Carbohydrate Research, 51 (1976) 249–252

A HEMICELLULOSIC β-D-GLUCAN FROM THE ENDOSPERM OF SORGHUM GRAIN*

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(Received July 28th, 1976; accepted for publication, August 7th, 1976)

ABSTRACT

A hemicellulosic β-D-glucan of $\overline{\text{d.p.}}$ ~26 has been isolated from the endosperm of sorghum grain. Methylation analysis, partial hydrolysis with acid, and periodate-oxidation studies showed that the glucan is linear and has both (1→3)- and (1→4)-linked D-glucopyranose residues in the ratio of 3:2. The low, positive, specific rotation and chromium trioxide oxidation studies indicated that the D-glucose residues are β-linked.

INTRODUCTION

Hemicellulosic β-D-glucans containing both (1→3)- and (1→4)-linked D-glucose residues have been isolated from stem[2] and leaf[2–4] tissues of oat plants, and from the stems of maize[5], barley[6], rye[6], and wheat[6]. A glucan has also been isolated from oat coleoptiles[7]. Water-soluble glucans containing both (1→3)- and (1→4)-linkages have been reported in barley endosperm[8,9]. In a previous paper[10], the extraction of the hemicelluloses from the chlorite holocellulose[11] prepared from the endosperm of sorghum grain was reported. Hemicellulose A had $[\alpha]_D^{20}$ +5° and contained glucose, arabinose, and xylose in the molar ratios of 15:1:1. The purification and structure analysis of the β-D-glucan from hemicellulose A is described herein.

RESULTS AND DISCUSSION

A sample of hemicellulose A from the endosperm of sorghum grain was resolved into a glucan and an arabinoglucoxylan by column chromatography on Bio-Gel P-100. These polysaccharides were recovered in the ratio of 7.8:1 (w/w).

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

The total recovery of polysaccharide material was 88%. The glucan accounts for 4.8% of total endosperm hemicellulose, and 0.015% of the whole grain; it is soluble in water, and is eluted as a single, symmetrical band from Bio-Gel P-30. The polysaccharide gives no coloration with iodine–potassium iodide solution, and is not attacked by *alpha*- or *beta*-amylase.

The acid hydrolyzate of the glucan contained only D-glucose. The low, positive, specific rotation of the glucan indicated that the D-glucose residues are β-linked. The degree of polymerization (d.p.) was found to be 26, corresponding to a molecular weight of 4230.

Formolysis and hydrolysis of the permethylated glucan gave 2,3,4,6-tetra- and 2,4,6- and 2,3,6-tri-*O*-methyl-D-glucose in the molar ratios of 1:15:10. The absence of dimethyl ethers in the hydrolyzate indicates that the glucan is linear, and that the 2,3,4,6-tetra-*O*-methyl-D-glucose arises from the nonreducing end-group. The occurrence of 2,4,6- and 2,3,6-tri-*O*-methyl-D-glucose shows that the glucan contains both β-(1 →3)- and β-(1 →4)-linked sugar residues in the ratio of 3:2.

A glucan having the structural features indicated by the methylation analysis would be expected to consume 0.46 mole of periodate per average sugar residue. The reduction of 0.42 mole of periodate by the glucan is in close agreement with the results of the methylation analysis. Paper chromatography and g.l.c. analysis of the hydrolyzate of the derived polyalcohol showed glucose and erythritol in the molar ratio of 3:2.

The nature of the glycosidic linkages was determined by chromium trioxide oxidation[12] of the peracetylated glucan. The low recovery of glucose confirmed that the sugar residues of the glucan are β-linked.

Partial hydrolysis of the glucan with 25mM oxalic acid[3,5] gave a mixture of glucose, disaccharides containing β-(1 → 3) or β-(1 → 4) linkages, and trisaccharides having both β-(1 → 3) and β-(1 → 4) linkages.

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. Gas–liquid chromatography (g.l.c.) was performed on a Packard 805 chromatograph with nitrogen as the carrier gas at a flow rate of 40 ml/min at 185°. Columns (180 × 0.3 cm i.d.) 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 that of 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). Descending partition-chromatography was carried out on Whatman No. 1 paper in the following solvent systems: (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 5:3:1:3 1-butanol–pyridine–benzene–water, (*C*) 4:1:1 ethyl acetate–pyridine–water, and (*D*) 18:4:1:3 ethyl acetate–acetic acid–formic acid–water. Components were detected with *p*-anisidine hydrochloride or alkaline silver nitrate. Samples were hydrolyzed with M sulfuric acid in sealed tubes for 7 h at 95°.

Isolation of the hemicellulosic material. — The alkaline extraction of the hemicelluloses from the chlorite holocellulose[11], prepared from the endosperm of Barnard Red sorghum grain, and the separation of hemicelluloses A and B, have been described[10].

Purification of the glucan. — A sample (2.3 g) of hemicellulose A was applied to a column (90 × 2.5 cm) of Bio-Gel P-100 and eluted with water. Fractions (6 ml) were collected and analyzed[13] for carbohydrate. *Fraction I* (tubes 21–45; 230 mg) had $[\alpha]_D^{20}$ − 35° (*c* 1.0), and g.l.c. analysis (alditol acetates, column *A*) of the hydrolyzate showed arabinose (*T* 0.37), xylose (*T* 0.54), and glucose (*T* 1.37) in the molar ratios of 2:2:1. *Fraction II* (tubes 91–140; 1.8 g) had $[\alpha]_D^{20}$ +9° (*c* 1.1), and g.l.c. analysis (alditol acetates, column *A*) of the hydrolyzate showed only glucose (*T* 1.37). This sugar had $[\alpha]_D^{20}$ +52° (*c* 1.0), and was characterized as *N*-*p*-nitrophenyl-α-D-glucosylamine[14], m.p. and mixed m.p. 183°. The glucan was eluted as a single, symmetrical band from Bio-Gel P-30. It gave no color when treated with iodine–potassium iodide solution, and was not degraded by *alpha*- or *beta*-amylase.

Determination of $\overline{\text{d.p.}}$ — A sample (5.2 mg) of the glucan was treated according to the method of Momose et al.[15], with D-glucose as the standard. This sample contained 200 μg of reducing end-group, corresponding to a $\overline{\text{d.p.}}$ of 26 ($\overline{M}_n$ 4230).

Methylation analysis. — An aqueous solution of the glucan (400 mg) was successively methylated by the methods of Haworth, Hakomori, and Purdie. The permethylated glucan (315 mg) was purified by precipitation from a chloroform solution with petroleum ether. The i.r. spectrum of this product showed no absorption attributable to OH.

A sample of the methylated glucan was hydrolyzed by the formic acid–sulfuric acid method[16], and the hydrolyzate made neutral with barium carbonate. Paper chromatography (solvents *A* and *D*) showed the presence of 2,3,4,6-tetra- and 2,3,6- and 2,4,6-tri-*O*-methylglucose. G.l.c. analysis (partially methylated alditol acetates, column *A*) showed 2,3,4,6-tetra- and 2,4,6- and 2,3,6-tri-*O*-methylglucose (*T* 1.00, 1.97, and 2.56, respectively) in the molar ratios of 1:15:10. A portion of the permethylated glucan was methanolyzed with 4% methanolic hydrogen chloride in a sealed tube for 16 h at 95°. G.l.c. analysis (methyl glycosides, column *B*) then showed methyl 2,3,4,6-tetra- and 2,4,6- and 2,3,6-tri-*O*-methylglucosides (*T* 1.00, 1.45, 3.48, and 4.80). The trimethyl ethers of the methyl glucosides were unresolved.

Periodate oxidation. — The glucan (97 mg) was treated with 0.03M sodium metaperiodate (50 ml) at 5° in the dark. The oxidation was complete after 25 h, as monitored titrimetrically[17] (0.42 mole of periodate consumed per glucose residue). The polyaldehyde was reduced with sodium borohydride, and the product hydrolyzed. Paper chromatography (solvent *D*) showed glucose and erythritol as the major components. G.l.c. analysis (alditol acetates, column *A*) showed glucose (*T* 1.37) and erythritol (*T* 0.11) in the molar ratio of 3:2.

Chromium trioxide oxidation. — The glucan (100 mg) in formamide (5 ml) was peracetylated 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 glucan (105 mg) was treated[12] with chromium trioxide (390 mg) in acetic acid (5 ml, containing hexa-*O*-acetylgalactitol as the internal standard) at 50°. Aliquots were removed at intervals, poured into water, and the products extracted into chloroform. The solvent was evaporated, the residues were hydrolyzed, and the products were analyzed by g.l.c. (alditol acetates, column *A*). The recovery of glucose was 81% (3 h), 38% (16 h), and 12% (24 h).

Partial, acid hydrolysis. — The glucan (50 mg) was heated with 25mM oxalic acid (20 ml) for 18 h at 95°. The solution was made neutral ($BaCO_3$), the suspension filtered, and the filtrate examined by paper chromatography[3,5] (solvents *B*, *C*, and *D*). Glucose, cellobiose, laminarabiose, cellotriose, and 3-*O*-β-cellobiosylglucose were identified by comparison with authentic compounds.

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