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A HETEROXYLAN FROM THE HUSK OF *SORGHUM* GRAIN*

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Key Word Index—*Sorghum bicolor*; Gramineae; grain; husk; hemicellulose B; heteroxylan; uronic acid; structure.

Abstract—Polysaccharide H-1 from the husk of *Sorghum bicolor* contains xylose, arabinose, glucose, galactose, glucuronic acid and 4-*O*-methylglucuronic acid in a molar ratio of 20:15:11:1:2:1. The polysaccharide has a primary chain of $\beta(1 \rightarrow 4)$ linked xylopyranose residues. Arabinofuranose units are attached at 0-3, or in some cases both 0-2 and 0-3, of certain xylose residues. Galactose occurs as non-reducing end-groups and is present in the pyranose form. Linear chains of glucose residues (10–12 sugar units), containing $\beta(1 \rightarrow 4)$ and $\beta(1 \rightarrow 3)$ linkages in a ratio of 10:1, are present in H-1. The uronic acid units are bonded through 0-2 of certain xylose moieties.

INTRODUCTION

The isolation of the polysaccharides from the grain husk [2] and endosperm [3] of *Sorghum bicolor* have been previously described as have the structural features of a hemicellulosic β-D-glucan from the endosperm [1]. *Sorghum* grain husk hemicellulose B was separated into 13 fractions (H-1 to 13) by DEAE-cellulose chromatography [2]. The MW of fractions H-1 to 13, determined by ultracentrifugation, have been reported [4] and some structural features of polysaccharide H-3, a glucuronoarabinoxylan, have been described [5]. In this paper the structure of polysaccharide H-1, a heteroxylan which could be an acidic glucoarabinoxylan, is now discussed.

RESULTS AND DISCUSSION

Polysaccharide H-1 was shown by electrophoresis and Bio-Gel chromatography to possess a high degree of homogeneity. The DP of H-1 was found to be 107 (MW 15 200) by Bio-Gel A-0.5 m chromatography, and 77 (MW_n 11 000) by reducing end-group analysis [6]. Previously [4] the DP of H-1 was found to be 97 (MW_w 13 600) by ultracentrifugation. The specific optical rotation of H-1 is negative [2] indicating a similarity to other cereal husk hemicelluloses [7].

The neutral sugars in the hydrolysate of H-1 were separated, and identified as D-xylose, L-arabinose, D-glucose and D-galactose. The glucose content in H-1 was not reduced when the polysaccharide was treated with either α- or β-amylase, and it did not give a coloration with I_2–KI solution. A sample of H-1, chromatographed on Bio-Gel A-0.5 m, was collected in 3 bulked fractions and the sugar compositions and optical rotations of these fractions were similar to those of H-1. Thus it is concluded that the glucose residues are constituents of H-1, and do not arise from a contaminating glucan. The acidic fraction from the hydrolysate of H-1 was methanolysed, then methylated, reduced, methanolysed and shown by GLC to contain equimolar amounts of Me 3,4-di-*O*-Me xylopyranoside and Me 2,3,4-tri-*O*-Me glucopyranoside. The remainder of the methanolysate after reduction and GLC analysis was shown to contain xylose, glucose and 4-*O*-Me glucose in a molar ratio of 3:2:1. These results indicate that the acid moiety from H-1 comprises the two aldobiouronic acids, 2-*O*-(glucopyranosyluronic acid)-xylose and 2-*O*-(4-*O*-Me glucopyranosyluronic acid)-xylose, in a molar ratio of 2:1. The neutralization equivalent of H-1, estimated as 3100, indicated a uronic acid content of 5.8%; a uronic acid content of 6% was found by the carbazole colorimetric method [8]. Polysaccharide H-1 therefore contains xylose, arabinose, glucose, galactose, glucuronic acid and 4-*O*-Me glucuronic acid in a molar ratio of 20:15:11:1:2:1.

Formolysis and hydrolysis of permethylated H-1, followed by GC–MS, gave the products listed in Table 1. Methylation analysis shows that H-1 is composed of xylose, arabinose, glucose and galactose in a molar ratio of 18:17:12:1. However, the uronic acid residues, and the

Table 1. Composition of the hydrolysate of permethylated H-1

Alditol acetate of:	R_t	Mole ratio
2,3,5-Tri-*O*-methylarabinose	0.48	15
2,3,4-Tri-*O*-methylxylose	0.65	1
3,5-Di-*O*-methylarabinose	0.89	1
2,3,4,6-Tetra-*O*-methylglucose	1.00	1
2,5-Di-*O*-methylarabinose	1.06	1
2,3,4,6-Tetra-*O*-methylgalactose	1.25	1
2,3-Di-*O*-methylxylose	1.51	6
2,4,6-Tri-*O*-methylglucose	1.97	1
2,3,6-Tri-*O*-methylglucose	2.66	10
2-*O*-Methylxylose	3.04	6
Xylose	5.50	5

* Part 7 in the Series '*Sorghum* Polysaccharides'. For Part 6, see ref. [1].

xylose units associated with them, are not determined by such analysis. The ratio of end-groups, branch-points and chain-units was found to be 1.1:1.2:1. The arabinose residues occur in the furanose form and are essentially non-reducing end-groups. Some arabinofuranose residues carry other sugars through either 0-2 or 0-3. The occurrence of 2,3,4-tri-*O*-Me xylose indicates that H-1 has two non-reducing xylose end-groups per molecule (DP *ca* 100). The remaining xylose residues occur in the primary chain. Some xylose units are unbranched, others are branched through 0-3, and the remainder are substituted at both 0-2 and 0-3. The ratio of unsubstituted, singly- and doubly-branched xylose residues is 1.2:1.2:1. The galactose units and a small proportion of the glucose residues, occur as non-reducing end-groups. Most of the glucose residues occur as chain units, and are linked through 0-4 or 0-3; the ratio of 1 → 4 to 1 → 3 linkages is 10:1.

Polysaccharide H-1 consumed 0.74 moles of periodate per average anhydro-sugar residue. GLC of the polyol showed the presence of glycerol, xylitol, erythritol, arabinitol and glucitol in a molar ratio of 24:14:9:3:2. Methylation analysis of H-1 suggests that this polysaccharide would consume 0.77 moles of periodate per average anhydro-sugar residue and give a polyol containing glycerol, xylitol, erythritol, arabinitol and glucitol in a molar ratio of 23:11:10:2:1. The polyol was subjected to Smith degradation [9] and the following components identified on PC by comparison with authentic samples: 2-*O*-β-D-xylopyranosylglycerol, *O*-β-D-arabinofuranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol, *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol, *O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-glycerol, *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.

Treatment of peracetylated H-1 with CrO_3 resulted in low recoveries of xylose (2%) and glucose (21%), indicating [1, 5, 10] that the glycosidic linkages of these sugars are of the β-configuration.

Partial acid hydrolysis of H-1 resulted in selective cleavage of most of the arabinofuranose linkages, with the formation of polysaccharide A, the dialysate containing arabinose, together with trace amounts of xylose.

Table 2. Composition of the hydrolysate of permethylated polysaccharide A

Methyl ether	Alditol acetate		Methyl glycoside	
	R_t	Mole ratio	R_t	Mole ratio
2,3,4-Tri-*O*-methylxylose	0.63	2	0.49;0.62	2
3,5-Di-*O*-methylarabinose	0.91	0.2	1.14;2.57	0.2
2,3,4,6-Tetra-*O*-methylglucose	1.00	1	1.0; 1.36	1
2,3,4,6-Tetra-*O*-methylgalactose	1.27	1	1.52;1.81	10
2,3-Di-*O*-methylxylose	1.54	9		
2,4,6-Tri-*O*-methylglucose	1.98	2	3.56;4.8	12
2,3,6-Tri-*O*-methylglucose	2.69	9		
2-*O*-Methylxylose	3.02	2	4.1; 6.14	1

Table 3. Composition of the hydrolysate of permethylated polysaccharide B

Methyl ether	Alditol acetate		Methyl glycoside	
	R_t	Mole ratio	R_t	Mole ratio
2,3,4,6-Tetra-*O*-methylglucose	1.0	1	1.0; 1.41	2
2,3 (or 3,4)-Di-*O*-methylxylose	1.5	1	1.41;1.74	
2,4,6-Tri-*O*-methylglucose	1.95	2	3.48;4.76	13
2,3,6-Tri-*O*-methylglucose	2.62	10		

Polysaccharide A comprised xylose, glucose, galactose, arabinose and uronic acid in a molar ratio of 24:20:2:1:4. Methylation analysis (Table 2), compared with that of H-1, shows a decrease in the proportion of 2-*O*-Me xylose, the absence of xylose and a corresponding increase in the amount of 2,3-di-*O*-Me xylose. Polysaccharide H-1 therefore has a linear framework of β(1→4) linked D-xylopyranose residues.

Polysaccharide H-1 was partially hydrolysed for an extended period, and dialysed. The dialysate was shown by PC to contain arabinose, xylose and a mixture of xylo-oligosaccharides (DP 2–5). Polysaccharide B (non-dialysable material) contained glucose and xylose in a molar ratio of 9:1, and had DP 12. The sugar composition of polysaccharide B was unaltered on further partial acid hydrolysis. Methylation analysis of polysaccharide B (Table 3) showed it to be essentially a linear glucan containing both β(1→4) and β(1→3) linked glucose residues in a molar ratio of 5:1. The perMe ether contained equimolar amounts of 2,3 (or 3,4)-di-*O*-Me xylose and 2,3,4,6-tetra-*O*-Me glucose in addition to the tri-*O*-Me glucose residues. The ratio of tri-*O*-Me glucose to 2,3,4,6-tetra-*O*-Me glucose is 12:1; a similar ratio was obtained for H-1. This result, and the absence of glucose in the dialysate obtained during the preparation of polysaccharide B, shows that the glucosidic bonds are not cleaved by dilute acid.

EXPERIMENTAL

A sample of *Sorghum* grain of the Barnard Red variety [*Sorghum bicolor* (L.) Moench] was used. Details of the origin of the grain sample, separation of the husk shavings, isolation of polysaccharides and fractionation of hemicellulose B have been reported previously [2]. Polysaccharides (*ca* 50 mg) were permethylated successively by the procedures of refs [11] and [12].

GLC was performed at N_2 flow rates of *ca* 40 ml/min at 175°, using FID, on columns (1.8 m × 0.3 mm) of (1) 3% ECNSS–M on Gas–Chrom Q (alditol acetates and partially methylated alditol acetates), and (2) 15% butan-1,4-diol succinate polyester on Chromosorb W (Me glycosides). R_t are quoted relative to hexa-*O*-acetyl-D-mannitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-Me-D-glucitol, or Me 2,3,4,6-tetra-*O*-Me-β-D-glucopyranoside.

GC–MS. Partially methylated alditol acetates were separated at He flow rates of *ca* 25 ml/min at 165° on a column (2 m × 3 mm) of 3% ECNSS-M on Gas–Chrom Q. MS were recorded at 70 eV.

PC. Carried out on Whatman no. 1 or 3 MM paper in the organic phase of the following systems (a) EtOAc–C_5H_5N–H_2O (10:4:3); (b) EtOAc–HOAc–HCO_2H–H_2O (18:4:1:3); (c) *n*-BuOH–EtOH–H_2O (4:1:5); (d) *n*-BuOH–C_5H_5N–C_6H_6–

H_2O (5:3:1:3); (e) *iso*-PrOH–HOAc–H_2O (7:1:2); (f) *n*-BuOH half satd with H_2O; (g) EtOAc–C_5H_5N–H_2O (8:2:1).

Electrophoretic analysis. Polysaccharide H-1 migrated as a single band on electrophoresis on Millipore PhoroSlides and cellulose acetate films, using 0.1 M $(NH_4)_2CO_3$ buffer (pH 8.9) and 0.05 M NaOAc buffer (pH 5) at 20 V/cm. Moving free-boundary (Tiselius) electrophoresis of H-1 in 0.05 M $Na_2B_4O_7$ (pH 9.2) at 10 mA and 150 V gave a single, symmetrical peak.

Bio-Gel chromatography. Samples (*ca* 8 mg) of H-1 were chromatographed on columns (90 × 1.5 cm) of Bio-Gel A-0.5 m, P-300 and P-100, using 0.5 M NaCl as eluant. In each case a single, symmetrical elution pattern was obtained. H-1 (200 mg) was chromatographed on a column (90 × 2.5 cm) of Bio-Gel A-0.5 m and eluted with 0.5 M NaCl. Fractions were combined (tubes 50–75; 76–95; 96–120) and dialysed. Samples of the 3 polysaccharides were hydrolysed and analysed as alditol acetates by GLC.

Composition. H-1 (1 g) was hydrolysed (M H_2SO_4, 8 hr, 95°), neutralized ($BaCO_3$), filtered, deionized [Amberlite IR-120 (H^+)] and separated into neutral and acidic components by passage through Amberlite IR 45 (OAc^-). The neutral sugars were separated on a column of cellulose (solvent f) into 3 fractions. Fractions 1 and 2 were identified as D-xylose and L-arabinose, respectively, by the methods previously described [5]. Fraction 3 (185 mg) was separated by preparative PC (solvent g) into two components. Fraction 3a had $[\alpha]_D^{20}$ + 48° (H_2O; *c* 1.1), and was chromatographically identical to D-glucose (solvents a, b, c and d). The derived alditol acetate had R_t 1.37, the same as that for hexa-*O*-acetyl-D-glucitol. The sugar was characterized as the *N*-*p*-nitrophenyl α-D-glucosylamine [13], mp and mmp 183°. Fraction 3b had $[\alpha]_D^{20}$ + 76° (H_2O; *c* 0.7) and was chromatographically identical with D-galactose (solvents a, b, c and d). The alditol acetate had R_t 1.16, identical with that of hexa-*O*-acetylgalactitol. The acidic moiety (148 mg) was methanolysed and a portion was methylated, reduced ($LiAlH_4$ in THF), methanolysed and analysed by GLC. The remainder of the Me ester Me glycosides was reduced ($LiAlH_4$ in THF), hydrolysed and analysed by GLC.

Estimation of DP. (i) H-1 (8 mg) was eluted in 0.5 M NaCl from a column (90 × 1.5 cm) of Bio-Gel A-0.5 m, calibrated with dextrans of known MW_w [14]. The peak ht corresponded to MW_w 15 200. Based on a MW of 142.1 for the average anhydro-sugar residue, DP of H-1 was calculated as 107. (ii) The average number of reducing end-groups was estimated colorimetrically [6], using D-xylose as standard. H-1 (2.65 mg) contained 32 μg reducing end-group, indicating a DP of 77 (MW_n 11 000).

Methylation analysis. Permethylated H-1 and $[\alpha]_D^{20}$ − 28° ($CHCl_3$; *c* 0.73), and showed no IR absorption attributable to OH. A portion (25 mg) was subjected to formolysis (98% HCO_2H, 1 hr, 95°), evaporated to dryness, and hydrolysed (M H_2SO_4, 10 hr, 95°). The hydrolysate was converted to the partially methylated alditol acetates and analysed by GC–MS.

Smith degradation. Oxidation of H-1 (480 mg) with 300 ml of 0.035 M $NaIO_4$ at 5° in the dark was complete after 24 hr, as monitored titrimetrically [15]. A portion of the reduced polyaldehyde was hydrolysed and analysed by GLC (alditol acetates). The remainder of the polyol was partially hydrolysed (0.5 M H_2SO_4, 3.5 hr, 22°), neutralized and examined by PC (solvents a and d).

Linkage analysis. Peracetylayed H-1 was treated with CrO_3 in HOAc (containing hexa-*O*-acetyl-D-mannitol) for 6 hr at 50° [10]. The soln was poured into H_2O and extracted into $CHCl_3$. The product was hydrolysed and analysed as the alditol acetates by GLC.

Preparation of polysaccharide A. H-1 (2 g) was partially hydrolysed in 5 mM H_2SO_4 (100 ml, 11 hr, 95°), neutralized and dialysed. The dialysate was examined by PC (solvent a). The non-dialysable material (polysaccharide A) was analysed for uronic acid content [8]. A portion of polysaccharide A was hydrolysed and analysed by GLC (alditol acetates).

Methylation analysis. Portions of the permethyl ether of polysaccharide A were hydrolysed (98% HCO_2H, followed by M H_2SO_4) or methanolysed (4% methanolic HCl, 12 hr, 95°), and the products analysed by GLC (alditol acetates and Me glycosides).

Preparation and analysis of polysaccharide B. H-1 (200 mg) was partially hydrolysed (20 ml, 5 mM H_2SO_4, 95°). Aliquots were removed at intervals, and dialysed. The non-dialysable materials were hydrolysed, and the products analysed as the derived alditol acetates by GLC. H-1 (2.4 g) was subjected to partial acid hydrolysis for 38 hr, neutralized and dialysed. The dialysate was examined by PC (solvent e). The non-dialysable material (polysaccharide B, 500 mg) had $[\alpha]_D^{20}$ + 64° (H_2O; *c* 0.74). The hydrolysate of polysaccharide B was analysed by GLC (alditol acetates). A sample of polysaccharide B (8 mg) was eluted from a column (95 × 1.5 cm) of Bio-Gel P-60 in 0.5 M NaCl. The elution pattern had peak ht corresponding to MW_w 1900 (DP 12). The mixtures of partially methylated alditol acetates and Me glycosides, derived from permethylated polysaccharide B, were each analysed by GLC.

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