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A HEMICELLULOSIC GLUCAN FROM OAT LEAF

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Abstract—A glucan of high D.P. has been isolated from the total hemicellulose of the young leaves of the oat plant, *Avena sativa* (var. Blenda). The glucan has $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked D-glucopyranose residues in the molar ratio of 1·00:1·65. The glucan accounted for 3·3% of the total hemicellulose from the tissues. Oligosaccharides having both $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked residues were isolated from partial hydrolysates.

INTRODUCTION

THIS paper continues a series concerned with the relationship between plant maturity and hemicellulosic composition with particular reference to the non-endospermic tissues of the oat plant, *Avena sativa*. The terms *total* and *pure* hemicellulose have been defined earlier.[1]

The structures of two pure hemicelluloses of importance in the present studies have been established; namely, those of an acidic arabinoxylan[2] and of an acidic galactoarabinoxylan.[3] Glucose residues are often present in hemicellulosic materials from members of the Gramineae, but the parent polysaccharide is generally eliminated during the fractionation procedure leading to the isolation of a pure hemicellulose. A glucan has been isolated from oat coleoptiles[4] and a non-cellulosic glucan has been found in maize stalks.[5] A glucoarabinoxylan has been reported in oat hulls.[6] The present studies were carried out on a glucan, which was neither of starch nor of cellulose type, from the leaves of young oat plants.

RESULTS

The aerial portion of each of the oat plants was harvested in early June and the enzymes in the tissues rapidly inactivated. The material consisted of leaf tissues only. The total hemicellulose, 19% of the air-dry tissue, was isolated by the method of Reid and Wilkie.[1] On hydrolysis of a sample, it gave galactose, glucose, arabinose and xylose in the ratio of 2:4:7:10, and a trace of rhamnose. The water-soluble material from a sample of the total hemicellulose and the water-insoluble material gave these sugars in the ratios of 3:7:8:10 and 0:4:5:10 respectively; rhamnose was not detected in the latter case. Difficulty was experienced in isolating the pure hemicellulose containing the glucose residues; the method was based on one used for the isolation of a glucan from maize stalks.[5] By a series of graded precipitations by ethanol a homoglucan was isolated from the oat leaf tissues. Many other

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fractionation methods were used, but there was no evidence of any heteroglycan containing glucose residues. The ethanolic precipitation method had the great merit that it was mild and led to high recoveries of all fractions.

The glucan was insoluble in water, and a solution in 2·5 M NaOH was electrophoretically homogeneous on silica gel thin-layers.[3] The material yielded only glucose on hydrolysis and had no methoxyl groups. The glucan accounted for 3·3% of the total hemicellulose and for *ca.* 20% of the glucose residues present in the total hemicellulose used as starting material. It gave no coloration with iodine and was not attacked by α- or β-amylases. The presence of β-D-glucosidic linkages was indicated by the low specific rotation, −5·2°, and by the presence of a peak at 895 cm^{-1} in the i.r. spectrum. The occurrence of (1→3) and (1→4) linked residues was indicated by the detection of glucose and erythritol in hydrolysates of the polyalcohol isolated after Smith degradation procedures.[7] GLC of the derived acetates,[8] and also estimation of the derived glycitols by the chromotropic acid method,[9] both showed that the ratio of (1→3) to (1→4) linkages was 1·00:1·65. The glucan was permethylated by the procedures of Haworth,[10] Anderson,[11] and Purdie[12] and yielded *ca.* 50% of product that displayed no peak in the i.r. spectrum attributable to hydroxyl groups. After hydrolysis of the methylated glucan by use of HCOOH and H_2SO_4[13] only two products were detected, namely, 2,3,6-, and 2,4,6-, tri-*O*-methylglucoses. The mixture gave only glucose on demethylation using BCl_3.[14] A sample of the permethylated glucan was methanolyzed and the products, on pertrideuteriomethylation by the method of Purdie,[12] using CD_3I, yielded compounds indistinguishable from methyl 2,3,4,6-tetra-*O*-methyl glucosides by TLC and GLC. The mass spectrum[15] was consistent with the presence of trideuteriomethylated methyl 2,3,6-, and 2,4,6-, tri-*O*-methylglucosides. The absence of tetra-*O*-methyl glucose and of any di-*O*-methylglucoses from the hydrolysate, and the absence of their methyl glucosides from the methanolysate, indicated that the glucan was unbranched and of high degree of polymerisation. Samples of the glucan were subjected to various treatments leading to partial hydrolysis. After carefully controlled acetolysis[16] the products were deacetylated and examined chromatographically; oligosaccharides having β(1→3) and (1→4) linkages and others with only β(1→3) or β(1→4) linkages were detected. Similar results were obtained when partial hydrolysis was effected using 0·025 M oxalic acid for 18 hr at 100°. Glucose and cellobiose alone were detected when the glucan was treated with 13 M HCl for 2 hr at room temp. All of the oligosaccharides reacted with 2,3,5-triphenyltetrazolium chloride indicating that none of the oligosaccharides had a residue glycosidically attached to the 2-position of its reducing residue.

The results indicate that the hemicellulose is pure and is a β-glucan with D-glucopyranose residues linked glycosidically β(1→3) and β(1→4) and in these respects, and in the specific rotation, the glucan is similar to those isolated from various cereal endosperms.

Endospermic water-soluble glucans have been isolated from many cereal seeds. A

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structurally similar glucan has also been isolated from oat endosperm[17] and shown to have $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ linked D-glucopyranose residues and to be degraded by "cellulase" and "laminarase".[18] The site of synthesis of the non-endospermic glucan from oats and the way in which it is metabolized are of interest. Recent work shows that a $\beta(1\rightarrow4)$ glucan, from the cell-walls of etiolated pea seedlings, is synthesized on the Golgi membranes.[19] Auxin promotes synthesis of cell-wall non-cellulosic glucan and of other hemicelluloses in oat coleoptile segments in which cell-wall elongation and the accompanying cellulose synthesis has been inhibited by Ca^{2+}.[20]

EXPERIMENTAL

General Methods

Paper chromatography was on Whatman No. 1 and 3MM papers, and TLC on silica gel (Kieselgel G or GF). The irrigants (v/v) were: A, EtOAc–pyridine–H_2O (360:100:115); B, EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4); C, EtOAc–HOAc–H_2O (3:1:3); D, EtOAc–benzene (3:7); E, *n*-BuOH–EtOH–H_2O–NH_4OH (4:1:5:trace); F, MeCOEt–H_2O–NH_4OH (10:1:trace); G, benzene–EtOH–H_2O–HOAc (200:47:15:1); H, *n*-BuOH–pyridine–benzene–H_2O (5:3:1:3). Chromatographic spray reagents were *p*-anisidine HCl, alkaline $AgNO_3$, 2,3,5-triphenyltetrazolium chloride, or 1-napthol-sulphuric acid. A Perkin–Elmer F11 gas chromatograph was used for GLC. The columns (2 m × 3 mm inside dia.) contained A, 3% ECNSS-M on Gas-Chrom Q (100–120 mesh); B, 8% butan-1,4-diol succinate on AW-HMDS Chromosorb W (80–100 mesh). The nitrogen carrier gas flow-rate was 50–80 ml/min. Electrophoretic examination of polysaccharides was carried out by the method of Reid and Wilkie[3] on TLC plates coated with Kieselgel G sprayed with 2·5 M NaOH. Quantitative estimations of sugars in hydrolysates were made by densitometry. Polysaccharides were hydrolyzed in sealed tubes with 0·5 M H_2SO_4 (12–16 hr at 100°); the hydrolysates were neutralized with $BaCO_3$.

Isolation of the Total Hemicellulose

The oat plants were grown, as before,[21] on the University Farm at Tillycorthie, Aberdeenshire; the seeds were sown on 27 March and the plants harvested on 11 June 1965. They were then *ca.* 40 cm high and the aerial portion consisted of leaf tissue only. The plants were rapidly immersed in boiling EtOH to inactivate the enzymes and the tissues air-dried and stored below 0°. The total hemicellulose was isolated by the method of Reid and Wilkie;[1] 17 and 35 g of total hemicellulose was isolated from 100 and 170 g respectively of air-dried leaf.

Examination of Fractions of Total Hemicellulose Soluble and Insoluble in Water

The first batch of total hemicellulose (17 g) was stirred vigorously with water (1·2 l.) for 2 hr and the insoluble material recovered, washed and dispersed in water (500 ml). The suspension and the solution plus washings were separately freeze-dried and gave Fractions I (3·1 g) and S (8·7 g) respectively.

The Fraction I material was dissolved in 1 M NaOH and clarified by centrifugation. Various precipitation techniques were used. The methods are listed briefly: 1, graded precipitation by neutralization of the alkaline solution of Fraction I material with HOAc; 2, addition of saturated $Ba(OH)_2$ and comparison of the soluble and precipitated materials; 3, addition of 0·5 M Cetavlon and comparison of the precipitated and soluble materials; 4, addition of 7% copper acetate and comparison of the precipitated and soluble materials after acidic decomposition of complexes. In all cases examination of the hydrolysate of the various precipitates and of the soluble materials revealed no significant fractionation.

A sample (1 g) of Fraction I in 1 M NaOH was treated with Fehling's solution and the gel collected and treated with 13 M HCl for 1 min. The resultant white precipitate was washed with EtOH and acetone and freeze-dried after dispersal in water (Fraction P). The acidified (HOAc) supernatant liquid was desalted and the soluble material isolated and dissolved in 1 M NaOH; insoluble material was removed (Fraction I′). No precipitate formed on adding Fehling's solution; the solute was recovered by desalting and freeze-drying (Fraction S′).

Attempts were made to further fractionate the hemicellulosic solute in aqueous solutions of Fraction S material. No precipitates were obtained on the addition of Fehling's solution, or of $Ba(OH)_2$, nor when

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TABLE 1. DENSITOMETRIC DETERMINATION OF THE SUGARS IN HYDROLYSATES OF FRACTION I HEMICELLULOSIC MATERIAL AFTER FRACTIONATION USING FEHLING'S SOLUTION*

Fraction	Weight, mg	Glucose	Arabinose	Xylose
P	523	3	3	10
I′	trace	0	3	10
S′	43	6	5	10

* Uronic acids were present in these fractions; galactose and rhamnose were absent.

0·05 M Cetavlon was added to the solution taken to pH2 with H_2SO_4. No fractionation took place when 7% copper acetate was used nor when ethanol was added afterwards to the point of turbidity. No fractionation was achieved using either basic lead carbonate, or $(NH_4)_2SO_4$. Some fractionation took place when 0·05 M Cetavlon was added to a sample (50 mg) of Fraction S in water (5 ml) until there was no further change in turbidity. The ratios of glucose, galactose, arabinose and xylose in hydrolysates of the precipitated and soluble materials were 1:1:6:10 and 7:0:4:10 respectively; acidic sugars and traces of rhamnose were present in each fraction. Similar procedures using 0·1 M cetyltrimethylammonium borate led to slight fractionation. No fractionation was achieved on columns of DEAE-cellulose irrigated successively with water and with aq. NaOH of increasing concentration nor with DEAE-cellulose in borate form irrigated with water and with increasingly concentrated solutions of sodium tetraborate.

Isolation of the Glucan

The glucan was isolated from two batches (TH and TH′) of total hemicellulose by the procedure illustrated in Fig. 1 with reference to sample TH (Fractions E_1–E_3 and E'_1–E'_3 were combined at the point shown). All aqueous solutions were freeze-dried. The glucan (1·4 g) that was isolated accounted for 3·3% of the total hemicellulose.

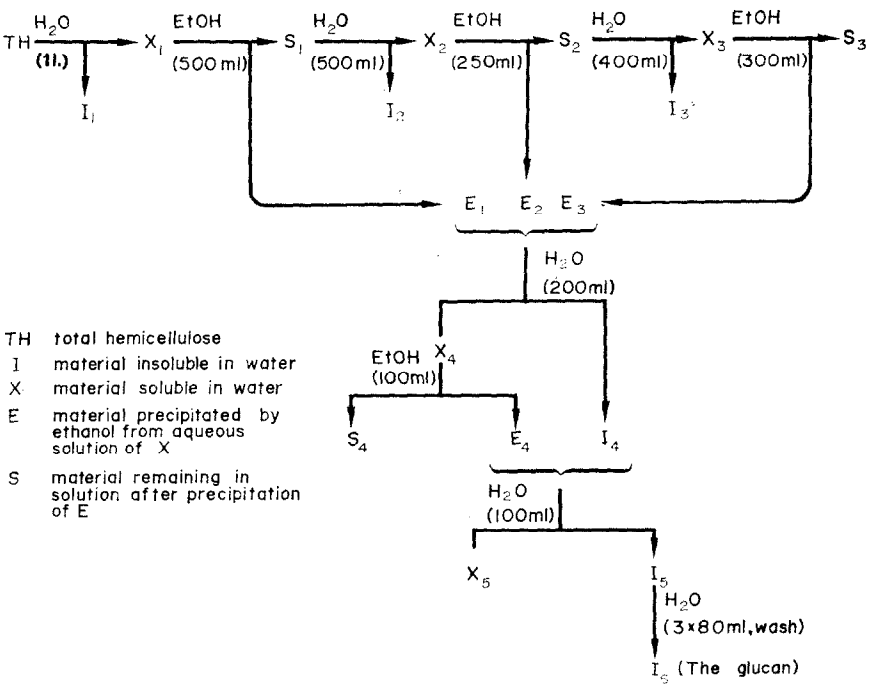

FIG. 1.

TABLE 2. DENSITOMETRIC DETERMINATION OF THE SUGARS IN HYDROLYSATES OF THE FRACTIONS OBTAINED DURING ISOLATION OF THE GLUCAN FROM OAT LEAF TOTAL HEMICELLULOSE*

Fraction	Weight, g	Galactose	Glucose	Arabinose
TH/TH′	20/23	2	4	7
I/I′	4·5/7·4	1/1	2/2	5/6
E_1/E'_1	0·61/1·4	tr/tr‡	10/18	2/4
E_2/E'_2	0·36/0·16	tr/tr	11/12	2/5
E_3/E'_3	0·78/0·43	tr/2	5/6	4/5
S_1/S'_1	9·5/11·8	2/tr	2/3	6/3
S_2/S'_2	8·4/10·1	tr/2	tr/3	4/6
S_3/S'_3	6·8/9·4	3/3	2/3	7/8
I_4†	1·7	0	105	tr
E_4†	0·2	0	31	2
S_4	0·7	4	4	6

* Densitometric determination relative to xylose = 10.
† Uronic acid and traces of rhamnose were present in all hydrolysates *other* than these two.
‡ tr = trace.

Study of the Glucan

The glucan had $[\alpha]_D$,-5·2° (c, 1·74 in 1M NaOH). Samples on hydrolysis gave glucose and, on heavily spotted chromatograms, a trace of xylose (Irrigants A,B and C). The glucan travelled as a single zone on electrophoresis. It gave no coloration when iodine was added to an aqueous suspension nor was it attacked by α nor by β amylases. It absorbed in the i.r. at 895 cm^{-1} (KBr disc). X-Ray diffraction studies showed the glucan was non-crystalline.

Periodate Oxidation

The glucan (31 mg) was oxidized with 0·1 M $NaIO_4$ (35 ml) at 5° in the dark. After 28 days, the suspension was dialysed, and the polyaldehyde reduced with $NaBH_4$ (50 mg). After 70 hr the excess borohydride was destroyed with Zeo-Karb 225 (H^+) ion-exchange resin, and borate removed by codistillation with methanol. The polyalcohol (26 mg) was isolated by freeze-drying and then hydrolyzed with 0·02 M HCl (10 ml; 8 hr at 100°). The hydrolysate was neutralized with Ag_2CO_3, filtered, and water removed by evaporation. Paper chromatography (Irrigants A–C) revealed glucose, erythritol and traces of glycolaldehyde. A sample (10 mg) of the hydrolysate was acetylated with Ac_2O and NaOAc.[8] TLC (Irrigant D) and GLC (Column A) revealed components indistinguishable from α and β glucose pentaacetates and erythritol tetraacetate. The ratio of glucose pentaacetates to erythritol tetraacetate was estimated to be 1·00:1·64 by triangulation of GLC peak areas. A sample (6 mg) of the hydrolysate was reduced with $NaBH_4$, and paper chromatographic examination (Irrigant A) revealed glucitol and erythritol, they were separated by thick paper chromatography (Irrigant A). Glucitol and erythritol were found in the molar ratio of 1·00:1·66 by the chromotropic acid method (determination at 570 nm).

Preparation and Analysis of the Methylated Glucan

A sample (150 mg) of the glucan was methylated twice by the Haworth method,[10] three times by that of Anderson,[11] and finally by that of Purdie.[12] The methylated glucan (120 mg) showed no absorption at 3400 cm^{-1} in the i.r. spectrum. A sample (60 mg) of the methylated glucan was hydrolysed by the HCOOH–H_2SO_4 method.[13] The hydrolysate was neutralized ($BaCO_3$), filtered, and the water evaporated. Paper chromatography (Irrigants E and F) and TLC (Irrigants G and H) revealed 2,3,6-, and 2,4,6-, tri-*O*-methylglucoses in the molar ratio of 1·7:1·0 (determination by densitometry). On demethylation by BCl_3 the mixture gave glucose alone on paper chromatography (Irrigant A). A sample (15 mg) of the methylated glucan was methanolysed with 4 per cent methanolic HCl and the product was permethylated with CD_3I and Ag_2O. The residue was removed by filtration and the solvent evaporated. Examination of the syrup by TLC (Irrigants G and H) and by GLC (Column B) revealed methyl 2,3,4,6-tetra-*O*-methylglucopyranosides alone. The mass spectrum was consistent with the presence of trideuteriomethylated methyl 2,3,6-, and 2,4,6-, tri-*O*-methylglucopyranosides. A sample (15 mg) of the methylated glucan was methanolysed with 4 per cent methanolic HCl. The methyl glycosides were examined by GLC. Three peaks, indistinguishable from those given by methyl 2,3,6-, and 2,4,6-, tri-*O*-methylglucopyranosides, were detected (Column B; 170°) having retention times of 2·12, 2·40 and 2·96 (relative to α-methyl 2,3,4,6-tetra-*O*-methylglucopyranoside).

Partial Hydrolysis

A sample (75 mg) of the glucan was acetylated by the method of Hess and Dziengel.[16] The glucan was stirred in a mixture of HOAc (3·4 ml), Ac_2O (3·4 ml) and H_2SO_4 (0·05 ml) and kept at 30° for 4 days. The filtrate and wash liquors were neutralized ($NaHCO_3$) and the precipitate dispersed in water (20 ml); the water was removed by freeze-drying and a sample (20 mg) of the white solid (53 mg) which remained was treated with 0·2 M NaOMe (7 ml; 1 hr at 37°). The suspension was acidified with HOAc and the filtrate and MeOH wash liquors (5 × 4 ml) combined. The solvent was removed and an aqueous solution of the solute deionized on Zeo-Karb 225 (H^+) resin. The water was removed and the mixture examined by paper chromatography. The following $R_{glucose}$ values were obtained in Irrigants A, B and H respectively—the tentative identity of the oligosaccharide is in parenthesis 0·04, 0·03, 0·16 (cellotriose); 0·17, 0·25, 0·27 (βGpl→3βGpl→4G); 0·36, 0·34, 0·40 (βGpl→4βGpl→3G); 0·52, 0·53, 0·56 (cellobiose); 0·62, 0·62, 0·69 (laminaribiose). The main and minor components were respectively the first and the last. All the sugars gave a coloration with 2,3,5-triphenyltetrazolium chloride.

Another sample (45 mg) of the glucan was treated with 0·025 M oxalic acid (25 ml; 18 hr at 100°). The solution was neutralized ($BaCO_3$) and the solution examined on paper chromatograms. The hydrolysate was qualitatively identical to the previous one but for the absence of the second component, namely, βGpl→3βGpl→4G; the other 4 were present in the ratio of *ca.* 2:1:3:1 respectively.

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