Note

Preparation of Glucuronoxyloligosaccharides from an Acid Hydrolysate of Corn-hulls

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Various kinds of aldobiouronic acids, which are disaccharides consisting of xylose and GlcUA or 4-*O*-Me-GlcUA, are essential as a standard sample for the structural study of glucuronoxylan and as a substrate for study of the substrate specificity of α -glucuronidase.^{1–3)} We report on the preparation of four kinds of aldobiouronic acids and two kinds of aldotriouronic acids from an acid hydrolysate of corn-hulls.

Acid hydrolysis of corn-hulls. Corn-hulls from *Zea mays* (3 kg) were hydrolyzed with 2 *N* H₂SO₄ (24 l) at 100°C for 3 hr. After filtration, the filtrate was neutralized with NaOH. The solution (24 l) contained 648 g of total sugar (as xylose).

Isolation of glucuronoxyloligosaccharides

i) **Charcoal and ion exchange chromatographies.** One-

sixth of the sugar solution (4 l) was put on a charcoal column (70 × 680 mm, 500 g of activated charcoal for chromatography, Wako Pure Chemical Ind.) to remove salts. After the column was washed well with water, the oligosaccharides were eluted from the column with 30% ethanol (10 l). Then, the eluents from six batches were concentrated to 500 ml (35.3 g of total sugar). The concentrate was adjusted to pH 8 with 6 *N* NaOH and left at room temperature for 1 hr to cleave the lactone ring of the glucuronic acid residue.

The sugar solution was put on a column (50 × 250 mm) of Dowex 1 × 2 (100–200 mesh) equilibrated with 0.1 *M* CH₃COOH. After this was washed with 0.1 *M* CH₃COOH (2 l) to remove neutral sugars, the glucuronoxyloligosaccharides were eluted with 2 *M* CH₃COOH (2.5 l). The eluent was concentrated to 1 l and neutralized with NaOH.

The sugar solution (2.0 g of total sugar) was desalted through a charcoal column (50 × 250 mm, 100 g of activated charcoal). The adsorbed glucuronoxyloligosaccharides were eluted with 35% ethanol (2 l). The eluent was concentrated to 45 ml (1.65 g of total sugar).

ii) **HPLC.** The sugar solution (800 μ l) was put on HPLC (M&S GILSON Auto-Preparative System) with LiChrosorb NH₂ column (10 × 250 mm), and chromatographed with the solvent system of CH₃CN–H₂O–CH₃COOH (30:70:1.5, v/v) at a flow rate of 4 ml/min.

Figure 1 shows six peaks (fractions A–F). After repeating the chromatographic operation automatically, each of the fractions ("—" in Fig. 1) was concentrated and lyophilized. The yields were A (84.3 mg), B (223.2 mg), C (40.0 mg), D (195.0 mg), E (280.4 mg), and F (480.0 mg). Each of A–E showed a single spot on TLC, which was done with Merck TLC plate silica gel 60 with the solvent system of 1-butanol–CH₃COOH–H₂O (2:1:1, v/v).

iii) **Silica gel chromatography of Fraction F.** Fraction F (50 mg) dissolved in 1 ml of water was put on a silica gel 60 (70–230 mesh, Merck) column (30 × 380 mm) followed by

Fig. 1. HPLC of Glucuronoxyloligosaccharides.

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Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; TFA, trifluoroacetic acid; 4-*O*-Me-GlcUA, 4-*O*-methyl-D-glucuronic acid; 4-*O*-Me-GlcUAX₁, *O*-4-*O*-methyl- α -D-glucuronopyranosyl-(1→2)-D-xylopyranose.

Table I. COMPONENT SUGARS AND METHYLATION ANALYSIS OF GLUCURONOXILO-OLIGOSACCHARIDES

	Oligosaccharides					
	A	B	C	D	E	F-I
Component sugars						
Xylp	2.22 ^b	1.10	1.20	2.26	0.99	1.19
4- <i>O</i> -Me-GlcUA	1	1	1			
GlcUA				1	1	1
Methylation analysis						
[Linkages]						
1,2,3,5-Me-xylitol ^a	1.09			0.93		
[→4]-Xylp]						
1,3,4,5-Me-xylitol		1.02			1.16	
[→2]-Xylp]						
1,2,4,5-Me-xylitol			1.29			1.31
[→3]-Xylp]						
3,4-Me-Xylp	1.21			1.04		
[→2]-Xylp-(1→]						
2,3,4,6-Me-Glcp	1	1	1	1	1	1
[GlcUA-(1→]						
or						
[4- <i>O</i> -Me-GlcUA-(1→]						

^a 1,2,3,5-Me-xylitol denotes 4-mono-*O*-acetyl-1,2,3,5-tetra-*O*-methyl-D-xylitol.

^b Each number in this table indicates the molar ratio obtained from the results of GLC analysis of each oligosaccharide. Xylp, xylopyranose; GlcUA, glucuronic acid; Me, methyl.

ascending development with the above solvent system. The chromatography separated fraction F into two kinds of sugars, fractions F-I and F-II. After repeating the chromatography twice, fraction F-I, 40.2 mg, was obtained.

Characterization of glucuronoxilo-oligosaccharides. Since the acidic saccharide was resistant to acid hydrolysis, glucuronic acid residues in the oligosaccharide were reduced to glucose residues with EDC and NaBH₄ by the method of Taylor and Conrad,⁴⁾ followed by hydrolysis with 10% TFA at 100°C for 2 hr. The resultant monosaccharides were converted into the corresponding alditol-acetates, and analyzed by GLC.⁵⁾ The methylation of glucuronoxilo-oligosaccharide was done by the method of Ciucanu⁶⁾ after reduction of the sugar by the above procedure.

Table I shows the results of analyses of component sugar and methylation.

i) **Fraction A.** The results of Table I suggest that fraction A was 2'-4-*O*-methyl-α-D-glucuronopyranosylxylobiose. Moreover, the partial acid hydrolysis of the parent fraction A showed 4-*O*-Me-GlcUAX₁⁷⁾ and xylose (data not shown).

ii) **Fraction B.** The results of Table I suggest that fraction B was *O*-4-*O*-methyl-α-D-glucuronopyranosyl-(1→2)-D-xylopyranose. Additionally, the sugar had the same *R_f* value as authentic 4-*O*-Me-GlcUAX₁⁸⁾ (data not shown).

Fig. 2. Structures of Glucuronoxilo-oligosaccharides A-F-I.

iii) **Fraction C.** The results of Table I suggest that fraction C was *O*-4-*O*-methyl-α-D-glucuronopyranosyl-(1→3)-D-xylopyranose.

iv) **Fraction D.** The results of Table I suggest that fraction D was 2'-α-D-glucuronopyranosylxylobiose. Moreover, the methylated derivative of parent fraction D showed the same retention time as that of parent fraction A (data not shown), analyzing by GLC with an OV-17 column.

v) **Fraction E.** The results of Table I suggest that

fraction E was *O*- α -D-glucuronopyranosyl-(1 $\rightarrow$ 2)-D-xylopyranose. Moreover, the methylated derivative of parent fraction E showed the same retention time as that of parent fraction B.

vi) *Fraction F-I*. The results of Table I suggest that fraction F-I was *O*- α -D-glucuronopyranosyl-(1 $\rightarrow$ 3)-D-xylopyranose. Moreover, the methylated derivative of parent fraction F-I showed the same retention time as that of parent fraction C. Figure 2 showed the structures of glucuronoxyloligosaccharides A-F-I. This is the first paper showing that corn-hull glucuronoxylan had both 1,3-linked glucuronosyl and 4-*O*-Me-glucuronosyl residues.

Corn-hulls had two kinds of GlcUA (GlcUA and 4-*O*-Me-GlcUA) and also two kinds of linkages (1,2- and 1,3-glucuronosyl linkages). Moreover, LiChrosorb NH₂ chromatography was more suitable for the isolation of glucuronoxyloligosaccharides than other methods such as gel filtration,⁸⁾ preparative paper⁹⁾ and ion-exchange¹⁰⁾ chromatographies. Therefore, our method is convenient for the preparation of glucuronoxyloligosaccharides.

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