rhamnose	0.97	14.34	3.57
Reduced fiber (RJ-3)	Galactose Glucose Mannose Arabinose Xylose Rhamnose 4-*O*-Methylglucose[c]	0.29	11.07	0.00
Lignin–carbohydrate complex (LCC)	Galactose Glucose Mannose Arabinose Xylose Rhamnose 4-*O*-Methylglucose	—	—	—

[a]By chromatography, solvents *A* and *B*, staining reagents (*a*) and (*b*); a series of oligosaccharides appeared above galactose in all of the chromatograms. [b]The values are expressed on o.d. weight of control fiber (CJ). [c]Identified also by g.l.c.

methyl glycosides. On hydrolysis (method *A*, 6 h) and chromatography (solvent *A* and staining reagents *a* and *b*), fraction f_3 (separated from fraction f_4 by column chromatography as already described) furnished xylose and 4-*O*-methylglucose (R_{Glc} 1.72 in solvent *A*) as the sole products. The latter sugar was obtained as a syrup (AMG) by preparative chromatography (solvent *B*) of the hydrolyzate. AMG had $[\alpha]_{589.5}$ +60.4° (*c* 1.0, water); lit.[30] +59.0°.

Identification of 4-O-methyl-D-glucose in reduced, jute fiber (RJ-3) and lignin–carbohydrate complex (LCC). — The control (CJ; 0.2 g) and reduced (RJ-3; 0.5 g) jute fibers, and LCC (~5 mg) were separately hydrolyzed (method *A*, 10 h), and the respective hydrolyzates (h_1, h_2, and h_3) were evaporated to dryness. Chromatographic examination (solvents *A* and *B*, and staining reagents *a* and *b*) showed the presence of several neutral sugars, *viz.*, galactose, glucose, mannose, arabinose, xylose, and rhamnose, along with a few oligosaccharides appearing above galactose in h_1, and

TABLE II

ANALYSIS OF POLYSACCHARIDE FRACTIONS A_1, A_2, A_3, AND A_4

Polysaccharide fraction	*Sugars detected*[a]	*Relative proportions*[b]	*Composition (%)*				*Number of uronic acid residues per 100 xylosyl residues*
			Ash	*Uronic acids*[c]	*Total pentose*	*Total hexose*	
A_1	Galactose	++					
	Glucose	++					
	Mannose	++	4.9	12.2	63.4	7.6	14.4
	Xylose	+++					
	Arabinose	++					
	Rhamnose	++					
A_2	Galactose	+					
	Glucose	+	4.8	15.8	74.7	traces	15.9
	Mannose	+					
	Xylose	+++					
A_3	Galactose	++					
	Glucose	++					
	Mannose	++					
	Xylose	+++	2.6	8.5	60.2	14.5	10.7
	Arabinose	+					
	Rhamnose	+					
	4-*O*-Methylglucose	++					
A_4	Galactose	+					
	Glucose	+					
	Mannose	+	0.4	10.6	76.0	10.7	10.5
	Xylose	+++					
	4-*O*-Methylglucose	++					

[a]By chromatography (solvent *A*, staining reagent *a*); a series of oligosaccharides appeared above galactose in all of the chromatograms. [b]+++, Major; ++, medium; and +, traces. [c]The values are corrected for interference due to hexoses and pentoses (*vide* Experimental section).

a new sugar (EMG) in h_2 and h_3 (in addition to those found in h_1). The last-named sugar had an identical chromatographic mobility (R_{Glc} 1.75 and 2.67 in solvents *A* and *B*, respectively) as that of AMG. Both EMG and AMG took yellow stains with staining reagent *b*. On heating with hydrobromic acid[31], followed by chromatography (solvent *A*, and staining reagents *a* and *b*), a portion (~1 mg) of a homogeneous preparation of EMG {$[\alpha]_{589.5}$ +61.3° (*c* 0.6, water)}, obtained by preparative chromatography (solvent *B*) of h_2, gave a mixture of glucose and the unreacted sugar. G.l.c. of another portion (~100 μg) of EMG as the alditol acetate derivative, furnished a peak corresponding to 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl-D-glucitol (prepared from AMG), which had a retention time of 0.94 and 6.51, respectively, relative to hexa-*O*-acetyl-D-glucitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity.

Isolation of D-*xylan from the control and from reduced, jute fibers, and fractionation of the crude polysaccharides by complexing with aqueous barium hydroxide*[32]. — The control fiber (~5 g) was delignified with sodium chlorite, and the hemicelluloses in it were isolated by extraction of the holocellulose, under nitrogen, with aqueous sodium hydroxide (2.5M, fiber to liquor ratio, 1:50, 1 h, 20–25°). The extract was centrifuged at 4° (12,000 r.p.m., 40 min), and the clear supernatant liquor, acidified to pH 4.5 with ice-cold acetic acid (6M), was dialyzed extensively against distilled water. A small amount of polysaccharide, appearing as a flocculent precipitate during dialysis, was removed by centrifugation. Precipitation of the polysaccharides in the dialyzed solution (adjusted to pH 4.5 with HOAc) with 95% ethanol (2 vol.) afforded a polysaccharide fraction A_1 (0.73 g). The hexosans in A_1, comprising residues of galactose, glucose, and mannose (detected chromatographically in solvent *A*; *vide* Table II), were precipitated as the barium complexes by treatment of an alkaline solution of the polysaccharide mixture (~0.5 g) with baryta[32]. Dialysis of the acidified (pH 4.5; HOAc), supernatant liquor, and precipitation of its contents with ethanol (2 vol.) furnished a polysaccharide fraction that, after another treatment with barium hydroxide, yielded an enriched D-xylan (polysaccharide A_2; 0.31 g), containing traces of hexoses. The reduced fiber (RJ-3; ~3 g) was delignified, and then extracted with alkali as described earlier, to afford a crude D-xylan (polysaccharide A_3; 0.56 g). This fraction (~0.5 g), on subsequent purification by using aqueous barium hydroxide, yielded polysaccharide A_4 (0.30 g). The polysaccharides A_1–A_4 were hydrolyzed by method *C* (8 h) for performing chromatographic tests, which revealed that fractions A_2 and A_4 contained xylose as the only pentose.

RESULTS AND DISCUSSIONS

The fiber cuttings obtained from the middle portion of strands of jute fiber (*Corchorus capsularis*, or White jute) Grade[19] W_5, were pulverized, and then dewaxed by solvent extraction to afford the starting material (control fiber) for the present studies. This had ash 0.97%, lignin 14.34%, and *O*-acetyl 3.57% of its oven dry (o.d.) weight. To achieve reduction of the ester linkages (completion being guided

by estimation of the *O*-acetyl content in the treated fiber), the control fiber was treated at room temperature with aqueous potassium borohydride. The pulp thus obtained was filtered off, and the adhering salts in it were removed by washing successively with water, oxalic acid (1%), and water. The filtrate and the first aqueous washings were pooled, decationized, and then centrifuged at 4°, and the clear, supernatant liquor was dialyzed. Concentration, followed by lyophilization of the concentrate, afforded a lignin–carbohydrate complex (LCC) as a pinkish-white powder, in ~3% yield. The complex (LCC) responded positively to a test for carbohydrates[28], but failed to respond to the characteristic test for the cinnamaldehyde end-groups of lignin[33], with phloroglucinol–hydrochloric acid reagent[27], possibly due to reduction of the aldehyde groups during the treatment with borohydride. The presence of lignin in LCC could, however, be presumed from its characteristic, u.v.-absorption curve[34], which showed a peak at ~280 nm (λ_{max} 274 nm, $E^{1\%}_{1cm}$ 29.5). This peak disappeared, and two new peaks (due to ionization of the free phenolic groups[34]), having absorption maxima at 248 and 300 nm, appeared when the spectrum of an alkaline solution of LCC, against a neutral solution of the same concentration as reference, was recorded.

The residual ester groups in the aforementioned, borohydride-treated fiber, containing 0.84% of *O*-acetyl, were further reduced by two successive treatments with borohydride solution, to furnish a pale-yellow fiber, RJ-3 (hereafter, reduced fiber). The final product was free from *O*-acetyl groups, and contained ash 0.33% and lignin 12.38% (of its o.d. weight). The ash and lignin contents, expressed as per cent o.d. weight of control fiber, were found to be 0.29 and 11.07%, respectively. These were calculated on the basis of total loss in weight (10.6%) encountered by control fiber during the borohydride treatments.

With a view to examining the effect of borohydride reduction, control and reduced fibers, and LCC, were hydrolyzed (*method A*, 10 h). Chromatographic identification of the component sugars revealed the presence of a new sugar (EMG) in both the reduced fiber and LCC, in addition to those present in the control fiber (*vide* Table I). A few oligosaccharides, known[4,7] to be comprised of xylo-oligosaccharides containing 4-*O*-methyl-D-glucuronic acid, also appeared in all of the chromatograms. EMG took a yellow stain with staining reagent *b* (a characteristic color produced only by hexoses), and moved faster than each of the sugars listed in Table I. In order to characterize EMG, a sufficient quantity of the sugar was isolated by repeatedly chromatographing the hydrolyzate obtained from reduced fiber. On demethylation[31], a homogeneous preparation of EMG $\{[\alpha]_{589.5}$ +61.3°$\}$ produced glucose (detected chromatographically) as one of the products, showing that the unknown sugar is a methylated derivative of glucose. EMG was finally identified as 4-*O*-methyl-D-glucose by comparing its staining behavior with staining reagent *b*, its specific rotation, its chromatographic mobility, and its g.l.c. profile (as the alditol acetate derivative), with those of the authentic sugar. The latter sugar was prepared by methyl esterification [using Dowex-50 (H^+) resin as the acid catalyst] of a mixture of D-xylo-oligosaccharides containing 4-*O*-methyl-D-glucuronic

acid (obtained from control fiber), followed by reduction of the resulting esters with aqueous potassium borohydride. Fractionation of the product on an ion-exchange resin afforded a mixture of neutral oligosaccharides as their methyl glycosides, which, on mild hydrolysis with acid, yielded xylose and 4-*O*-methylglucose. The latter was purified by preparative chromatography, and used as authentic 4-*O*-methylglucose in the foregoing experiments.

The identification, in the reduced fiber and in LCC, of 4-*O*-methyl-D-glucose, which is otherwise conspicuously absent from the control fiber, unambiguously demonstrates that at least some of the 4-*O*-methyl-D-glucuronic acid side-chains of the D-xylan in the control fiber participate in an ester linkage through the carboxyl groups. It is noteworthy that the reduced fiber retains only ~77% of the original lignin (*vide* Table I), suggesting that the portion of lignin (~23%) removed during the borohydride treatments contributes the alcohol counterpart of these ester bonds; consequently, saponification of these ester bonds should release similar amounts of lignin from the fiber. However, treatment of the fiber with alkali[35], under conditions known[10] to hydrolyze most of the ester linkages present therein, does not cause the removal of any appreciable amount of lignin[35]. This discrepancy may be explained by considering that lignin is hydrophobic in nature[36,37], and therefore remains within the fiber, even after it is detached from the carbohydrate core as a consequence of saponification of the ester bonds. On the other hand, the portion of lignin removed during the reductive treatments is rendered hydrophilic, due to conversion of the constituent aromatic carbonyl compounds into phenolic compounds that pass into solution in the aqueous medium as their potassium salts.

The nature of the ester linkages in jute thus having been established, an attempt was made to quantify the extent of their occurrence in the fiber. Because the reduced fiber is devoid of *O*-acetyl groups, it would be reasonable to presume that the fiber does not contain any more residual, ester bonds. The reduced fiber, however, retained a part of the original uronic acid (appearing as oligoglycuronic acids in the chromatogram of its hydrolyzate, *vide* Table I), indicating that D-xylan is only partially esterified in its native state.

In order to ascertain the number of acidic side-chains reduced during the borohydride treatments, and, hence, the number of esterified uronic acid residues in the control fiber, crude D-xylans were isolated from chlorite-holocelluloses, prepared from control and reduced fibers, by extraction with aqueous alkali. Ethanol precipitation of the alkaline extracts, after acidification (HOAc) and extensive dialysis, afforded polysaccharide fractions A_1 and A_3, respectively. Repeated fractionation of A_1, using barium hydroxide as the complexing reagent, yielded polysaccharide A_2, containing uronic acids 15.8%, pentose 74.7%, and traces of hexoses. An enriched D-xylan (polysaccharide A_4) was also prepared from A_3 by employing the same purification steps; this contained uronic acid 10.6%, pentose 76.0%, and hexose 10.7%. The constituent monosaccharides of polysaccharide fractions A_1, A_2, A_3, and A_4, and the spectrophotometric analyses of contents of uronic acid, pentose, and hexose of the respective fractions are summarized in Table II. As fractions A_2 and

A_4 are comprised of xylose as the sole pentose, at least within the limits of detectability, the number of uronic acid residues per 100 xylosyl residues in these fractions may be shown to be 15.9 and 10.5, respectively, implying that ~34% of the acidic side-chains of D-xylan in the unreduced fiber participate in ester linkages. The degree of esterification (d.e.), calculated from the uronic acid and pentose contents of A_1 and A_3, was found to be a little lower (~26%). This is quite to be expected, because the proportion of arabinosyl residues present in these preparations was not accounted for, in calculating the molar ratio of uronic acid to xylose. The average d.e. of the D-xylan in the control fiber may, therefore, be accepted as being 34%, which falls short of earlier reports[10] by ~15%. This difference may be attributed to (*a*) variation in the species, or quality, of the fiber used in our studies, or (*b*) the presence of ester linkages in the lignin itself, or both. The latter possibility remains an open question, and needs further investigation.

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