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Partial Chemical and Physical Characterisation of (1→3),(1→4)-β-D-Glucans from Oat (*Avena sativa* L.) Aleurone

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ABSTRACT

(1→3),(1→4)-β-D-glucans from oat aleurone extracted at different temperatures generally showed increasing intrinsic viscosities with increasing extraction temperatures. Purification by ammonium sulphate precipitation resulted in β-glucans with much smaller differences in intrinsic viscosities than the unpurified extracts. No sequences of two or more adjacent (1→3)-linkages were found in the β-glucans. O-carboxymethylation of the glucan was performed, and the resulting product had a stiffness parameter of 0·116, corresponding to a Kuhn-length in the unperturbed state of about 5 nm. A titration curve of the β-glucans showed the presence of two different charged groups in the samples, one with p K_a*-values around 4·5 and the other with* p K_a*-values between 5 and 7·5. The presence of the charged groups was also seen from the determination of osmotic pressures of β-glucans in pure water solutions. Aqueous 1 M LiI was shown to be a good solvent for the β-glucan, as revealed from the osmotic second virial coefficients. A series of β-glucans of decreasing molecular weight and intrinsic viscosities were prepared by ultrasonication (solvent: 1 M aqueous LiI). The Mark–Houwink–Sakurada viscosity equation was determined for samples having number average molecular weights from* $6{\cdot}3 \times 10^4$ *to* $3{\cdot}3 \times 10^5$ *daltons as*

$$[\eta] = 5{\cdot}0 \times 10^{-4} \overline{M}_n^{0{\cdot}75} \text{ (in 100 ml g}^{-1}\text{) (20°C, 1 M LiI)}$$

INTRODUCTION

Mixed-linkage (1→3),(1→4)-β-D-glucans (referred to as β-glucans) are major components of cereal endosperm and aleurone cell walls from oat and barley (Wood & Fulcher, 1978). Barley β-glucans have been extensively studied because they can lead to filtration difficulties in the brewing process (Luchsinger, 1967; Bathgate & Palmer, 1974; Bathgate & Dalgliesh, 1974), and because they are responsible for decreased growth rates in poultry (Hesselmann & Åman, 1985). β-glucans are constituents of 'dietary fibre' (Bailey *et al.*, 1978), and may be the active component in oats and barley responsible for the hypocholestomeric effect in man (Gould *et al.*, 1980).

β-glucans from different sources have been subjected to extensive structural analysis, especially with respect to the arrangement of (1→3)- and (1→4)-linkages in the chains. Most water-soluble β-glucans contain approximately 30% (1→3) and 70% (1→4)-linkages which occur predominantly as cellotriosyl and cellotetraosyl residues separated by single (1→3)-linkages (Parrish *et al.*, 1960; Dais & Perlin, 1982; Woodward *et al.*, 1983). Evidence for the occurrence of two or more adjacent (1→3)-linkages has been reported (Fleming & Manners, 1966; Igarashi & Sakurai, 1966; Bathgate *et al.*, 1974; Fleming & Kawakami, 1977), although it has not been found in all preparations (Luchsinger *et al.*, 1965*a*,*b*; Dais & Perlin, 1982; Woodward *et al.*, 1983).

Physical properties, molecular shape and solution configuration of a β-glucan isolated from barley have been reported (Woodward *et al.*, 1983; Buliga *et al.*, 1986). Viscosity and molecular weight data in aqueous solution of the β-glucan was discussed in terms of an ellipsoidal configuration with an axial ratio of 100 (Woodward *et al.*, 1983). However, this view was questioned by Buliga *et al.* (1986) who, by using a random coil model, showed that considerable chain flexibility arises from the isolated (1→3)-linkages in the chains.

In the present study the chemical structures of a series of β-glucans from oat harvested in Scandinavia have been investigated and are compared with a β-glucan from Hinoat, a high-protein cultivar of oat from Canada. Carboxymethylation of the β-glucan was performed, and the dependence of the intrinsic viscosity on the ionic strength of the solvent determined, from which the stiffness parameter (B) can be calculated (Smidsrød & Haug, 1971). Furthermore, the relationship between the intrinsic viscosity and the number average molecular weights of a series of β-glucan samples is reported, and the coefficients of the Staudinger equation determined.

During the study it was observed that the β-glucans from oat showed polyelectrolytic behaviour in osmometry. The cause of this behaviour was studied in some detail.

MATERIALS AND METHODS

Materials

All extractions were carried out at the Technical Research Centre of Finland (Helsingfors) by Dr Olavi Myllymaki, as part of a Nordic co-operative project ('Cereals as Raw Materials for Industry'). Extractions were carried out at different temperatures at pH 9. The water-soluble β-glucan was extracted from the aleurone layer which was previously separated from oat grain. Since the aleurone layer contains much less starch than the endosperm, the extracts were relatively pure after protein was removed by lowering the pH to 4 (between 60–80% β-glucan content, the other major component was protein). The details of the extractions are reported elsewhere (Autio *et al.*, 1987).

β-glucan from Hinoat was kindly supplied by Dr P. Wood, Food Research Centre, Ottawa, Canada. Glycoaldehyde was supplied by Sigma (St Louis, USA), and erythritol by Aldrich (Steinheim, FRG). 2-*O*-β-D-Glucosyl-erythritol was kindly provided by Prof. Perlin, McGill University, Canada. Lithium iodide trihydrate was supplied by Merck (Darmstadt, FRG). $Na_2S_2O_3$ (5 mM) was added to prevent the formation of a brown colour due to iodine in the LiI solutions.

Purification

The β-glucans were purified by precipitating the β-glucan from a 0·3% (w/v) β-glucan solution with addition of solid ammonium sulphate (30% (w/v) solution), left at 0°C for 2 h and finally centrifuged at 4°C. The precipitate was washed three times with 30% ethanol, and the final precipitate was homogenised in water with a tissue homogeniser. This product was lyophilised.

Solubilisation of β-glucans

Solubilisation of β-glucans in aqueous solvents involved heating on a boiling water bath for 30 min followed by gentle shaking overnight. To investigate if this procedure led to degradation of the polysaccharide, the

intrinsic viscosities of two different samples that were heated for 0·5, 1·5 and 3 h were determined. No significant decrease in the intrinsic viscosities was found. Solubilisation of the β-glucan in 1 M LiI needed only gentle shaking overnight (no heating).

Chemical analysis of β-glucans

The purity of the β-glucans was analysed by an enzymic procedure (McCleary & Glennie-Holmes, 1985), and nitrogen content was determined in a Carlo Erba Elemental Analyser, Model 1101. Protein was calculated as nitrogen content × 6·25.

Periodate oxidation and Smith degradation

A solution of the β-glucan (733 mg) in water (500 ml) containing $NaIO_4$ (20 mmol) was oxidised in the dark at 5°C for 135 h. Excess periodate was destroyed by addition of 0.98 g ethylene glycol, and after dialysis the polyaldehyde was reduced conventionally with sodium borohydride overnight. The solution was neutralised by dropwise addition of glacial acetic acid at 0°C, and dialysed. Partial acid hydrolysis was performed with 0·3 M sulphuric acid for 48 h at room temperature, and followed by neutralisation with solid barium carbonate. Insoluble material was removed by centrifugation, and the supernatant applied to a column (13 mm × 1720 mm) of Sephadex G-10. The column was eluted with water at a constant flow rate of 12 ml h^{-1}. The elution was monitored by continuously measuring the UV absorbance at 206 nm (ISCO model 1840 detector). Fractions of 10 ml were collected and assayed by the phenol–sulphuric acid method (Dubois *et al.*, 1956).

^{13}C-NMR spectroscopy

The ^{13}C-NMR spectra were recorded at 90°C on solutions in D_2O (external standard Me_4Si) with a Bruker WM-400 (intact β-glucan) or JEOL JNM-FX 100 FT spectrometer (Smith degradation products).

O-Carboxymethylation of β-glucan

The polysaccharide was reduced conventionally with sodium borohydride and ground (mesh size < 0·8 mm) prior to carboxymethylation.

A slurry of the reduced β-glucan (1 g) in 27 ml isopropanol was stirred vigorously while 2·7 ml 30% aqueous NaOH was added dropwise during 0·5 h at room temperature. The mixture was stirred for 60 min,

and 1·2 g monochloroacetic acid was added over 30 min, and left at 55°C for 3·5 h. The surplus liquid was decanted, and the precipitate was stirred with 70% ethanol (50 ml) while the mixture was neutralised with 90% acetic acid. The precipitate was washed twice with 70% isopropanol and with pure isopropanol, and then dried at 60°C, to give 0·9 g carboxymethylglucan. This procedure is essentially as described by Green (1964) to prepare fibrous sodium *O*-carboxymethylcellulose.

Charge density measurements

Solutions of the *O*-carboxymethylglucan were dissolved in distilled water and dialysed extensively against 0·1 M $MgCl_2$ and then distilled water. Polymer-bound magnesium was released by dialysing four times against 0·2 M HCl. These dialysates were then combined, the volume was measured and the magnesium content analysed by atomic absorption spectroscopy (Perkin Elmer 560 atomic absorption spectrometer).

Depolymerisation of β-glucans

As different extraction temperatures yielded β-glucans with only small differences in intrinsic viscosities after purification with ammonium sulphate precipitation, the β-glucan was depolymerised by ultrasonic irradiation using a Labsonic 2000 equipped with a standard titanium probe (B. Braun, Melsungen, FRG). A 0·8% (w/v) solution of β-glucan in 1 M LiI (50 ml) was degraded for 3, 6, 8, 15 and 25 min. The solution was kept cold on an ice bath during depolymerisation.

Depolymerisation by ultrasonic radiation was also performed prior to recording the ^{13}C-NMR spectra of intact β-glucans.

Viscosity measurements

Dilutions of the β-glucans were performed manually. Relative viscosities were measured at 20°C in a Micro-Ubbelohde viscometer (Schott-Gerate, Type No. 53610), and the intrinsic viscosities ($[\eta]$) obtained by extrapolation to infinite dilution. The intrinsic viscosity of the carboxymethyl-glucan was determined as a function of ionic strength with NaCl concentrations of 0·01, 0·02, 0·1 and 0·5 M.

Osmotic pressure measurements

The osmotic pressure of solutions of β-glucan in the actual solvent was determined at 25°C in a Knauer Membrane Osmometer (Type 01.00;

membrane diameter 40 mm) fitted with a Sartorius SM 11736 cellulose acetate membrane. Concentrations of 0·1–0·8% (w/v) were used. The number average molecular weights ($\overline{M}_n$) were determined by extrapolation to infinite dilution.

Titration curves

Prior to titration, the β-glucan was converted to the H^+-form by lowering the pH of an aqueous solution of the β-glucan to 2. This solution was dialysed against 0·01 M HCl at 4°C, and then against Milli-Q water (Millipore, Bedford, USA).

Titration curves were obtained on a 1·5% solution of the polysaccharide in Milli-Q water, using 0·0100 M HCl to lower the pH to 3·7, and then titrating with 0·01M NaOH solution (freshly prepared). The titration curve of the same volume of water was subtracted from the β-glucan titration curve.

RESULTS

Extractions

Extraction conditions, such as temperature and pH, are known to affect both yields and intrinsic viscosities of the extracted β-glucans (Wood *et al.*, 1978). Table 1 shows the effect of extraction temperature on the intrinsic viscosities, purity and protein content before and after purification by ammonium sulphate precipitation. Generally, higher temperatures gave extracts with higher intrinsic viscosities. However, purification by ammonium sulphate precipitation resulted in β-glucans with much smaller differences in $[\eta]$, which may indicate selection of a specific type of β-glucans, with either the same chemical structure in terms of fine structure or with less variation in molecular weight.

Periodate oxidation and Smith degradation

To investigate if sequences of two or more adjacent (1 → 3)-linkages were present in the β-glucans, the fractions were subjected to Smith degradation (periodate oxidation, reduction with sodium borohydride followed by mild acid hydrolysis). Gel filtration of the products and subsequent identification by ^{13}C-NMR spectroscopy revealed that glycolaldehyde, D-erythritol and 2-*O*-β-D-glucosyl-D-erythritol were the major products. No 2-*O*-β-D-laminaribiosyl-D-erythritol or 2-*O*-β-D-laminaridextrin-D-

TABLE 1
Extraction Conditions, Purity and Properties of Extracts Before and After Purification by Ammonium Sulphate Precipitation

Fraction	*Extraction conditions*		*Purity (%)*		*Protein content (%) (after purification)*	*Intrinsic viscosity (100 ml g^{-1}) (solvent: distilled water)*	
	Temperature	*pH*	*Before*	*After*		*Before*	*After*
1	30	9·2	85	94	4·7	2·8	3·8
2	70	9·2	78	98	6·8	12·5	8·4
3	90	9·2	73	92	4·3	13·2	6·2
4	50	9·0	55	99	8·0	nd	6·2
5[a]	45	8·5	nd	98	4·0	8·5	4·8

[a] From Hinoat grown in Canada.
nd = Not determined.

erythritol derivatives could be identified among the Smith degradation products.

^{13}C-NMR Spectroscopy

^{13}C-NMR Spectroscopy of the intact β-glucan in D_2O (degraded by ultrasonication to reduce the relative viscosity) at 100 MHz gave spectra (not shown) essentially identical to those reported by Dais & Perlin (1982).

O-carboxymethyl-glucan

The product had a Degree of Substitution (DS) of 0·81 (see Materials and Methods) and was readily soluble in water. The intrinsic viscosity of the carboxymethyl-glucan at different ionic strengths (using NaCl) was determined. The intrinsic viscosity plotted against the reciprocal of the square root of the ionic strength yielded a straight line. The slope of the line (S) was 1·70. The stiffness parameter (B), defined by Smidsrød and Haug (1971) as

$$B = S/([\eta])^{1\cdot3}$$

where S is the slope of the straight line (see above) and $[\eta]$ is the intrinsic viscosity at an ionic strength of 0·1 M, could then be calculated to 0·116, corresponding to a Kuhn-length in the unperturbed state of about 5 nm.

Titration curve

The protein content of the fractions suggested the presence of charged groups on the β-glucans. A titration curve of the polymer is shown in Fig. 1. The number average molecular weight (osmotic pressure measurements) of this particular fraction was determined to be 270 000 with 1 M LiI as solvent. The titration curve of the same volume of water is subtracted from the β-glucan titration curve. From the titration curve, the presence of about five charged groups (per polymer molecule) with pK_a-values between 4 and 4·5 can be seen. Furthermore, the titration curve showed the presence of about four additional charged groups with pK_a-values between 5 and 7·5.

Osmotic pressure measurements

Distilled water was first chosen as solvent, because solubilisation of β-glucans involved wetting with ethanol and heating on a boiling water bath for 30 min. Figure 2 shows π/c plotted as a function of concentration of polymer (c) for one of the β-glucan fractions (Fraction 5). In distilled water, π/c increases as concentration decreases, while π/c decreases with decreasing polymer concentration in 1 M aqueous LiI. Obtaining a stable osmotic pressure at a given concentration in distilled water and returning to the baseline after a measurement both took several hours. With salt, 'normal' behaviour is observed, and the number average molecular weight of this fraction and the second virial coefficient were estimated to be 270 000 daltons and $4{\cdot}5 \times 10^{-4}$ ml mol g^{-2},

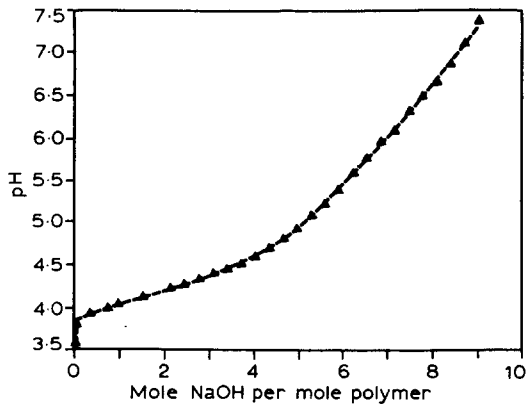

Fig. 1. Titration curve of β-glucan.

respectively, from this experiment. The presence of charged groups on a polymer, from which counterions dissociate as the polymer concentration is lowered, can explain these observations. Furthermore, the pH of solutions of the β-glucan in water was measured at different concentrations, showing an increase in pH with decreasing polymer concentration (Table 2). The calculated values for π/c in Fig. 2 are obtained assuming that the β-glucan is a polyelectrolyte (for details, see Discussion).

Due to the presence of charged groups on polymers in the samples, the number average molecular weights could not be determined in distilled water. Addition of a salt such as sodium chloride would solve this problem, but because of difficulties with the solubilisation of the β-

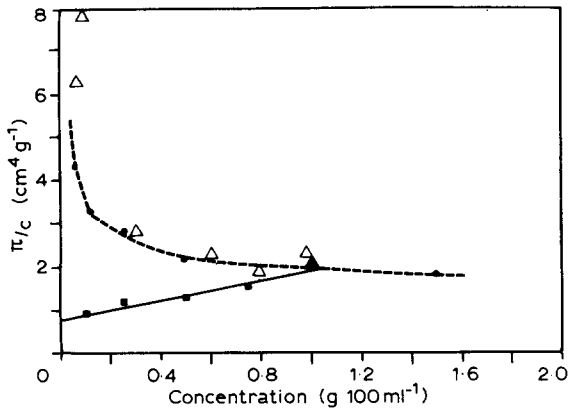

Fig. 2. Osmotic pressure measurements of β-glucan in distilled water and in 1 M aqueous LiI: △, Experimental values in distilled water; ●, calculated values in distilled water; ■, experimental values in 1 M aqueous LiI.

TABLE 2
Measured pH-Values of β-Glucan (Fraction 5) in Water at Different Concentrations

Concentration (%)	*pH*
0·065	4·85
0·125	4·65
0·25	4·46
0·50	4·16
1·00	4·09
1·50	4·06

glucans in this solvent (see Materials and Methods) and the possibility of aggregation at the highest polymer concentrations, it was attempted instead to dissolve the β-glucans in 1 M LiI. Gentle shaking overnight at room temperature completely solubilised the polysaccharide, and the osmotic pressure at different polymer concentrations could be measured relatively quickly (equilibrium after 15–20 min) and with much higher accuracy.

Number average molecular weights from 63000 to 330000 were determined after ultrasonic irradiation, and the second virial coefficients in 1 M aqueous LiI ranged from 5 to $8{\cdot}2 \times 10^{-4}$ ml mol g^{-2} (see Table 3). The second virial coefficient in 0·5 M NaCl was from 1 to 2×10^{-4} ml mol g^{-2} (data not shown).

Dependence of the intrinsic viscosity upon molecular weight

A series of β-glucans of decreasing molecular weight and intrinsic viscosities were prepared by ultrasonication in 1 M aqueous LiI (Table 3). A double logarithmic plot of $[\eta]$ versus $\overline{M}_n$ is shown in Fig. 3. The points could be fitted with a straight line, giving the Mark–Houwink–Sakurada (MHS) equation

$$[\eta] = 5{\cdot}0 \times 10^{-4} \overline{M}_n^{0{\cdot}75} \text{ (100 ml g}^{-1}\text{) (20°C, 1 M aqueous LiI)}$$

To the authors' knowledge, this is the first report of values for the Mark–Houwink–Sakurada coefficients for β-glucans.

DISCUSSION

Sequences of two or more adjacent (1→3)-linkages in the oat aleurone β-glucan appear to be absent. The experimental procedure for Smith degradation was as described by Woodward *et al.* (1983), who found that the hydrolysis of labile acetal linkages in the periodate-oxidised, reduced polysaccharide was incomplete unless the treatment with sulphuric acid was extended. Goldstein *et al.*(1965) reported sequences of two and three (1→3)-linked glucose units in β-glucan extracted from oat flour (endosperm). It is possible that the β-glucan from oat aleurone is different. However, Goldstein *et al.* (1965) allowed the partial acid hydrolysis to proceed for only 8 h, which may have resulted in incomplete hydrolysis. Furthermore, the ^{13}C-NMR spectrum of intact β-glucan showed that the signals attributed to C-2 to C-6 of 3-*O*-substituted residues were all sharp singlets (for details, see Dais & Perlin, 1982), confirming that these residues occur isolated in the polymer.

TABLE 3

Number Average Molecular Weights ($\overline{M_n}$), Second Virial Coefficients (B; ml mol g^{-2}) and Intrinsic Viscosities ([η]; in 100 ml g^{-1}) of the β-Glucan (Fraction 5) After Ultrasonic Degradation

Degradation time (min)	$\overline{M_n}$	$B(\times 10^4)$	$[\eta]$
3	330000	5·0	7·4
6	219000	8·0	4·3
8	170000	6·1	4·4
15	123000	8·2	3·3
25	63000	7·9	2·0

Solvent: 1M aqueous LiI

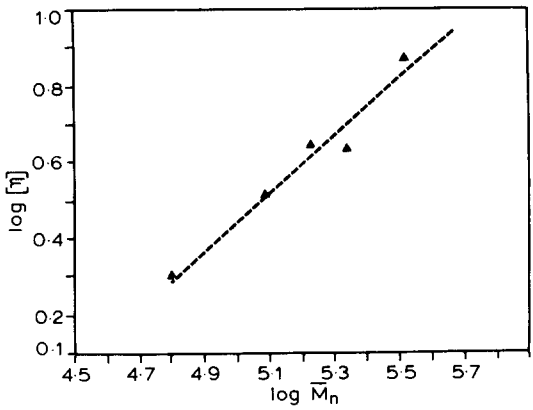

Fig. 3. Double logarithmic plot of $[\eta]$ versus $\overline{M_n}$ for β-glucan fractions.

Reduction of the β-glucan prior to *O*-carboxymethylation was essential to avoid extensive degradation ('alkaline peeling') during the strong alkaline reaction conditions used. Non-reduced β-glucan which was subjected to *O*-carboxymethylation gave low yields, probably because the degraded product was solubilised during washing with 70% ethanol.

The stiffness parameter (B) of the *O*-carboxymethyl-glucan was 0·116, reflecting the increased flexibility of the (1→3)-linkages in this polymer compared to carboxymethylcellulose ($B = 0{\cdot}065$; Smidsrød & Haug, 1971).

The titration curve clearly showed the presence of two different charged groups in Fraction 5. A similar titration curve was obtained with Fraction 4 (data not shown), although the number of charged groups per

polymer molecule may be different. The pK_a-values indicate that the γ-carboxyl group of glutamic acid and the amino-group of histidine are possible candidates for the charged groups.

However, from the present data, the possibility that the charged groups in the fractions are present as polymeric impurities (probably proteins) not covalently linked to the polysaccharide cannot be excluded. Preliminary results from fractionation of β-glucans using gel permeation chromatography indicate that fractions with different molecular weights all contain protein, which indicates that the protein is covalently linked to the β-glucans. It should be noted that β-glucans from barley endosperm have been shown to contain firmly-linked peptide sequences (Forrest & Wainwright, 1977). Furthermore, the total content of phosphorus (see Materials and Methods) in Fraction 5 was below 0·07% (w/w), as determined by the method of Koroleff (1976). This does not, however, exclude the possibility of up to five phosphate groups per polymer molecule.

The second virial coefficients determined in the osmotic pressure measurements with 1 M aqueous LiI as solvent are rather high, contrary to the value of approximately zero for the osmotic second virial coefficient of barley β-glucans (solvent: 0·15 M NaCl) reported by Woodward *et al.* (1983). This difference may be explained by (a) the different solvents used; (b) real differences in the solubility of β-glucans from barley endosperm and oat aleurone and (c) the presence of charged groups on the β-glucans studied in this laboratory.

The presence of charged groups may be used to obtain some quantitative understanding of the results from the osmotic pressure experiments (Fig. 2). It can be seen that the experimental values from osmotic pressure measurements in distilled water are in reasonable agreement with the calculated values of the osmotic pressures which would be measured, with the following assumptions:

(1) The polysaccharide contains five negatively charged groups with protons as counterions (i.e. the polymer is an acidic polyanion).
(2) The acidic groups have a pK_a-value of 4·5.

The total charge on the β-glucan will depend on the pH of the solution, with the polyanion going from fully charged to fully discharged within a rather narrow pH-range around the pK_a-value. An increase in the pH of the β-glucan solution from 4·06 (1·5% solution) to 4·85 (0·065% solution), implies dissociation of protons, which contributes to the observed osmotic pressure, and which may dominate over the contribution from the polymer itself at low polymer concentration. This

can be seen from the equation

$$\pi/c = RT/M(1 + \alpha z) \quad \text{(at low polymer concentrations)}$$

where α is the fraction of counterions which are dissociated from the polymer and z is the number of counterion binding sites on the polymer. From the Henderson–Hasselbach equation, α may be calculated at the different concentrations (and pH-values, see Table 2), giving the calculated values of π/c in Fig. 2. It should be stressed that the calculated values require non-dialysable material with fixed weak acid groups as either separate polymer molecules (protein contamination) or linked to the β-glucan chains.

There are several reports indicating that β-glucans from barley endosperm exist as extended, wormlike chains in aqueous solutions (Woodward *et al.*, 1983; Buliga *et al.*, 1986). The value (0·75) of the MHS-exponent found in this work indicates that β-glucans exist as random coils in 1 M LiI. Furthermore, 1 M LiI is a good solvent for β-glucans, as the value of the exponent is rather close to the theoretical upper limit of 0·8 for flexible linear chains and different from the theoretical value of 0·5 for a random coil in a theta solvent (Tanford, 1961). To the authors' knowledge, there are no other reports of the K and a-values for β-glucans. However, Woodward *et al.* (1983) have reported intrinsic viscosities and number average molecular weights for two different barley endosperm β-glucans. From their values the exponent can be calculated to be 0·81 and the constant to be $2{\cdot}5 \times 10^{-4}$, which are in reasonable agreement with values reported in this paper, considering that the former values are calculated from only two points.

Ultrasonic irradiation has been used to depolymerise several polysaccharides (Szu *et al.*, 1986; Yanaki *et al.*, 1982). For schizophyllan, a rodlike trimer with a triple helical structure, it was shown that the probability of chain breaking was higher at the centre than near the ends and that the polydispersity of the samples gradually decreased with sonication time, resulting in a minimum molecular length below which no further breaking occurs (Yanaki *et al.*, 1982). It is therefore possible that the molecular weight distribution in each of the present samples is different. However, the ultrasonic irradiation used was not extensive, and by prolonged irradiation it was still possible to degrade the polysaccharide further (data not shown), which means that the molecular weight distribution of the most degraded samples is not totally different from the less degraded samples.

Only the number average molecular weights ($\overline{M_n}$) of the samples were determined, and no attempt was made to characterise the molecular weight distribution of the samples. In the literature it is more common to

report the relationship between the weight average molecular weight ($\overline{M_w}$) and the intrinsic viscosity. It can be predicted that a lower constant (K) of the Mark–Houwink–Sakurada equation would result from determinations of the relationship between $\overline{M_w}$ and $[\eta]$ in any polydisperse system (see Molyneux, 1984). Work is in progress at this laboratory to prepare more narrowly distributed β-glucan fractions of different molecular weights by gel permeation chromatography.

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