Note

Chemical Structure of Xylan in Cotton-seed Cake

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Cotton-seed cake, which is a by-product on the process of oil extraction from cotton-seeds and contains large amounts of xylan (40-45% as pentosan), has been used as an industrial raw material for xyllose production. However, the chemical structure of xylan in the cake has been unsolved until now. This paper deals with a structural study of the xylan based on fragmentation analysis.

Enzymatic hydrolysis of xylan, and isolation of hydrolysis products. Cotton-seed cake, 1000 g of air-dried matter (containing 458 g of total pentose analyzed by the orcinol–HCl method), was soaked in 600 ml of 2% NaOH solution at room temperature for 4 days, to promote the enzymatic susceptibility of xylan in the cake. The alkali-treated cake (790 g of air-dried matter containing 394 g of total pentose), after it was washed with water and air-dried, was added to a solution of xylanase from *Streptomyces* sp. E-86 (800 ml containing 220,000 units), and the reaction was done at pH 5.7 and 55°C for 24 hr. Solid material was filtered off, and the resultant filtrate was concentrated to 4500 ml (containing 234 g of total pentose).

E in Fig. 1 shows that the concentrate (sugar solution) contained xylose, xylobiose, xylotriose, and oligosaccharides with *R* values lower than xylotriose. On the other hand, the complete hydrolysis of the sugar solution, treated with 10% trifluoroacetic acid (TFA) for 3 hr followed by *x*-glucuronidase of *Aspergillus niger* (unpublished data) to hydrolyze the resultant aldo-biouronic acid, gave xylose (1 mol) and glucuronic acid (0.05 mol, analyzed by Milner's method).

One-third of the sugar solution was put on a charcoal column (70 x 680 mm, 550 g of activated charcoal for chromatography, Wako Pure Chemical Ind.). The column was well washed with water to remove xylose and salts, followed by 30% aqueous ethanol to elute the adsorbed oligosaccharides. The chromatography was repeated 3 times. The oligosaccharide fraction thus obtained was concentrated to 267 ml (containing 142 g of total pentose). The sugar solution, adjusted to pH 8.0 with NaOH solution, was then put on a Dowex 1 × 2 column (44 x 170 mm) pre-equilibrated with 1 m acetic acid. After the column was washed with water to remove neutral sugars, the adsorbed sugar was eluted with 2 m acetic acid, and desalting was done by passing the eluate through a charcoal column by the method described above. The salt-free sugar solution (A in Fig. 1) contained 37.1 g of total pentose.

The sugar solution (0.7 ml containing about 50 mg as total pentose) was put on HPLC (M & S GILSON Auto-Preparative System) with a Hiber LiChrosorb NH2 column (10 x 250 mm, Merck), and chromatographed with a solvent system of CH3CN-H2O-CH3COOH (40:60:20).

Fig. 1. TLC of Acidic-oligosaccharides Isolated from the Enzymatic Hydrolysate of Cotton-seed Xylan.

TLC was done Merck TLC plate silica gel 60 with the solvent system of 1-butanol-CH3COOH-water (2:1:1, v/v). The sugars were detected by heating after spraying with sulfuric acid. X, authentic xylose to xylohexaose from top to bottom; E, enzymatic hydrolysate of the xylan; A, acidic-oligosaccharides fraction after eliminated neutral sugars from E; I–IV, acidic-oligosaccharides I–IV.

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Abbreviations: 4-O-Me-GlcUA, 4-O-methyl-glucuronic acid; 4-O-Me-GluUAX, O-4-O-methyl-*x*-D-glucuronosyl-(1→2)-*D*-xylopyranose.
1.5, v/v) at a flow rate of 5 ml/min. The sugar was separated into four kinds of oligosaccharides, which were designated as acidic-oligosaccharides I, II, III, and IV in the order of elution. The yields of I, II, III, and IV, after 15 repeats of HPLC, were 40 mg, 227 mg, 84 mg, and 336 mg, respectively, and each of them was homogeneous on TLC (I, II, III, and IV in Fig. 1).

**Characterization of acidic-oligosaccharides I, II, III, and IV.**

(1) **Acidic-oligosaccharide I.** This sugar gave 4-O-Me-GlcUA (1 mol) and xylose (4.0 mol) on complete hydrolysis by the method described above, and also gave 4-O-Me-GlcUA and xylose on hydrolysis with a mixture of α-glucuronidase and β-xylosidase (originating from Aspergillus niger, data in our laboratory). This sugar gave 4-O-Me-GlcUA and xylose on partial acid hydrolysis with 10% TFA at 100°C for 2 hr, and the resultant 4-O-Me-GlcUA was further hydrolyzed to 4-O-Me-GlcUA and xylose by the α-glucuronidase. On the other hand, the methylation analysis of this sugar after hydrogenation with 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride and NaBH₄ gave 1,2,3,5-Me-xylitol (1 mol), 2,3-Me-Xylp (2 mol), 3,4-Me-Xylp (1 mol), and 2,3,4,6-Me-Glcp (1 mol). Thus, we conclude that acidic oligosaccharide I has the structure of 2³,4-O-methyl-α-D-glucuronopyranosyl-1,4-β-D-xylotetraose (Fig. 2).

(2) **Acidic-oligosaccharide II.** This sugar gave 4-O-Me-GlcUA (1 mol) and xylose (2.8 mol) on complete hydrolysis, gave the same sugars as above on hydrolysis with the mixture of enzymes, and gave 4-O-Me-GlcUA and xylose on partial acid hydrolysis. The α-glucuronidase further hydrolyzed 4-O-Me-GlcUA, to 4-O-Me-GlcUA and xylose. The methylation analysis of acidic-oligosaccharide II after hydrogenation gave 1,2,3,5-Me-xylitol, 2,3-Me-Xylp, 3,4-Me-Xylp, and 2,3,4,6-Me-Glcp in an equimolar ratio. Thus, we conclude that acidic-oligosaccharide II had the structure of 2³,4-O-methyl-α-D-glucuronopyranosyl-1,4-β-D-xylotriose (Fig. 2).

(3) **Acidic-oligosaccharide III.** This sugar gave GlcUA (1 mol) and xylose (4.2 mol) on complete hydrolysis, gave the same sugars as above on enzymatic hydrolysis, and also gave GlcUA and xylose on partial acid hydrolysis. GlcUA was further hydrolyzed to GlcUA and xylose by the α-glucuronidase. The methylation analysis of hydrogenated acidic-oligosaccharide III gave 1,2,3,5-Me-xylitol (1 mol), 2,3-Me-Xylp (2 mol), 3,4-Me-Xylp (1 mol), and 2,3,4,6-Me-Glcp (1 mol). Thus, we conclude that the structure of acidic-oligosaccharide III was 2³,4-α-D-glucuronopyranosyl-1,4-β-D-xylotetraose (Fig. 2).

(4) **Acidic-oligosaccharide IV.** This sugar gave GlcUA (1 mol) and xylose (3.2 mol) on complete hydrolysis, gave the same sugars as above on enzymatic hydrolysis, and also gave GlcUA and xylose on partial acid hydrolysis. The α-glucuronidase hydrolyzed GlcUA, to GlcUA and xylose. The methylation analysis of hydrogenated acidic-oligosaccharide IV gave 1,2,3,5-Me-xylitol, 2,3-Me-Xylp, 3,4-Me-Xylp, and 2,3,4,6-Me-Glcp in an equimolar ratio. Thus, we conclude that the structure of acidic-oligosaccharide IV was 2³,4-α-D-glucuronopyranosyl-1,4-β-D-xylotriose (Fig. 2).

**Structure of cotton-seed xylan.** The neutral sugar analysis of xylan, which was extracted with 10% NaOH solution from cotton-seed cake and analyzed by GLC, gave rhamnose (0.7%), arabinose (0.8%), xylose (96.9%), galactose (0.9%), and glucose (0.3%). The enzymatic hydrolysis of xylan in cotton-seed cake obtained xylose, 1,4-β-xylo-oligosaccharides (DP; 2 and 3), two kinds of acidic-oligosaccharides with 4-O-Me-GlcUA, and two kinds of acidic-oligosaccharides with 4-O-Me-GlcUA, and two

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4-O-Me-GlcUA GlcUA

Fig. 3. Structure of Glucuronoxylan in Cotton-seed Cake. X, 1,4-linked xylopyranose; GlcUA, α-1,2-linked GlcUA; 4-O-Me-GlcUA, α-1,2-linked 4-O-Me-GlcUA.
kinds of acidic-oligosaccharides with GlcUA. From these results, we consider that the xylan is a glucuronoxylan having both 4-O-Me-GlcUA and GlcUA, and the glucuronoxylan is composed of a main-chain of (1→4)-linked β-D-xylosyl residues to which are directly attached 4-O-methyl-α-glucuronosyl and α-D-glucuronosyl stubs at the O-2 of the xylosyl residues of the main-chain (Fig. 3). This is similar to the chemical structure of rice-straw arabinogl glucuronoxylan except the xylan has α-L-arabinofuranosyl stubs on the main-chain. Thus, cotton-seed cake is suitable as a starting material for the production of xylose or xylo-oligosaccharides by enzymatic hydrolysis because sources of xylan at a high concentration, with a high level of purity, and with simple chemical structure are limited in nature.

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References
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