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STRUCTURAL STUDIES ON A GLUCURONOARABINOXYLAN FROM THE HUSK OF SORGHUM GRAIN*

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ABSTRACT

Polysaccharide H-3 is composed of L-arabinose, D-xylose, glucuronic acid, and 4-*O*-methylglucuronic acid in the molar ratios of 15:18:2:1. The polysaccharide contains a primary chain of β-D-(1→4)-linked D-xylopyranose residues. The identification of 2-*O*-(glucopyranosyluronic acid)-D-xylose and 2-*O*-(4-*O*-methylglucopyranosyluronic acid)-D-xylose revealed that the acid residues are bonded through O-2 of some D-xylopyranose moieties. Most of the L-arabinofuranose residues occur as nonreducing end-groups, attached through O-3, and in some cases through both O-2 and O-3, of certain D-xylose residues. Some L-arabinofuranose residues are substituted at O-2 or O-3. P.m.r. spectroscopy suggested that most of the L-arabinose units are α-linked.

INTRODUCTION

In an earlier paper[2], the isolation of hemicellulose B from the husk shavings of the Barnard Red variety of sorghum grain was reported. Studies[3] on the hemicellulose B extracts from malted sorghum grain indicated that only minor modifications occur in this polysaccharide during malting. Hemicellulose B was separated[2] by DEAE-cellulose chromatography into thirteen fractions (H-1 to H-13). The $\bar{M}_w$ values of polysaccharides H-1 to H-13 have been reported[4]. Some structural features of polysaccharide H-3 are described herein.

RESULTS AND DISCUSSION

Polysaccharide H-3 is electrophoretically homogeneous, and gives a single, symmetrical peak in chromatography on Bio-Gel A-1.5 m. The degree of polymeri-

*Sorghum polysaccharides, Part V. For Part IV, see ref. 1.

zation ($\overline{d.p.}$) of H-3 was found to be 441 by chromatography[5] on Bio-Gel A-1.5 m, and 457 by reducing end-group analysis[6]. The specific rotation of H-3 is negative, indicating that this hemicellulose fraction is similar to the arabinoxylans from the husks of other cereals[7].

The neutral moiety of the acid hydrolyzate contained equimolar quantities of D-xylose and L-arabinose. Methanolysis of the reduced, permethylated acid component afforded equimolar amounts of methyl 3,4-di-*O*-methylxylopyranoside and methyl 2,3,4-tri-*O*-methylglucopyranoside. Hydrolysis of the reduced methyl ester methyl glycosides gave xylose, glucose, and 4-*O*-methylglucose in the molar ratios of 3:2:1. The hydrolyzate of the methylated, reduced acid fraction contained 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylxylose. The acid moiety therefore comprises 2-*O*-(glucopyranosyluronic acid)xylose and 2-*O*-(4-*O*-methylglucopyranosyluronic acid)xylose in the molar ratio of 2:1. The isolation of these acidic fragments indicated that the glucuronic acid and 4-*O*-methylglucuronic acid units are linked through O-2 of certain xylopyranose residues of the primary chain. A total acid content of 9% was determined by the carbazole colorimetric method. The neutralization equivalent was found to be 2,100 (8.4% of uronic acid). Polysaccharide H-3 therefore contains L-arabinose, D-xylose, glucuronic acid, and 4-*O*-methylglucuronic acid in the molar ratios of 15:18:2:1. The properties of polysaccharide H-3 are listed in Table I.

TABLE I

PROPERTIES[a] OF POLYSACCHARIDE H-3

Property	*Value*
Ash (%)	0.01
Nitrogen (%)	0.10
$[\alpha]_D^{20}$ (degrees)	−106
Equivalent weight[b]	2,100
Uronic anhydride[b,c] (%)	8.4
Uronic anhydride[d] (%)	9
$\overline{D.p.}$[e]	441
$\overline{D.p.}$[f]	457
Composition (mole ratio):	
L-arabinose	15
D-xylose	18
glucuronic acid	2
4-*O*-methylglucuronic acid	1

[a]All data corrected to a dry-weight basis. [b]Titration with 0.01M sodium hydroxide. [c]Assuming that all acidity arises from uronic acid groups. [d]Carbazole colorimetric method. [e]Bio-Gel A-1.5m chromatography. [f]Reducing end-group analysis.

Formolysis and hydrolysis of the permethyl ether of H-3, and analysis of the neutral sugars by g.l.c.-mass spectrometry gave the products listed in Table II. The occurrence of 2-*O*-methylxylose in the hydrolyzate indicates branching through O-3,

whereas the presence of D-xylose shows that some residues are branched through both O-2 and O-3. The presence of 2,3,5-tri- and 2,5- and 3,5-di-*O*-methyl-L-arabinose indicates that the arabinose units are in the furanose form. Branches are terminated by L-arabinose residues. Some L-arabinose units are substituted at O-2 or O-3. Permethylated H-3 was successively methanolyzed and hydrolyzed, and the resulting neutral and acidic sugars separated. The acidic fraction was methanolyzed, and the product reduced. G.l.c. analysis showed equimolar quantities of methyl 2,3,4-tri-*O*-methylglucopyranoside and methyl 3-*O*-methylxyloside. P.m.r. analysis of permethylated H-3 showed anomeric signals at τ 4.72 and 5.75.

TABLE II

COMPOSITION OF HYDROLYZATE OF PERMETHYLATED POLYSACCHARIDE H-3

Component	T^a	*Mole ratio*
2,3,5-Tri-*O*-methyl-L-arabinose	0.48	10
2,3,4-Tri-*O*-methyl-D-xylose	0.66	~0.1
3,5-Di-*O*-methyl-L-arabinose	0.90	1
2,5-Di-*O*-methyl-L-arabinose	1.06	1
2,3-Di-*O*-methyl-D-xylose	1.50	5
2-*O*-Methyl-D-xylose	3.04	6
D-Xylose	5.40	2

[a]Column *A*.

Treatment of peracetylated H-3 with chromium trioxide in acetic acid resulted in almost complete oxidation of the D-xylose residues, indicating[8] that H-3 contains β-linked D-xylopyranose units. Oxidation of H-3 with sodium periodate resulted in the uptake of 0.64 mole of periodate per sugar residue. The polyalcohol contained D-xylose and L-arabinose in the molar ratio of 4.3:1. The presence of L-arabinose in the hydrolyzate of the polyalcohol is in accordance with the methylation analysis; this shows that the di-*O*-methylarabinoses in the hydrolyzate of permethylated H-3 do not result either from undermethylation or *O*-demethylation. The presence of 2,5-di-*O*-methyl-L-arabinose in the hydrolyzate of permethylated, wheat-bran hemicellulose[9] and of 3,5-di-*O*-methyl-L-arabinose in the hydrolyzate of permethylated, barley-husk hemicellulose[10] has been reported. A hemicellulose having the structural features indicated by the results of the methylation analysis of H-3 would consume 0.60 mole of periodate per sugar residue, and afford a polyalcohol having xylose and arabinose in the molar ratio of 4:1. Smith degradation[11] of the polyalcohol gave a mixture which was chromatographed on Dowex 50W-X8 (Ba^{2+}) ion-exchange resin, and purified by paper chromatography. The components were identified[12,13] as 2-*O*-β-D-xylopyranosylglycerol (**1**), *O*-β-D-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→2)-glycerol (**2**), *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-glycerol (**3**), *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-glycerol (**4**), and *O*-β-D-xylopyranosyl-(1→4)-*O*-

β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-glycerol (5).

Controlled, acid hydrolysis of polysaccharide H-3 resulted in selective cleavage of most of the L-arabinofuranose linkages, with the formation of a degraded polymer. The L-arabinose content in the degraded polysaccharide (~6%) could not be lowered further without extensive hydrolysis of the xylopyranose linkages. The resistance of these L-arabinose residues to acid hydrolysis has not been established, but could result from those arabinose units that are β-linked. G.l.c. analysis of the permethylated, degraded polymer (see Table III) showed it to consist essentially of 2,3-di-*O*-methylxylose. This result and the methylation analysis of H-3 show that H-3 has a linear,

TABLE III

COMPOSITION OF PERMETHYLATED, DEGRADED H-3

Component	*Column A*		*Column B*	
	T	*Mole ratio*	*T*[a]	*Mole ratio*
2,3,5-Tri-*O*-methyl-L-arabinose	0.48	2	(0.57), 0.73	3
2,3,4-Tri-*O*-methyl-D-xylose	0.66	1	0.46, (0.57)	
3,5-Di-*O*-methyl-L-arabinose	0.90	2	1.05, 2.43	2
2,3-Di-*O*-methyl-D-xylose	1.50	26	1.50, 1.79	27
2-*O*-Methyl-D-xylose	3.04	2	4.21, 6.31	2
2,3,4-Tri-*O*-methylglucuronic acid[b]	—	n.d.	2.53, 3.24	4

[a]Figures in parentheses indicates *T* values of components not completely resolved. [b]Present as methyl ester methyl glycoside; n.d., not determined.

xylan chain, and that only L-arabinofuranose residues are linked through O-3, or both O-2 and O-3, of certain xylose units of this chain. The occurrence of 3,5-di-*O*-methyl-L-arabinose in the hydrolyzate of permethylated, degraded H-3 shows that some L-arabinofuranosyl residues are substituted at O-2. Periodate oxidation of degraded H-3 resulted in extensive breakdown of the polysaccharide, consistent with the methylation analysis. Chromium trioxide oxidation of peracetylated, degraded H-3 resulted in low recoveries of D-xylose, indicating[8] that these residues are β-linked. P.m.r. analysis of the permethylated, degraded polymer showed a signal at τ 5.73 attributable to the anomeric protons of β-linked D-xylopyranosyl residues. The signal at τ 4.72 in the p.m.r. spectrum of permethylated H-3 can therefore be assigned to the anomeric protons of the L-arabinofuranose units. The chemical shift of these protons suggests that the residues are α-linked[14]. Partial, acid hydrolysis of degraded H-3 gave xylose and four components which moved slower than xylose in paper chromatography. The component having R_{Xyl} 0.60 (solvent *E*) was isolated, and identified[15] as 4-*O*-β-D-xylopyranosyl-D-xylose. The minor components were identified by their R_{Xyl} values in paper chromatography[16] to be β-(1→4)-linked xylo-oligosaccharides containing 3, 4, and 5 xylose residues. This result confirms that polysaccharide H-3 contains a primary chain of β-(1→4)-linked D-xylopyranose residues.

EXPERIMENTAL

General. — Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer 237 spectrophotometer. Optical rotations were measured with a Bendix–NPL Automatic Polarimeter Type 143D for aqueous solutions, unless otherwise stated. P.m.r. spectra were recorded with a Varian HA-100 instrument for solutions in chloroform-*d*, with tetramethylsilane as the internal standard. Gas–liquid chromatography (g.l.c.) was performed with a Packard 805 chromatograph, with nitrogen as the carrier gas at a flow rate of 40 ml/min at 175°. Columns (180 × 0.3 cm) were packed with (*A*) 3% of ECNSS-M on Gas-Chrom Q (100–120 mesh), or (*B*) 15% of 1,4-butanediol succinate polyester on Chromosorb W (60–80 mesh). Retention times (*T*) are given relative to hexa-*O*-acetylmannitol (alditol acetates), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (partially methylated alditol acetates), or methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside (methyl glycosides). G.l.c.–mass spectrometry (m.s.) was performed with a Varian Aerograph 1200 chromatograph attached to a Varian CH7 mass spectrometer. The mass spectra were recorded at an ionizing voltage of 70 eV. Electrophoresis was carried out on Millipore PhoroSlide electrophoresis strips (7.5 × 2.5 cm) and on strips (17 × 2.5 cm) of cellulose acetate in 0.1M ammonium carbonate buffer (pH 8.9), and 0.05M acetate buffer (pH 5.0), at 20 V/cm. Polysaccharides were detected[17] by spraying with periodate–rosaniline hydrochloride. Moving free-boundary (Tiselius) electrophoresis was conducted in a Beckman Model H apparatus using 0.05M sodium borate (pH 9.2) at 10 mA and 150 V. Descending partition chromatography was carried out on Whatman No. 1 or 3 MM paper in the following solvent systems: (*A*) 10:4:3 ethyl acetate–pyridine–water, (*B*) 18:4:1:3 ethyl acetate–acetic acid–formic acid–water, (*C*) 4:1:5 1-butanol–ethanol–water (upper layer), (*D*) 5:3:1:3 1-butanol–pyridine–benzene–water, (*E*) 7:1:2 2-propanol–acetic acid–water, or (*F*) 1-butanol, half saturated with water. Components were detected with *p*-anisidine hydrochloride or alkaline silver nitrate. Polysaccharides were methylated successively by the Hakomori and Purdie procedures. Samples were methanolyzed with 4% methanolic hydrogen chloride in sealed tubes for 12 h at 95°, or hydrolyzed with M sulfuric acid in sealed tubes for 7 h at 95°.

Extraction of polysaccharide H-3. — The isolation of the polysaccharides from the husk shavings of Barnard Red sorghum grain, and the fractionation of hemicellulose B by DEAE-cellulose chromatography, have been reported[2]. Fraction H-3 was eluted from the DEAE-cellulose in 0.01M sodium borate buffer (pH 9.0), after elution of H-1 and H-2 in water and 2.5mM sodium borate, respectively.

Electrophoretic analysis. — The polysaccharide migrated as a single band on electrophoresis on Millipore PhoroSlides and on cellulose acetate films. Moving free-boundary electrophoresis gave a single, symmetrical peak.

Determination of $\overline{d.p.}$ of polysaccharide H-3. — (*a*). The average number of reducing end-groups was estimated colorimetrically[6] by using D-xylose as the standard. A 20-mg sample of H-3 contained 42.5 μg of reducing end-group. Based on

a molecular weight of 136 for the average sugar residue, the $\overline{d.p.}$ was calculated to be 457 ($\overline{M}_n$ 62, 000).

(*b*). Chromatography of a sample (8 mg) of H-3 on a column (90 × 1.5 cm) of Bio-Gel A-1.5 m, calibrated[5] with dextrans of known $\overline{M}_w$, gave a symmetrical elution-pattern with peak height corresponding to $\overline{M}_w$ 60,000; $\overline{d.p.}$ 441.

Composition of polysaccharide H-3. — The polysaccharide (1 g) in M sulfuric acid (50 ml) was heated for 8 h on a steam bath. The cooled solution was neutralized (barium carbonate), filtered, de-ionized [Amberlite IR-120 (H^+) resin], and separated into the neutral and acidic components by passage through a column of Amberlite IR-45 (OAc^-) resin. The neutral fraction (690 mg) was resolved into the component sugars on a column (80 × 4 cm; solvent *F*) of cellulose.

Fraction I (270 mg) had m.p. and mixed m.p. (D-xylose) 150°, $[\alpha]_D^{20}$ +19° (*c* 1), and was chromatographically identical (solvents *A*, *B*, and *D*) with D-xylose. The sugar was identified as its di-*O*-benzylidene dimethyl acetal derivative, m.p. and mixed m.p. 210°. The derived alditol acetate had the same retention time (*T* 0.54, column *A*) as penta-*O*-acetylxylitol.

Fraction II (295 mg) had m.p. and mixed m.p. (L-arabinose) 158°, $[\alpha]_D^{20}$ +101° (*c* 1.6), and the same chromatographic mobility (solvents *A*, *B*, and *D*) as L-arabinose. The sugar was characterized as its phenylosazone, m.p. and mixed m.p. 163°. The alditol acetate had *T* 0.37 (column *A*), the same as that of penta-*O*-acetyl-L-arabinitol.

The acid moiety (125 mg) was eluted from the Amberlite IR-45 resin with 5% formic acid. This fraction was methanolyzed, and a portion of the product was methylated, the product reduced with lithium aluminum hydride in tetrahydrofuran, and the product methanolyzed. G.l.c. (column *B*) showed the presence of equimolar quantities of methyl 3,4-di-*O*-methylxylopyranoside (*T* 1.32, 1.60) and methyl 2,3,4-tri-*O*-methylglucopyranoside (*T* 2.58, 3.74). The rest of the methyl ester methyl glycosides was reduced with lithium aluminum hydride in tetrahydrofuran. Acid hydrolysis of a portion of this product was succeeded by paper chromatography (solvents *A* and *B*), which showed the presence of xylose, glucose, and 4-*O*-methyl-glucose. G.l.c. (alditol acetates, column *A*) showed three components, having *T* 0.54, 1.13, and 1.37, identical with the retention times of the acetates of xylitol, 4-*O*-methyl-glucitol, and glucitol respectively, in the molar ratios of 3:1:2. The rest of the reduced, acid fraction was methylated, and the product hydrolyzed. G.l.c. analysis (alditol acetates, column *A*) showed equimolar amounts of the acetates of 2,3,4,6-tetra-*O*-methylglucitol (*T* 1.00) and 3,4(2,3)-di-*O*-methylxylitol (*T* 1.52).

The neutralization equivalent of H-3 was found to be 2,100. The uronic acid content (9%) was determined[18] by the carbazole colorimetric method.

Methylation analysis of polysaccharide H-3. — The polysaccharide (2 g) was converted into the permethyl ether. The product (1.7 g), purified by precipitation from a chloroform solution with petroleum ether (b.p. 60–80°), had $[\alpha]_D^{20}$ −102° (*c* 0.6, chloroform), and showed, in the i.r. spectrum, no absorption attributable to OH. A solution of permethylated H-3 (30 mg) in 98% aqueous formic acid (2 ml) was heated for 1 h at 95°, cooled, and evaporated to dryness. The residue was

hydrolyzed in M sulfuric acid, and the solution made neutral (barium carbonate), and analyzed by g.l.c.–m.s. (partially methylated alditol acetates, column *A*). The results are summarized in Table II.

The rest of the methylated polysaccharide was hydrolyzed successively with 4% methanolic hydrogen chloride (100 ml, 3 h, reflux) and 0.5M sulfuric acid (100 ml, 4 h, 95°). The acidic and neutral sugars were separated as already described. The acid moiety was converted into the methyl ester methyl glycosides, and these were reduced with lithium aluminum hydride. G.l.c. (methyl glycosides, column *B*) showed equimolar amounts of methyl 2,3,4-tri-*O*-methylglucopyranoside and methyl 3-*O*-methylxyloside.

Smith degradation of polysaccharide H-3. — A sample of H-3 (1.0 g) was treated with 0.03M sodium periodate (500 ml) at 4–5° in the dark. Aliquots were removed at intervals, and the amount of periodate consumed was determined titrimetrically[19]. The oxidation was complete after 27 h (0.64 mole of periodate consumed per sugar residue). The excess of periodate was decomposed with ethylene glycol (3 ml), and the solution was dialyzed. The polyaldehyde was reduced with sodium borohydride, and the reaction was terminated by the addition of acetone. The solution was dialyzed, and the product (870 mg) recovered by freeze-drying. G.l.c. (alditol acetates, column *A*) of the hydrolyzate of a sample of the polyalcohol showed xylose and arabinose in the molar ratio of 4.3:1. The polyalcohol was treated with 0.5M sulfuric acid (60 ml) for 3.5 h at 22°, and the solution made neutral (barium carbonate), and evaporated to a syrup (740 mg). Paper chromatography (solvents *A* and *D*) showed the presence of glycerol, glycolaldehyde, and five glycosidic components having R_{Xyl} (solvent *A*) 1.00, 0.75, 0.62, 0.35, and 0.10. The mixture was applied to a column (74 × 4 cm) of Dowex 50W-X8 (Ba^{2+}) resin (100–200 mesh), and this was eluted with water. The components were further purified by paper chromatography (solvent *D*), and identified as compounds **1** to **5** by methods previously reported[12,13].

Chromium trioxide oxidation of peracetylated H-3. — A solution of H-3 (48 mg) in formamide (5 ml) was treated with acetic anhydride (4 ml) and pyridine (3 ml) for 24 h at 20°. The solution was poured into water, dialyzed, and freeze-dried. The peracetylated polysaccharide (53 mg) was treated[8] with chromium trioxide (150 mg) in acetic acid (5 ml, containing hexa-*O*-acetylmannitol) at 50°. Aliquots were removed at intervals, poured into water, and extracted into chloroform. The solvent was evaporated, and the products hydrolyzed, and analyzed by g.l.c. (alditol acetates, column *A*). The recovery of xylose was 67 (2 h), 34 (3 h), and 4% (6 h).

Partial, acid hydrolysis of polysaccharide H-3. — Polysaccharide H-3 (200 mg) in 5mM sulfuric acid (20 ml) was heated at 95°. Samples (3 ml) were removed at intervals, made neutral (barium carbonate), and dialyzed. G.l.c. analyses (alditol acetates, column *A*) of the hydrolyzates of the dialyzates and degraded polysaccharides showed that the arabinose content was lowered to ~6% after 11 h, without cleavage of the xylopyranose linkages. Continued hydrolysis resulted in the appearance of xylose in the dialyzate. A sample of H-3 (1.5 g) in 5mM sulfuric acid (200 ml) was

heated for 11 h at 95°, made neutral (barium carbonate), the suspension filtered, and the filtrate dialyzed. Paper chromatography (solvents *A* and *C*) showed the dialyzate to contain only arabinose. The degraded polysaccharide (640 mg) contained xylose, arabinose, and uronic acid in the molar ratios of 26:2:5.

Methylation analysis of degraded H-3. — The degraded polysaccharide (58 mg) was converted into the permethyl ether. Portions were hydrolyzed or methanolyzed, and the products analyzed by g.l.c. (alditol acetates, column *A*; methyl glycosides, column *B*). The results are summarized in Table III.

Periodate oxidation of degraded H-3. — A sample of the degraded polysaccharide reduced 0.95 mole of periodate per sugar residue. G.l.c. (alditol acetates, column *A*) of the hydrolyzate showed ethylene glycol and glycerol, with minor amounts of arabinose and xylose.

Chromium trioxide oxidation of the acetyl derivative of degraded H-3. — The degraded polysaccharide was acetylated, the acetate oxidized with chromium trioxide, and the product analyzed by g.l.c. as already described. The recovery of xylose was 68 (1 h), 22 (2 h), and 5% (3 h).

Partial, acid hydrolysis of degraded H-3. — The degraded polysaccharide (500 mg) was hydrolyzed successively for 8-h periods in 5mM sulfuric acid at 95°. After each hydrolysis, the solution was made neutral, and material of low molecular weight was removed by dialysis. The dialyzates were combined and evaporated, and the neutral and acidic sugars were separated as already described. Paper chromatography (solvent *E*) showed the neutral fraction to contain xylose, and four slower-moving components having R_{Xyl} 0.60, 0.32, 0.11, and 0.06. The sugar having R_{Xyl} 0.60 was separated from the mixture by preparative paper-chromatography (solvent *E*). The syrup (35 mg) had $[\alpha]_D^{20}$ −22° (*c* 0.8), and gave xylose on hydrolysis. A sample was methylated and hydrolyzed; g.l.c. (partially methylated alditol acetates, column *A*) showed equimolar amounts of 2,3,4-tri- and 2,3-di-*O*-methylxylose.

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