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Water-Soluble (1→3), (1→4)-β-D-Glucans from Barley (*Hordeum vulgare*) Endosperm. II. Fine Structure

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ABSTRACT

Water-soluble (1→3),(1→4)-β-D-glucans isolated from barleys grown in Australia and the UK were depolymerised using a purified (1→3),(1→4)-β-D-glucan 4-glucanohydrolase (EC 3.2.1.73). Oligomeric products were quantitatively separated by high resolution gel filtration chromatography and their structures defined by methylation analysis. Approximately 90% (w/w) of each polysaccharide consists of cellotriosyl and cellotetraosyl residues separated by single (1→3)-linkages but blocks of 5–11 (1→4)-linked glucosyl residues are also present in significant proportions. Periodate oxidation followed by Smith degradation suggested that contiguous (1→3)-linked β-glucosyl residues are either absent, or present in very low frequency. The potential for misinterpretation of data due to incomplete Smith degradation was noted.

The irregularly-spaced (1→3)-linkages interrupt the relatively rigid, ribbon-like (1→4)-β-glucan conformation and confer a flexibility and 'irregular' shape on the barley (1→3),(1→4)-β-D-glucan, consistent with its solubility in water. Molecular models incorporating the major structural features confirm that the polysaccharide is likely to assume a worm-like conformation in solution. Non-covalent interactions between long blocks of (1→4)-linkages in (1→3),(1→4)-β-D-glucans, or between these blocks and other polysaccharides, offer a possible explanation for the organisation of polysaccharides in the framework of the cell wall.

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1. INTRODUCTION

The 40°C water-soluble (1→3),(1→4)-β-D-glucans (hereafter referred to as β-glucans) from barley grain, which account for up to 20% (w/w) of total β-glucan in endosperm cell walls (Fincher, 1975), are linear polymers consisting of (1→3)- and (1→4)-β-linked D-glucosyl residues (Parrish *et al.*, 1960) with small amounts of associated protein (Forrest & Wainwright, 1977) and molecular weights in the range 150 000–300 000 (Woodward *et al.*, 1983). The polysaccharide moiety has been the subject of extensive structural analysis, in particular with respect to the arrangement of (1→3)- and (1→4)-linkages in the chain. Although some variation is observed, most water-soluble preparations contain approximately 30% (1→3)- and 70% (1→4)-linkages, arranged predominantly as cellotriosyl and cellotetraosyl residues separated by single (1→3)-linkages (Parrish *et al.*, 1960; Dais & Perlin, 1982). However, these structures account for only 75–85% of the molecule and there is evidence for the occurrence, in relatively low frequency, of blocks of four or more adjacent (1→4)-linkages (Luchsinger *et al.*, 1965*a*). The presence of two or more adjacent (1→3)-linkages has also been suggested, based on both chemical (Fleming & Manners, 1966; Igarashi & Sakurai, 1966; Fleming & Kawakami, 1977) and enzymic (Bathgate *et al.*, 1974) evidence, although adjacent (1→3)-linkages have not been detected in all preparations (Luchsinger *et al.*, 1965*b*; Dais & Perlin, 1982).

In the present work we have made use of the specificity of a purified (1→3),(1→4)-β-D-glucan endohydrolase from germinating barley (Woodward & Fincher, 1982*a*,*b*) to define and quantitate the structural features of water-soluble β-glucans isolated from barleys grown in Australia and in the UK. In addition, the Smith degradation procedure has been used in attempts to identify blocks of adjacent (1→3)-linkages. The fine structure of the polysaccharides is discussed in relation to the physicochemical parameters and solution behaviour described in part I in this series (Woodward *et al.*, 1983) and to their organisation in cell walls.

2. EXPERIMENTAL

2.1 Materials

The water-soluble β-glucan was prepared from barley (*Hordeum vulgare* L. cv. Clipper) harvested in Victoria, Australia (Woodward *et al.*, 1983).

Commercial β-glucan (batch number 80147) was a gift from Mr C. J. Dowzer, Biocon (Australia) Pty. Ltd. The (1→3),(1→4)-β-D-glucan 4-glucanohydrolase (EC 3.2.1.73) was enzyme II purified from germinating barley by Woodward & Fincher (1982*a*). The enzyme hydrolyses (1→4)-β-glucosyl linkages only where the glucosyl residue is substituted at the C(O)3 position and thus specifically hydrolyses (1→3),(1→4)-β-D-glucans (Woodward & Fincher, 1982*b*). 2-O-β-D-Glucosyl-erythitol and 2-O-β-D-laminaribiosyl-erythritol were prepared by lead tetra-acetate oxidation of cellobiose and 4-O-β-D-laminaribiosyl-D-glucose respectively (Parrish *et al.*, 1960). An additional sample of 2-O-β-D-laminaribiosyl-erythritol was generously provided by Professor A. S. Perlin (McGill University, Montreal, Canada).

2.2 Chemical analysis of β-glucans

The polysaccharides (5 mg) were hydrolysed in 4 ml of 0·5 M HNO_3 containing 0·5% (w/v) urea for 4 h at 100°C (Jermyn & Isherwood, 1956). Monosaccharides in neutralised hydrolysates were determined as their alditol acetates (Albersheim *et al.*, 1967) by gas–liquid chromatography on a column (2·7 m × 3 mm internal diameter) packed with 3% SP 2340 on Supelcoport 100/120 mesh (Supelco Inc., Pennsylvania, USA).

Linkage positions were defined by the methylation procedure of Hakomori (1964) as described by Björndal *et al.* (1970). Permethylated alditol acetates were chromatographed on a glass column (1·8 m × 3·2 mm internal diameter) packed with 3% OV 225 on Gas Chrom Q (100/120 mesh), using a Jeol (Japanese Electron Optics Co., Tokyo, Japan) JGC-20K gas–liquid chromatograph interfaced to a Jeol JMS-D100 double focusing mass spectrometer.

Nitrogen, ash, methoxy, acetoxy and uronic acid contents of the polysaccharide preparations were determined by the Australian Microanalytical Service, Australian Mineral Development Laboratories, Fishermen's Bend, Victoria 3207, Australia.

2.3 Fractionation of β-glucan with ethanol

Barley β-glucan (5 mg ml^{-1} in water containing 2 mM sodium azide) was stirred vigorously and ethanol added dropwise to a final concentration in 19% (v/v). After 16 h at room temperature precipitated material was removed by centrifugation and washed. Materials

precipitating between 19–30% (v/v), 30–60% (v/v) and 60–80% (v/v) ethanol were prepared in a similar fashion. Precipitates were dried by solvent exchange through ethanol, methanol and n-pentane (Green, 1963) and stored in a vacuum desiccator. Trace amounts of the polysaccharide were recovered in the 60–80% ethanol fraction but were not examined further.

2.4 Enzymic hydrolysis

Barley β-glucans were dissolved in 50 mM-sodium acetate buffer, pH 4·8, at 5 mg ml^{-1} and incubated at 35°C, in the presence of toluene, with barley (1→3),(1→4)-β-D-glucan 4-glucanohydrolase II (8 μg ml^{-1}; 26·8 nkat ml^{-1}). After 24 h, additional enzyme was added to a final concentration of 16 μg ml^{-1} and the incubation continued for a further 24 h. The reaction was stopped by heating at 100°C for 10 min. Insoluble material was removed by centrifugation and thoroughly washed with water prior to analysis.

2.5 Structural analysis of oligosaccharides

Oligosaccharides released during enzymic hydrolysis of β-glucans were purified by preparative scale gel filtration chromatography (Anderson & Stone, 1975; Woodward & Fincher, 1982*b*) on Bio-Gel P-2 (batch 188434 minus 400 mesh, Bio-Rad Laboratories, Richmond, California). The column (0·9 cm × 170 cm) was eluted with water at 60°C and 2×10^5 N m^{-2} pressure at a flow rate of ~34 ml h^{-1}. Cellodextrins and laminaridextrins were used to calibrate the column. Linkage positions and sequence were defined by methylation analysis of unmodified oligosaccharides and of oligosaccharides pre-reduced with sodium borodeuteride (Woodward & Fincher, 1982*b*).

2.6 Periodate oxidation

Barley β-glucan (338·8 mg) was dried to constant weight and dissolved in 75 mM-sodium acetate buffer, pH 4·5, at a concentration of 17·1 mM with respect to anhydroglucose residues. Sodium metaperiodate (BDH, Poole, Dorset, UK) was added in 2·5 fold excess (42·75 mM) and the polysaccharide oxidised in the dark at 5°C for 750 h. Aliquots were removed for estimations of periodate consumption by the sodium arsenite titrimetric method (Fleury & Lange, 1933). Excess periodate

was destroyed by addition of 3 ml of ethylene glycol. After 16 h at room temperature the oxidised polysaccharide solution was dialysed exhaustively against water and reduced with 500 mg of sodium borohydride (from BDH) for 12 h at room temperature. An additional 200 mg of sodium borohydride was added and after a further 12 h excess sodium borohydride destroyed with glacial acetic acid. Borate was removed by repeated washing with anhydrous methanol and evaporation of the volatile methyl borate.

Controlled hydrolysis of the oxidised, reduced polysaccharide (Smith degradation; Goldstein *et al.*, 1965) was performed using the procedure of Igarashi & Sakurai (1966). The polymer was dissolved in 30 ml of 0·515 M sulphuric acid and sampled after various times up to 110 h at room temperature. Barium carbonate was added to neutralise the acid. Insoluble material was removed by centrifugation and ~200 μg soluble carbohydrate applied to the Bio-Gel P-2 gel filtration column.

2.7 Paper chromatography

Products of Smith degradation were also separated by descending chromatography on Whatman No. 1 paper with pyridine–ethyl acetate–water (2 : 5 : 7 v/v) (Goldstein *et al.*, 1965). Components were located with alkaline silver nitrate (Trevelyan *et al.*, 1950).

3. RESULTS

3.1 Composition of β-glucans

The compositions of the two β-glucans are shown in Table 1. Monosaccharide analysis revealed that while glucose accounted for ~98% of recovered sugars, small but detectable amounts of arabinose and xylose and traces of mannose and galactose were also present. The ratios of (1→4)- to (1→3)-linkages were 72:28 for the Clipper β-glucan and 70:30 for the commercial β-glucan, although it was noted that the proportions of the linkages varied by ±1% between methylation analyses.

The β-glucans contained small amounts of nitrogen and acetyl groups (Table 1). Whether or not the nitrogen represents covalently associated protein (cf. Forrest & Wainwright, 1977) was not investigated.

TABLE 1
Composition of Barley β-Glucans

Composition (units)	*Source*	
	Clipper	*Commercial*
Monosaccharides (% w/w)		
glucose	98·3	97·9
arabinose	1·1	1·3
xylose	0·6	0·8
mannose	trace	trace
galactose	trace	trace
Linkage positions (% mol/mol)		
(1→3)-glucosyl	28	30
(1→4)-glucosyl	72	70
terminal glucosyl	trace	trace
Nitrogen (% w/w)	0·18	0·22
Protein (% nitrogen x 6·25)	1·2	1·4
Ash (% w/w)	1	1
Methoxy (% w/w)	0·2	0·3
Acetoxy (% w/w)	1·5	1·6
Uronic acid (% w/w)	<0·1	<0·1

3.2 Soluble and insoluble components in enzymic hydrolysates

During enzymic hydrolysis of the β-glucans flocculent precipitates were formed. This material represented ~5% by weight of the β-glucans and was collected by centrifugation, washed thoroughly with water and its structure examined without further fractionation. The soluble oligosaccharides released during hydrolysis were fractionated on Bio-Gel P-2 prior to the analysis of individual components.

3.3 Structure of soluble oligosaccharides

Gel filtration chromatography of soluble hydrolysis products on Bio-Gel P-2 revealed components with degrees of polymerisation (DP) 2–6, with some higher oligosaccharides eluting in a position corresponding to DP 7–9 (Fig. 1). Salt was eluted near the void volume of the column (Luchsinger & Stone, 1976).

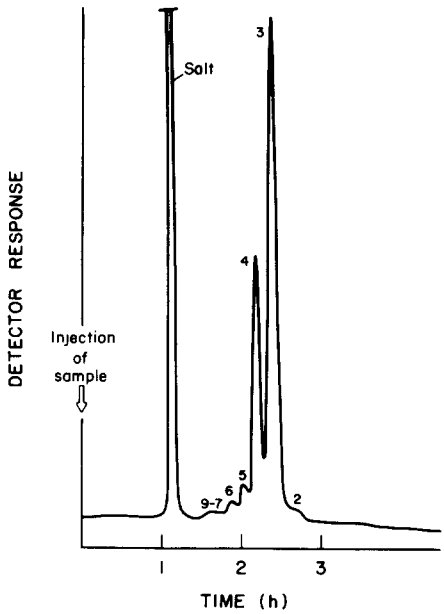

Fig. 1. Bio-Gel P-2 elution profile of soluble oligosaccharides released from barley β-glucan (Clipper) by barley (1→3),(1→4)-β-D-glucan 4-glucanohydrolase II. Numbers correspond to the DP of the oligosaccharides.

There was insufficient material of DP 2 for methylation analysis, but its mobility on paper chromatograms was similar to cellobiose and we conclude that it is predominantly cellobiose. The major oligosaccharides were identified by methylation analysis as 3-O-β-D-cellobiosyl-D-glucose and 3-O-β-D-cellotriosyl-D-glucose respectively (see Woodward & Fincher, 1982*b* for details). The penta- and hexa-saccharides were also (1→3),(1→4)-β-oligoglucosides containing a single (1→3)-linkage adjacent to the reducing end.

The unfractionated water-soluble oligosaccharides from the hydrolysate were made 80% (v/v) with respect to ethanol and the precipitated material examined by methylation analysis. The 80% ethanol-insoluble oligosaccharides were also (1→3),(1→4)-β-oligoglucosides containing a single (1→3)-linkage adjacent to the reducing end, but their average DP was 6 (data not shown). These oligosaccharides are likely to correspond, in part, to the higher DP components seen in Fig. 1.

The weight and molar proportions of oligosaccharides are shown in Table 2. The proportion of each in the two β-glucans is similar,

TABLE 2
Composition of Enzymic Hydrolysates of Barley β-glucan

Component	*DP*	*Clipper*		*Commercial*	
		% (w/w)	*% (mol/mol)*	*% (w/w)*	*% (mol/mol)*
(a) Water-Soluble:					
$G4G_R$	2	trace	trace	trace	trace
$G4G3G_R$	3	56	65	61	70
$G4G4G3G_R$	4	31	27	28	24
$G4G4G4G3G_R$	5	5	4	4	3
$G4G4G4G4G3G_R$	6	3	2	2	1
$(G4G)_3G3G_R$-$(G4G)_4G3G_R$	7-9	<1	<1	<1	<1
(b) Water-Insoluble:					
$(G4G)_4G3G_R$	9 (average)	5	2		
$G4(G4G)_4G3G_R$	10 (average)			5	2

although the commercial preparation has a higher percentage 3-O-β-D-cellobiosyl-D-glucose.

3.4 Structure of insoluble material precipitated during hydrolysis

The monosaccharide compositions of the precipitates, which accounted for 5·4% and 5·3% by weight of the Clipper and commercial β-glucans respectively, are compared in Table 3. In addition to glucose, significant amounts of arabinose, xylose, mannose and galactose were detected.

The average DP of the glucose-containing component estimated from methylation data (Woodward & Fincher, 1982*b*) was 9 for the fraction from Clipper β-glucan and 10 for the commercial preparation (Table 2). When the precipitated material was reduced with sodium borodeuteride prior to methylation, negligible amounts of the derivative arising from (1→3)-linked glucosyl residues (1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-glucitol) were present but 3-O-acetyl-1,2,4,5,6-penta-O-methyl-glucitol, which is derived from 3-linked glucitol, was detected. This indicated that all the (1→3)-linkages were adjacent to the reducing terminus of the oligosaccharide (Woodward & Fincher, 1982*b*).

TABLE 3
Monosaccharide Composition of Water-insoluble Material[a] from Enzyme Hydrolysates

Monosaccharide	*%(w/w)*	
	Clipper	*Commercial*
Arabinose	8	10
Xylose	8	7
Mannose	6	8
Galactose	7	9
Glucose	71	66

[a] Represents 5·4% (w/w) and 5·3% (w/w) of the total Clipper and commercial β-glucans respectively.

3.5 Composition of ethanol fractions

In order to assess heterogeneity in molecular size and in linkage proportions, the polysaccharides were fractionated into 0–19%, 19–30% and 30–60% (v/v) ethanol-insoluble fractions. The yields and linkage ratios of the fractions are compared with the unfractionated β-glucans in Table 4. Significantly more β-glucan from Clipper barley was precipitated in the 0–19% ethanol-insoluble fraction, but no major differences could be detected in the ratios of (1→4)- to (1→3)-linkages in the fractions.

TABLE 4
Ethanol Fractionation of Barley β-Glucans

	Unfractionated	*Glucan fraction (% ethanol)*		
		0–19	*19–30*	*30–60*
Yield (%)				
Clipper	100	74	20	6
commercial	100	41	54	5
Linkage ratio (1→4)/(1→3)[a]				
Clipper	72/28	72/28	73/27	71/29
commercial	70/30	70/30	70/30	70/30

[a] From methylation analyses.

During hydrolysis of the ethanol fractions with (1→3),(1→4)-β-D-glucan 4-glucanohydrolase, precipitates were formed. The precipitated material was relatively more abundant and of higher average DP in the 0–19% ethanol fraction than in the 19–30% ethanol fraction (Table 5).

The water-soluble oligosaccharides released by enzymic hydrolysis were separated by gel filtration chromatography on Bio-Gel P-2, and proportions of the various oligosaccharides were almost identical to those obtained for the unfractionated β-glucans (Fig. 1) (data not shown).

TABLE 5
Water-Insoluble Oligosaccharides Precipitated during Enzymic[a] Hydrolysis of Barley β-glucans

	Unfractionated	*Glucan fraction (% ethanol)*		
		0–19	*19–30*	*30–60*
Percent of β-glucan precipitated				
Clipper	5·4	6·0	3·1	2·0
commercial	5·3	5·4	2·4	2·0
Average DP				
Clipper	9·3	13·8	8·6	ND
commercial	9·9	11·9	8·9	7·0

ND, not determined.
[a] Hydrolysis with (1→3),(1→4)-β-D-glucan 4-glucanohydrolase II.

3.6 Periodate oxidation of unfractionated β-glucans

Consumption of periodate was essentially complete after 250 h, although it continued to rise slowly to 750 h. Extrapolation of the curve from the region 350–750 h to zero time indicated a net periodate consumption of 0·65 mol mol^{-1} of anhydroglucose for the Clipper β-glucan and 0·67 mol mol^{-1} for the commercial preparation. These compare with values of 0·68 mol mol^{-1} (Clarke & Stone, 1966), 0·73 mol mol^{-1} (Bathgate *et al.*, 1974), 0·74 mol mol^{-1} (Igarashi & Sakurai, 1965) and 0·75 mol mol^{-1} (Parrish *et al.*, 1960).

Gel filtration chromatography of the products of Smith degradation showed that after 5 h hydrolysis in 0·515 M H_2SO_4, more than 30% of the oligomers were of higher molecular weight than 2-O-β-D-glucosyl-D-erythritol (Fig. 2(a)). Examination of the elution profile indicated that retention times of the higher molecular weight components did not correspond with those expected for the laminaridextrin-erythritol series.

When the hydrolysis period was extended to 72 h, the higher molecular weight peaks disappeared, leaving 2-O-β-D-glucosyl-D-erythritol and D-erythritol as the major products (Fig. 2(b)). The

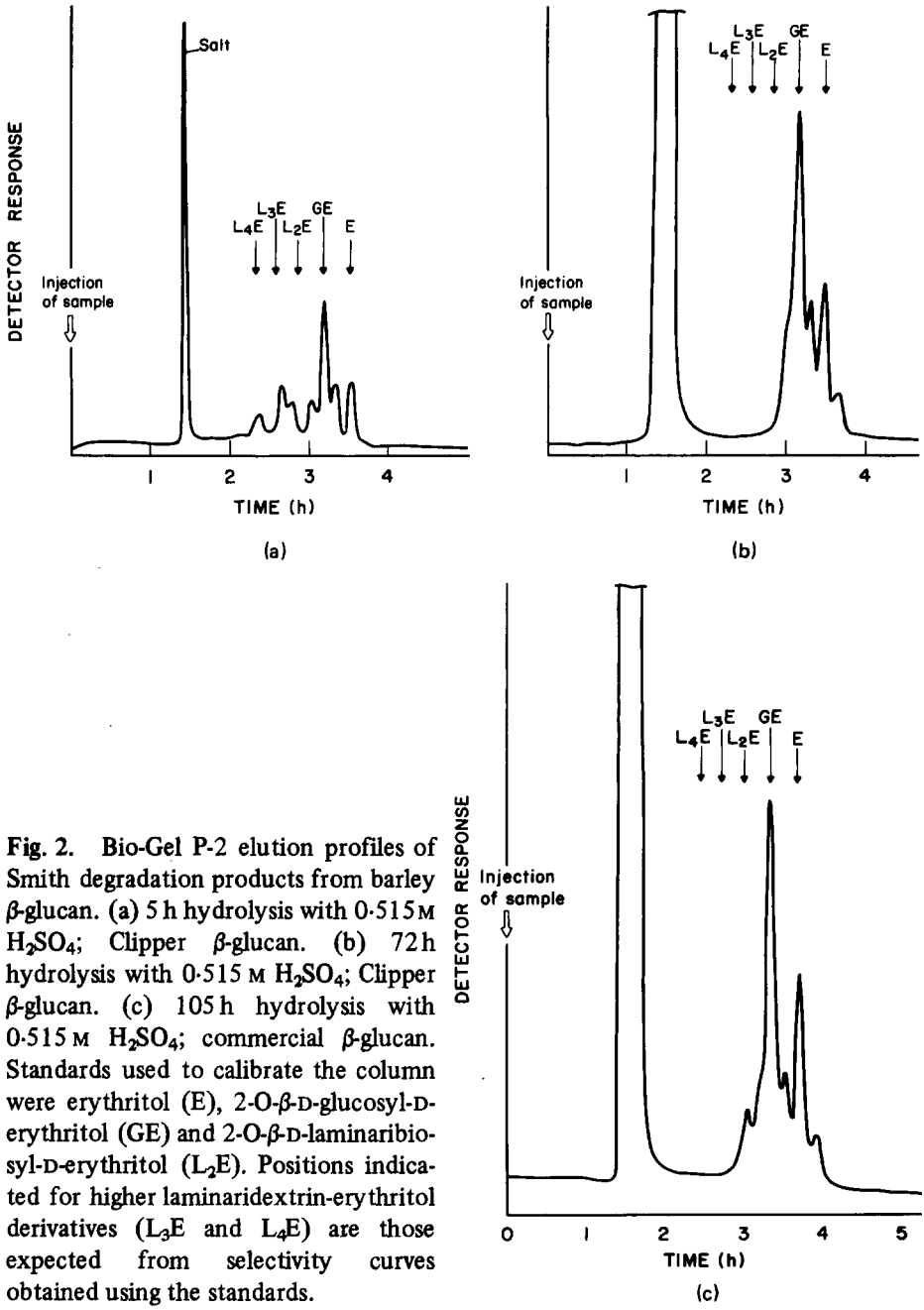

Fig. 2. Bio-Gel P-2 elution profiles of Smith degradation products from barley β-glucan. (a) 5 h hydrolysis with 0·515 M H_2SO_4; Clipper β-glucan. (b) 72 h hydrolysis with 0·515 M H_2SO_4; Clipper β-glucan. (c) 105 h hydrolysis with 0·515 M H_2SO_4; commercial β-glucan. Standards used to calibrate the column were erythritol (E), 2-O-β-D-glucosyl-D-erythritol (GE) and 2-O-β-D-laminaribiosyl-D-erythritol (L_2E). Positions indicated for higher laminaridextrin-erythritol derivatives (L_3E and L_4E) are those expected from selectivity curves obtained using the standards.

identities of these were confirmed by paper chromatography. No 2-O-β-D-laminaribiosyl-D-erythritol or higher 2-O-β-D-laminaridextrin-D-erythritol derivatives were present in the Smith degradation products from Clipper β-glucan, although unidentified components appeared on the leading and trailing edges of the 2-O-β-D-glucosyl-D-erythritol peak, and a small peak eluted after erythritol (Fig. 2(b)).

To check whether the extended acid hydrolysis led to degradation of (1→3)-β-glucosyl linkages, laminaritetraose and 2-O-β-D-laminaribiosyl-D-erythritol were hydrolysed for 95 h under identical conditions. No depolymerisation of these standards could be detected as judged by Bio-Gel P-2 profiles. However, acid treatment of 2-O-β-D-laminaribiosyl-D-erythritol and 2-O-β-D-glucosyl-D-erythritol resulted in small peaks appearing on the trailing edges of the main peaks, and it is possible that the minor peaks seen in Fig. 2(b) have a similar origin. Whether these components correspond to the glycoaldehyde acetals of the glycosyl-erythritol derivatives (Goldstein *et al.*, 1965) was not investigated.

The degradation products of the commercial preparation were similar, except for an additional small peak of mobility similar to 2-O-β-D-laminaribiosyl-D-erythritol (Fig. 2(c)). The component did not co-chromatograph exactly with 2-O-β-D-laminaribiosyl-D-erythritol standards during paper chromatography and its identity remains uncertain.

4. DISCUSSION

Hydrolysis of two water-soluble barley β-glucan preparations by the specific β-glucan endohydrolase enabled the relative abundance of blocks of adjacent (1→4)-linkages to be quantified. While nearly 90% (w/w) of the polysaccharide is comprised of cellotriosyl and cellotetraosyl residues separated by single (1→3)-β-linkages, the remainder consists of blocks of up to 10 or more adjacent (1→4)-linkages. This agrees with the suggestion of Luchsinger *et al.* (1965*a*) that blocks of more than three contiguous (1→4)-linkages were present in their samples. Based on number average molecular weights of 210 000 and 150 000 for the Clipper and commercial β-glucans respectively (Woodward *et al.*, 1983), the average number of each block per molecule can be estimated (Table 6).

TABLE 6
Blocks of Adjacent (1→4)-linkages in Barley β-Glucans

Number of adjacent (1→4)-linkages	*DP of corresponding (1→4)-β-oligoglucosides*	*Approximate number of blocks per molecule*[a]	
		Clipper	*commercial*
2	3	242	188
3	4	100	65
4	5	13	7
5	6	6	3
9 (average)	10 (average)	7	
10 (average)	11 (average)		5

[a] Calculated from number average molecular weights of 210 000 (Clipper) and 150 000 (commercial).

It would be expected, by analogy with the very low solubility of cellodextrins of DP 7 and higher, that oligosaccharides containing six or more adjacent (1→4)-linkages and a single (1→3)-linkage at the reducing terminus would be sparingly soluble in aqueous media. This is presumably why the insoluble oligosaccharides precipitate after their enzymic excision from the β-glucans. Methylation analysis suggests an average DP of 9–10 and a single (1→3)-linkage adjacent to the reducing end of the oligoglucoside moiety. However, the insoluble material is likely to consist of a mixture of oligosaccharides with varying DPs. Arabinose, xylose, mannose and galactose sugars found in the parent polysaccharides before enzymic hydrolysis (Table 1), are also detected in the precipitated material. The recovery of these sugars in this fraction might be due to coprecipitation or retrogradation of admixed arabinoxylan and other polysaccharides in the β-glucan preparations, but it also raises the possibility that the sugars are associated covalently with specific regions of the water-soluble barley β-glucans. Whether protein is also involved in the structure of the insoluble material is not known, although microanalysis of the precipitates revealed no enrichment of protein or acetyl groups. Sedimentation-equilibrium density-

gradient ultracentrifugation has shown that some protein is covalently associated with carbohydrate in water-soluble β-glucans isolated from barley endosperm cell walls (Forrest & Wainwright, 1977).

To identify polydispersity with respect to linkage disposition in the polysaccharides, the water-soluble preparations were fractionated with ethanol prior to enzymic hydrolysis. No significant differences could be detected in overall linkage ratios of the fractions (cf. Parrish *et al.*, 1960) or in gel filtration profiles of the water-soluble oligosaccharides released by the enzyme. However, significantly more water-insoluble oligosaccharides were recovered after enzymic hydrolysis of the 0–19% ethanol fraction and their average DP was higher than the equivalent precipitates from other ethanol fractions (Table 5). Although this represents a relatively low proportion of the total molecule, it is clear that there is some polydispersity in DP of the water-insoluble oligosaccharides formed during enzymic hydrolysis. The major difference in the ethanol fractions was that 74% of the Clipper β-glucan was precipitated in the 0–19% ethanol fraction and 20% in the 19–30% fraction, compared with 41% and 54% respectively for the commercial preparation. It is concluded that the lower ethanol solubility of the Clipper β-glucan is related mainly to its higher molecular weight (Woodward *et al.*, 1983), although the slightly higher frequency of long blocks of adjacent (1→4)-linkages (Tables 2 and 6) may also contribute.

Blocks of two or more adjacent (1→3)-linkages were apparently absent in the Clipper β-glucan. We estimate that 0.2% (w/w) of the polysaccharide as adjacent (1→3)-linked glucosyl residues would be detected in these experiments. Results for the commercial preparation are not as conclusive due to the presence of an unidentified component in the Smith degradation products (Fig. 2(c)). This component could not be positively identified as 2-O-β-D-laminaribiosyl-D-erythritol, due to its different mobility on paper chromatograms. No higher laminaridextrin-erythritol derivatives were present (Fig. 2).

The presence of adjacent (1→3)-linkages in barley β-glucans may depend on the source of the barley and the procedure used to isolate the polysaccharide. Luchsinger *et al.* (1965*b*) could not detect contiguous (1→3)-linkages in their preparation and none were reported in ^{13}C-NMR studies (Dais & Perlin, 1982). On the other hand, Fleming & Manners (1966) separated Smith degradation products by paper chromatography and showed the presence of (1→3)-linkages by methy-

lation analysis. Fleming & Kawakami (1977) used similar procedures to show that a water-soluble β-glucan contained approximately 1% (mol mol^{-1}) each of 2-O-β-D-laminaritriosyl- and 2-O-β-D-laminaritetraosyl-D-erythritol and Igarashi & Sakurai (1966) reported even higher levels of laminaridextrin-erythritol derivatives after Smith degradation.

In the present study hydrolysis of labile acetal linkages in the periodate-oxidised, reduced polysaccharide was incomplete unless treatment with 0·515 M H_2SO_4 was extended from 3 h (Igarashi & Sakurai, 1966) to 72 h (Fig. 2). Others have also noted the critical importance of ensuring complete acid hydrolysis during Smith degradation (Churms *et al.*, 1977; Krusius & Finne, 1981). Incomplete degradation might lead to errors in the estimation of adjacent (1→3)-linkages in barley β-glucans, since methylation of incompletely degraded material would result in the detection of 1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-D-glucitol, even if no adjacent (1→3)-linkages were present in the polysaccharide.

From a biological viewpoint, the structural detail of the barley β-glucan may provide clues of fundamental importance for understanding the supermolecular organisation of these polysaccharides in endosperm cell walls. In Fig. 3 a space-filling model incorporating the major structural features of the barley β-glucan is compared with models of a (1→4)-β-D-glucan, a (1→3)-β-D-glucan and a (1→3),(1→4)-β-D-glucan containing (1→3)- and (1→4)-linkages arranged in a strictly repeating sequence (Anderson & Stone, 1975). The (1→4)-β-D-glucan is a fully-extended ribbon-like molecule, while the (1→3)-β-D-glucan forms a 'large amplitude' helix (Rees & Scott, 1971). Both interact over extended regions of the molecule and at a DP comparable with the barley β-glucan (Woodward *et al.*, 1983) are insoluble in water.

The (1→3),(1→4)-β-D-glucan with alternating (1→3)- and (1→4)-linkages, designated RSIII, was prepared by reduction of carboxyl groups on the pneumococcal type III polysaccharide (Anderson & Stone, 1975). The molecule has an extended conformation and is insoluble in water. This is consistent with the crystalline conformation of the parent type III polysaccharide, which is an almost fully extended helix (Marchessault & Deslandes, 1981).

In contrast, the barley β-glucan is a relatively flexible molecule. Molecular models can be stretched into a fully extended conformation (Fig. 3(d)) or arranged as a worm-like chain (Fig. 3(e)). Based on molecular weight and axial ratio determinations, Woodward *et al.*

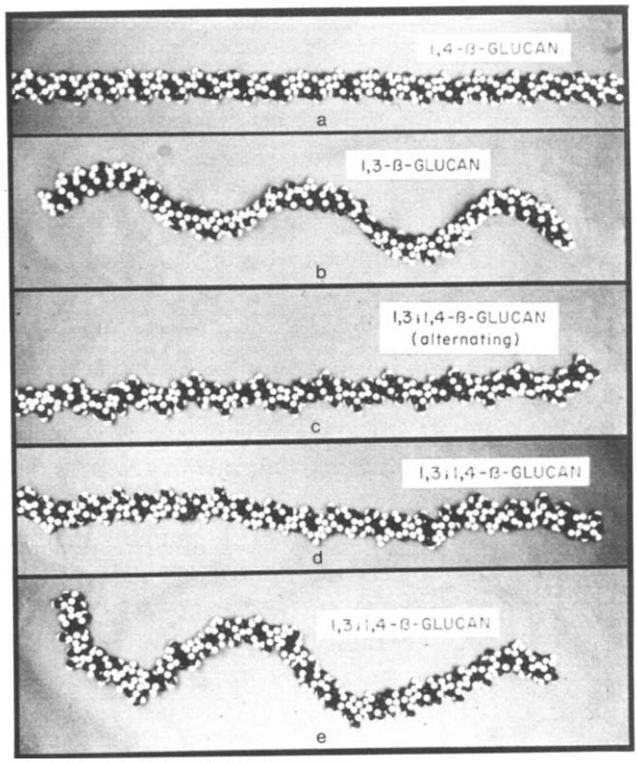

Fig. 3. Space-filling molecular models of β-glucans (DP approximately 30): (a) (1→4)-β-glucan; (b) (1→3)-β-glucan; (c) (1→3),(1→4)-β-glucan with alternating (1→3)- and (1→4)-linkages, i.e. RSIII polysaccharide; (d) barley (1→3),(1→4)-β-glucan – model stretched into extended conformation; (e) barley (1→3),(1→4)-β-glucan – model arranged as a worm-like chain.

(1983) concluded that the polysaccharide has a worm-like conformation in aqueous solution. Thus the insertion of (1→3)-linkages at irregular intervals in the barley β-glucan results in an irregular shape and precludes extensive intermolecular association. This is likely to explain the molecule's solubility in water.

The water-soluble barley β-glucan, which is a component of barley endosperm and aleurone cell walls (Fincher, 1975; Bacic & Stone, 1981), has the potential to form intermolecular junction zones (Rees, 1972) by hydrogen-bonding between the 'cellulosic' blocks of adjacent (1→4)-linked β-D-glucosyl residues. The extent of these interactions is

not sufficient to render this polysaccharide insoluble, although retrogradation does occur (Letters, 1977). It should be noted that of the total cell wall (1→3),(1→4)-β-D-glucan, approximately 20% (w/w), is soluble in water at 40°C (Fincher, 1975). The presence of even longer blocks of adjacent (1→4)-linkages, in greater abundance, may explain the lower solubility of other cell wall (1→3),(1→4)-β-D-glucans. Such polydispersity with respect to the lengths and proportions of (1→4)-β-glucosyl blocks is already demonstrated in the β-glucan studied here (Table 5).

The presence of extended blocks of (1→4)-linked residues may indeed offer an explanation for the binding of those polysaccharides in the primary cell wall matrix (Fincher & Stone, 1981). Thus the regions of adjacent (1→4)-linkages might associate non-covalently with corresponding blocks in other β-glucan molecules. Similarly, they could interact with unsubstituted regions of arabino-(1→4)-β-xylan chains or with cellulose microfibrils in a manner analogous to the interactions involved in co-gelation of other polysaccharides (Rees, 1972), and provide a structurally firm matrix for the cell wall while retaining the porosity and flexibility necessary for endosperm development.

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