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Structural Investigations on the Non-starchy Polysaccharides of Oat Bran

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SUMMARY

Digestion of oat bran with hog pancreatic α-amylase to hydrolyze starch (~50%) results in solubilization of much β-D-glucan (9%) which is the main non-starchy polysaccharide. This soluble β-D-glucan has been shown by methylation analysis and specific enzymic hydrolysis to contain linear chains with (1→3) and (1→4) linkages in the proportions 1 : 2·6. Compositional and linkage analysis studies on the water-insoluble residue have shown the presence of further β-D-glucan (5%) and arabinoxylan (3%), but only traces of cellulose (<0·5%).

INTRODUCTION

The non-starchy polysaccharides of plant foods, which are largely of cell wall origin, are the main compounds included in the chemically heterogeneous group of substances referred to as 'dietary fibre'. These materials are only slowly digested in the upper gastro-intestinal tract and exert various beneficial physiological effects (Burkitt & Trowell, 1975; Kay, 1982). The nature of these materials varies substantially from source to source as do the physiological effects which may be ascribed to them. As a cereal product oat bran is of particular interest since its physiological effects in humans (Wyman *et al.*, 1976; Kirby *et al.*, 1981) are markedly different from those of wheat bran (Anderson & Chen, 1979). In addition, previous studies, based on compositional

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analysis, have indicated substantially different proportions of polysaccharide components in these materials (Selvendran & Du Pont, 1980). We describe here a structural investigation of the non-starchy polysaccharides of oat bran in which a central problem is that of selective removal of starch without loss of other polysaccharides. For some plant materials starch may be selectively extracted with aqueous dimethyl sulfoxide (Selvendran, 1975; Selvendran & Du Pont, 1980) but a careful check must be made for the solubilization of other polysaccharides. Alternatively, amylolytic digestion may be the method of choice, but in addition to accompanying polysaccharide solubilization, special attention must be given to the catalytic specificity of the enzyme preparation. For example, a common preparation of *Bacillus subtilis* α-amylase contains an impurity which degrades β-D-glucans of the mixed linkage type (Huber & Nevins, 1977) and has been used for that purpose in the present study.

RESULTS AND DISCUSSION

Oat bran was extracted with boiling ethanol–water (9:1) to remove soluble sugars which might interfere with the analysis of sugar formed on hydrolysis of polysaccharide components. The overall carbohydrate composition of the extractive-free bran was determined (a) by hydrolysis with trifluoroacetic acid and (b) after digestion with 72% sulfuric acid followed by dilution and further heating. The lack of difference between the results from the two methods, which gave values of glucose (67%), xylose (4%) and arabinose (3%), with traces (~1%) of mannose and galactose, indicated that only very small amounts of cellulose were present in the bran. Digestion of the bran with amyloglucosidase gave glucose corresponding to the presence of 50% by weight of starch in the extractive-free bran.

Attempts to account for the non-starchy polysaccharides in oat bran as insoluble residues from (a) preparative-scale digestion of the bran with amyloglucosidase or (b) preparation of cell wall material (CWM) by Selvendran's procedure (Selvendran, 1975) involving sequential extraction with aqueous 1% sodium deoxycholate, phenol–acetic acid–water and dimethylsulfoxide–water gave materials amounting to less than 5% by weight of the bran. The water-soluble, but non-dialyzable, fraction from the former extraction sequence only contained approxi-

mately one-third polysaccharide, but nevertheless provided the most satisfactory source of the arabinoxylan for linkage analysis. The extraction sequence failed to account for non-starchy glucan in the bran because the enzyme preparation, as was shown later, acted on oat β-D-glucan with the formation of dialyzable fragments. Recoveries of soluble polysaccharides from the supernatant liquors in the latter extraction sequence were unsatisfactory. Subsequently the following procedures were shown to provide a reasonable balance sheet for the polysaccharide components.

Digestion of the bran with hog pancreatic α-amylase gave glucose and maltose corresponding to 50% starch in the bran. Non-dialyzable polysaccharide was isolated in 10% yield and gave on hydrolysis glucose (90%) with small amounts (2% each) of xylose and arabinose. Recovery of this polysaccharide after precipitation with 20% ammonium sulfate (McLeod & Preece, 1954) failed to effect any significant change in sugar composition. For subsequent studies this polysaccharide is referred to as oat bran β-D-glucan. The water-insoluble residue from the amylase digestion gave on hydrolysis the following sugars expressed as percentages of the original bran: glucose (8·8%), xylose (1·9%), arabinose (1·2%) and galactose and mannose in traces.

Oat bran β-D-glucan was characterized and shown to be of the essentially linear mixed linkage type in the following experiments. Hydrolysis of the permethylated polysaccharide gave the following sugars, which were characterized by reduction and acetylation to partially methylated alditol acetates and analysis by gas–liquid chromatography (g.l.c.)–mass spectrometry: 2,3,4,6-tetra- (1·1%), 2,3,6-tri- (64·0%) and 2,4,6-tri-*O*-methylglucose (24·2%), with small amounts of methylated sugars arising from unremoved methylated arabinoxylan. Treatment of the β-D-glucan with a *Bacillus subtilis* α-amylase preparation containing a specific β-D-glucanase or 'lichenase' impurity (Huber & Nevins, 1977) gave a mixture of a trisaccharide and a tetrasaccharide, each containing a 3-*O*-substituted D-glucopyranose reducing group, which were characterized as 3-*O*-β-cellobiosyl-D-glucose (1) and 3-*O*-β-cellotriosyl-D-glucose (2). The oligosaccharides were separated by preparative paper chroma-

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-D-Glc

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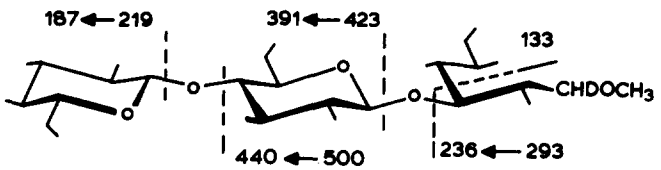
β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-D-Glc

2

tography, and each was reduced with sodium borodeuteride, and the resulting oligosaccharide alditols were methylated. The ^1H nuclear magnetic resonance (n.m.r.) spectrum of the permethylated trisaccharide alditol (3)* showed signals for two anomeric protons at $\delta = 4{\cdot}30$ and 4·50, each with coupling constants $J_{1,2} = 7{\cdot}8$–$7{\cdot}9$ Hz characteristic of β-D-glucopyranosyl linkages. The ^{13}C n.m.r. spectrum showed only one signal for anomeric carbon at $\delta_C = 103{\cdot}2$, again consistent with the β-D-glucopyranosyl configuration. Hydrolysis of the permethylated derivative (3), followed by reduction ($NaBH_4$) and acetylation, gave a



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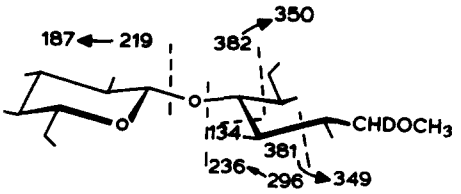
mixture of partially methylated alditol acetates characterized by g.l.c.–mass spectrometry as 1,2,4,5,6-penta-*O*-methylglucitol-1-*d* monoacetate, 2,3,4,6-tetra-*O*-methylglucitol diacetate and 2,3,6-tri-*O*-methylglucitol triacetate. In addition to these products consistent with formation from the permethylated trisaccharide alditol 3, 1,2,3,5,6-penta-*O*-methylglucitol-1-*d* monoacetate was detected as a minor component of the mixture. The most probable explanation for the formation of this compound is that the basic conditions resulting from the use of unbuffered sodium borohydride solution for the reduction of the trisaccharide 1 resulted in alkaline erosion of the 3-*O*-substituted reducing group to give cellobiitol-1-*d*. Support for this possibility was obtained from examination of the mass spectrum of the permethylated trisaccharide alditol preparation which showed fragment ions consistent with structure 3 together with fragment ions shown for permethylated cellobiitol-1-*d* (4). In a similar manner the permethylated tetrasaccharide alditol (5) formed from 3-*O*-β-cellotriosyl-D-glucose (2) was characterized from (i) the p.m.r. spectrum with anomeric protons at $\delta = 4{\cdot}32$, 4·33 and 4·49 ($J_{1,2} = 7{\cdot}8$–$7{\cdot}9$ Hz), (ii) the c.m.r. spectrum with anomeric carbon at $\delta_C = 103{\cdot}20$ and 103·22, (iii) g.l.c.–mass spectrometry of the

* In formulae **3**, **4**, and **5** for permethylated oligosaccharide alditols substituents are —— = OCH_3 and ___/ = CH_2OCH_3. *m/e* Values are shown for structurally significant fragment in the mass spectra.



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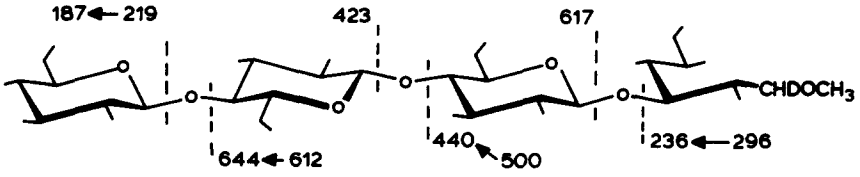

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derived partially methylated alditol acetates which showed the same compounds as from the trisaccharide derivative (3) but with an increased proportion of 2,3,6-tri-*O*-methylglucitol triacetate and (iv) direct insertion mass spectrometry of the tetrasaccharide alditol derivative (5). The formation of derivative 5 was again accompanied by some base-catalyzed degradation, presumably with production of permethylated cellotriitol-1-*d*.

These observations show that the β-D-glucan from oat bran has the same general structure as the corresponding polysaccharide from oat flour (Parrish *et al.*, 1960), oat coleoptiles (Labavitch & Ray, 1978), and several other monocotyledonous plants (Wilkie, 1979). On the basis of the present evidence the oat bran β-D-glucan contains unbranched chains of 4-*O*- and 3-*O*-substituted β-D-glucopyranose residues in the ratio of 2·6 : 1 as shown in the general structure **6**.

→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1

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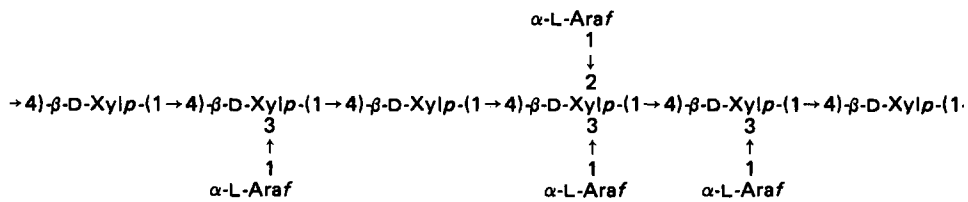
The water-insoluble residue, which remained after digestion of oat bran with α-amylase, furnished a preparation containing β-D-glucan on extraction with dimethylsulfoxide corresponding to 5% of the dry weight of oat bran. Methylation analysis showed that the polysaccharide was indistinguishable from the water-soluble β-D-glucan with trace amounts of accompanying arabinoxylan. The dimethylsulfoxide-insoluble material which remained after the above extraction contained

arabinoxylan corresponding to 3% by weight of the bran. Differences in the proportions of glucose liberated from this residue on (a) hydrolysis with trifluoroacetic acid and (b) digestion with 72% sulfuric acid followed by dilution and hydrolysis pointed to the presence of very small amounts of cellulose and further β-D-glucan, each less than 0·5% by weight of the bran.

The nature of the arabinoxylan in oat bran was established by methylation analysis of the water-soluble fraction obtained from amyloglucosidase digestion of the bran. Although the polysaccharide material contained a mixture of polysaccharides, the methylated polysaccharide preparation consisted solely of arabinoxylan derivative. Hydrolysis afforded the following sugars which were identified by g.l.c.–mass spectrometry of the derived partially methylated alditol acetates: 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*- and a mixture of 2-*O*- (mainly) and 3-*O*-methylxylose, together with xylose from doubly branched residues. Assuming the same enantiomeric and anomeric configurations as in the similar arabinoxylans from several cereals and grasses (Wilkie, 1979), the general structure (7) may be advanced.



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The results of this study show that extractive-free oat bran is composed of ~75% carbohydrate polymers based on glucose (67%), xylose (4%) and arabinose (3%) with traces of galactose and mannose. The major portion of the polysaccharide is accounted for as starch (50%), β-D-glucan (>14%) of which more than half was directly isolated and arabinoxylan (>3%). Analyses indicate that cellulose is present in surprisingly small amounts (see also Selvendran & Du Pont, 1980) and no account can yet be given of the nature of the carbohydrate polymers giving galactose and mannose on hydrolysis. The polysaccharide composition of oat bran is greatly different from that of wheat bran in which cellulose and associated arabinoxylan are the major components (Ring & Selvendran, 1980). The differences in non-digestible poly-

saccharide composition between wheat bran and oat bran are therefore probably responsible for the markedly different physiological effects of these two materials. Thus wheat bran is recognized for its role in increasing fecal bulk through its high water-binding capacity, but has no apparent effect on serum cholesterol levels (Anderson & Chen, 1979). In contrast oat bran is reported to selectively lower serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men (Kirby *et al.*, 1981), but to have little effect on fecal bulk (Wyman *et al.*, 1976).

The present results have particular significance in emphasizing that a full account of the non-digestible polysaccharides of food materials must include those which may be rendered water-soluble during the preparation of cell wall material. For some such materials loss of water-soluble polysaccharide may be of little consequence, but for oat bran a major polysaccharide component could be substantially lost. Insofar as β-D-glucan is the main non-starchy polysaccharide of oat bran and is strongly implicated in the physiological effects of this material, it is noteworthy that the polysaccharide is rendered water-soluble during treatment of the bran with pancreatic α-amylase. This operation is probably similar to the digestive process occurring with human salivary α-amylase and suggests therefore that β-D-glucan may exert its physiological effects in solution rather than as a component of an insoluble material, whether fibrous in physical nature or present as a highly swollen gel.

EXPERIMENTAL

Materials

Oat bran was a generous gift of the Quaker Oats Co. The bran as received was exhaustively extracted with boiling ethanol–water (9:1) to remove soluble sugars and to inactivate enzymes. Hydrolysis of the resulting bran, either with trifluoroacetic acid or with sulfuric acid after initial digestion with 72% sulfuric acid, gave glucose (67%), xylose (4%), arabinose (3%), together with traces (~1%) each of mannose and galactose.

Enzyme preparations purchased from Sigma Chemical Co. Ltd were: hog pancreatic α-amylase, Type 1-A, DFB treated; *Bacillus subtilis*

α-amylase, Type XI-A, known to contain 'lichenase' activity (Huber & Nevins, 1977); and amyloglucosidase (glucoamylase), Grade II from a *Rhizopus* mold.

General methods

Unless otherwise stated experimental methods were those described in a previous paper (Aspinall & Fanous, 1984). For starch estimations the sample (100 mg) in water (50 ml) was digested with amyloglucosidase (7 mg) at room temperature until glucose liberation, as monitored by the Somogyi micro copper method (Hodge & Hofreiter, 1962), was complete (4 h for soluble material but for longer periods with insoluble substrates). Alternatively, the sample (100 mg) in water (50 ml) was digested with pancreatic α-amylase (1 mg) until liberation of reducing sugars (glucose and oligosaccharides) was complete. Reducing sugar was determined as maltose by the Somogyi method and the procedure was calibrated with respect to corn starch.

Preparation of oat bran CWM

Oat bran (100 g) was treated by the procedures of Selvendran (1975) involving (i) extraction with aqueous 1% sodium deoxycholate (700 ml) in a ball mill at 0°C, (ii) three extractions with phenol–acetic acid–water (2:1:1, w/v/v, 700 ml) at 2°C, and after sonication, (iii) three times with dimethylsulfoxide–water (9:1) to ensure complete removal of starch (negative iodine test). Insoluble material was separated by centrifugation and was washed thoroughly with water to remove dimethylsulfoxide and freeze-dried to give CWM (5·8 g). Hydrolysis of CWM (sulfuric acid) gave glucose (20%), xylose (17%), arabinose (17%), mannose (3%) and galactose (2%).

Digestion of oat bran with amyloglucosidase

For analytical purposes glucose liberation on digestion of oat bran with amyloglucosidase reached a constant value of 50% after 12 h. On a preparative scale bran (18 g) in water (750 ml) was digested with amyloglucosidase (500 mg) in the presence of a few drops of toluene for 48 h before reaching the same value of 50% glucose liberation. The insoluble residue was separated by centrifugation and then extracted

twice with dimethylsulfoxide–water (9 : 1). The DMSO-soluble (225 mg) and DMSO-insoluble fractions (2·5 g) were each treated to remove solvent, dispersed in water and freeze-dried. Sugar analyses of both materials indicated 10% or lower carbohydrate content. The water-soluble fraction from the enzyme digestion was heated at 100°C for 10 min to inactivate enzyme, dialyzed to remove reducing sugars and freeze-dried to give a water-soluble residue (1·5 g) which gave, on hydrolysis, arabinose (10%), xylose (13%), glucose (6%), mannose (1%) and galactose (3%). Methylation of the water-soluble residue by the Hakomori procedure gave a methylated arabinoxylan with no detectable derivatives of other polysaccharides. Hydrolysis of the methylated polysaccharide, followed by reduction and acetylation, gave alditol acetates from the following sugars: 2,3,5-tri-*O*-methylarabinose (34%), 2,3-di-*O*-methylxylose (22%), a mixture (12%) of 2- and 3-*O*-methylxylose and xylose (10%). The mass spectrum of the chromatographically inseparable mixture of 2- and 3-*O*-methylxylitol tetracetates showed that the former was the major component.

Digestion of β-D-glucan with amyloglucosidase

β-D-Glucan (100 mg) in suspension in water (50 ml) was digested with amyloglucosidase (7 mg) at room temperature and estimation of liberated reducing sugar (as glucose) showed 5% after 24 h and a final value of 60% after 5 days. The solution was dialyzed and analysis of the dialyzate using the phenol–sulfuric acid reagent showed the presence of carbohydrate corresponding to 74% of the original glucan. Thin-layer chromatography examination of the dialyzate in butan-1-ol–acetic acid–water (4 : 3 : 2) showed glucose but no chromatographically mobile oligosaccharides. No non-dialyzable carbohydrate remained.

Digestion of oat bran with pancreatic α-amylase

Oat bran (20 g) in water (700 ml) in the presence of a few drops of toluene was digested with α-amylase (25 mg) for 48 h until reducing sugar liberation reached a constant value corresponding to the hydrolysis of 50% starch. Two further batches of oat bran (20 g each) were similarly digested. Insoluble residues (23 g) were separated by centrifugation, washed with water and freeze-dried in suspension. The supernatant liquids were heated at 100°C for 10 min to inactivate enzyme,

dialyzed to remove reducing sugars and freeze-dried to give a water-soluble fraction (6 g). Hydrolysis of this material gave glucose (90%), arabinose (2%) and xylose (2%). Ammonium sulfate to a 20% (w/v) concentration was added to the water-soluble fraction (3 g) in water (1000 ml) and the gel-like precipitate was separated by centrifugation. No further precipitate was obtained on addition of ammonium sulfate up to a 50% concentration. The precipitated gel on dispersion in water was dialyzed to remove ammonium sulfate and the solution was freeze-dried to give oat bran β-D-glucan (2·7 g), which gave on hydrolysis glucose (92%) with small amounts (2% each) of arabinose and xylose from residual arabinoxylan.

Hydrolysis of the water-insoluble residue gave glucose (23%), xylose (5%), arabinose (3%) and traces (1%) of mannose and galactose. A portion (100 mg) of the residue was extracted with dimethylsulfoxide (25 ml) for 3 h at room temperature. The soluble (70 mg) and insoluble (30 mg) fractions were freed from dimethylsulfoxide by dialysis and washing, respectively, and then isolated by freeze-drying. Sugar analysis showed that the DMSO-soluble fraction contained glucan as the major polysaccharide component in an amount corresponding to 3–5% by weight of the bran. Methylation analysis, as for the β-D-glucan, showed that this polysaccharide fraction was indistinguishable. Compositional and methylation analyses of the DMSO-insoluble fraction showed the presence of further arabinoxylan with a small amount of residual glucan.

Characterization of oat bran β-D-glucan

(a) *Methylation*

Glucan was methylated by the Hakomori procedure and hydrolysis of the permethylated polysaccharide, followed by reduction and acetylation, afforded partially methylated alditol acetates derived from the following sugars: 2,3,4,6-tetra- (1·1%), 2,3,6-tri- (64·0%) and 2,4,6-tri-*O*-methylglucose (24·25%) which were characterized by g.l.c.–mass spectrometry.

(b) *Enzymic hydrolysis*

Glucan (50 mg) in water (10 ml) was digested with *Bacillus subtilis* α-amylase with 'lichenase' activity (Type IIIA, Sigma Chemical Co.) (3 mg) for 24 h. At this time reducing sugar liberation, as measured by

the Somogyi micro copper method (Hodge & Hofreiter, 1962), had reached a constant value. After centrifugation the supernatant liquid was concentrated (to 2 ml), spotted onto Whatman 3MM filter sheets, and chromatographed using butan-1-ol–pyridine–water (6:4:3) as eluant. Extraction of the appropriate sections of the filter sheets afforded oligosaccharides 1 (15 mg) and 2 (14 mg) as the only detectable hydrolysis products. Each oligosaccharide was reduced with sodium borodeuteride in water, and the resulting oligosaccharide alditols were methylated. The permethylated oligosaccharides were isolated from dimethylsulfoxide by partitioning between chloroform and water, the chloroform layer was washed several times with water, dried, and final purification was effected by passage through columns of Sephadex LH-20 and silica gel. The permethylated oligosaccharides were characterized by mass spectrometry (direct insertion) and ^{1}H- and ^{13}C-n.m.r. spectroscopy and samples were then hydrolyzed, reduced and acetylated, the resulting alditol acetates being examined by g.l.c.–mass spectrometry.

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