Structural Characterization of a Pentosan from the Water-Insoluble Portion of Durum Wheat Endosperm[1]

D. G. MEDCALF and **K. A. GILLES**, North Dakota State University, Fargo 58103

ABSTRACT

The arrangement of branching has been determined for a pentosan fraction isolated from the water-insoluble portion of durum wheat endosperm. Data from chemical and physical analysis, methylation and hydrolysis studies, periodate oxidation, and Smith degradation experiments permit formulation of a detailed picture of the structure of this polysaccharide. The polymer consists of a main chain of D-xylopyranosyl units linked beta-1,4. L-Arabinofuranosyl side-chains are found attached to the 3- (single branches) and to the 2- and 3-positions (double branches) of the xylose chain. Three of five D-xylose units are branched, and almost half of these are branched at both the 2- and 3-positions. Branches occur predominantly on alternating D-xylose units. Occasionally two branched D-xylose units occur in succession. Very infrequently, three or four contiguous D-xylose units are branched.

The pentosans of wheat endosperm can be generally classified into two types, the water-soluble pentosans and the pentosans associated with the water-insoluble or "sludge" portion of the endosperm. The chemical structure of the water-soluble pentosans of wheat flour *(Triticum aestivum)* has been studied in some detail by Perlin and co-workers (1–4). The general structure of a pentosan isolated from the "sludge" fraction of *T. aestivum* wheat has been investigated by Montgomery and Smith (5). It was found to have the same general type of structure reported for the soluble pentosans. Wheat endosperm pentosans and *Gramineae* endosperm pentosans in general (6,7) consist of a main chain of D-xylopyranose units linked beta-1,4. Single-unit L-arabinofuranosyl side-chains are found attached to the 2- and 3-positions of the xylose chain. Degree of branching and detailed arrangement of the branches differ among the various pentosan preparations.

Recently, this laboratory reported on a comparison of the chemical composition and physical properties between endosperm pentosans of hard red spring (*T. aestivum*) and durum (*T. durum*) wheat (8). Durum pentosans from both the water-soluble and "sludge" fractions were more highly branched than comparable pentosans from hard red spring (HRS) wheat.

This paper reports the detailed structural characterization of the major pentosan fraction isolated from the water-insoluble ("sludge") portion of the endosperm of durum wheat (variety Wells).

MATERIALS AND METHODS

Isolation and Purification of Pentosan Fraction

The isolation, purification, and chemical composition of the pentosan fraction used in this work has been described previously (8). The mucilaginous "sludge" fraction was isolated from Wells durum wheat. After pan-

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Authors: Assistant Professor and Professor, respectively, Department of Cereal Chemistry and Technology, North Dakota State University, Fargo.

Present address of Medcalf, Department of Chemistry, University of Puget Sound, Tacoma, Washington.

creatin digestion (9), alkali extraction, and precipitation with ethanol, a crude "sludge" pentosan fraction was isolated in about 4% yield from the "sludge." Protein content was 1.8%. After acetylation and fractional precipitation from acetone with petroleum ether (b.p. 30°–60°C.), one acetone-insoluble and three acetone-soluble fractions were isolated. Soluble fraction I was isolated in the highest yield (35% of the crude sludge pentosans) and was used for the structural characterization reported in this paper. After deacetylation (8) this fraction was isolated as a white, stringy polymer which was soluble in warm water. It contained <1% protein and consisted of D-xylose (53%), L-arabinose (41%), and D-glucose (6%). Its physical properties as reported previously (8) included: intrinsic viscosity, 7.0; molecular weight, 148,000; $[\alpha]_D^{25}$–126°.

Graded Hydrolysis

Mild acid hydrolysis was performed on the pentosan fraction with 0.05*N* sulfuric acid at 75°–77°C. for 4.5 hr. (2). After this had cooled to 0°C., a precipitate was isolated (fraction I). Ethanol was added to the remaining solution to a concentration of 85%. The precipitated material was collected (fraction II) and the remaining solution was concentrated to dryness (fraction III). All three fractions were analyzed for their component sugars by gas chromatography according to the procedure described previously (8).

Methylation

Sludge pentosan (200 mg.) was methylated by the one-step procedure described by Srivastava *et al.* (10). After chloroform extraction, the methylated polymer was isolated as an amorphous solid in about 50% yield. Infrared analysis of the methylated polymer showed complete absence of the strong OH absorption band at 3,600–3,200 $cm.^{-1}$. This indicated that methylation had been completed.

Hydrolysis of Methylated Polymer and Quantitative Estimation of Methylated Sugars

A portion of the methylated polymer (10 mg.) was dissolved in 0.5 ml. of 72% sulfuric acid at 0°C. The solution was kept at 25°C. for 1 hr., then diluted with 2 ml. of water and placed in a boiling-water bath for 4 hr. After the solution had cooled, it was neutralized with barium carbonate and centrifuged, and the solids were removed and washed with water and ethanol. The combined supernatants were concentrated to a syrup under reduced pressure and examined by paper chromatography. Papers were developed with 1-butanol-ethanol-water (50:10:40:v./v., upper layer) as solvent, and spots were visualized with *p*-anisidine spray reagent (11). Four distinct compounds were detected; these were identical in rate of movement with authentic samples of D-xylose, 2-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2,3,5-tri-O-methyl-L-arabinose.

Further qualitative identification and quantitative estimation of the ratio of methylated sugars was performed by the procedure described by Rees and Richardson (12).

Methylated polysaccharide was hydrolyzed with 45% formic acid at 100°C. for 16 hr. After adjustment to pH 8 with ammonia, the methylated sugars were reduced with sodium borohydride, and the alditols were acetylated before analysis by gas chromatography.

Gas chromatography was performed with a Beckman GC-2A chromatograph equipped with a flame ionization detector. Separations were made on a ⅛-in. Al column, 10 ft. in length, packed with 3% XE-60 on 100- to 120-mesh Gas Chrom P (Applied Science Laboratories, Inc.). Nitrogen was used as the carrier gas with a column temperature of 190°C. and a flow rate of 25 cc./min.

Four major peaks were detected from the methylated polysaccharide. These were identical in retention time with the reduced and acetylated products from authentic D-xylose, 2-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2,3,5-tri-O-methyl-L-arabinose.

The relative molar ratios of these four products were estimated quantitatively by the procedure of Rees and Richardson (13). Peak areas were determined by triangulation.

Periodate Oxidation and Smith Degradation

Periodate uptake by Wells sludge pentosan was determined by the procedure of Hay, Lewis, and Smith (13). Periodate consumption was determined by the iodometric method. Periodate uptake was constant after 48 hr. and corresponded to 0.67 mole of periodate consumed per anhydropentose unit.

Smith periodate degradation studies were performed as described by Rees and Richardson (12). Pentosan (200 mg.) was dissolved in 0.04*M* sodium metaperiodate solution (60 ml.) and allowed to stand at 25°C. for 72 hr. Ethylene glycol was added to destroy excess periodate, and an excess of sodium borohydride was added. After this had stood 2 days at room temperature, a mixture of IR 120 (H^+) and IR 45 (OH^-) resins was added, and the solution was filtered and concentrated to dryness under reduced pressure at 35°–40°C. Methanol was repeatedly evaporated from the residue to remove boric acid. The residual syrup was taken up in 25 ml. 1*N* sulfuric acid, allowed to stand at room temperature overnight, and neutralized with barium carbonate. The solution was centrifuged to remove insolubles and concentrated to a syrup under reduced pressure.

Paper chromatography with ethyl acetate-pyridine-water (10:4:3 v./v.) solvent and silver nitrate (11) spray reagent indicated the presence of glycerol, glycolaldehyde, and four unidentified components having R_{xylose} values as shown in the table below. The four unidentified components were isolated in pure form by paper chromatography on Whatman 3MM paper.

Component	R_{xylose} *Value*	$[\alpha]_D^{25}$
I	1.0	−38° (*c* 0.4, water)
II	0.67	−53° (*c* 0.6)
III	0.37	−58° (*c* 0.4)
IV	0.12	−66° (*c* 0.3)

The molar ratio of the four components was determined in the original Smith degradation mixture by quantitative paper chromatography, according to the phenol-sulfuric acid method (14).

A portion (2–3 mg.) of each of the unidentified components was hy-

drolyzed with 1*N* sulfuric acid at 100°C. for 7 hr. After neutralization with barium carbonate, paper chromatography indicated the presence of only D-xylose and glycerol in each case. A portion of each hydrolysate was separated quantitatively on paper. After elution from the paper, the amount of D-xylose was determined by the phenol-sulfuric acid method (14), and glycerol was estimated by oxidation with periodate followed by determination of the formaldehyde formed; the acetylacetone-ammonia method was used (15).

A portion (10–20 mg.) of each component (I-IV) was methylated (10) and the molar ratio of the methylated sugars was determined after acid hydrolysis as described above (12). Peaks were identified by comparison of retention times with authentic samples.

RESULTS AND DISCUSSION

Pentosans from durum wheat appear to be more highly branched than those from HRS wheat (8). The sludge pentosan isolated in this work from Wells durum wheat was similar in general structural features to the sludge pentosan from common bread wheat characterized by Montgomery and Smith (5). The only previous detailed structural characterizations of cereal endosperm arabinoxylans have been performed on pentosans from the soluble portion of the endosperm (3,4,7). The pentosan fraction from the sludge portion of durum endosperm, though more highly branched, was similar in structure to the water-soluble pentosans from bread wheat (3,4) and rye (7).

The pentosan studied in this work was composed of D-xylose (53%), L-arabinose (41%), and D-glucose (6%). The D-glucose was not detected after methylation, and was not considered in the structural characterization of the main pentosan polymer.

The ratio of pentose units (arabinose:xylose) was 1:1.3. This indicated approximately four L-arabinose units for every five D-xylose units, a relatively high degree of branching. The large negative optical rotation was similar to that reported for other cereal pentosans and is indicative of a main chain of D-xylose units linked beta-1,4. The results of mild acid hydrolysis were generally similar to those reported by Perlin (2) for wheat pentosans from the water-soluble portion of the endosperm. The soluble fraction (III) contained 60% of the arabinose and very little xylose. Fractions I and II contained over 90% of the xylose. The labile condition of the L-arabinose units suggested that they existed primarily in the furanose form. Periodate uptake was 0.67 mole of periodate per anhydropentose unit. This value was consistent with a general structure composed of D-xylopyranosyl units linked 1,4 with single-unit L-arabinofuranosyl side-chains attached to the 2- and 3-positions of the xylose units.

Hydrolysis of the methylated polymer and quantitative estimation of the molar ratio of methylated sugars gave the following results: 2,3,5-tri-O-methyl-L-arabinose, 20 moles; 2,3-di-O-methyl-D-xylose, 12 moles; 2-O-methyl-D-xylose, 9 moles; D-xylose, 6 moles. These results were consistent with

the chemical composition and periodate oxidation data. These data indicate that the polymer is composed of D-xylopyranose units linked beta-1,4. Single-unit L-arabinofuranosyl branches occur attached to the 3-position of the xylose units (single branches) and to the 2- and 3-positions of the xylose units (double branches). Approximately three of every five xylose units are branched and six of every 15 branched xylose units contain double branches. The isolation of 9 moles of 2-O-methyl-D-xylose required nine side-chains, whereas 6 moles of D-xylose requires 12 side-chains. This requirement of 21 side-chains is in good agreement with the 20 moles of 2,3,5-tri-O-methyl-L-arabinose found.

The above data serve to indicate the average structure of the polymer. They do not, however, permit an exact picture of the structure to be constructed, since several branching arrangements could conceivably result in the same "average" structure. For the highly branched polymer being studied in this work, the choices are primarily only two: a reasonably random distribution of branched xylose units, or an arrangement in which some areas of the polymer chain contain a very high concentration of branched units and some areas of the polymer are relatively free of branching.

Use of the Smith degradation procedure (16) permitted a more exact determination of the structure of the pentosan from the sludge portion of durum endosperm. In this method, the units which are not attacked by periodate can be isolated as glycerol glycosides. For the polysaccharide in this work, only those xylose units which are branched are resistant to periodate attack.

Four unidentified components were detected after the Smith degradation reaction. All yielded xylose and glycerol upon acid hydrolysis. Components I, II, and III were essentially identical in R_{xylose} values and specific optical rotation to 2-O-β-D-xylopyranosylglycerol, O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→2)-glycerol, and O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→2)-glycerol, respectively. These were identified previously in the Smith degradation of rye arabinoxylan by Aspinall and Ross (7). The R_{xylose} value and optical rotation of component IV was consistent with its being the next higher member of the polymer-homologous series, namely 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. The molar ratio of xylose to glycerol and the molar ratio of methylated sugars from the methylated components I-IV were consistent with these conclusions (Table I). The molar ratio of these four components in the Smith degradation mixture was 21:8:2.5:1 for components I, II, III, and IV, respectively.

These data indicate that the L-arabinose branches occur predominantly on isolated D-xylose units. Occasionally, two branched D-xylose units are found in succession. Very infrequently, three or four branched D-xylose units occur together. No more than four contiguous branched D-xylose units are indicated.

The predominance of branching on isolated D-xylose units and the low number of areas where several branched units occur in succession were

TABLE I

RESULTS FROM ACID HYDROLYSIS AND METHYLATION STUDIES ON SMITH DEGRADATION PRODUCTS

COMPONENT	ACID HYDROLYSIS PRODUCTS, MOLAR RATIO		HYDROLYSIS PRODUCTS FROM METHYLATED COMPONENTS, MOLAR RATIO	
	Xylose	Glycerol	2,3,4-Me_3-Xylose	2,3-Me_2-Xylose
I	1.0	1	1	....
II	2.1	1	1	1.2
III	2.8	1	1	1.7
IV	3.7	1	1	2.8

strong indications that the polymer consists of a relatively random distribution of branched and unbranched D-xylose units. No large areas where every xylose unit is branched were indicated, and the over-all data are not consistent with the presence of large areas of unbranched D-xylose units. The over-all data suggest that the polymer consists largely of alternating branched xylose units (or two contiguous branched units) and unbranched xylose units. The predominance of alternating branched units was consistent with the high degree (6 of 15) of doubly branched xylose units. For steric reasons, a doubly branched D-xylose unit would probably favor unbranched units on either side.

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