Carbohydrate Polymers **4** (1984) 237–252

Alginate Block Structure in Phaeophyceae from Nova Scotia: Variation with Species, Environment and Tissue-type*

J. S. Craigie

Atlantic Research Laboratory, National Research Council of Canada, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada

E. R. Morris†, D. A. Rees† & D. Thom

Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ, UK

(Received: 7 October 1983)

SUMMARY

The block composition of alginate from a number of Phaeophyceae from Nova Scotia, Canada, has been determined by circular dichroism analysis. Laminaria longicruris *from three different ecological locations shows appreciable morphological variation, which is reflected in differences in alginate composition of the blades. Stipe material shows less variability, but is higher in polyguluronate, consistent with the greater mechanical rigidity of this tissue. Similarly, differences in alginate composition with tissue type and growth location have been observed for* L. digitata. Agarum cribrosum *shows little tissue-to-tissue variation in alginate composition, material from both blades and mid-ribs being very similar in block structure to commercially available alginate from* Macrocystis pyrifera *and* Ascophyllum nodosum. Stilophora rhizodes *and* Leathesia difformis, *from the order Ectocarpales, both yield alginate of very high polyguluronate content, consistent with the brittle texture of both species.* Stilophora rhizoides *harvested during two consecutive growing seasons showed no appreciable variation in alginate block structure. Two alginate samples*

* NRCC No. 22530.

† Present address: National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

analysed in this work (from Leathesia difformis *and from blades of* Laminaria longicruris *from one of the populations studied) show respectively higher and lower levels of the structure-forming polyguluronate sequences than any previously reported alginate samples from mature plant tissue.*

1. INTRODUCTION

The major structural polysaccharide of marine brown algae (Phaeophyceae) is alginate (Fig. 1), a linear 1,4-linked block copolymer of α-L-guluronate and β-D-mannuronate (Rees & Samuel, 1967), with residues arranged (Haug *et al.*, 1966, 1967) in homopolymeric sequences of both types, and in heteropolymeric mixed sequences that were formerly referred to as 'alternating blocks', but have been shown by detailed enzymic (Boyd & Turvey, 1978) and n.m.r. (Grasdalen *et al.*, 1981) studies to deviate substantially from a regular disaccharide repeating sequence. The mechanism of structure formation by intermolecular association of alginate chains has been explored extensively *in vitro* (Haug, 1964, 1974; Haug *et al.*, 1966, 1967; Smidsrød & Haug, 1968, 1972; Grant *et al.*, 1973; Morris *et al.*, 1973, 1977, 1978, 1982; Bryce *et al.*, 1974) , and the conclusions may illuminate the biological role of these polymers in the plant (Rees, 1972; Haug, 1974; Smidsrød, 1974; Morris *et al.*, 1977; Rees & Welsh, 1977).

The primary mode of interaction is by dimerisation (Morris *et al.*, 1978) of polyguluronate sequences in the regular two-fold chain geometry characteristic of the solid state (Atkins *et al.*, 1970, 1973; Mackie, 1971), with interchain chelation of arrays of calcium or related divalent cations on specific binding sites periodically spaced along each of the participating chains (Grant *et al.*, 1973). Under suitable conditions of ionic environment further association of polyguluronate dimers, or of mixed chain sequences, may occur (Morris *et al.*, 1978; Liang *et al.*, 1980). There is no evidence of any cation-induced association of polymannuronate (Morris *et al.*, 1978, 1982). Thus, the structure forming ability of alginate is critically dependent upon block composition, and in particular upon the level of polyguluronate present.

In the present work we have used a recently developed circular dichroism method (Morris *et al.*, 1980) to analyse the block composition of alginate from a number of brown algae from Nova Scotia.

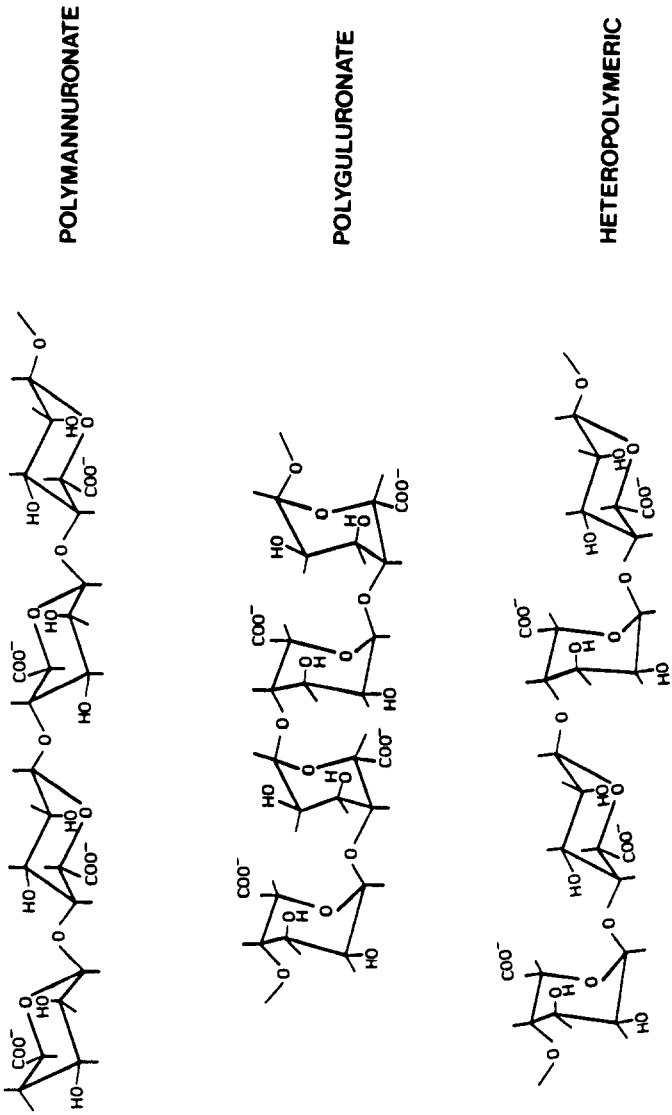

Fig. 1. Alginate block structure.

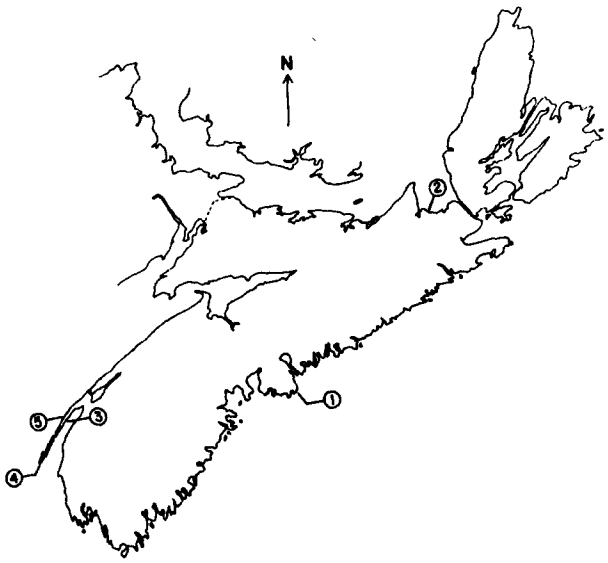

Fig. 2. Locations in Nova Scotia where algae were collected: 1, Atlantic coast near Halifax; 2, Pomquet Harbour; 3, E. Sandy Cove, St Mary's Bay; 4, Pond Cove; 5, Sandy Cove, Bay of Fundy.

2. EXPERIMENTAL

Laminaria longicruris de la Pylaie was taken near Fink Cove on the open Atlantic Coast (site 1, Fig. 2) at 9–12 m depth, at site 3 (between E. Sandy Cove and Mink Cove, St Mary's Bay) from 3–5 m depth at low tide, and at site 5 (near Sandy Cove, Bay of Fundy) from *c.* 3 m depth at low tide. Only specimens with hollow stipes were selected. *Laminaria digitata* (L.) Lamour. was collected only at site 5 from 1–2 m depth while *Agarum cribrosum* (Mert.) Bory was taken from site 1 at a depth of 13–17 m. *Leathesia difformis* (L.) Aresch. was collected from the intertidal zone at Pond Cove, site 4. *Stilophora rhizodes* (Turn.) J. Ag. was harvested from Pomquet Harbour, site 2. All measurements were recorded as soon as possible on the fresh tissue. Portions of the thalli sampled are indicated in Table 1.

Representative alginate samples were derived by combining material from a large number of plants (typically ~ 40–60). The extraction

procedure involved soaking the fresh plants in formalin solution (2%) overnight, followed by acid (pH 2) and alkali (3% Na_2CO_3) extraction, and ethanol precipitation. The precipitate was then redissolved in aqueous ammonia (1%), reprecipitated with calcium (0·1 M $CaCl_2$) and finally converted to the sodium salt form by repeatedly (three times) precipitating as alginic acid (0·1 M HCl), and redissolving by neutralisation with alkali (0·01 M NaOH) in the presence of excess sodium ions (~ 1 M NaCl). The final solution was dialysed extensively against deionised water, and freeze-dried. The total uronate content of the freeze-dried material was determined by infrared (Bociek & Welti, 1975).

Solutions for circular dichroism (c.d.) analysis (~ 3 mg ml^{-1}) were accurately neutralised, and filtered to optical clarity (3 μm Millipore). Spectra were recorded at 25°C on a Cary 61 CD Spectropolarimeter, using a 1 mm pathlength cell and 10 s integration period. Block analysis involved matching the observed spectra by linear combination of the known c.d. spectra for the component block-types, using a least-squares computer method (Morris *et al.*, 1980). Figure 3 illustrates the quality of fit obtained.

3. ADVANTAGES AND LIMITATIONS OF ALGINATE BLOCK ANALYSIS BY CIRCULAR DICHROISM

The earliest and most widely exploited approach to alginate block analysis involves cleavage of the polysaccharide into chain segments approximating in structure to the three block-types present in the parent molecule (Haug *et al.*, 1966, 1967, 1974). On partial hydrolysis under acid conditions, alginate separates rapidly into a soluble fraction of heteropolymeric material and an insoluble fraction consisting predominantly of homopolymeric sequences of both types. The precipitate may then be fractionated at higher pH (2·85) where polymannurate is soluble while polyguluronate is not (Haug *et al.*, 1974), or analysed by ^1H n.m.r. (Penman & Sanderson, 1972). One disadvantage of this approach, pointed out by the original authors (Haug *et al.*, 1974), is that the results obtained depend on the mechanism of hydrolysis as well as on the primary structure of the polysaccharide. The analysis is also laborious and time-consuming and uses comparatively large amounts of material.

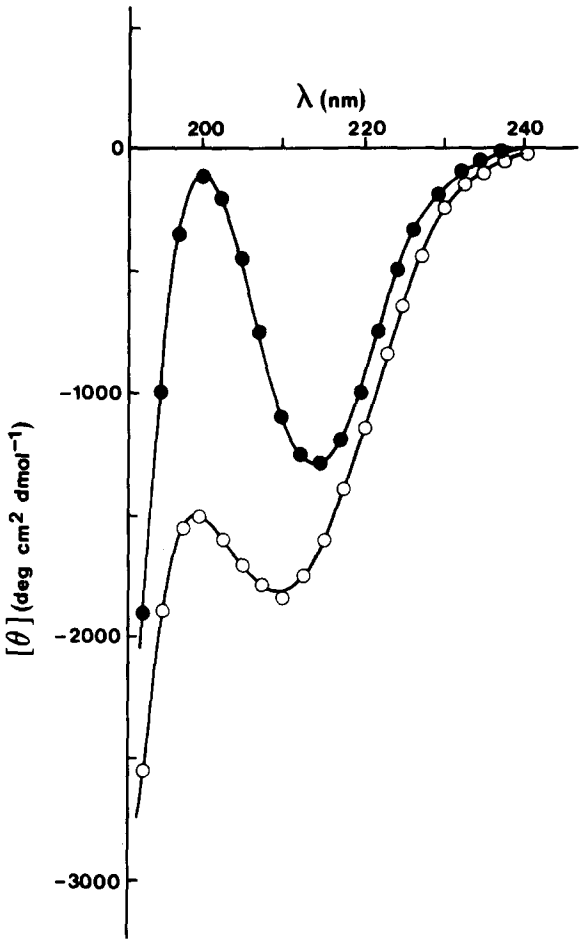

Fig. 3. Alginate block determination by computer curve fitting of c.d. spectra. Comparison of fitted c.d. ellipticities for alginate from *Leathesia difformis* (o) and *Laminaria longicruris* blades (Sandy Cove) (•), with observed spectra (—). The samples shown represent the compositional extremes of the materials studied in this work, and we believe them to show, respectively, the highest and lowest levels of structure-forming polyguluronate sequences so far reported for mature algal tissue.

It has been known for some time that isolated chain sequences approximating to the three block-types present in alginate show very different c.d. behaviour (Morris *et al.*, 1973, 1975), and that in principle this should offer a route to block determination without hydrolysis

of the sample. Subsequently the c.d. approach was developed as a simple, rapid and non-destructive technique for block analysis on a few milligrams of material (Morris *et al.*, 1980; Stockton *et al.*, 1980*b*).

More recently, however, it has been demonstrated that the fine structure of alginate heteropolymeric sequences shows substantial variation, ranging from almost regularly alternating to almost statistically random (see for example Grasdalen *et al.*, 1981). Although the c.d. method for alginate analysis is essentially empirical and the mechanism by which contiguous residues interact to modify spectral form is not yet fully understood, it seems reasonable to suppose that such variations in primary sequence must be accompanied by changes in c.d. behaviour and that no single model spectrum can accurately reflect all possible arrangements of residues within heteropolymeric sequences from different botanical sources.

The published method for c.d. analysis of alginate block structure (Morris *et al.*, 1980) used chain sequences isolated by partial hydrolysis of alginate from *Ascophyllum nodosum*. Polyguluronate blocks prepared from this source were essentially free of mannuronate, but the polymannuronate preparation contained 7% guluronate and the heteropolymeric sequences contained mannuronate and guluronate in the ratio 60 : 40. 'Idealised' block spectra were constructed on the assumption that 'excess' mannuronate residues in the heteropolymeric preparation were present as polymannuronate, and that guluronate residues in the polymannuronate preparation occurred in alternating sequences.

Full evaluation of the likely error introduced by these assumptions must await comparison of the c.d. spectra of heteropolymeric sequences with different fine structure, as characterised by enzymic hydrolysis or n.m.r. For the moment, however, some indication of the validity of the c.d. approach may be obtained from comparison with results by other methods. In a previous analysis of commercial alginates from different plant sources (Morris *et al.*, 1980) relative percentages of each block type (polymannuronate : heteropolymeric : polyguluronate) found by c.d. were in good agreement with the values expected from independent determination by hydrolysis and n.m.r. (Penman & Sanderson, 1972):

Ascophyllum nodosum:
found (37·8 : 40·8 : 21·4); expected (38·4 : 41·0 : 20·7);
Laminaria hyperborea:
found (23·1 : 33·7 : 43·3); expected (20·2 : 30·4 : 49·3);

Macrocystis pyrifera:
found (36·5 : 45·0 : 18·5); expected (40·6 : 41·7 : 17·7);
L. hyperborea stipes:
found (22·0 : 13·8 : 64·2); expected (18·7 : 22·7 : 58·6).

The ratio of mannuronate : guluronate calculated from the c.d. block analysis agreed closely (± 2·5%) with results from complete hydrolysis of the polymer. On the basis of this comparison it therefore appears that the c.d. technique used in the present work gives results which agree to within about ± 10% with values obtained by less convenient techniques with considerably greater sample requirements.

The c.d. method for *structural* analysis of alginate is closely analogous to the widely accepted c.d. approach to *conformational* analysis of proteins (Markussen & Bølund, 1975). In both cases observed spectra are matched by linear combination of varying proportions of two model spectra for well-characterised structures (polymannuronate and polyguluronate in the case of alginate, and alpha helix and beta sheet in proteins) and a third spectrum for an ill-defined structure (alginate heteropolymeric sequences and protein disordered sequences, respectively). The likely reason why, despite this, both c.d. methods appear to give reasonable results, is that in each case the well-defined spectra are of considerably greater magnitude than that of the third, ill-defined component, and will therefore dominate the fitted spectrum. Variations in the exact form of the third model spectrum (corresponding to variations in the fine structure of alginate heteropolymeric sequences, or conformational differences between disordered chain sequences in different proteins) may therefore have comparatively little effect on the fitted parameters for the proportion of each structural type present, except in cases where the ill-defined component predominates.

We therefore suggest that c.d. analyses yielding high levels of polymannuronate and/or polyguluronate are likely to be very reliable, but that results showing high levels of heteropolymeric structure should be treated with more caution.

4. RESULTS AND DISCUSSION

4.1 *Laminaria* spp.

Our most extensive studies are on *Laminaria longicruris*, which is not reported in European waters although the species may be conspecific

TABLE 1

Measurements of *Laminaria longicruris* and *Agarum cribrosum* from which Samples were Taken for Alginate Analysis

	Laminaria longicruris			*Agarum cribrosum*
	Bay of Fundy[a]	*St Mary's Bay*[b]	*Atlantic*[c]	*Atlantic*[c]
Blade[d]				
length (m); mean (range)	3·86 (1·2–6·2)	2·42 (0·7–4·0)	1·72 (0·5–2·6)	0·72 (0·42–1·14)
width (m); mean (range)	0·57 (0·37–0·70)	0·43 (0·29–0·55)	0·24 (0·08–0·35)	0·35 (0·15–0·44)
thickness (mm); mean ± standard deviation	1·53 ± 0·22	1·29 ± 0·09	2·64 ± 0·11	–[f,g]
fresh wt (mg cm^{-2})	165	149	259	35
Stipe[e]				
length (m); mean (range)	~3·6	2·13 (1·3–2·8)	1·13 (0·6–1·7)	–
diameter (cm); mean (range)	2·58 (2·1–3·6)	1·58 (1·0–2·7)	1·85 (1·4–2·8)	–
wall thickness (mm); mean (range)	5·87 (4·0–9·0)	3·77 (3·0–5·0)	5·46 (4·0–8·0)	–
Number of plants	46	44	42	56

[a] Collected 6 August 1971.

[b] Collected 7 August 1971.

[c] Collected 18 August 1971.

[d] Samples were excised near the median line as near to 0·8 m from the base of the blade as possible to ensure that mature tissues were selected. Blades shorter than 0·8 m were sampled at their distal end.

[e] 2-3 cm samples were taken near the point of maximum diameter (recorded above) of the hollow stipe.

[f] The blade was sampled at a point 1/3 its length above the base, at approximately the position of maximum width. The blade thickness was not measured, but was very thin.

[g] The solid mid-rib, sampled at the point of maximum blade width, was 2·47 ± 0·32 mm thick and weighed 216 mg cm^{-1} fresh weight.

with *L. agardhii* and *L. saccharina* (Chapman, 1974). We have investigated tissue-to-tissue variation in alginate structure by analysis of material from blades and stipes, and the effect of growth conditions by comparing plant populations from three different locations: Fink Cove, Sandy Cove and St Mary's Bay. The first of these is a relatively exposed site on the Atlantic seaboard near Halifax where a deficiency of nitrogenous nutrients in the summer months inhibits the growth of *Laminaria* (Chapman & Craigie, 1977). The Bay of Fundy site at Sandy Cove is subjected to strong tidal currents, but is only a moderately exposed site. The nitrogenous nutrients here show a seasonal fluctuation, but the levels remain sufficiently high that growth of *Laminaria* is not impaired during summer (Gagné *et al.*, 1982). The nutrient conditions and tidal currents at site 3, St Mary's Bay, are not known but the *L. longicruris* plants are distinguishable from those at the other two sites (Table 1).

TABLE 2
Block Composition of Alginate from *Laminaria* Species

Species	*Block content (%)*[a]							
	Stipe				*Blade*			
	GG	*GM*	*MM*	*M/G*	*GG*	*GM*	*MM*	*M/G*
L. longicruris								
Bay of Fundy	18	55	27	1·20	3	57	40	2·17
St Mary's Bay	15	58	27	1·27	13	44	43	1·86
Atlantic	20	56	24	1·08	15	52	33	1·44
L. digitata								
Bay of Fundy[b]	29	46	25	0·92	22	41	37	1·35
East Yorkshire (UK)[c]	16	54	30	1·33	15	35	50	2·08

[a] Sources of error in the levels of polyguluronate (GG), polymannuronate (MM), and heteropolymeric chain sequences (GM) are discussed in Section 3.
[b] Sections were taken from the mid-stipe region below the obviously flattened portion. Blade tissue was excised from the frond just distal to the region of cleft initiation.
[c] From Stockton *et al.* (1980*a*).

Plants from Sandy Cove, Bay of Fundy, are longer, broader and thicker than those from St Mary's Bay, and their blades appear more flaccid and pliable than those of plants from the other two sites. *Laminaria longicruris* from the open Atlantic coast, site 1, show smaller blades, but these are rather thick (Table 1).

As shown in Table 2, these differences in morphology are paralleled by significant differences in alginate structure. Most spectacularly, the broad flexible blades from Sandy Cove have only ~ 3% polyguluronate which, to our knowledge, is the lowest value so far recorded for any mature phaeophycean tissue. Blade samples from the other two sites show a substantially higher polyguluronate content (~ 14%). In plants from St Mary's Bay this is principally at the expense of mixed sequences, while those from Fink Cove show a corresponding reduction in polymannuronate. Stipes from all three locations have approximately the same alginate composition (~ 18% polyguluronate, ~ 26% polymannuronate and ~ 56% mixed sequences).

In vitro studies of the mechanical properties of alginate gels (Mitchell & Blanshard, 1976) indicate that, as anticipated from a molecular understanding of the mechanism of interchain association (Haug, 1974; Smidsrød, 1974; Morris *et al.*, 1977; Rees & Welsh, 1977), structural rigidity increases with increasing polyguluronate content. The degree of deformation which can be accommodated before rupture increases with polymannuronate, consistent with the presence of these sequences as unassociated connecting regions between junctions. The role of mixed sequences is more complex. Although probably themselves incapable of crosslinking a cohesive network, they do show limited cation chelation and association (Morris *et al.*, 1978, 1982), and may therefore reinforce polyguluronate junctions. It has also been demonstrated (Bailey *et al.*, 1977; Mitchell & Blanshard, 1979) that while the mechanical rigidity of alginate gels is determined primarily by the short, stiff chain sequences present, yield stress (i.e. toughness) is enhanced by the presence of long, flexible chain sequences which continue to crosslink the network after initial rupture of the primary junctions. Since the flexibility of mixed chain sequences is substantially greater than that of polyguluronate or polymannuronate (Smidsrød *et al.*, 1973), they are likely to have a predominant role in imparting toughness, rather than brittleness, to algal tissue. We therefore regard polyguluronate and, to a lesser degree, mixed sequences as conferring mechanical strength, and polymannuronate as introducing flexibility.

In these simple terms we would expect, on the basis of alginate composition, a gradation of mechanical properties from extremely flexible to relatively strong and stiff through the series blades (Sandy Cove), blades (St Mary's Bay), blades (Fink Cove), stipes (all locations), which correlates well with visual inspection of *L. longicruris* tissues. We have also examined alginate from the blades and stipes of *L. digitata* harvested from Sandy Cove. As shown in Table 2, these have a significantly higher polyguluronate content than *L. longicruris* alginate, principally at the expense of mixed sequences. Comparison with *L. digitata* samples from the east coast of Britain (Stockton *et al.*, 1980*a*) shows a substantial difference in alginate composition in both blades and stipes. We therefore conclude that in addition to the species-to-species variation in the structural polysaccharide of *Laminaria*, there is evidence of large, and sometimes overriding, differences between plants of the same species harvested from different growth locations.

One possible explanation of these differences is in terms of genetic variations between separate plant populations. Studies with *L. longicruris* showed little correlation between parents and offspring, at least for alginate content (Chapman & Doyle, 1979). In recent experiments, *L. longicruris* was transplanted from the Bay of Fundy to the open Atlantic coastline where it survived for only a limited time suggesting that there may be genetic differences peculiar to this population (Gagné *et al.*, 1982). Alternatively, the observed differences in alginate composition might reflect environmental adaptation of genetically similar plants. While all three populations of *L. longicruris* studied were entirely sublittoral, each was exposed to different local conditions of current, irradiance level, and nutritional status. Alginate is believed to be biosynthesised initially as polymannuronate, with subsequent conversion to polyguluronate and mixed sequences by enzymic action at the polymer level (Lin & Hassid, 1966; Madgwick *et al.*, 1973). The extent of conversion might therefore offer a mechanism for biological control of tissue structure in response to environmental needs, a thesis recently discussed by Larsen (1981). Enzymic control of the mechanical properties of algal tissue has been suggested for other structural polysaccharides, including porphyran (Rees & Conway, 1962) and carrageenan (Lawson & Rees, 1970).

A further possibility is that variations in alginate composition with local environment might simply reflect differences in growth rate. Thus,

in rapidly growing plants, enzymic conversion of mannuronate to guluronate may be less complete than under slower growth conditions. Comparison of the size attained by *L. longicruris* blades in different growth locations with the observed ratios of mannuronate and guluronate residues (Table 2) provides some support for this interpretation. It may be no coincidence that the plants with the lowest polyguluronate content were taken from an area in which *L. longicruris* showed the highest growth rate of several populations investigated (Gagné *et al.*, 1982). Fuller and more definitive understanding of the factors determining alginate composition and structure, however, must remain the subject of further experiments.

4.2 Other phaeophyceae

Analysis of alginate from blades and mid-ribs of *Agarum cribrosum* (order Laminariales) harvested from Fink Cove, shows little variation

TABLE 3
Block Composition of Alginate From Other Phaeophyceae

Species	*Block content (%)*[a]			
	GG	*GM*	*MM*	*M/G*
Agarum cribrosum				
blades	23	41	36	1·30
mid-ribs	26	41	33	1·15
Stilophora rhizodes[b]				
whole plant (1970)	51	37	12	0·44
whole plant (1971)	51	34	15	0·47
Leathesia difformis				
whole plant	67	12	21	0·37

[a] Sources of error in the levels of polyguluronate (GG), polymannuronate (MM) and heteropolymeric chain sequences (GM) are discussed in Section 3.

[b] Average dry matter content of samples collected during October and November was 8·0%. The total ash was 54·9%; alginate = 12·9% and protein = 5·5%, all on a dry weight basis.

in block composition with tissue type (Table 3). The alginate from both parts of this plant has essentially the same structure as typical commercial alginate from *Macrocystis pyrifera* or *Ascophyllum nodosum*. We have also examined alginate derived from whole plants of the summer annuals, *Stilophora rhizodes* and *Leathesia difformis*, both of the order *Ectocarpales*. The former is a relatively small (up to ~ 30 cm), extensively branched, filiform plant, which inhabits protected, warm shallow bays. It attaches itself by a small holdfast, but the plant is relatively stiff and brittle, and readily fragments to give a bed of unattached, tangled plants. *Leathesia difformis* has a small (up to ~ 10 cm diameter), hollow 'puff ball' shaped thallus, which is brittle like *Stilophora* and easily fragmented.

As shown in Table 3, both species are very high in polyguluronate and low in polymannuronate, consistent with the stiff, brittle character of the plants. *Stilophora rhizodes* harvested during two consecutive growing seasons (1970 and 1971)* showed no appreciable variations in alginate composition. The polyguluronate content of the *L. difformis* material (~ 67%) is, we believe, the highest so far reported for intact alginate from mature, vegetative algal tissue. However, in this regard it is comparable to alginate from embryos and zygotes of *Fucus* spp. and from *Ectocarpus* although the latter sources show lower levels of mixed sequences (Larsen, 1981). The samples studied in the present work therefore extend our knowledge of the structure of native alginates and establish that the block composition of this structural polymer may vary widely in the same species harvested from different geographical locations.

ACKNOWLEDGEMENTS

We thank W. Crosby and the late Tom Moore for expert technical assistance.

* The algae studied were harvested during 1970 and 1971 and c.d. spectra were recorded at that time on the freshly extracted alginate. The technique for quantitative analysis of alginate c.d. to give block composition, however, was developed only comparatively recently (Morris *et al.*, 1980).

REFERENCES

Atkins, E. D. T., Mackie, W. & Smolko, E. E. (1970). *Nature (London)* **255**, 626.

Atkins, E. D. T., Niedusynski, I. A., Mackie, W., Parker, K. D. & Smolko, E. E. (1973). *Biopolymers* **12**, 1865.

Bailey, E., Mitchell, J. R. & Blanshard, J. M. V. (1977). *Colloid Polym. Sci.* **255**, 856.

Bociek, S. M. & Welti, D. (1975). *Carbohydr. Res.* **42**, 217.

Boyd, J. & Turvey, J. R. (1978). *Carbohydr. Res.* **66**, 187.

Bryce, T. A., McKinnon, A. A., Morris, E. R., Rees, D. A. & Thom, D. (1974). *Faraday Discuss. Chem. Soc.* **57**, 221.

Chapman, A. R. O. (1974). *Mar. Biol.* **24**, 85.

Chapman, A. R. O. & Craigie, J. S. (1977). *Mar. Biol.* **40**, 197.

Chapman, A. R. O. & Doyle, R. W. (1979). *Proc. Int. Seaweed Symp.* **9**, 125.

Gagné, J. A., Mann, K. H. & Chapman, A. R. O. (1982). *Mar. Biol.* **69**, 91.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C. & Thom, D. (1973). *FEBS Lett.* **32**, 195.

Grasdalen, H., Larsen, B. & Smidsrød, O. (1981). *Carbohydr. Res.* **89**, 179.

Haug, A. (1964). Report No. 30, Norwegian Institute of Seaweed Research, Trondheim.

Haug, A. (1974). Chemistry and biochemistry of algal cell-wall polysaccharides, *MTP Int. Rev. Sci., Biochem. Ser. One* **11**, 51.

Haug, A., Larsen, B. & Smidsrød, O. (1966). *Acta Chem. Scand.* **20**, 183.

Haug, A., Larsen, B. & Smidsrød, O. (1967). *Acta Chem. Scand.* **21**, 691.

Haug, A., Larsen, B. & Baardseth, E. (1969). *Proc. Int. Seaweed Symp.* **6**, 443.

Haug, A., Larsen, B. & Smidsrød, O. (1974). *Carbohydr. Res.* **32**, 217.

Larsen, B. (1981). *Proc. Int. Seaweed Symp.* **10**, 7.

Lawson, C. J. & Rees, D. A. (1970). *Nature (London)* **227**, 392.

Liang, J. N., Stevens, E. S., Frangou, S. A., Morris, E. R. & Rees, D. A. (1980). *Int. J. Biol. Macromol.* **2**, 204.

Lin, T.-Y. & Hassid, W. Z. (1966). *J. Biol. Chem.* **241**, 3283.

Mackie, W. (1971). *Biochem. J.* **125**, 89P.

Madgwick, J., Haug, A. & Larsen, B. (1973). *Acta Chem. Scand.* **27**, 3592.

Markussen, J. & Bølund, A. (1975). *Int. J. Peptide and Protein Res.* **7**, 47.

Mitchell, J. R. & Blanshard, J. M. V. (1976). *J. Text. Stud.* **7**, 219.

Mitchell, J. R. & Blanshard, J. M. V. (1979). In: *Food Texture and Rheology*, ed. P. Sherman, Academic Press, London, p. 425.

Morris, E. R., Rees, D. A. & Thom, D. (1973). *Chem. Commun.* 245.

Morris, E. R., Rees, D. A., Sanderson, G. R. & Thom, D. (1975). *J. Chem. Soc. Trans. Perkin II* 1418.

Morris, E. R., Rees, D. A., Thom, D. & Welsh, E. J. (1977). *J. Supramol. Struct.* **6**, 259.
Morris, E. R., Rees, D. A., Thom, D. & Boyd, J. (1978). *Carbohydr. Res.* **66**, 145.
Morris, E. R., Rees, D. A. & Thom, D. (1980). *Carbohydr. Res.* **81**, 305.
Morris, E. R., Rees, D. A. & Young, G. (1982). *Carbohydr. Res.* **108**, 181.
Penman, A. & Sanderson, G. R. (1972). *Carbohydr. Res.* **25**, 273.
Rees, D. A. (1972). *Biochem. J.* **126**, 257.
Rees, D. A. & Conway, E. (1962). *Biochem. J.* **84**, 411.
Rees, D. A. & Samuel, J. W. B. (1967). *J. Chem. Soc. (C)* 2295.
Rees, D. A. & Welsh, E. J. (1977). *Angew. Chem. Int. Ed. Engl.* **16**, 214.
Smidsrød, O. (1974). *Faraday Discuss. Chem. Soc.* **57**, 263.
Smidsrød, O. & Haug, A. (1968). *Acta Chem. Scand.* **22**, 1989.
Smidsrød, O. & Haug, A. (1972). *Acta Chem. Scand.* **26**, 79.
Smidsrød, O., Glover, R. M. & Whittington, S. G. (1973). *Carbohydr. Res.* **27**, 107.
Stockton, B., Evans, L. V., Morris, E. R., Powell, D. A. & Rees, D. A. (1980*a*). *Bot. Mar.* **23**, 563.
Stockton, B., Evans, L. V., Morris, E. R. & Rees, D. A. (1980*b*). *Int. J. Biol Macromol.* **2**, 176.