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STRUCTURAL STUDIES ON THE WATER-SOLUBLE GUMS FROM THE ENDOSPERM OF SORGHUM GRAIN*

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ABSTRACT

Purification by Bio-Gel P-100 chromatography of the cold-water-soluble gum from the endosperm of sorghum grain gave an electrophoretically homogeneous D-glucan. This polysaccharide (glucan A) had $[\alpha]_D^{20}$ +88° and $\overline{\text{d.p.}}$ 23. Methylation analysis and periodate-oxidation studies indicated that glucan A has both (1→4)- and (1→6)-linked D-glucopyranose residues, in the ratio of 2:1. On average, three D-glucose residues per molecule of glucan A are branched. Chromium trioxide oxidation analysis of glucan A indicated a preponderance of α-D-glucosidic linkages. P.m.r. spectroscopy of permethylated glucan A showed the presence of α- and β-linkages in the ratio of 4:1. Purification of the hot-water-soluble gum by Bio-Gel P-100 chromatography gave glucan B, having $[\alpha]_D^{20}$ +134° and $\overline{\text{d.p.}}$ 20. Structural studies showed glucan B to be similar to glucan A, differing in the ratio of (1→4)- to (1→6)-linkages; glucan B has these D-glucosidic linkages in the ratio of 6:1.

INTRODUCTION

In an earlier paper[2], the isolation of hemicelluloses A and B and the water-soluble gums from the endosperm of the Barnard Red variety of sorghum grain was reported. The structural features of a β-D-glucan, the major component of hemicellulose A, have been described[3]; this polysaccharide was shown to be a linear D-glucan containing β-(1→3)- and β-(1→4)-glucosidic linkages. Hemicellulose B from the endosperm has been separated[2] into thirteen fractions by DEAE-cellulose chromatography. Studies[2] on the hemicellulose A preparations, and on the DEAE-cellulose fractions of hemicellulose B from the endosperm of sorghum malts, indicated that major modifications occur in these polysaccharides during malting. This may be

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contrasted with an analysis of sorghum-husk polysaccharides in which it was shown[4] that only minor changes occur in hemicelluloses A and B during malting.

The cold- and hot-water-soluble gums from the endosperm of sorghum grain are essentially D-glucans, containing minor proportions of arabinose, xylose, and galactose[2]. The positive optical rotations of these gums indicated a preponderance of α-linked D-glucosyl residues. The gums gave no coloration with iodine–potassium iodide solution, and were not degraded by *alpha*- or *beta*-amylase. Some structural features of glucans A and B, respectively prepared from the cold- and hot-water-soluble gums, are described herein.

RESULTS AND DISCUSSION

The cold- and hot-water-soluble gums from the endosperm of sorghum grain[2] were each resolved by chromatography on Bio-Gel P-100 into two fractions, one of higher and the other of lower molecular weight. The materials of higher molecular weight, containing arabinose, xylose, galactose, and glucose residues, were not further investigated. The recoveries of the lower molecular-weight fractions from the cold- and hot-water-soluble gums (glucans A and B) were 80 and 75%, respectively. Glucan A represents 0.14% of the endosperm, whereas glucan B accounts for 0.06%. The elution patterns of glucans A and B on Bio-Gel P-6 indicated that each polysaccharide possessed a high degree of homogeneity. These glucans were electrophoretically homogeneous, gave no coloration with iodine–potassium iodide solution, and were not degraded by *alpha*-amylase. G.l.c. analysis of the alditol acetate derivatives from glucans A and B showed single components having retention time (T) 1.39, identical with that of hexa-*O*-acetyl-D-glucitol. The component sugar in each of the hydrolyzates of glucans A and B was further characterized as D-glucose. A determination of the proportions of reducing end-groups[5] in each of glucans A and B showed these polysaccharides to have $\overline{\text{d.p.}}$ 23 ($\bar{M}_n$ 3,700) and 20 ($\bar{M}_n$ 3,200), respectively.

Formolysis and hydrolysis, or methanolysis, of permethylated glucans A and B gave the products listed in Table I. The methylation analysis of glucan A shows that this polysaccharide contains both (1→4)- and (1→6)-glucosidic linkages in the ratio of 2:1. Branching occurs through O-3, O-4, or O-6 of certain D-glucose residues. The proportion of 2,3,4,6-tetra-*O*-methyl-D-glucose in the hydrolyzate of permethylated glucan A indicates a $\overline{\text{d.p.}}$ of 25, which is in close agreement with the results obtained by reducing end-group analysis. Glucan A therefore has, on average, three branch-points per molecule. Methylation analysis of glucan B showed the presence of (1→4)- and (1→6)-glucosidic linkages in the ratio of 6:1, and a d.p. of 21. Glucan B also contains, on average, three branch-points per molecule.

Periodate oxidation of glucans A and B resulted in the respective uptake of 1.30 and 1.19 moles of periodate per mole of D-glucose residue. Glucans having the structural features suggested by the methylation analyses would be expected to consume 1.32 (glucan A) and 1.19 (glucan B) moles of periodate per mole of D-glucose

TABLE I

COMPOSITION OF THE HYDROLYZATES AND METHANOLYZATES OF PERMETHYLATED GLUCANS A AND B

Methyl ethers of D-*glucose*	*Glucan A*				*Glucan B*			
	Column A		*Column B*		*Column A*		*Column B*	
	T	*Mole ratio*	T[a]	*Mole ratio*	T	*Mole ratio*	T[a]	*Mole ratio*
2,3,4,6-Tetra-	1.00	4	1.00; 1.43	1	1.00	4	1.00, 1.44	1
2,3,4-Tri-	2.46	6	2.58; (3.56)	5	2.49	2	2.65; (3.58)	4
2,3,6-Tri-	2.75	12	(3.56); 4.71		2.76	12	(3.58); 4.71	
2,6-Di-	3.80	2	n.d.[b]		3.73	2	n.d.	
2,3-Di-	5.46	1	n.d.		5.42	1	n.d.	

[a]Figures in parentheses indicate *T* values not completely resolved. [b] Not determined.

residue. Small proportions of D-glucose were present in the hydrolyzates of periodate-oxidized glucans A and B, arising from those units which afford 2,6-di-*O*-methyl-D-glucose on hydrolysis of the permethylated glucans.

Chromium trioxide oxidation[5] of the peracetylated glucans resulted in the recoveries of 79 (glucan A) and 74% (glucan B) of glucose after reaction for 1 h. It has been shown[6] that the acetates of cellobiitol and gentiobiitol are completely oxidized by treatment with chromium trioxide for 1 h, whereas maltitol peracetate is not oxidized under these conditions. Previous studies[1,3] on D-glucose-containing polysaccharides from sorghum grain indicated that extended periods of time are needed in order to oxidize the component, β-linked, D-glucose residues. Peracetylated glucans A and B were treated with chromium trioxide for 24 h, and the recoveries of glucose were 68 (glucan A) and 70% (glucan B). These results indicate that glucans A and B contain mainly α-glucosidic linkages. The p.m.r. spectra of permethylated glucans A and B each showed signals at τ 4.38 and 4.83, corresponding to the anomeric protons of α- and β-linked D-glucopyranose residues, respectively. Quantitative estimations of these anomeric signals showed both glucans to have α- and β-glucosidic linkages in the ratio of 4:1.

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 Perkin–Elmer 241 polarimeter for aqueous solutions, unless otherwise stated. Gas–liquid chromatography (g.l.c.) was performed with a Packard 805 chromatograph, with nitrogen as the carrier gas at flow rates of 40 ml/min, at 185°. Columns (180 × 0.3 cm) were packed with (*A*) 3% of ECNSS-M on 100–120-mesh Gas-Chrom Q (alditol acetates and partially methylated alditol acetates), or (*B*) 15% of 1,4-butanediol succinate polyester on 60–80-mesh Chromosorb W (methyl glycosides). Retention times (*T*) are given relative to those of 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). G.l.c.–mass spectrometry was performed with a Hewlett–Packard 5710A gas chromatograph coupled to a Hewlett–Packard 5981A mass spectrometer by means of a silicone membrane. Mass spectra were recorded at 70 eV. P.m.r. spectra were recorded with a Varian HA-100 instrument for solutions in C_6D_6 with tetramethylsilane as the internal standard. Descending, partition chromatography was conducted on Whatman No. 1 paper in the following solvent systems: (*A*) 5:3:1:3 1-butanol–pyridine–benzene–water, or (*B*) 18:4:1:3 ethyl acetate–acetic acid–formic acid–water. Components were detected with a spray of *p*-anisidine hydrochloride. Polysaccharides were methylated successively by the Hakomori and Purdie procedures.

Gel chromatography. — A sample (2 g) of the cold-water-soluble gum[2] was applied to a column (90 × 2.5 cm) of Bio-Gel P-100, and eluted as two well-defined

fractions in 0.5M sodium chloride. The higher molecular-weight material (180 mg) contained arabinose, xylose, galactose, and glucose. The fraction of lower molecular weight (glucan A, 1.6 g) was shown by Bio-Gel P-6 chromatography to possess a high degree of homogeneity.

Similarly, the hot-water-soluble gum[2] (2 g) was separated into two fractions by chromatography on Bio-Gel P-100. The lower molecular-weight material (glucan B, 1.5 g), when chromatographed on Bio-Gel P-6, gave an elution pattern which was essentially the same as that obtained for glucan A.

Electrophoretic studies. — Electrophoresis of glucans A and B was conducted on Millipore PhoroSlide electrophoresis strips (7.5 × 2.5 cm) and on strips of cellulose acetate (17 × 2.5 cm) at 20 V/cm. Glucans A and B migrated as single bands in 0.01M sodium borate buffer (pH 9.2).

Composition of glucans A and B. — Glucans A and B had $[\alpha]_D^{20}$ +88° (*c* 1.0) and +134° (*c* 1.2), respectively. These polysaccharides gave no coloration when treated with iodine–potassium iodide solution, and were not degraded by *alpha*-amylase. Samples of glucans A and B were hydrolyzed (M sulfuric acid, 7 h, 95°), neutralized ($BaCO_3$), and analyzed by g.l.c. (alditol acetates). The sugar in the hydrolyzate of glucan A was chromatographically identical (solvents *A* and *B*) with D-glucose, had $[\alpha]_D^{20}$ +50° (*c* 1.0), and was characterized as *N*-(*p*-nitrophenyl)-α-D-glucosylamine[7], m.p. and mixed m.p. 183°.

A similar study on the hydrolyzate of glucan B showed D-glucose to be the component sugar of this polysaccharide.

Estimation of d.p. — Samples of glucans A and B were analyzed for reducing end-groups, according to the modified Nelson procedure[5], using D-glucose as the standard. Glucans A (625 μg) and B (460 μg) contained 27 and 23 μg of reducing end-group, respectively.

Methylation analysis. — Glucans A and B (~400 mg) were each permethylated, and the products purified by precipitation from chloroform solution with petroleum ether. The i.r. spectra of the permethyl ethers showed no absorption attributable to OH. Permethylated glucans A and B had $[\alpha]_D^{20}$ +106° (*c* 1.3, chloroform) and +130° (*c* 1.7, chloroform), respectively.

A sample of each permethylated glucan was hydrolyzed (98% formic acid, 1 h, 95°; followed by M sulfuric acid, 7 h, 95°), neutralized ($BaCO_3$), and analyzed by g.l.c.–m.s. as the partially methylated alditol acetates. Portions of the permethylated glucans were methanolyzed (4% methanolic hydrogen chloride, 12 h, 95°), neutralized (Ag_2CO_3), and the methyl glycosides analyzed by g.l.c.

Periodate oxidation. — Samples of glucans A (300 mg) and B (290 mg) were treated with 0.04M sodium periodate (100 ml) in the dark at 20°. The oxidation was monitored titrimetrically[8]. The reaction was complete after 25 h. Ethylene glycol was added to decompose the excess of periodate, the solutions were dialyzed, and the products reduced (sodium borohydride), hydrolyzed, and analyzed by g.l.c. (alditol acetates).

Chromium trioxide oxidation. — Samples of the glucans (~150 mg) in for-

mamide (3 ml) were peracetylated with acetic anhydride (4 ml) and pyridine (3 ml) for 24 h at 20°. Aqueous solutions of the products were dialyzed and freeze-dried. To the peracetylated derivatives of glucans A and B, each in acetic acid (5 ml, containing hexa-*O*-acetylgalactitol as the internal standard), was added chromium trioxide (~400 mg), and the solutions were stirred at 50°. Aliquots were removed at intervals, and partitioned between water and chloroform. The organic phases were evaporated, and the products hydrolyzed, and analyzed by g.l.c. (alditol acetates).

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