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ISOLATION AND ANALYSIS OF NEUTRAL GLUCANS FROM *Ecklonia radiata* AND *Cystophora scalaris*

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ABSTRACT

Neutral glucans were isolated from the stipes and fronds of *Ecklonia radiata* and *Cystophora scalaris*. Partial acid hydrolysis revealed the presence of gentiobiose and laminara-oligosaccharides. Methylation analysis, periodate oxidation, and enzyme studies indicated that the glucans contain β-(1→3) and β-(1→6) linkages. Methylation studies showed that branching in these glucans occurs *via* a 1,3,6-tri-*O*-substituted residue with a frequency of one branch point per seven glycosyl residues. In contrast to laminaran from *Laminaria digitata*, the intrachain (1→3)- and (1→6)-glucopyranoside occur in a molar ratio of 1:1. Enzymic hydrolysis confirmed the absence of long segments of (1→3)-linked residues in the glucans.

INTRODUCTION

Brown seaweeds contain starches, mannitol, and laminarans as reserve carbohydrate[1]. Seaweeds of the northern hemisphere have been studied in detail for the relative abundance, composition, and structure of these carbohydrates Laminaran from *Laminaria* species, for example, has been described in some detail[2,3]. Less is known, however, of the structure of neutral glucans from brown seaweeds of the southern hemisphere. Two examples of the brown seaweeds that proliferate around the coastal waters of New Zealand, *Ecklonia radiata* and *Cystophora scalaris*, were therefore examined. Laminaran has been reported in acid extracts of *Ecklonia radiata*[4,5], but no structural studies on the polysaccharide have been performed. In this study, the neutral polysaccharides obtained from *Ecklonia radiata* and *Cystophora scalaris* were compared with laminaran from the northern hemisphere Phaeophyceate *Laminaria digitata*.

EXPERIMENTAL

Materials. — Hog pancreatin, gentiobiose, and 3-*O*-methyl-D-glucose were

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obtained from Sigma Chemical Company, MO, U.S.A. Zymolase 5000, obtained from the Kirin Brewery Co. Ltd., Takasaki, Japan, contained an endo-(EC 3.2.1.6) and an exo-(1→3)-β-D-glucan hydrolyase activity. Purified (1→3)-β-D-glucan exo-hydrolase (EC 3.2.1.58) from *Euglena gracilis*[6] was a gift from Professor B. Stone, La Trobe University, Bundoora, Vic., Australia. A purified (1→3,1→4)-β-D-glucan exo-hydrolase from the culture filtrate of the thermophile *Thermoascus aurantiacus*[7] was available in the laboratory. Laminaran was purchased from the U.S. Biochemical Corporation, Cleveland, OH. Pachyman and laminara-oligosaccharides were gifts from Dr. R. J. Sturgeon, Heriot-Watt University, Edinburgh, Scotland. 2,3,4,6-Tetra-*O*-methyl-D-glucose was obtained from the Koch-Light Co., Colnbrook, England; mannitol hexa-acetate was purchased from B.D.H., Poole, England. Fresh samples of *Ecklonia radiata* and *Cystophora scalaris* were kindly supplied by Dr. P. R. Bergquist and the staff of the Leigh Marine Station, University of Auckland.

General methods. — Solvents were routinely evaporated under diminished pressure at 37–40°. Descending paper chromatography was performed on Whatman No. 1 or 3MM paper with one of the following systems: Solvent A, 12:5:4 ethyl acetate–pyridine–water; Solvent B, 6:4:3 1-butanol–pyridine–water. Detection was effected with alkaline silver nitrate[8]. Carbohydrate was determined by the phenol–sulphuric acid method of Dubois *et al.*[9]. D-Glucose was estimated by the glucose oxidase method with a Clarke-type oxygen electrode[10]. Reducing sugars were measured colorimetrically with the *p*-aminobenzoic acid hydrazide method of Lever[11], as described by Hurst *et al.*[12]. Optical rotation was measured in water with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer 357 grating spectrophotometer. Gas–liquid chromatography (g.l.c) was performed on a Shimadzu GC-5A gas chromatograph equipped with a flame-ionization detector and a glass column (0.2 × 180 cm) containing 3% of ECNSS-M on 100–120 mesh Gas Chrom Q at 190°. G.l.c.–mass spectra (m.s.) were recorded with a Varian 1400 gas chromatograph fitted with a stainless-steel column (0.2 × 180 cm, 3% of ECNSS-M on 100–120 mesh Gas Chrom Q) linked to a Varian MAT CH7 mass spectrometer. Spectra were recorded at an ionizing potential of 70 eV.

Isolation and purification of seaweed glucans. — Coarsely ground fronds and stipes (2.5 kg of *Ecklonia radiata* and 0.5 kg of *Cystophora scalaris*) were extracted with 10 vol. of 0.1M hydrochloric acid overnight at 4°. Neutralized extracts were evaporated to 1 vol. and the polysaccharides precipitated with aqueous ethanol (85% v/v). The ethanol-washed precipitates were dissolved in warm water (60°) and passed through columns (4.0 × 50.0 cm) of Zeolite DM-F mixed resin. Neutral polysaccharides in the column eluates were precipitated with aqueous ethanol (85% v/v). The ethanol-washed precipitates were dissolved in warm water and reprecipitated with ethanol prior to freeze drying.

Monosaccharide analysis. — Polysaccharide preparations (47.2 mg of laminaran, 15.7 mg of *Ecklonia* glucan, and 53 mg of *Cystophora* glucan) were boiled under reflux in 5.0 mL of 2.5M sulphuric acid for 6 h. The hydrolysate was made neutral with barium carbonate, filtered, and concentrated to 3.0 mL. Monosaccharides were

separated as their alditol peracetate derivatives[13]. For the g.l.c. identification of alditol acetates, standards were prepared from D-rhamnose, D-xylose, D-arabinose, D-glucose, D-mannose, *myo*-inositol, D-fucose, 3-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose.

Partial acid hydrolysis. — Samples (1.0 mg) of the three seaweed glucans were hydrolysed for 45 min at 100° with M sulphuric acid. Another set of samples was hydrolysed under reflux for 120 min. The hydrolysates were made neutral with barium carbonate and evaporated. Oligomers were separated on paper in Solvents A and B and compared with gentiobiose, cellobiose, maltose, and laminara-oligosaccharides as standards.

Enzymic hydrolysis of glucans. — Incubations of enzymes and glucans were conducted at 37° in a volume of 1.0–1.2 mL. Unless otherwise stated, each incubation mixture contained 2.0 mg of substrate. Chloramphenicol (100 μg) and azide (0.1% w/v) were included in each assay. Incubations with Zymolase (2.0 mg), and the mixed (1→3,1→4)-β-D-glucan exo-hydrolase from *T. aurantiacus* (2.0 mg) were made in 0.2M acetate buffer, pH 5.6. Incubations with pancreatin (1 0 mg) were made in 0.05M Tris–HCl buffer, pH 8.1, and those with (1→3)-β-D-glucan exo-hydrolase were performed in 0.2M acetate buffer, pH 5.2. Assays with pancreatin contained calcium chloride (2mM). Incubations were conducted for up to 90 h Aliquots (0.2 mL) were withdrawn at various time-intervals, heated for 20 min at 100°, and analysed for glucose or reducing sugar Maltose was used as the standard where the product of hydrolysis was known to be a disaccharide [as with pancreatin and (1→3,1→4)-β-D-glucan exo-hydrolase]; otherwise, release of reducing sugar was estimated with respect to glucose as the standard.

Periodate oxidation. — Aqueous suspensions of each polysaccharide (25 mg) were treated with sodium metaperiodate (final concentration of 0.13M) at 4° in the dark. Oxidation was monitored at 222 5 nm[14] and the formic acid produced was determined by titration with mM sodium hydroxide[15].

Methylation analysis. — Laminaran (45 7 mg), *Ecklonia* glucan (35.8 mg), and *Cystophora* glucan (38.0 mg) were reduced with 0.1 g of potassium borohydride in an aqueous suspension (15 mL) (overnight, 18°), made neutral with acetic acid, and dialysed (3 changes of double-distilled water) for 24 h. The samples were freeze-dried and left to dissolve in 10 mL of dimethyl sulphoxide for 48 h. Complete dissolution was achieved by intermittent sonication for 4 h at 40°. Methylation was effected by the modified method of Hakomori[16]. Methylsulphinyl anion was prepared as described by Conrad[17] and the methylation performed as described by Bouveng and Lindberg[18]. Two methylations were performed on each sample and the reaction was monitored by the loss of hydroxyl absorption at 3200–3500 nm in the infrared. Each methylated polysaccharide was hydrolysed[18], the hydrolysate was acetylated as already described, and the products were analysed by g.l.c.–m.s.

Nuclear magnetic resonance spectra. — Samples of glucan (100 mg) were dissolved in 1 mL of deuterium oxide, and sodium 4,4-dimethyl-4-silapenta-1-sulphonate was added as the internal standard. The ^{1}H-decoupled ^{13}C-n.m.r. spectra were

recorded at 15.04 MHz with a JEOL FX-60 FTNMR spectrometer at 90°. The ^{13}C chemical shifts are expressed in p.p.m. downfield from external tetramethylsilane.

RESULTS

The yield of polysaccharide from *Ecklonia radiata* was 2% on a fresh-weight basis. This polysaccharide preparation had $[\alpha]_D^{18}$ −35.5° (*c* 0.1) and was 96% carbohydrate (found: ash, <0.1; C, 40; H, 6; N, 0%); it was not fractionated further by sequential ethanol precipitation. No loss occurred in the reducing end-groups after dialysis (reducing end-groups, measured as glucose equivalents, were 0.07 mg per 1.00 mg of polysaccharide). The polysaccharide preparation from *Cystophora scalaris* (1.2% yield) was 54% carbohydrate (ash, 12; C, 31; H, 5; N, 0.5; and S, 5.53%) and exhibited $[\alpha]_D^{18}$ −78.0° (*c* 0.1). There was no loss in reducing sugar after dialysis (reducing end-groups, measured as glucose equivalents per mg of polysaccharide, = 0.063). The commercial preparation of laminaran (95% carbohydrate) had $[\alpha]_D^{18}$ −12.5° (*c* 0.1) and there was a 9% loss in reducing sugar after dialysis (reducing end-groups per mg of polysaccharide = 0.15). Analysis showed an ash value of 0.1; C, 43; H, 6; and N, 0.6%: theoretical values for laminaran are: C, 44.4; H, 6.22%).

Acid hydrolysis (see Table I) revealed glucose as the major product in all preparations. Mannose occurred in all preparations to the extent of ~3%. Galactose was present in the *Ecklonia* (4%) and *Cystophora* (2%) preparations, but was absent from laminaran. The *Cystophora* polysaccharide was different in having a high content (33%) of fucose.

Enzymic hydrolysis. — Preliminary experiments were conducted to determine the specificity of several glucan hydrolase preparations. Zymolase contained exo- and endo-(1→3)-β-D-glucan hydrolase activity. This is consistent with the findings of Scott and Schekman[19]. When laminaran was degraded with zymolase, nearly 60% was released as glucose (Table II), together with other oligosaccharides, as revealed

TABLE I

G L.C. ANALYSES (% COMPOSITION) OF HYDROLYSATES[a] FROM *Ecklonia* AND *Cystophora* POLYSACCHARIDES

Component	Ecklonia *preparation*	Cystophora *preparation*	*Laminaran*
Glucose	93.0	60.0	94.0
Mannose	3 3	2.7	2 8
Xylose			3.0
Galactose	4.0	2.0	
Fucose		33.0	

[a]Isothermal separation of alditol acetates at 190° was performed on Gas Chrom Q coated with 3% of ECNSS-M, and percent composition was determined from the area under the curve of g.l c. traces.

TABLE II

ENZYMIC HYDROLYSIS[a] OF THE GLUCANS

Enzyme	Ecklonia *glucan*	Cystophora *glucan*	*Laminaran*
Zymolase:			
exo- and endo-[a]	41.3	24 2	51.0
exo-[b]	0 0	0 0	54 0
E. gracilis (1→3)-β-D-glucan exo-hydrolase	4.6	7.4	46 0
(1→3,1→4)-β-D-glucan exo-hydrolase from *T. aurantiacus*	14 0	24.0	80 0

[a]Hydrolysis is expressed as percent of reducing sugar released from the glucan either as glucose equivalents [Zymolase, (1→3)-β-D-glucan exo-hydrolase] or as maltose equivalents [in the case of (1→3, 1→4)-β-D-glucan exo-hydrolase]. [b]Percentages refer to glucose released as a ratio of hexose residue present in the glucan.

by paper chromatography (Solvents A and B). The hog pancreatin preparation used in this work liberated from starch 5.0 mg of reducing sugar as maltose equivalents/30 min/mg enzyme under the assay conditions defined. No reducing sugar was released from laminaran by this preparation. The mixed (1→3,1→4)-β-D-glucan hydrolase from *T. aurantiacus* was, as reported previously[7], active toward cellulose, *O*-(carboxymethyl)cellulose, and laminaran. This enzyme preparation liberates cellobiose from cellulose[7]. With maltose as a standard, the reducing sugar liberated from laminaran represented 80% of the yield for complete hydrolysis (Table II). The (1→3)-β-D-exo-glucan hydrolase from *E. gracilis*[6] was active with laminaran (the enzyme released 77 μg of glucose/min/mg protein).

The enzymic hydrolysis of glucans from *Ecklonia* and *Cystophora* was compared with the hydrolysis of laminaran. The results are summarized in Table II. Zymolase caused partial hydrolysis of the three glucans; only with laminaran was glucose detected in the digests. No reducing sugar was liberated with pancreatin, and there was only a small amount of reducing sugar released from either *Ecklonia* or *Cystophora* glucan (7.4 and 4.6%, respectively, of the yield on complete hydrolysis) after incubation with the (1→3)-β-D-glucan exo-hydrolase. Partial degradation of these polysaccharides was observed after incubation with the (1→3,1→4)-β-D-glucan hydrolase from *T. aurantiacus*.

Partial acid hydrolysis. — Partial acid hydrolysis of laminaran and the glucans from *Ecklonia* and *Cystophora* produced a number of oligosaccharides, glucose, and a sugar that co-chromatographed with gentiobiose in solvents A and B. Laminara-oligosaccharides, prepared from pachyman, showed four components in both solvents (R_{Glc} 0.49, 0.57, 0.67, and 0.85 in Solvent A and R_{Glc} 0.19, 0.39, 0.62, and 0.82 in Solvent B). Hydrolysates from *Ecklonia* and *Cystophora* glucans and from laminaran contained oligosaccharides having the same mobilities. The *Ecklonia* and *Cystophora*

TABLE III

PERIODATE OXIDATION OF GLUCANS[a]

	Time (h)	Ecklonia *glucan*	Cystophora *glucan*	*Laminaran*
A. Formic acid produced (mol/mol hexose residues)	0	0.00	0 00	0 00
	24	0.48	0.52	0.13
	50	0.46	0 52	0.16
	72	0.48	0.52	0.16
	120	0 51	0 59	0.17
B. Periodate consumed (mol/mol hexose residues)		0 83	0.91	0.38

[a]Data are not corrected for non-glucose polysaccharide constituents present.

preparations also contained several other oligosaccharides having lower R_{Glc} values, but these were not identified.

Periodate oxidation. — All glucans produced formic acid on periodate oxidation. The reactions were complete in 24 h (Table IIIA). Production of acid was fractionally higher for the *Cystophora* glucan when compared with the *Ecklonia* sample, but both of these glucans exhibited three-times more acid (mole/mole of hexose residues) than laminaran. Consistent with the high acid-production, the glucans from *Ecklonia* and *Cystophora* consumed 2–2.4-times more periodate (mole/mole of hexose residues) compared with laminaran (Table IIIB). Following complete periodate oxidation, the oxidised glucans were hydrolysed (data not shown). Twice as much glucose was recovered from the periodate-oxidised laminaran as from the oxidised glucans from *Ecklonia* and *Cystophora.*

In a separate periodate-oxidation experiment with *Ecklonia* glucan, pustulan, and laminaran, the oxidised glucans were reduced with borohydride and the products hydrolysed with mild acid[14]. Each sample was neutralised, deionised, and concentrated before applying to a column (28 × 2 cm) of Sephadex G-25. The column was eluted with 50mM Tris–HCl, pH 7.3. No polymeric material was eluted in the void volume when the acid-treated oxidised glucans from either *Ecklonia* or pustulan were chromatographed. In contrast, acid-treated oxidised laminaran and the native glucans were eluted with the void volume.

Methylation analysis. — After hydrolysis, each methylated glucan yielded four *O*-methylated alditol acetates (Table IV). The methylated sugars in the hydrolysates from laminaran and *Ecklonia* were identified by g.l.c.–m.s., whereas the methylated sugars in the *Cystophora* preparation were identified by g.l.c. retention-time only. Laminaran afforded 2,4,6-tri-*O*-methylglucose in a high molar yield (89 % by composition), compared with 2,4-di-*O*-methyl-D-glucose (4.1%) and 2,3,4,6-tetra-*O*-methylglucose (6.1%). The molar ratios of the components were 26.0:1.0:1.7, respectively. A further peak (1.3% by composition, *T* of 2.6) in the hydrolysates of

TABLE IV

COMPOSITION OF THE HYDROLYSATES OF PERMETHYLATED GLUCANS FOLLOWING REDUCTION AND ACETYLATION

Methylated D-*glucose*	*Link indicated*	% *Composition*[a]	*Molar ratio*[b]
Laminaran			
2,3,4,6-Tetra-*O*-	Glc*p*-(1→	6.1	1.7
2,4,6-Tri-*O*-	→3)-Glc*p*-(1→	89.0	26.0
2,4-Di-*O*-	→3,→6)-Glc*p*-(1→	4 1	1 0
4,6-Di-*O*-	→4,→6)-Glc*p*-(1→	1.0	0 3
Ecklonia glucan			
2,3,4,6-Tetra-*O*-	Glc*p*-(1→	14 6	1 4
2,4,6-Tri-*O*-	→3)-Glc*p*-(1→	36 0	3.1
2,3,4-Tri-*O*-	→6)-Glc*p*-(1→	35.7	2 9
2,4-Di-*O*-	→3,→6)-Glc*p*-(1→	13.7	1 0
Cystophora glucan			
2,3,4,6-Tetra-*O*-	Glc*p*-(1→	13 6	1.1
2,4,6-Tri-*O*-	→3)-Glc*p*-(1→	36.0	2.7
2,3,4-Tri-*O*-	→6)-Glc*p*-(1→	36.0	2.7
2,4-Di-*O*-	→3,→6)-Glc*p*-(1→	14.4	1.0

[a]Composition (%) was determined as the area under the curve from g.l c traces [b]Molar ratios were calculated from the percentage-composition data.

the methylated laminaran was identified by g l c–m s. as 4,6-di-*O*-methylglucose This could have arisen from incomplete methylation or through demethylation during the subsequent manipulations. The methylated glucan from *Ecklonia* contained 2,4,6-tri-*O*-methylglucose and an equimolar content of 2,3,4-tri-*O*-methylglucose (35.7 and 36.0% by composition, respectively). Furthermore, the molar ratio of 2,3,4,6-tetra-*O*-methylglucose to 2,4-di-*O*-methylglucose was lower (1.4: 1.0; 14.6 and 13.7%, respectively). Methylation analysis of the *Cystophora* glucan revealed equimolar amounts of 2,4,6-tri-*O*-methylglucose and 2,3,4-tri-*O*-methylglucose (36.0% by composition of each). The yields of 2,4-di-*O*-methylglucose and 2,3,4,6-tetra-*O*-methylglucose were also equimolar (molar ratio of 1.0:1.1: 13.5 and 14.4%, respectively).

DISCUSSION

The glucans from *Ecklonia* and *Cystophora* were polymers of high molecular weight with large, negative optical rotations. Commercial laminaran, used for comparison in the present study, lost 9% of the reducing sugar on dialysis, and the $[\alpha]_D$ value was consistent with previously reported values[2,3]. The commercial laminaran and *Ecklonia* preparations were essentially pure neutral glucans, as judged

from monosaccharide analysis. The *Cystophora* preparation, however, contained a significant content (33%) of fucose. This probably arises from contaminating fucoidan. The high ash value (12%) for the *Cystophora* preparation is consistent with the presence of this sulphated polysaccharide. Mannitol, detected in all three preparations by g.l.c., reflects the presence of either mannose or mannitol in these glucans. Mannitol (2–3%) has been detected previously in laminaran[2,3]. Further studies would be necessary to determine the significance of galactose (3%) in the *Ecklonia* glucan. The neutral glucans present in *Cystophora* (60% w/w) were analysed, even though the preparation contained fucoidan. Fucoidan contains 60% L-fucose, 32% sulphate, 4% D-galactose, and negligible glucose[3], and therefore would not complicate analyses of a neutral glucan by g.l.c., periodate oxidation, and enzymic hydrolysis.

Consistent with the structure reported for laminaran[3,20,21], three major products were detected by methylation analysis. The major component (2,4,6-tri-*O*-methylglucose) is consistent with a (1→3)-linked glucan, and the presence of 2,4-di-*O*-methylglucose indicates that (1→6) branches occur. These data indicate a polymer having one branch point per 26 residues and 1.7 non-reducing end-groups per branch point. The methylated glucan from *Ecklonia* contained equimolar amounts of 2,4,6-tri-*O*-methyl- and 2,3,4-tri-*O*-methylglucose, consistent with a polymer containing equal numbers of (1→6) and (1→3) links. Furthermore, the molar ratios of the methylated derivatives summarized in Table IV indicate that this glucan has seven glucopyranose residues per branch point. The *Cystophora* glucan also shows an equimolar frequency of (1→3)- and (1→6)-linked glucopyranose residues, and the methylation data indicate a highly branched polymer.

The periodate-oxidation data were consistent with the methylation analyses. The three-fold higher acid production with *Ecklonia* and *Cystophora* glucans can be accounted for by the intrachain (1→6) links and the high frequency of non-reducing end-groups. Previously reported results for periodate oxidation on laminaran gave values of 0.08–0.12 mol of formic acid/mol hexose residues[20–22]. When the values in Table III are corrected for acid production from mannose and mannitol, values of 0.06–0.07 mol/mol hexose residues were obtained. No evidence for α-(1→4) linkages was found from enzymic analyses. Hydrolysis of the glucans by Zymolase and (1→3, 1→4)-β-D-glucan exo-hydrolase indicated β-(1→3)-linked polymers. Laminaran was found to be susceptible to attack by exo-glucanase with either Zymolase (54% as glucose) or (1→3)-β-D-glucan exo-hydrolase. In contrast, the glucans from *Ecklonia* and *Cystophora* yielded only 5 and 7%, respectively, of the substrate as free glucose; these highly branched polymers are probably excluded from the active sites of the exo-glucanases. The inability of exo-glucanases to degrade either the *Ecklonia* or the *Cystophora* glucan eliminates the possibility that the preparations analysed contained either mixtures of (1→3) and (1→6) homopolymers. Furthermore, the glucans cannot contain extensive segments of β-(1→3)-linked residues, as there was no glucose produced on incubation with Zymolase (a preparation containing both exo- and endo-(1→3)-β-D-glucan hydrolase activity).

This study indicates that the glucans from both *Ecklonia* and *Cystophora* are

highly branched polymers having (1→3) and (1→6) intrachain linkages. Branching occurs only *via* a 1,3,6-*O*-substituted residue with a frequency of one branch point per seven glycosyl residues. The pattern of enzyme hydrolysis showed that the structure does not contain long segments of either β-(1→3)- or β-(1→6)-linked residues. This conclusion was supported by the observation that 74% of the *Ecklonia* glucan was resistant to alkaline degradation (data not shown). Chromatography on Sephadex G25 of the acid-treated oxidised glucans from *Ecklonia* confirmed the presence of intrachain (1→6) linkages in this glucan.

The ^{13}C-n.m.r. spectrum of the *Ecklonia* glucan showed at least three anomeric signals in the 103.7–104.1 p.p.m. region. The absence of a signal at ~100 p.p.m. clearly demonstrated that there were no α linkages in the glucan. The other major resonances exhibited were characteristic of unsubstituted polysaccharides[23–27]. In particular, the regions about 62.1, 69.6, 74.3, 76, and 85.8 p.p.m. may be assigned to C-6, C-4, C-2, C-5, and C-3, respectively, in a (1→3)-substituted β-D-glucose residue. The signals in the C-1 and substituted C-3 region, however, are complex and support the concept of a highly branched polymer. Highly branched β-D-glucans containing unsubstituted (1→3)- and (1→6)-linked glucopyranoside residues occur in extracellular polysaccharides obtained from *Rhizobium japonicum*[23]. From comparison of the ^{13}C-n.m.r. spectra of the *Rhizobium* and *Ecklonia* glucans, we conclude that the polymers are similar: that is, they are highly branched and contain intrachain β-(1→3) and β-(1→6) glucopyranoside residues.

Several species of the Chrysophyceae and Bacillariophyceae contain chrysolaminarans[1–3], which are (1→3)-β-D-glucans having (1→6) branch points and which do not contain mannitol. A linear (1→3)-β-D-glucan from *Eisenia bicyclis*[28], a northern-hemisphere relative of the *Laminariales*, contained inter-residue (1→6)- and (1→3)-β-D links (ratio of 1:4 after methylation). A branched glucan has been isolated from *Ischige okamurai*[29], an alga unrelated to the *Laminariales*. The *Ischige* laminaran contains more-frequent (1→6) inter-residue links than the *Eisenia* glucan [the ratio of (1→6) to (1→3) links is 1:3 after methylation] Clearly, laminarans from different sources vary considerably in structure.

The glucans from *Ecklonia* and *Cystophora* are similar, but in comparison with laminaran from *L. digitata* they are highly branched and contain both (1→3) and (1→6) intrachain links. Branching in all three glucans only occurs *via* 1,3,6-*O*-substituted residues.

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