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STRUCTURAL STUDIES ON THREE HEMICELLULOSE B FRACTIONS FROM THE HUSK OF SORGHUM GRAIN*

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ABSTRACT

Hemicelluloses H-4, H-8, and H-9 each contains L-arabinose, D-xylose, D-galactose, D-glucose, glucuronic acid, and 4-*O*-methylglucuronic acid. The degrees of polymerization of these polysaccharides are 993 (H-4), 2,380 (H-8), and 851 (H-9). These polysaccharides have backbones of β-(1→4)-linked D-xylopyranosyl residues to which are attached side chains of L-arabinofuranosyl or glucosyluronic acid groups. L-Arabinofuranosyl groups are attached at O-3, or O-2 and O-3, of certain D-xylopyranose residues. Glucosyluronic acid side-chains are attached to the primary xylan chain at O-2 of some D-xylose residues. Hemicelluloses H-4, H-8, and H-9 differ from one another in their degree of branching.

INTRODUCTION

Hemicelluloses and water-soluble gums have been isolated from the husk shavings[2] and endosperm[3] of sorghum grain. The water-soluble gums[4] from the endosperm are D-glucans containing both (1→4)- and (1→6)-linkages. Hemicellulose A from the endosperm of sorghum grain is a β-D-glucan having β-(1→3)- and β-(1→4)-glycosidic linkages[5], and is similar to the glucans from other cereals[6–9]. Hemicellulose B from the husk of sorghum grain has been separated[2] by DEAE-cellulose chromatography into thirteen fractions (H-1 to H-13). Some structural features of hemicelluloses H-1 (ref. 10) and H-3 (ref. 11) have been described. In this paper, the structures of hemicelluloses H-4, H-8, and H-9 are discussed.

RESULTS AND DISCUSSION

Polysaccharides H-4, H-8, and H-9 each gave a single, symmetrical peak in chromatography[1,2] on Bio-Gel A-15 m. The properties of H-4, H-8, and H-9 are

*Sorghum Polysaccharides, Part X. For Part IX, see ref. 1.

summarized in Table I. The high, negative, optical rotations of H-4 and H-9 are similar to that obtained[11] for the arabinoxylan H-3. Polysaccharide H-8 has a low, positive, optical rotation, indicating that this polymer is structurally different from H-4 and H-9. The low contents of uronic acid groups in H-4 (6.3%) and H-8 (5.4%) are similar to the values obtained[10,11,13] for the other polymers in the B(A) groups[2]. Polysaccharide H-9 has a higher content of uronic acid groups (12.1%), which is representative[13] of the polymers of the B(B) group[2]. This difference in the uronic acid content accounts for the separation[2] of hemicellulose B into the B(A) and B(B) groups by DEAE-cellulose chromatography. The degrees of polymerization of H-4, H-8, and H-9 were found by reducing end-group analysis[14] to be 993, 2,380, and 851, respectively.

TABLE I

PROPERTIES[a] OF POLYSACCHARIDES H-4, H-8, AND H-9

Property	*Value*		
	H-4	*H-8*	*H-9*
Nitrogen (%)	0.10	0.41	0.05
$[\alpha]_D^{20}$ (degrees)	−101	+19	−98
Uronic group[b] (%)	6.3	5.4	12.1
Equivalent weight[c]	2,800	3,300	1,500
$\overline{D.p.}^d$	993	2,380	851
$\bar{M}_n{}^d$	136,000	333,000	117,000
Periodate consumed[e]	0.59	0.64	0.75
Composition (mole %)			
arabinose	50	43	38
xylose	37	34	47
galactose	4	9	2
glucose	3	8	1
glucuronic acid	4	4	10
4-*O*-methylglucuronic acid	2	2	2

[a]All data corrected to a dry-weight basis. [b]Carbazole, colorimetric method. [c]Calculated from content of uronic group. [d]Reducing end-group analysis. [e]Molecule of periodate consumed per average sugar residue.

The neutral fraction of the acid hydrolyzates of H-4, H-8, and H-9 contained arabinose, xylose, galactose, and glucose. The xylose, galactose, and glucose residues were assumed to have the D, and arabinose the L, configuration; these assumptions are based on previous studies[10,11] on the hemicelluloses from sorghum grain in which these sugars were fully characterized. Methanolysis of the reduced, permethylated acid fraction gave, in each case, equimolar amounts of methyl 3,4-di-*O*-methylxylopyranoside and methyl 2,3,4-tri-*O*-methylglucopyranoside. The glucosyluronic acid groups are, therefore, linked 1→2 to certain xylose residues. Hydrolysis of the reduced methyl ester methyl glycosides gave xylose, glucose, and 4-*O*-methylglucose, showing

that these polysaccharides contain both glucosyluronic acid and 4-*O*-methylglucosyluronic acid groups as end groups. The sugar compositions of polysaccharides H-4, H-8, and H-9, which include the neutral and acid sugars of the aldobiouronic acids (not detected by the g.l.c. method previously used[2]), are given in Table I. The polysaccharides are essentially arabinoxylans containing various proportions of arabinose and xylose. The total content of neutral hexose units increases from 3% (H-9) to 17% (H-8). Polysaccharides H-4 and H-8 have glucosyluronic acid and 4-*O*-methylglucosyluronic acid groups in the ratio of 2:1, whereas H-9 has these acid groups in the ratio of 5:1.

Methylation analysis of H-4, H-8, and H-9 gave the products listed in Table II. Xylose and its methyl ethers arise from the primary chain, and indicate branching through O-3, or both O-2 and O-3. The proportions of xylose and 2-*O*-methylxylose

TABLE II

COMPOSITION OF HYDROLYZATES OF PERMETHYLATED H-4, H-8, AND H-9

Component	T^a	*Mole ratio* (%)		
		H-4	*H-8*	*H-9*
2,3,5-Tri-*O*-methyl-L-arabinose	0.48	39	39	38
2,3,4-Tri-*O*-methyl-D-xylose	0.65	—	1	1
3,5-Di-*O*-methyl-L-arabinose	0.93	4	2	2
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	—	5	1
2,5-Di-*O*-methyl-L-arabinose	1.08	6	11	7
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.27	3	2	3
2,3-Di-*O*-methyl-D-xylose	1.54	12	5	19
2,4,6-Tri-*O*-methyl-D-glucose	1.94	2	—	1
2,3,6-Tri-*O*-methyl-D-glucose	2.66	1	3	tr[b]
2-*O*-Methyl-D-xylose	3.06	25	3	19
2,3,4-Tri-*O*-methyl-D-galactose	3.37	—	2	—
2,6-Di-*O*-methyl-D-glucose	3.77	—	2	—
3,6-Di-*O*-methyl-D-galactose/glucose	4.42	—	4	—
D-Xylose	5.42	8	21	9

[a]With column *A*. [b]tr = trace.

show that H-8 is more highly branched than H-4 and H-9. Side chains are terminated by arabinofuranosyl, galactopyranosyl, and glucopyranosyl groups. Certain arabinofuranose residues are substituted at either O-2 or O-3. Polysaccharides H-4, H-8, and H-9 also contain small proportions of hexose residues as chain units, and, in addition, H-8 has some of these sugar residues as branch-points. The proportion of arabinose, xylose, galactose, and glucose in polysaccharides H-4, H-8, and H-9, as calculated from the results of the methylation analysis (corrected for those xylose residues combined in the aldobiouronic acids, and not detected by the g.l.c. procedure used), are in close agreement with the neutral sugar compositions of these polysaccharides, shown in Table I. The ratio of end groups to branch-points (calculated from Table II) for each permethylated polysaccharide is ~1:1.

Treatment of H-4, H-8, and H-9 with sodium periodate resulted in the uptake of 0.59, 0.64, and 0.75 molecule of periodate per sugar residue, respectively. The polyalcohols contained xylose and arabinose in the ratios of 3.0:1 (H-4), 1.8:1 (H-8), and 3.1:1 (H-9). Polysaccharides having the structural features indicated by the results of the methylation analyses would be expected to consume 0.58 (H-4), 0.67 (H-8), and 0.67 (H-9) molecule of periodate per sugar residue, and give polyalcohols having xylose-to-arabinose ratios of 3.3:1 (H-4), 1.8:1 (H-3), and 3.1:1 (H-9).

Oxidation of the peracetylated polysaccharides with chromium trioxide in acetic acid for 6 h resulted in low recoveries of xylose for H-4 (6%), H-8 (10%), and H-9 (6%). These results indicate[15] that H-4, H-8, and H-9 each has primary chains of β-linked D-xylopyranose residues.

EXPERIMENTAL

General. — I.r. spectra were recorded with a Perkin–Elmer 237 spectrophotometer. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. 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. 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*-acetyl-D-mannitol (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). Polysaccharides were successively methylated 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°.

Isolation of polysaccharides H-4, H-8, and H-9. — The extraction of the polysaccharides from the husk shavings of the Barnard Red variety of sorghum grain, and the fractionation of hemicellulose B by DEAE-cellulose chromatography into thirteen fractions (H-1 to H-13), have been reported[2].

Determination of $\overline{d.p.}$ — The average number of end groups in polysaccharides H-4, H-8, and H-9 was estimated colorimetrically[14], using D-xylose as the standard. Samples of polysaccharides H-4 (33.0 mg), H-8 (20.2 mg), and H-9 (36.5 mg) contained 32.0, 8.0, and 41.0 μg of reducing end-group, respectively. Based on molecular weights of 137 (H-4), 140 (H-8), and 138 (H-9) for the average sugar residues, the $\overline{d.p.}$ values for the polysaccharides were calculated to be 993 (H-4), 2380 (H-8), and 851 (H-9).

Composition of polysaccharides H-4, H-8, and H-9. — A sample (200 mg) of each polysaccharide was hydrolyzed, the acid neutralized (barium carbonate), the suspension filtered, the filtrate de-ionized [Amberlite IR-120 (H^+) resin], and the mixture separated into neutral and acidic components by passage through a column

of Amberlite IR-45 (OAc^-) resin. Portions of the neutral fractions were converted into the alditol acetate derivatives, and these were analyzed by g.l.c. (column *A*).

The acid fractions were eluted from the Amberlite IR-45 (OAc^-) resin with 5% formic acid, and methanolyzed, and portions of the products were methylated, reduced (lithium aluminum hydride in tetrahydrofuran), and methanolyzed. In each case, g.l.c. (column *B*) showed equimolar quantities of methyl 3,4-di-*O*-methyl-xylopyranoside (*T* 1.36 and 1.63) and methyl 2,3,4-tri-*O*-methylglucopyranoside (*T* 2.54 and 3.57). The rest of the methyl ester methyl glycosides was reduced (lithium aluminum hydride in tetrahydrofuran), hydrolyzed, and analyzed by g.l.c. (alditol acetates, column *A*). Each chromatogram had peaks with retention times corresponding to those of the acetates of authentic xylitol (*T* 0.54), 4-*O*-methylglucitol (*T* 1.13), and glucitol (*T* 1.37).

Methylation analyses. — Samples of polysaccharides H-4, H-8, and H-9 were converted into the permethyl ethers, and these were purified by precipitation from chloroform solution with petroleum ether (b.p. 100–120°). The permethyl ethers of H-4, H-8, and H-9 had $[\alpha]_D^{20}$ −26° (*c* 1.1, chloroform), −18° (*c* 1.1, chloroform), and −56° (*c* 1.2, chloroform), respectively, and showed, in their i.r. spectra, no absorption attributable to OH. Solutions of the permethylated polysaccharides in 98% formic acid were heated for 1 h at 95°, cooled, and evaporated to dryness. The residues were hydrolyzed (M sulfuric acid), and the solutions made neutral (barium carbonate), and analyzed by g.l.c. (partially methylated alditol acetates, column *A*). The results are summarized in Table II.

Periodate oxidation. — Samples of polysaccharides H-4, H-8, and H-9 were treated with 0.04M sodium periodate at 4–5° in the dark. Aliquots were removed at intervals, and the amounts of periodate consumed were determined titrimetrically[16]. After 24 h, when the oxidations were complete, the excess of periodate was decomposed with ethylene glycol, and the solutions were dialyzed. The polyaldehydes were reduced (sodium borohydride), the reactions terminated by the addition of acetone, and the solutions dialyzed. The polyalcohols were hydrolyzed, and analyzed by g.l.c. (alditol acetates, column *A*).

Chromium trioxide oxidation. — Solutions of polysaccharides H-4, H-8, and H-9 (50 mg) in formamide (5 ml) were treated with acetic anhydride (4 ml) and pyridine (3 ml) for 24 h at 20°. The solutions were poured into water, dialyzed, and freeze-dried. The peracetylated polysaccharides were oxidized[15] with chromium trioxide (150 mg) in acetic acid (5 ml, containing hexa-*O*-acetyl-D-mannitol) 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*).

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