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DMSO-SOLUBLE HEMICELLULOSES FROM THE HUSK OF *SORGHUM* GRAIN*

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Abstract—The chlorite holocellulose from the grain husk of *Sorghum bicolor* was extracted with DMSO and the hemicellulosic material separated into water-soluble and water-insoluble fractions. To determine the proportion of hemicellulosic material extractable with DMSO, the DMSO-extracted holocellulose was successively extracted with water and alkali. The proportion of hemicellulosic material extractable with DMSO was 23.8% of total hemicellulose. The water-soluble portion of the DMSO extract was separated into 13 fractions by chromatography on DEAE-cellulose. Structural differences amongst polysaccharides C-1 to C-8 and D-1 to D-5 were indicated by variations in their sugar compositions and specific optical rotations. Polysaccharide C-1 is essentially a glucan, having $[\alpha]_D^{20}$ +60°. The decrease in the relative glucose content from C-1 to C-3 is paralleled by a similar trend in the specific optical rotation. The relative glucose content of polysaccharides C-4 to C-8 increases from C-4 to C-7 then decreases for C-8. This trend in glucose content is accompanied by a similar trend in specific optical rotation. The polysaccharides of the D-group are highly-branched arabinoxylans, containing lesser amounts of mannose, galactose and glucose, and having negative specific optical rotations. The *O*-acetyl content of the polysaccharides of the C-group varies between 0.6% and 2.4%; D-1 to D-5 have *O*-acetyl contents in the range 0.6% to 2.2%.

INTRODUCTION

In previous papers the isolations of hemicelluloses A and B and the water-soluble gums from the grain husk [2] and endosperm [3] of *Sorghum bicolor* have been described. The husk and endosperm hemicellulose B fractions were each separated by chromatography on DEAE-cellulose into 13 fractions [2, 3]. Annual variations in the distribution of the *Sorghum* husk hemicellulose B fractions have also been reported [4].

DMSO was first used as a solvent for the isolation of hemicelluloses from plant materials by Hägglund *et al.* [5]. Neither the *O*-acetyl groups nor the glycosidic linkages of polysaccharides are degraded by this solvent [5]. However, complete recoveries of hemicellulosic materials are not attained with DMSO.

In this paper the isolation of hemicellulosic materials from *Sorghum* husk holocellulose with DMSO is reported. To determine the total hemicellulose content of the husk sample, the DMSO-extracted holocellulose was successively extracted with H_2O and alkali. The H_2O-soluble fraction of the DMSO extract was chromatographed on DEAE-cellulose and separated into 13 fractions.

RESULTS AND DISCUSSION

The chlorite holocellulose was prepared in 62.5% yield from a husk sample of the Barnard Red variety of *Sorghum* grain (1974 season) [4]. The holocellulose was extracted with DMSO, and the polysaccharides in this extract were separated into H_2O-soluble (1a) and H_2O-insoluble (1b) components. After an extraction with H_2O, the partially-extracted holocellulose was treated with M NaOH and this extract was separated into hemicelluloses A_1 and B_1. A final extraction of the holocellulose residue with 5 M NaOH afforded hemicelluloses A_5 and B_5. The analytical data for the DMSO-, H_2O- and NaOH-extracted polysaccharides are given in Table 1. The total recovery of polysaccharide materials was 100.9 g, representing 10.1% of the husk sample. The yield of DMSO-extractable hemicelluloses was 23.8% of the total hemicelluloses, of which 73.4% and 23.6% were H_2O-soluble and H_2O-insoluble components, respectively. The proportions of the various extracts, based on total hemicellulose content, were 2.9% (H_2O), 7.1% (A_1), 59.5% (B_1), 1.7% (A_5) and 5.1% (B_5).

Hemicelluloses A_1 and B_1 had sugar compositions which were similar to those of the corresponding hemicelluloses A and B isolated from the husk of the same grain sample with M NaOH, without prior extraction with DMSO. Comparisons of the sugar compositions of hemicelluloses A_1 with A_5, and hemicelluloses B_1 with B_5, showed that the hemicelluloses extracted with M NaOH are essentially similar to those isolated with 5 M NaOH. Hemicelluloses A_5 and B_5, however, have increased proportions of mannose and galactose compared with the corresponding polymers isolated with M NaOH. The higher, negative specific optical rotations of hemicelluloses A and B isolated previously [4], compared with those of the corresponding hemicelluloses of

* Part 9 in the Series '*Sorghum* Polysaccharides'. For Part 8, see ref. [1].

Table 1. Data on the polysaccharides from the husk of *Sorghum* grain by successive use of solvents

Polysaccharide	Recovery from 1 kg husk (g)	(%)	Mole ratio arab : xyl : man : gal : glu	$[\alpha]_D^{20}$
1. DMSO extract:	24	2.40		
1a Water-soluble	17.8	1.78	1.0 : 1.0 : 0.1 : 0.4 : 4.6	−14°
1b Water-insoluble	5.5	0.55	1.1 : 1.0 : 0.5 : 0.5 : 70	
2. H_2O extract	2.9	0.29	1.2 : 1.0 : — : 0.2 : 1.5	+7°
3. M NaOH extract:				
3a Hemicellulose A_1	7.2	0.72	1.0 : 1.0 : — : 0.1 : 0.6	−33°
3b Hemicellulose B_1	60.0	6.0	1.2 : 1.0 : — : — : 0.3	−86°
4. 5 M NaOH extract:				
4a Hemicellulose A_5	1.7	0.17	1.1 : 1.0 : 0.1 : 0.2 : 0.8	−10°
4b Hemicellulose B_5	5.1	0.51	1.5 : 1.0 : 0.1 : 0.2 : 0.4	−43°

the M NaOH and 5 M NaOH extractions, indicate structural differences amongst these polysaccharides.

The H_2O-insoluble fraction of the DMSO extract (1b) is a glucan, containing trace amounts of arabinose, xylose, mannose and galactose. The H_2O-soluble fraction (1a) contains glucose as the principal sugar. No precipitate was produced when an alkaline solution of 1a was acidified with HOAc, showing that this extract is related to the B-group of hemicelluloses. Fraction 1a gave a colouration with I_2–KI solution, but was not degraded when treated with α-amylase.

Fraction 1a was applied to a column of DEAE-cellulose (phosphate form) and separated into Fractions C and D by elution with 0.01 M Na phosphate buffer (pH 6.8) and 0.01 M Na phosphate buffer (pH 6.8) containing M NaCl, respectively. The recoveries of Fractions C and D from the DEAE-cellulose were 47.5% and 31.0%, respectively. The total recovery of Fractions C and D (78.6%) is slightly higher than that obtained on DEAE-cellulose chromatography from other *Sorghum* hemicellulose B extracts [2–4]. The irreversible adsorption of xylans on DEAE-cellulose and on cellulose has been reported [6–8]. Fraction C contained glucose as the principal sugar, with lesser amounts of arabinose, xylose, mannose and galactose. This polysaccharide gave a colouration with I_2–KI solution. Fraction D consists essentially of arabinoxylans, containing smaller proportions of mannose, galactose and glucose, and gave no colouration with I_2–KI solution.

Fraction C was further separated by chromatography on DEAE-cellulose (borate form) into 8 fractions (C-1 to C-8) using successively (a) H_2O, (b) a stepwise-increasing gradient of Na borate buffer, and (c) 0.01 M Na borate buffer (pH 9.2) containing a stepwise-increasing gradient of NaCl. Fraction D was chromatographed on DEAE-cellulose (phosphate form) using a

Table 2. Data on the DEAE-cellulose fractions of the DMSO extract *Sorghum* grain husk

Polysaccharide	Eluting buffer	Recovery (g) from 10 g Fraction 1a	Mole ratio arab : xyl : man : gal : glu	$[\alpha]_D^{20}$	*O*-Acetyl content (%)
C	0.01 M sodium phosphate	4.75	0.8 : 1.0 : 0.1 : 0.7 : 5.4	−13°	1.3
D	0.01 M sodium phosphate containing M NaCl	3.1	1.2 : 1.0 : 0.2 : 0.4 : 0.4	−68°	2.3
C-1	H_2O	1.78	0.4 : 1.0 : 0.1 : 0.8 : 7.2	+60°	2.4
C-2	0.0025 M $Na_2B_4O_7$	0.20	1.1 : 1.0 : 0.2 : 0.6 : 2.3	−23°	1.3
C-3	0.1 M $Na_2B_4O_7$	0.15	1.0 : 1.0 : 0.1 : 0.1 : 0.6	−43°	1.5
	0.01 M $Na_2B_4O_7$ containing:				
C-4	0.025 M NaCl	0.20	1.3 : 1.0 : 0.1 : 0.5 : 0.7	−49°	1.9
C-5	0.05 M NaCl	0.15	0.8 : 1.0 : 0.1 : 0.6 : 1.6	−13°	2.2
C-6	0.075 M NaCl	0.18	0.9 : 1.0 : 0.1 : 0.8 : 2.0	−10°	2.0
C-7	0.1 M NaCl	0.05	0.9 : 1.0 : 0.2 : 0.7 : 2.5	−9	0.7
C-8	1.0 M NaCl	0.13	0.7 : 1.0 : 0.1 : 0.2 : 1.4	−14°	0.6
	0.01 M sodium phosphate containing:				
D-1	0.025 M NaCl	0.44	1.5 : 1.0 : 0.2 : 0.3 : 0.3	−77°	2.2
D-2	0.05 M NaCl	0.20	1.0 : 1.0 : 0.1 : 0.2 : 0.3	−59°	1.0
D-3	0.075 M NaCl	0.08	1.1 : 1.0 : 0.1 : 0.2 : 0.3	−53°	2.1
D-4	0.1 M NaCl	0.20	1.2 : 1.0 : 0.1 : 0.3 : 0.4	−69°	1.4
D-5	1.0 M NaCl	0.95	1.3 : 1.0 : 0.2 : 0.6 : 0.3	−74°	0.6

stepwise-increasing gradient of NaCl in 0.01 M Na phosphate buffer (pH 6.8) to give polysaccharides D-1 to D-5. The recoveries and analytical data for polysaccharides C-1 to C-8 and D-1 to D-5 are listed in Table 2. Subfractions C-1 to C-8 and D-1 to D-5 were recovered from the DEAE-cellulose columns in yields of 63.1% and 64.9%, respectively. The total yield of C- and D-subfractions on DEAE-cellulose chromatography of Fraction 1a was 50%.

Polysaccharide C-1 is the most abundant subfraction of DMSO extract 1a and contains glucose as the principal sugar. Compared with the other C- and D-subfractions, C-1 has the lowest arabinose content, and a positive specific optical rotation. Polysaccharide C-1 is the only subfraction which gave a colouration when treated with I_2–KI solution. The analytical data for polysaccharides C-1, 2 and 3 indicate that there are structural differences amongst these polymers and the decrease in relative glucose content from C-1 to C-3 is accompanied by a corresponding decrease in specific optical rotation.

Polysaccharides C-4 to C-8 each contain arabinose, xylose, galactose and glucose as principal sugars, with the exception that C-8 has galactose as a minor component. In addition, they each contain small proportions of mannose. The glucose:xylose ratio increases from 0.7:1 (C-4) to 2.5:1 (C-7), then decreases to 1.4:1 (C-8). This trend in glucose content of polysaccharides C-4 to C-8 is accompanied by a similar trend in their specific optical rotations.

Polysaccharides D-1 to D-5 are essentially arabinoxylans, with varying arabinose-to-xylose ratios. The high proportions of arabinose indicate that these polymers are highly branched. Polysaccharides D-1 to D-5 also contain lesser amounts of mannose, galactose and glucose, and each have relatively high, negative specific optical rotations.

The *O*-acetyl content of Fractions C and D were found to be 1.3% and 2.3%, respectively. Polysaccharides C-1 to C-8 had *O*-acetyl contents varying between 0.6% (C-8) and 2.4% (C-1), while that of polysaccharides D-1 to D-5 ranged between 0.6% (D-5) and 2.2% (D-1).

EXPERIMENTAL

A sample of *Sorghum* grain of the Barnard Red variety [*Sorghum bicolor* (L. Moench)] was used in this investigation. Details of the origin of the grain sample, and separation of the husk shavings have been reported [4].

General methods. Extractions were made of *ca* 12.5% (wt/vol.) husk suspensions and they were repeated until extraction was complete. Polysaccharides were fractionated on columns (30 × 1.9 cm) of DEAE-cellulose. Optical rotations were measured on aq. solns. The acetate ester content of polysaccharides C-1 to C-8 and D-1 to D-5 were determined by alkaline hydrolysis [9], followed by distillation and titration of the liberated HOAc with 0.01 M NaOH. Polysaccharides were analysed for component sugars by hydrolysing a portion of each (M H_2SO_4, 7 hr, 95°), followed by GLC of the derived alditol acetates. GLC was performed at a N_2 flow rate of *ca* 40 ml/min at 185°, using FID, on a column (1.8 m × 0.3 mm) of 3% ECNSS–M on Gas-Chrom Q.

Extraction with DMSO. Husk shavings (1 kg) were extracted successively with 70% EtOH (reflux), $CHCl_3$–MeOH (2:1, reflux), and H_2O (20°) [4]. A suspension of the residue in H_2O was boiled to gelatinise the starch, and treated with α-amylase. The starch-free residue was delignified with HOAc and $NaClO_2$, and the holocellulose (625 g) washed with H_2O until acid-free. The holocellulose was then suspended in DMSO (3 l.) and agitated in a stream of N_2. Penetration of DMSO into the husk material was aided by periodic application of vacuum. The DMSO extract, recovered by centrifugation, was filtered and poured into 5 vols of EtOH. The ppt. (Fraction 1, 24 g) was suspended in H_2O, stirred at 30° for 6 hr, and centrifuged. The residue was re-extracted with H_2O, and the aq. extracts combined, filtered and freeze-dried (Fraction 1a, 17.8 g). The insoluble material (Fraction 1b, 5.5 g) was suspended in H_2O and freeze-dried. No ppt. was obtained when a sample of Fraction 1a (2 g) was dissolved in M NaOH and the clear soln was acidified to pH 5 with HOAc. The holocellulose residue from the DMSO extract was stirred with H_2O and centrifuged. The supernatant was filtered, concentrated, dialysed, and pptd in 5 vols of EtOH. The ppt. was dissolved in H_2O and freeze-dried (Fraction 2, 2.9 g).

Extraction with M NaOH. The DMSO-extracted holocellulose was extracted ×4 with M NaOH, each extraction being carried out for 16 hr in a N_2 atmosphere. The supernatant, recovered by centrifugation, was adjusted to pH 5 with HOAc. Hemicellulose A_1 was collected by centrifugation and washed with 5% HOAc. The polysaccharide was dissolved in M NaOH, and the soln was acidified with HOAc. Hemicellulose A_1 was redissolved in H_2O and pptd with EtOH. An aq. soln of hemicellulose A_1 was dialysed and freeze-dried (7.2 g). The supernatants and acid washings from the hemicellulose A_1 extracts were poured into 5 vols of EtOH. A soln of the ppt in M NaOH was adjusted to pH 5 with HOAc and the clear soln poured into 5 vols of EtOH. The ppt (hemicellulose B_1) was dissolved in H_2O, dialysed and freeze-dried (60 g).

Extraction with 5 M NaOH. The holocellulose residue from the M NaOH extraction was suspended twice in 5 M NaOH for 16 hr in a N_2 atmosphere. The supernatants were acidified with HOAc and hemicelluloses A_5 (1.7 g) and B_5 (5.1 g) were recovered as described for hemicelluloses A_1 and B_1.

Chromatography of Fraction 1a on DEAE-cellulose. A sample of the H_2O-soluble fraction of the DMSO extract (Fraction 1a, 10 g) was applied to a column of DEAE-cellulose (Pi form) and eluted with 0.01 M NaPi buffer (pH 6.8). The polysaccharide in the eluate was dialysed and freeze-dried (Fraction C, 4.75 g). An aq. soln of Fraction C (4.5 g) was applied to a column of DEAE-cellulose (borate form) and eluted successively with H_2O 0.0025 M $Na_2B_4O_7$ and 0.01 M $Na_2B_4O_7$ buffers; then with 0.01 M $Na_2B_4O_7$ buffer containing a stepwise-increasing gradient of NaCl. The polymeric material adsorbed on the DEAE-cellulose (Pi form) was eluted with 0.01 M NaPi buffer containing M NaCl, dialysed and freeze-dried (Fraction D, 3.1 g). A soln of Fraction D (2.9 g) in 0.01 M NaPi buffer (pH 6.8) was chromatographed on DEAE-cellulose (Pi form) using a stepwise-increasing gradient of NaCl in 0.01 M NaPi buffer (pH 6.8).

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