Molecular Characterization of Cereal β-D-Glucans. Structural Analysis of Oat β-D-Glucan and Rapid Structural Evaluation of β-D-Glucans from Different Sources by High-Performance Liquid Chromatography of Oligosaccharides Released by Lichenase[1]

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ABSTRACT

Oligosaccharides obtained by the action of (1→3),(1→4)-β-D-glucan-4-glucanohydrolase (lichenase) on the (1→3),(1→4)-β-D-glucan of oats (oat β-glucan) were characterized by methylation analysis. The polysaccharide was confirmed as composed mainly of β-(1→3)-linked cellotriosyl and cellotetraosyl units with a small amount of regions containing 4–8 consecutive (1→4)-linked units. ^{13}C-Nuclear magnetic resonance data confirmed these structural features. The oligosaccharides released by lichenase were analyzed by elution with water from a Bio-Rad HPX-42A high-performance liquid chromatography column with peak detection by an automated orcinol-sulfuric acid reaction, and the ratio of tri- to tetrasaccharide was determined. This ratio defines the major structural repeating units of the cereal (1→3),(1→4)-β-D-glucans and may be determined without prior purification of polysaccharide. The molar ratio of tri- to tetrasaccharide determined for oats was 2.1 ± 0.1, for barley 3.2 ± 0.3, and for wheat 3.5 ± 0.4. No difference was detected between the β-D-glucan from oat brans and the whole groat. In addition, the areas or heights of the major tri- and tetrasaccharide reaction products were used for quantitation by calibration with a β-D-glucan standard.

The (1→3),(1→4)-β-D-glucan (β-glucan) of barley plays a major role in the malting and brewing process and limits the utility of feed barley for chicks (Woodward and Fincher 1983). This has stimulated considerable research on structure and location (Fincher 1975, Woodward et al 1983b), physical properties (Forrest and Wainwright 1977, Woodward et al 1983a), and analysis of barley β-glucan (McCleary and Glennie-Holmes 1985). The less extensively studied oat β-glucan was the subject of a recent review (Wood 1986).

Purified β-glucans from oat and barley and lichenan from *Cetraria islandica* generally were shown to be linear, unbranched polysaccharides composed of 4-O-linked β-D-glucopyranosyl units (~70%) and 3-O-linked β-D-glucopyranosyl units (~30%) (Parrish et al 1960, Perlin and Suzuki 1962, Luchsinger et al 1965, Fleming and Manners 1966, Clarke and Stone 1966, Aspinall and Carpenter 1984).

The evidence suggests that the (1→3) linkages in each of these polysaccharides occur singly (Dais and Perlin 1982, Woodward et al 1983b, Vårum and Smidsrød 1988). Some previous reports of contiguous (1→3) linkages in barley β-glucan (Fleming and Manners 1966, Bathgate and Dalgliesh 1974, Fleming and Kawakami 1977) may have been based on misidentification of Smith degradation products (Woodward et al 1983b, Woodward and Fincher 1983). This structural feature may be present, however, in small (0.2–0.3%) amounts, as has been identified in the β-glucan from shoots of *Zea* (Kato and Nevins 1986).

Most of the (1→4)-linkages occur in groups of two or three (Perlin and Suzuki 1962, Woodward et al 1983b, Aspinall and Carpenter 1984), but longer runs of consecutive (1→4)-linked units have been identified in barley β-glucan (Luchsinger et al 1965, Woodward et al 1983b). The resultant structure for oat and barley β-glucan is a polysaccharide composed mainly of β-(1→3)-linked cellotriosyl and cellotetraosyl units. Oat and barley β-glucans have been categorized generally as structurally similar, and most reports have distinguished these β-glucans as containing more of the (1→3)-linked cellotetraosyl units than lichenan (Parrish et al 1960, Perlin and Suzuki 1962, Fleming and Manners 1966).

The current interest in oats as a source of soluble dietary fiber, the effectiveness of which might be related to viscosity (Jenkins et al 1978), has generated the need for knowledge of the properties and levels of β-glucan in oats similar to that available for barley. Rapid methods for analysis (McCleary and Glennie-Holmes 1985, Åman and Hesselman 1985, Jørgensen and Aastrup 1988) are now available, but before the development of these methods, our program on isolation of β-glucan-rich fractions from oats and the large-scale production of these for nutritional studies (Wood et al 1989) required a rapid, automated β-glucan analysis. A method using high-performance liquid chromatography (HPLC) of oligosaccharides released by (1→3),(1→4)-β-D-glucan-4-glucanohydrolase (lichenase) was reported in a preliminary communication (Wood and Weisz 1986). Lichenase specifically cleaves the β-(1→4)-linkage of a 3-O-substituted D-glucopyranose residue in the polysaccharide to give 3-O-β-cellobiosyl-D-glucose and 3-O-β-cellotriosyl-D-glucose as the major products.

Although β-glucan levels are clearly important, variations in molecular size and structure would affect physical and possibly physiological properties. Because the product of most medical (and present consumer) interest is oat bran, variations in the β-glucan throughout the groat requires consideration. Structural studies reported to date have used purified polysaccharide and techniques which, although necessary in rigorous structure assignment, are not useful for multiple samples and, because they concentrate on a particular fraction, might even be misleading (Wilkie 1979). The HPLC procedures initially developed (Wood and Weisz 1986) for β-glucan analysis also provide considerable structural information because the tri- and tetrasaccharide released by lichenase correspond to the main structural feature of the polysaccharide (Wood 1986). This article describes this methodology and its application to oat products, barley, wheat, and rye.

MATERIALS AND METHODS

Analytical-grade reagents and distilled water were used.

Lichenase was part of a β-glucan analysis kit supplied by Biocon (USA) Inc., Lexington, KY. Lichenan (*C. islandica*) was obtained from Sigma Chemical Co., St. Louis, MO (catalog L-8378). Barley β-glucan was obtained from Biocon (USA). Corn starch and soluble starch were from Fisher Scientific Co., Ottawa, ON, and cellobiose was from V Labs. Inc., Covington, LA. Laminaribiose was a gift from J. Erfle, Animal Research Centre, Agriculture Canada.

Oat gum was prepared as described by Wood et al (1989) at the POS Pilot Plant Corporation, Saskatoon, SK.

Purified oat β-glucan was prepared by dissolving oat gum in water (0.5%, w/v), centrifuging at 33,000 × *g* for 0.5 hr to remove a small amount of residue, and adding 50% (w/v) ammonium

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sulphate to the supernatant, slowly with stirring to make 20% (w/v) in ammonium sulphate. The precipitate was isolated by centrifuging (4,100 × *g*, 0.5 hr), disrupted in a Virtis homogenizer (Virtis Co., Gardiner, NY), and redissolved in water (0.5%, w/v). The ammonium sulphate isolation was repeated, and the pellet was dissolved again in water (0.5%, w/v). An equal volume of propan-2-ol (IPA) was added slowly with vigorous stirring, and the precipitate was allowed to settle and was collected by centrifugation (4,100 × *g*, 20 min). The precipitate was redissolved and again precipitated by IPA, washed with 100% IPA, and dried in a stream of air with gentle warming.

Before treatment with enzyme, flours were ground with a coffee grinder to pass a 30-mesh (600-μm) screen. To inactivate enzymes and remove low-molecular-weight sugars, samples (0.1 g) were treated with 50% (v/v) aqueous ethanol (5 ml) in a boiling water bath for 5 min, a further 5 ml of solvent was added, and the mixture was centrifuged. The supernatant was discarded. Then a further 10 ml of the solvent was added, the sample was recentrifuged, and the supernatant was discarded again. In some instances, dried ethanol-inactivated samples were prepared by refluxing in 75% (v/v) ethanol in water (liquid-solid, 20:1) for 4 hr and washing with 75% ethanol and then 95% ethanol, and the solvent was removed by air drying with gentle warming.

Preparation of Oligosaccharides for Methylation Analysis

Oat gum (3 g) was dissolved in 0.05*M* phosphate buffer (600 ml, pH 6.9) containing 10 m*M* NaCl by heating (60°C) and stirring for 3 hr. Insoluble material was removed by centrifugation (33,000 × *g*, 30 min), and the supernatant was treated with 30 units of hog pancreatic α-amylase (Type 1A, treated with diisopropyl fluorophosphate, catalog A-6255, Sigma Chemical Co.) for 1 hr at room temperature. The solution was heated (70–75°C) for 45 min and centrifuged (33,000 × *g*, 1 hr), and IPA (600 ml) was added by drops to the supernatant. The precipitate was collected by centrifugation (4,100 × *g*), redispersed (using a Virtis homogenizer), recentrifuged in 50% IPA, dispersed in 100% IPA, filtered, and air dried (79% yield).

The α-amylase-treated gum was wetted with ethanol (8 ml), dissolved in 20 m*M* phosphate buffer (200 ml, pH 6.5), and treated with lichenase (150 units) for 4 hr at 40°C. The solution was heated to 80°C for 15 min and stored at 5°C overnight, and the precipitate was recovered by centrifugation (33,000 × *g*, 30 min). The precipitate was washed twice with water, suspended in IPA, and recovered on a sintered glass filter (5% yield).

Three volumes of IPA were added to the supernatant; the mixture was centrifuged; and the supernatant was concentrated to 10 ml before fractionation by Biogel P-2 chromatography essentially as described by Woodward and Fincher (1982). Fractions corresponding to di-, tri-, tetra-, penta-, and hexasaccharide were collected, and their purity was assessed by HPLC on a Bio-Rad HPX-42A column (Bio-Rad Laboratories, Richmond, CA) as described below.

Methylation Analysis

Dried samples (1–3 mg) of oligosaccharide fractions were methylated, and the alditol acetates were prepared according to the method of Harris et al (1984) using sodium to generate the methylsulfinyl carbanion. Reduction with sodium borodeuteride before methylation was as described by Valent et al (1980). The methylated alditol acetates were analyzed on a gas-liquid chromatograph (8310, Perkin-Elmer Corp., Norwalk, CT) using a DB-225 capillary column (0.15-μm film thickness) with injection port at 270°C and flame ionization detector at 300°C. Helium carrier gas was used, and the column was programmed for 180–220°C at 4°C/min and held at 220°C for 10 min. Data were processed by Chrom 3 software (version 2.0) using the PE 7500 computer (Perkin-Elmer). Gas-liquid chromatography (GLC)-mass spectrometry was carried out on a Finnigan MAT 4500 (Finnigan Corp., San Jose, CA) under similar chromatographic conditions.

^{13}C-Nuclear Magnetic Resonance Spectroscopy

Oat and barley β-glucans (200 mg) were dissolved in water (40 ml) and centrifuged, and supernatants were sonicated using a microtip probe of a Vibra Cell VC 250 (Sonics and Materials Inc., Danbury, CT) with a 90% time pulse and at setting 4 for approximately 1.25 hr total time. The viscosity-reduced β-glucans were isolated by precipitation with 50% IPA (yields of 165 mg for oats, 159 mg for barley). Lichenan (200 mg) in water (40 ml) was stored at 5°C until opalescent, then centrifuged (33,000 × *g*, 1 hr). A soft gel was removed from a brown precipitate, and, after it was redissolved in water (40 ml), the solution was sonicated as above. After keeping overnight at 5°C (slight opalescence), 60 ml of IPA and 3 drops of glacial acetic acid produced a precipitate, which was isolated by centrifugation (yield of 123 mg).

The sonicated oat and barley β-glucans and purified lichenan (80 mg) were dissolved in 2 ml of deuterated dimethylsulfoxide (99.9% deuterium), and the ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded in 10-mm tubes at 62.8 MHz on a WM250 NMR spectrometer (Bruker Spectrospin Canada, Milton, ON) operating at 90°C. Spectra typically consisted of 50,000 scans acquired with a 12-μsec (60°) pulse and a 1.5-sec recycle time and were plotted using resolution enhancement.

Preparation of Oat Brans

Oat flours and bran were prepared by P. Fedec (POS Pilot Plant Corporation) as follows. Dehulled or hulless (Tibor and 03669) oats were milled at setting 0 in an AB Falling Number mill (model KT-30, Falling Number AB, Stockholm, Sweden). A portion of the flours obtained (10 g) was fractionated into coarse (bran) and fine fractions in an ATM sonic sifter (model L-38, ATM Sonic Sifter Division, Milwaukee, WI) fitted with a 40-mesh (U.S.) (425-μm) screen. Samples were sifted for 5 min at setting 5.

Brans also were prepared by milling at setting 1 in the AB Falling Number mill before fractionation in the ATM sonic sifter. The coarse fraction then was further enriched in β-glucan by sieving in 50% ethanol, after heating to inactivate enzymes as described above. The coarse fraction was retained on a 100-mesh (150-μm) screen. This process of fractionation in aqueous ethanol also was carried out on Quaker "quick" oats and oat bran (supermarket purchase).

Autoanalyzer Methods

A Technicon System II Autoanalyzer (Technicon Corp., Tarrytown, NY) was used for automated assays. For colorimetric monitoring of HPLC columns, methods based on the orcinol-sulfuric acid assay (Simatupang 1979) and potassium ferrocyanide determination of reducing sugar (Technicon industrial method 426-76A) were used (Fig. 1A and B). Two glucose oxidase assays were used: one based on oxidation of potassium ferrocyanide (Technicon industrial method 168-76A), and one based on the method described in the Biocon kit (McCleary and Glennie-Holmes 1985) (Fig. 1C). Reagents were as follows.

Orcinol-sulfuric acid. Orcinol (Baker Chemical Co., Phillipsburg, NJ) was dissolved (0.5 g/L) in 70% (v/v) analytical reagent-grade sulfuric acid in water. Freshly prepared reagent was used and was protected from light.

Potassium ferricyanide. Potassium ferricyanide was used at 0.18 g/L in water.

Potassium ferrocyanide. One gram of the trihydrate was used in 1 L of water containing 1.0 ml of Brij 35 (Fisher Scientific).

4-Aminophenazone. 4-Aminophenazone (Sigma Chemical Co.) was used at 0.2 g in 2 L of water containing 2 ml of Brij 35.

Glucose oxidase-peroxidase reagent for potassium ferrocyanide chemistry. Five milligrams of glucose oxidase (catalog 646423, Boehringer-Mannheim, Dorval, PQ) and 1.0 mg of horseradish peroxidase (catalog 108090, Boehringer-Mannheim) were dissolved in 50 ml of tris-phosphate-glycerol buffer (pH 7.0), which was prepared by dissolving 36.3 g of trishydroxymethyl aminomethane and 50 g of sodium dihydrogen phosphate monohydrate in water containing 400 ml of glycerol, diluting with water to 1 L, and adjusting to pH 7.0 with solid sodium

dihydrogen phosphate.

Glucose oxidase-peroxidase reagent for aminophenazone chemistry. The reagent was the same as for potassium ferrocyanide except for the buffer (pH 7.0), which was prepared by dissolving 20.64 g of disodium hydrogen phosphate heptahydrate, 10.97 g of sodium dihydrogen phosphate monohydrate, 4.0 g of benzoic acid, and 3.0 g of parabenzoic acid in 2 L of water.

Starch Analysis

To a sample containing 1–10 mg of starch, water (1.0 ml) and heat-stable α-amylase (50 μl) from *Bacillus subtilis* (2 mg/ml, catalog 171568, Calbiochem-Behring Corp., La Jolla, CA) were added, and the mixture was heated at 70–75°C for 1 hr with stirring. After cooling, 1.0 ml of 0.2*M* sodium acetate buffer, pH 4.8, and 0.1 ml of amyloglucosidase from *Aspergillus niger* (50 mg/ml, Agidex, catalog 39075, BDH Inc., Toronto, ON) were added, and the mixture was stirred at 48°C for 0.5 hr. Appropriately diluted samples were centrifuged, and aliquots were analyzed for glucose using glucose oxidase. Corn starch, used as a check of enzyme action in each analysis, showed 96–100% anhydroglucose. β-Glucan standards (oat and barley β-glucans, lichenan, laminaran) showed <0.5% anhydroglucose in the assay.

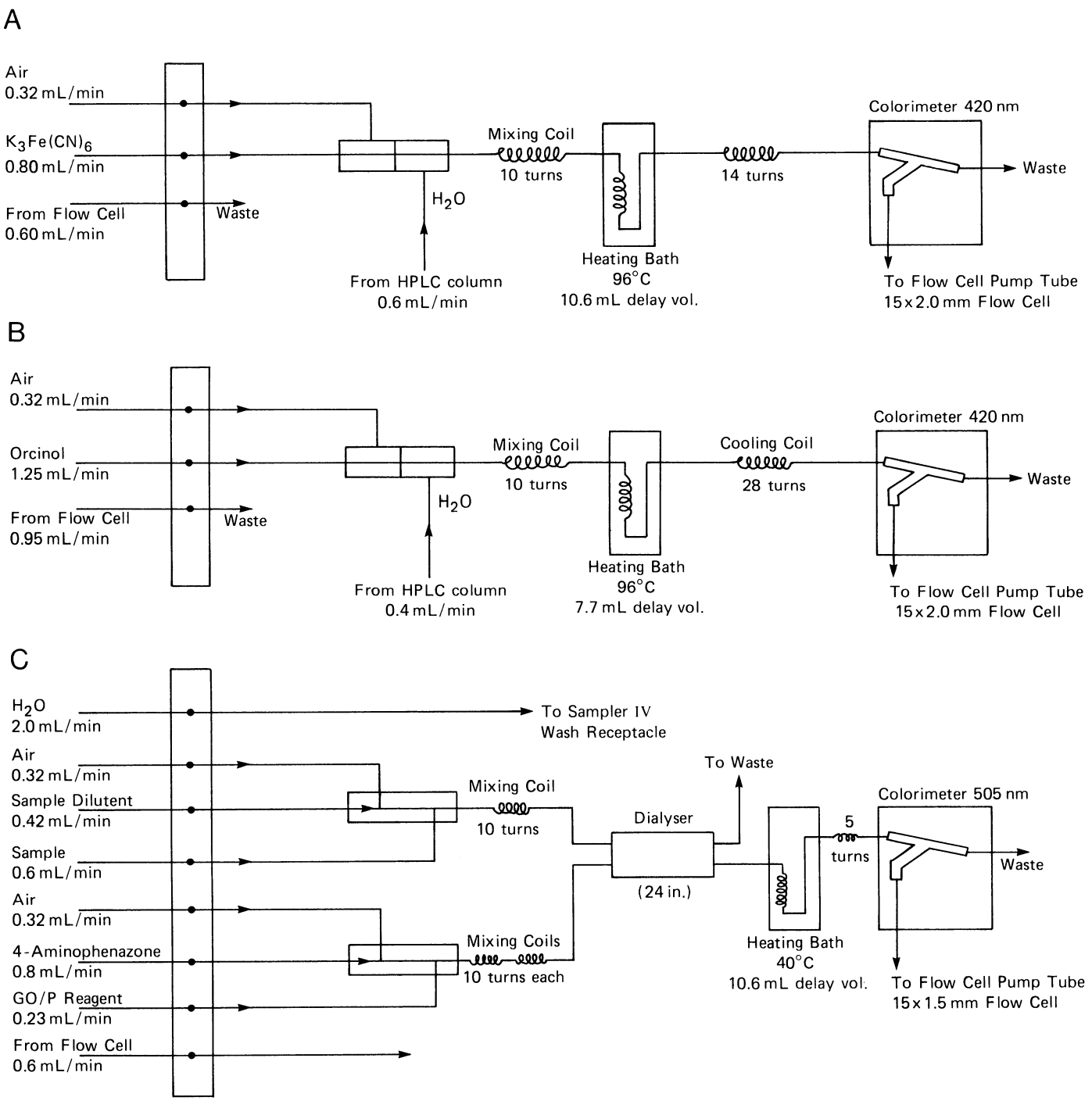

Fig. 1. Flow diagrams for Technicon Autoanalyzer II chemistries. **A,** High-performance liquid chromatography (HPLC) on an HPX-65A column with detection by the reducing sugar reaction. **B,** HPLC on an HPX-42A column with detection by the orcinol-sulfuric acid reaction. **C,** Automated version of the glucose oxidase assay of McCleary and Glennie-Holmes (1985). A sampler IV sample rate of 70/hr was used with a sample wash ratio of 8:1. Potassium ferrocyanide chemistry was similar with the following differences: $K_4Fe(CN)_6$ at 0.6 ml/min (instead of 4-aminiphenazone at 0.8 ml/min); line from flow cell at 0.8 ml/min; 12-in. dialyzer; colorimeter at 420 nm; flow cell of 15 × 2.0 mm; sample rate of 40/hr; sample-to-wash ratio of 12:1.

High-Performance Liquid Chromatography

Samples were filtered (0.45 μm, MST filters, Fisher Scientific Co.) and analyzed on a Bio-Rad HPX-42A carbohydrate analysis column (Bio-Rad, Mississauga, ON). Bio-Rad de-ashing guard columns were used. Glass-distilled, degassed water was used at 85°C and at a flow rate of 0.4 ml/min. In some experiments, a Bio-Rad HPX-65A column and a 0.6 or 0.8 ml/min flow rate were used. Routinely a 50-μl injection volume from a Perkin-Elmer ISS 100 autosampler was used with a Waters model 590 pump (Waters Scientific Ltd., Mississauga, ON). Detection was with a Waters R401 refractive index detector or with automated chemistry, as described. For data handling, a Perkin-Elmer 7500 computer with Chrom 3 (version 2.0) software was used.

Lichenase Digestion

For routine analysis, the Biocon kit lichenase digestion procedure (McCleary and Glennie-Holmes 1985) was followed but with a sample size of 100 mg. Enzyme amounts were adjusted accordingly. Isolated oat gums or β-glucan were predissolved in buffer before enzyme incubation. Aliquots from the lichenase digestion were analyzed by HPLC or were treated further with β-glucosidase, and the released glucose was analyzed by glucose oxidase. Blanks without enzyme also were analyzed. Quantitation by HPLC analysis required the use of a standard oat or barley β-glucan depending on the substrate to be analyzed. Starch analysis and acid hydrolysis showed that oat β-glucan was 99% pure and barley β-glucan was 94% pure. Duplicate samples (approximately 10 mg) were incubated with lichenase as described, the duplicate digests were combined, and dilutions were prepared to give four to five calibration points in the range 0.1–0.5 mg/ml. Routinely, the tri- and tetrasaccharide peak areas or heights were separately plotted against β-glucan concentration. Two regression equations then were used to calculate β-glucan contents on the basis of both the tri- and tetrasaccharide peaks, and the average of the two values was taken. This procedure requires the sample and the standard to have similar tri- and tetrasaccharide ratios and yields. Normally, triplicate enzyme digests were used.

RESULTS

Methylation Analysis of Oligosaccharides Released by Action of Lichenase on Oat β-Glucan

The results of the methylation analysis are summarized in Table I. Tentative identification of the methylated alditol acetates was made by comparison with the retention times of alditol acetates obtained from standards and were confirmed by the GLC mass spectra. Relative to the tetra-O-methyl alditol acetate from the nonreducing end of the oligosaccharides, the tri- and tetrasaccharide fractions gave one and two mole proportions, respectively, of the 2,3,6-tri-O-methyl alditol acetates derived from 4-O-linked residues. Losses (approximately 50%) occurred at the reducing end during methylation, as shown for a laminaribiose standard. The data shown were corrected for this loss on the basis of the 0.52 (±0.06) molar proportion yield observed for laminaribiose. The identity of the reducing end was established by methylation analysis of the borodeuteride-reduced oligosaccharide where, for the tri-, tetra-, and pentasaccharide, the molar proportion of the 2,4,6-tri-O-methyl alditol acetate was decreased to trace or very low amounts. This corresponded to the appearance of the 1,2,4,5,6-penta-O-methyl alditol acetate derived from the borodeuteride-reduced residue. The hexasaccharide appeared to be a mixture of products. The insoluble residue appeared to have an average degree of polymerization of eight to nine and contained mainly (1→4)-linked residues.

TABLE I
Methylation Analysis of Oligosaccharides Produced by the Action of Lichenase on the (1→3),(1→4)-β-D-Glucan of Oats (average of duplicates)

	Methylated Alditol Acetates (mole fraction)				
Oligosaccharide	**12456**	**12356**	**2346**	**246**	**236**
Disaccharide	nd[a]	nd	1	tr[a]	0.93[b]
Trisaccharide	nd	nd	1	1.03[b]	1.02
Reduced trisaccharide	0.40	0.10	1	tr	1.23
Tetrasaccharide	nd	nd	1	0.98[b]	1.97
Reduced tetrasaccharide	0.40	0.10	1	tr	2.27
Pentasaccharide	nd	nd	1	0.81[b]	2.66
Reduced pentasaccharide	0.56	0.18	1	0.09	3.24
Hexasaccharide	nd	nd	1	0.71	3.42
Reduced hexasaccharide	0.52	0.22	1	0.59	4.18
Water insoluble	nd	nd	1	0.43	6.19
Laminaribiose (n=8)	nd	nd	1	0.52	nd

[a]nd, not detected; tr, trace.
[b]Adjusted by correction factor (0.52 for laminaribiose, 0.48 for cellobiose).

^{13}C-NMR Spectroscopy

The spectra obtained from oat and barley β-glucans and lichenan (Fig. 2) appeared to be almost identical, but closer inspection revealed some differences in the relative signal intensities of the resonances assigned by Dais and Perlin (1982) to the C-4 involved in the glycosidic linkage (79.6–79.9 ppm, shown enlarged in the inset of Fig. 2). The two major lower field signals (79.6 and 79.7 ppm) were assigned by Dais and Perlin (1982) to the C-4 of 4-O-linked D-glucopyranosyl units attached, either glycosidically or through C-4, to 3-O-linked D-glucopyranose. The minor upfield signal (79.9 ppm) was assigned to the C-4 of 4-O-linked D-glucopyranosyl units flanked by further 4-linked D-glucopyranosyl units. The ratios of the integrals of the signals at 79.6 and 79.7 ppm to the signal at 79.9 ppm were 5.3 for oats, 8.1 for barley, and 5.9 for lichenan. Because these resonances were resolved only by the use of resolution enhancement, the signal-to-noise ratio thus produced prevented highly accurate integration of the three signals.

In all three β-glucans, only a single resonance was observed for the signal at 86.2 ppm assigned (Dais and Perlin 1982) to the C-3 involved in the glycosidic linkage.

Colorimetric Analysis for Total Glucan

The automated orcinol assay was used to determine total glucan content of purified β-glucan and soluble starch. For oat β-glucan, a value of 97.2 ± 2.6% was obtained. Soluble starch gave 99.4 ± 0.9% anhydroglucose.

Quantitative Analysis

Duplicate large-scale lichenase digestions of oat gum produced some insoluble glucose containing material that was 6.0% of the total β-glucan. The soluble oligosaccharide reaction products were separated and analyzed by HPLC with monitoring by orcinol, reducing sugar, or refractive index (Fig. 3). All solubilized carbohydrate material was detected by the orcinol-sulfuric acid reaction. Except when purified β-glucan was used, a peak from polymeric material was observed and was seen to increase in oat gum relative to purified β-glucan (Figs. 3 and 4). This peak presumably arose from solubilized starch and pentosan. The response of arabinose and xylose in the orcinol assay was about seven times that of glucose. The reducing sugar assay detected lower oligosaccharides only. Refractive index was less sensitive and selective but could be used with purified or ethanol-extracted material.

When aliquots from lichenase digests of ethanol-treated oat flour (cultivar Hinoat) were analyzed with detection by the orcinol chemistry and by the Biocon kit, there was no significant difference ($P > 0.5$, paired t-test) between the methods (5.66 ± 0.45% for HPLC and 5.65 ± 0.33% for the Biocon kit). The difference in values from HPLC and the Biocon kit ranged from −0.7 to +1.2. Quantitation by HPLC with detection using orcinol chemistry was less reproducible than quantitation by the Biocon kit or HPLC with reducing sugar chemistry. This may in part be due to temperature sensitivity of the orcinol reaction. Lowering the temperature of the reaction bath by 2°C reduced the peak height by approximately 30%.

Reducing sugar postcolumn chemistry was more reproducible than orcinol chemistry for quantitation of β-glucan and was the HPLC method of choice. Results obtained for a selection of oat products (Table II) showed that values obtained by HPLC were similar to those obtained by the Biocon kit (McCleary and Glennie-Holmes 1985). Analysis using refractive index gave similar values.

Determination of Ratios of Trisaccharide to Tetrasaccharide

The ratio of tri- to tetrasaccharide was determined using orcinol-sulfuric acid postcolumn chemistry because this directly measures glucose content. Summation of all oligosaccharide peaks gave a total anhydroglucose recovery of 83.7 ± 6.5% (n = 9). The tri- and tetrasaccharide peaks represented 89.9 ± 2.3% of this total. The tri- and tetrasaccharide yield from barley β-glucan was similar. Penta- and hexasaccharide peaks were also distinguishable and in lichenan were present in amounts that were larger than the amount of tetrasaccharide (Fig. 4). There were traces of disaccharide, which increased with extended hydrolysis time (20 hr). Elution profiles and peak area ratios were essentially the same on HPX-42A and HPX-65A (Figs. 3 and 4) when the same postcolumn detection was used. Peak retention volumes were less on HPX-65A than on HPX-42A, but real chromatographic values are obtained only with refractive index detection (Fig. 3C) because postcolumn chemistry introduces different delay times for reaction (Fig. 1).

The elution profiles of lichenase digests of oat gum, purified oat, and barley β-glucan and lichenan are shown in Figures 3 and 4. Peaks designated tri-, tetra-, penta-, and hexasaccharide had the same retention times as the trisaccharide-to-hexasaccharide fractions purified by Biogel P-2 chromatography. The "crude" oat gum and purified oat β-glucan had identical ratios of tri- to tetrasaccharide (2.0), whereas the ratio for barley β-glucan was 3.1 and for lichenan, 20.2. Profiles from lichenase digestion of barley, rye, and oat flours were similar to those from purified products (Fig. 5) except for increased peaks in the polymer region. Without ethanol extraction, low-molecular-weight sugars naturally present in the cereal grain were detected (Fig. 5D).

HPLC analysis of lichenase digests from four oat cultivars and brans obtained by dry sieving is summarized in Table III. Values obtained using the Biocon kit were similar to but somewhat lower than those obtained using HPLC. The ratio of tri- to tetrasaccharide was the same in the bran as in the original groat, and no significant cultivar variation was apparent. Brans containing higher levels of β-glucan were obtained by sieving in aqueous ethanol (Table IV). Reproducibility of the β-glucan values was poor in these brans because of sampling errors caused by coarse particles and small sample size. Peak area ratios were reproducible, however, and again showed values similar to those for the original groat.

Analysis of four barley cultivars, three commercial whole wheat flours, and a wheat and rye cultivar is summarized in Table V. These samples showed significantly higher ratios of tri- to tetrasaccharide than all oat samples studied. The range of values within barley (2.93–3.41) or wheat (3.04–3.84) was larger than that observed for oats (2.09–2.25).

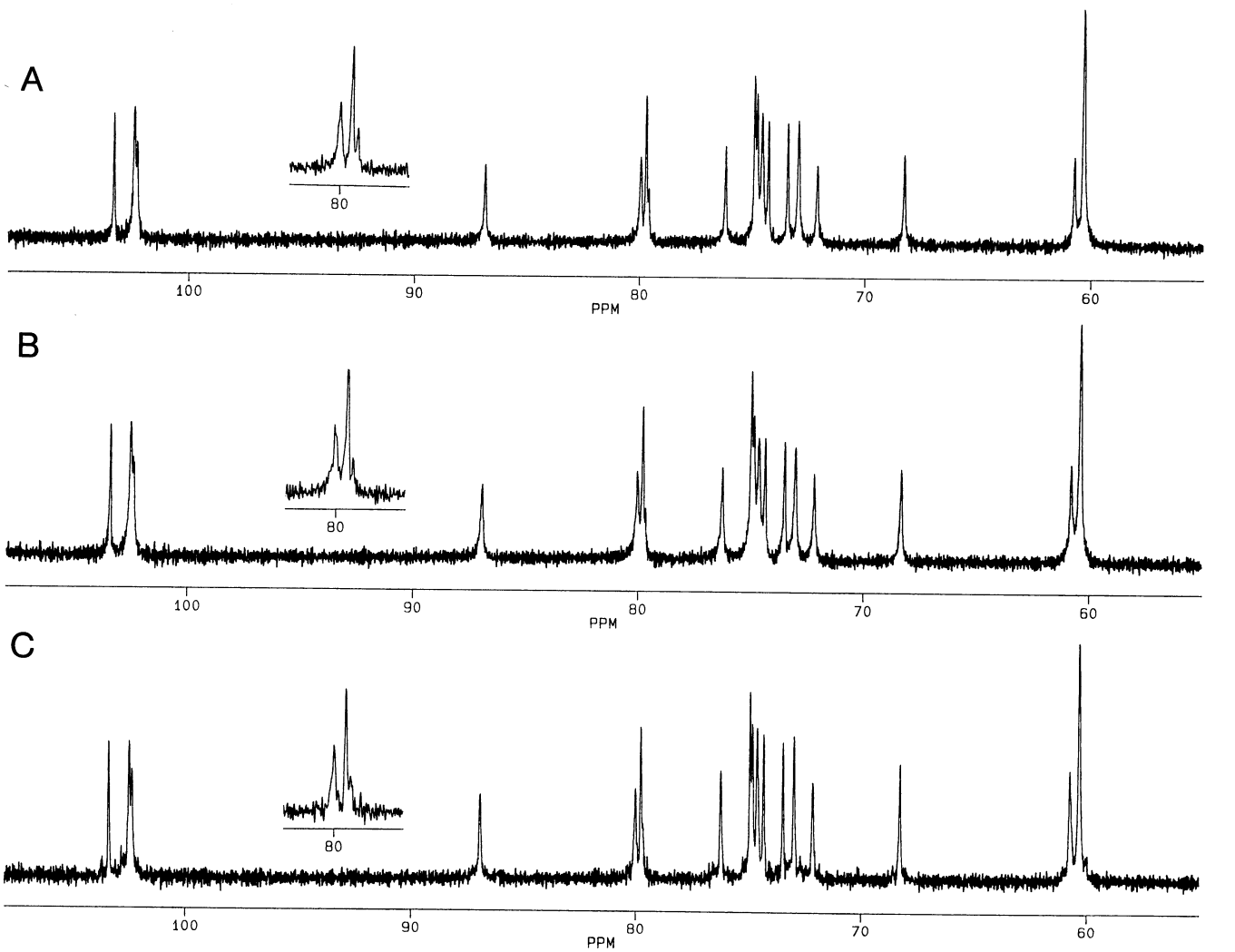

Fig. 2. ^{13}C-nuclear magnetic resonance (NMR) spectra obtained at 62.8 MHz on a Bruker WM250 NMR spectrometer. Samples (4%, w/v) in deuterated dimethylsulfoxide: **A,** oat β-glucan; **B,** barley β-glucan; **C,** lichenan.

DISCUSSION

Methylation analysis of oligosaccharides released by lichenase from oat β-glucan showed that the major products were 3-O-β-cellobiosyl-D-glucose and 3-O-β-cellotriosyl-D-glucose. This confirmed previous structural studies (Parrish et al 1960, Perlin et al 1962, Aspinall and Carpenter 1984) and extended these results to show that, like barley β-glucan (Woodward et al 1983b), a small (5–6%) portion of the oat β-glucan structure consists of cellulose like runs of consecutive (1→4)-linked β-D-glucopyranosyl residues. In our hands, the methylation techniques failed to give quantitative yields from the reducing end of the oligosaccharides. This was also true when the methylation procedure of Hakomori (1964) as modified by Jansson et al (1976) was used. Losses were consistent and could be corrected by the use of standards. Indeed, reduced molar yield appeared to be diagnostic of the reducing end. Thus, despite the failure to achieve quantitative recoveries as reported by other workers (Woodward and Fincher 1982, Harris et al 1984, Kato and Nevins 1986), consistent results were obtained. Because of the highly basic nature of the methylation medium, the reducing-end unit would be expected to be susceptible to losses. The identity of the reducing end was confirmed by prereduction, but once again alkaline sensitivity of this unit was noted. The appearance of small amounts of the 1,2,3,5,6-penta-O-methyl alditol acetate clearly suggests that an alkaline-peeling reaction, exposing the 4-linked glucose, briefly preceded the reduction. Aspinall and Carpenter (1984) commented about this previously. In our study, no products were found that would suggest the presence of runs of consecutive (1→3)-linked D-glucopyranose. However, methods used would not detect small amounts of this feature such as Kato and Nevins (1986) reported for the β-glucan of shoots of *Zea*.

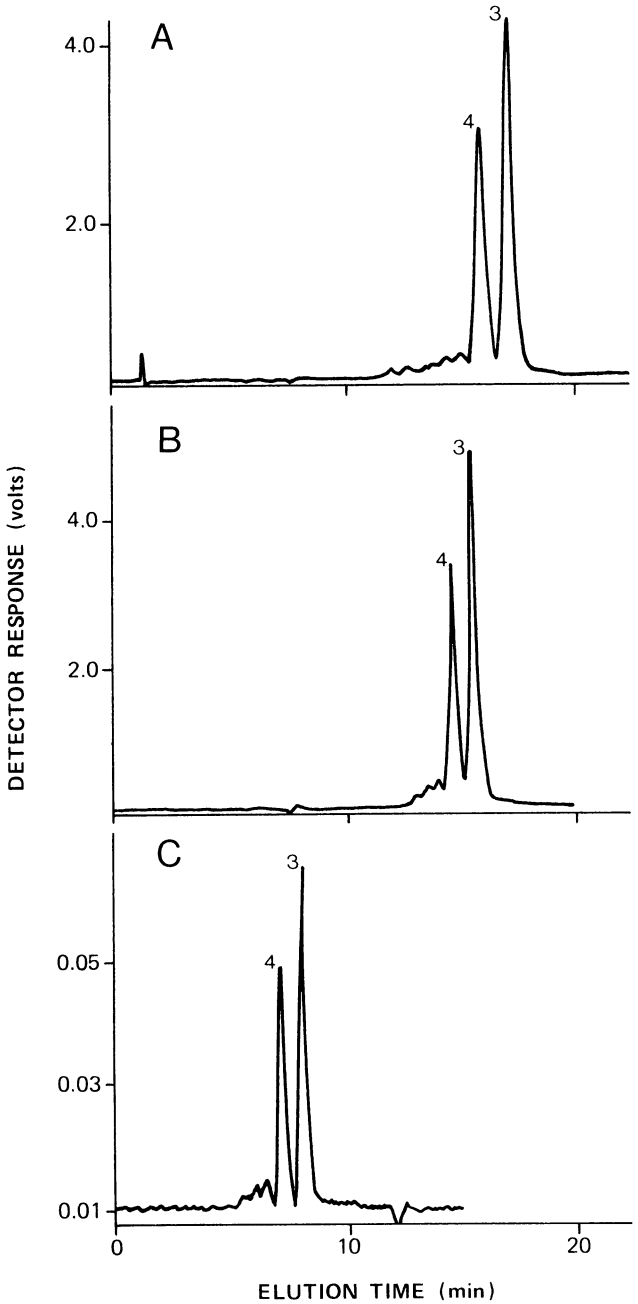

Fig. 3. High-performance liquid chromatography on HPX-65A of oligosaccarides released by lichenase from purified oat β-glucan. **A,** Orcinol-sulfuric acid detection, 0.6 ml/min flow rate. **B,** Reducing sugar detection, 0.8 ml/min flow rate. **C,** Refractive index detection, 0.8 ml/min flow rate. Peaks 3 and 4 are tri- and tetrasaccharide, respectively.

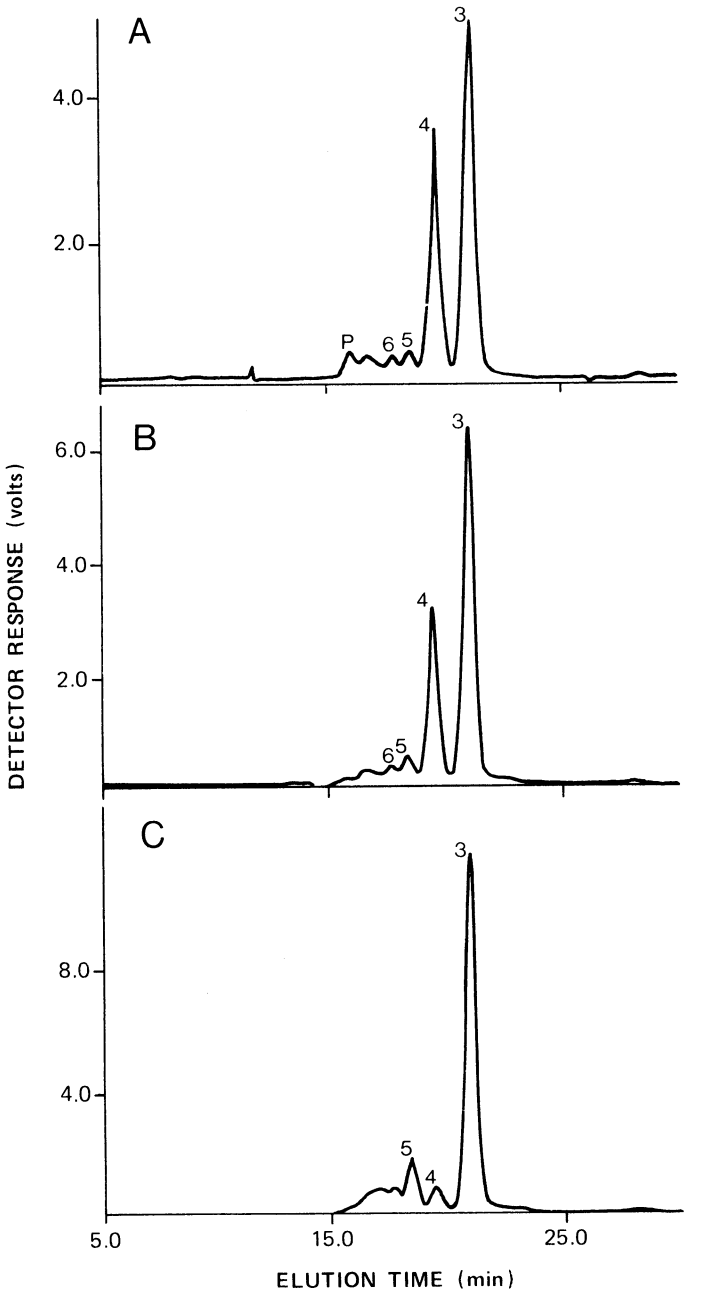

Fig. 4. High-performance liquid chromatography on HPX-42A (0.4 ml/min) of oligosaccharides released by lichenase from: **A,** oat gum; **B,** barley β-glucan; and **C,** lichenan. Detection by the orcinol-sulfuric acid reaction. Peaks 3, 4, 5, and 6 are tri-, tetra-, penta-, and hexasaccharide, respectively. P = polymer peak.

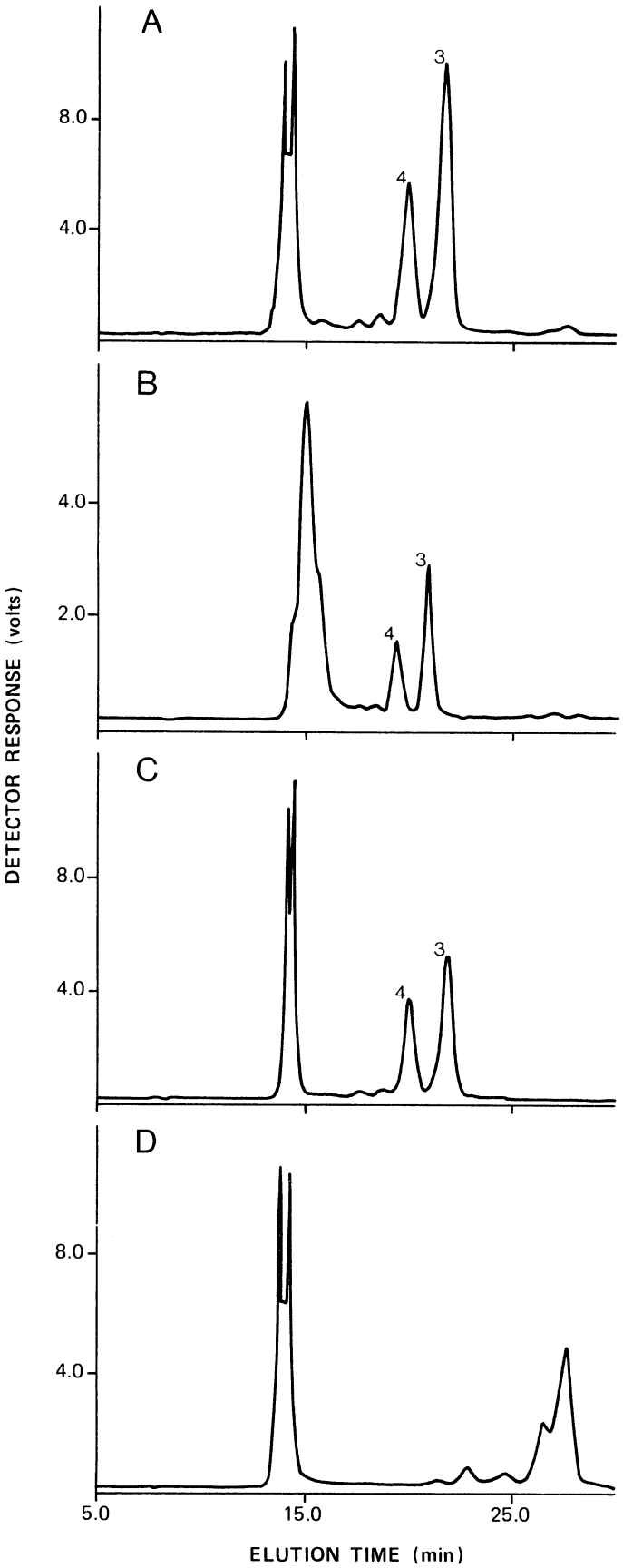

Fig. 5. High-performance liquid chromatography on HPX-42A (0.4 ml/min) of oligosaccharides released by lichenase from: **A,** barley flour; **B,** rye flour; **C,** oat flour; and **D,** oat flour that was not extracted with ethanol and was not treated with lichenase. Peaks 3 and 4 are tri- and tetrasaccharide, respectively.

^{13}C-NMR supported the lack of significant amounts (<5%) of contiguous (1→3)-linkages (because only one resonance was observed for linked C-3) and confirmed the similarity of oat and barley β-glucans. Despite the very similar ^{13}C-NMR spectral data, lichenan from *C. islandica* is structurally distinct from cereal β-glucans, as made clear by the HPLC studies. In a regular polymer of (1→3)-linked cellotriosyl units, only two environments exist (and hence only two resonances: those at 79.6 and 79.7 ppm) for the 4-O-substituted C-4 carbon. The minor upfield signal only arises from cellotetraosyl and higher units, and in the absence of significant proportions of the latter, the ratio of the two lower field signals to the upfield signal provides a measure of the cellotriosyl and cellotetraosyl units. This ratio should correspond to the ratio of tri- to tetrasaccharide found by HPLC. The value calculated from NMR data for oats was 1.6 (found 2.0), for barley 3.0 (found 3.1), and for lichenan 2.0 (found 20.2). In lichenan the assumption of the calculation, that the upfield signal arises mainly from the cellotetraosyl unit, clearly is not valid. HPLC shows a relative increase in lichenan of cellopentaosyl and higher units, which contribute proportionately more to the signal at 79.9 ppm. Furthermore, no attempt was made to isolate insoluble product, which is not detected by HPLC, from the lichenase digest of lichenan. These features would contribute, however, to the observed ^{13}C-NMR spectra. Further studies of this, using a higher magnetic field, are planned.

HPLC analysis of oligosaccharides released by lichenase from cereals gave results for β-glucan content that were similar to those from the method of McCleary and Glennie-Holmes (1985) in which the oligosaccharide reaction products are hydrolyzed by β-glucosidase to glucose, which is determined colorimetrically. The availability of a commercial kit, however, makes the method of McCleary and Glennie-Holmes more convenient, and it is more reproducible than HPLC, which requires a standard and calibration with chromatographic peaks that individually represent <50% of the β-glucan.

The HPLC technique is useful, however, for detecting structural differences between β-glucans. Data reported here clearly demonstrated a major difference between the cereal β-glucans and lichenan; the latter polysaccharide had a 10-fold higher proportion of the (1→3)-linked cellotriosyl unit. Within the oat samples studied, there was no essential structural variation of β-glucan between cultivars or between whole groat and bran. Approximately one third of the structure of oat β-glucan was made up of (1→3)-linked cellotetraosyl units, distinguishing it from other β-glucans from barley, wheat, and rye, which had approximately one fourth of their structure composed of this feature. There appeared to be a greater structural variability in the wheat and barley compared with oats.

These significant structural variations are not greatly reflected

TABLE II
Comparison of β-Glucan Content in Oat Samples as Determined by HPLC with Reducing Sugar Chemistry and by the Biocon Kit[a]

Sample	Biocon	HPLC
Hinoat (1979)[b]	5.42 ± 0.14	5.27 ± 0.15
Hinoat (1984)[b]	5.63 ± 0.03	5.49 ± 0.10
Hinoat (1985)[b]	4.68 ± 0.22	4.45 ± 0.25
Hinoat bran[c]	12.78 ± 0.08	12.57 ± 0.17
Hinoat bran[c]	13.69 ± 0.29	13.27 ± 0.17
Hinoat bran[c]	10.72 ± 0.22	10.40 ± 0.08
Mothers bran[d]	9.98 ± 0.08	9.86 ± 0.23
Quaker bran[d]	7.06 ± 0.13	6.73 ± 0.07
Quaker quick[d]	4.22 ± 0.07	4.18 ± 0.04
Quaker instant[d]	4.44 ± 0.08	4.44 ± 0.07
Ogilvy (old-fashioned thick flakes)[d]	4.13 ± 0.08	4.12 ± 0.06

[a]HPLC, high-performance liquid chromatography. Analysis with the Biocon kit was performed as described in McCleary and Glennie-Holmes (1985).
[b]Whole groat from dehulled seed.
[c]Prepared at the POS Pilot Plant, Saskatoon, SK (Wood et al 1989).
[d]Purchased from supermarket.

TABLE III
β-Glucan Content and Molar Ratio of Trisaccharide to Tetrasaccharide Released by Lichenase from Four Oat Cultivars and Brans Prepared by Dry Sieving (average of triplicates)

Sample	Yield of Bran (% of groat)	β-Glucan Content (% dry weight basis)		Molar Ratio of Trisaccharide to Tetrasaccharide	
		Groat	Bran	Groat	Bran
Donald	52	5.38 ± 0.29	8.07 ± 0.19	2.05 ± 0.01	2.12 ± 0.01
Marion	56	6.70 ± 0.12	9.45 ± 0.34	2.07 ± 0.01	2.09 ± 0.01
Tibor	39	4.34 ± 0.26	7.51 ± 0.08	2.19 ± 0.03	2.19 ± 0.01
03669	50	4.46 ± 0.15	7.43 ± 0.15	2.05 ± 0.01	2.25 ± 0.01

TABLE IV
β-Glucan Content and Molar Ratio of Trisaccharide to Tetrasaccharide Released by Lichenase from Oat Brans Prepared by Sieving in Aqueous Ethanol

Starting Material	Fraction Yield	β-Glucan Content (% dry weight basis)	Molar Ratio of Trisaccharide to Tetrasaccharide
Bran			
cv. Donald	33	11.42 ± 1.10	2.17 ± 0.02
cv. Marion	34	10.83 ± 0.52	2.13 ± 0.09
cv. Tibor	32	12.18 ± 1.44	2.22 ± 0.06
cv. 03669	31	12.90 ± 0.79	2.11 ± 0.09
Quaker quick oats	26	13.36 ± 0.47	2.20 ± 0.01
Quaker bran	61	14.89 ± 0.45	2.23 ± 0.01

TABLE V
β-Glucan Content and Molar Ratio of Trisaccharide to Tetrasaccharide Released by Lichenase from Barley, Wheat, and Rye (average of duplicates)

Sample	β-Glucan Content (% dry weight basis)	Molar Ratio of Trisaccharide to Tetrasaccharde
Barley		
cv. Bruce	5.29	3.41
cv. Rodeo	4.29	2.999
cv. Birka	4.73	2.93
cv. Mingo	4.72	3.39
Wheat		
Soft white winter	0.84	3.44
Hard red winter	0.82	3.84
Hard red spring	0.68	3.04
cv. Frederick	0.62	3.84
Rye, cv. Musketeer	1.41	2.73

by differences in the percentage of (1→4)-linkages which, in the absence of structural features other than (1→3)-linked cellotriosyl and cellotetraosyl units, can vary only from 66.7 to 75.0%. Methylation analysis of the intact polysaccharides showed 71% (1→4)-linkages in oat and barley β-glucans and 67% in lichenan.

It is not known whether these structural differences affect rheological or physiological properties. Viscosity is highly sensitive to molecular-weight differences, making evaluation of structural effects difficult. Furthermore, it may be that differences in minor structural features, such as consecutive (1→3)-linked units or celluloselike sequences of (1→4)-linkages (which, by virtue of their insolubility after lichenase action, are not detected by the HPLC methodology), have greater influence on physical properties. Theoretical studies suggest that this might be so (Buliga et al 1986). The presence of other minor structural features, such as peptide (Forrest and Wainwright 1977, Vårum and Smidsrød 1988), remains a possibility.

Nevertheless, the data reported here establish a methodology for rapid structural evaluation of (1→3),(1→4)-β-D-glucans without the need for purification. This should prove useful for assessing possible differences due to cultivar, tissue, maturity, and environment.

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