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ALGINATE COMPOSITION OF SOME NEW ZEALAND BROWN SEAWEEDS

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Key Word Index—Brown seaweeds; alginate composition; guluronic/mannuronic acids; block polymer structure.

Abstract—Guluronic: mannuronic acid ratios were assessed for the alginates from 11 New Zealand brown seaweeds. The highest contents of guluronic acid were found in alginates from two seaweeds from the Chordariales, which are mucilaginous, nonrigid and are winter/spring annuals; hence, high levels of guluronic acids do not appear to correlate with either plant rigidity or age. Conversely, the amounts of guluronic acid were highest in the species with the lowest yield of alginate. The percentage of the lowest uronic acid in the alternating polymer blocks was approximately constant within orders but varied between orders.

INTRODUCTION

It is generally agreed that all members of the Phaeophyceae contain alginates, which are polymers of D-mannuronic acid (M) and L-guluronic acid (G). Alginates are block copolymers of the various combinations of these units [1, 2], i.e. $(M)_n$, $(G)_{n'}$, or $(MG)_{n''}$, the last block representing heteropolymeric units [3]. Biosynthesis of alginates is thought to occur by a C-5 epimerase reacting with a polymannuronic acid to convert the D-mannuronic acid units into L-guluronic acid units. The conversion is irreversible and both types of blocks have been shown to be formed [4]. The ratio of (M):(G) units is known to vary between species, but also to some extent between parts of the plant and the season of collection [5], the age of the parts of the plant [6] and, by implication, between plants. The gelation of alginates is dependent on the number and length of polyguluronate sequences [7].

Although the formation of L-guluronic acid units is irreversible and alginates with high contents of guluronic acid have been observed, many alginates which have been examined for chemical composition appear to have a predominance of D-mannuronic units. Observed M:G ratios include, Laminariales—*Laminaria brasilliensis* 1.2 [8]; *Macrocystis integrifolia* and *Nereocystis luetkeana* 1.2–1.7 [9]; *Ecklonia cava* 1.4–3.1 [10]; *Laminaria digitata*, 1.45 [6], 1.35–2.08 (blade) [11], *Laminaria hyperborea* (fronds) 1.35 [6], 0.54 [12] 0.66 [11], 0.72 [14] *Laminaria longicruris* 1.44–2.17 [11] *Agarum cribosum* 1.30 (blade) [11] *Macrocystis pyrifera* 1.44 [11], 2.08 [12]; Fucales—*Sargassum ringgoldianum* 1.8–2.3 [13], *Ascophyllum nodosum* 1.85 [6], 1.39 [11], 1.44 [12], *Pelvetia canaliculata* 1.5 [6], *Durvillaea antarctica* 1.27 [14]; Dictyosiphonales—*Dictyosiphon foeniculaceus* 0.85 [6]; Scytosiphonales—*Scytosiphon lomentaria* 1.15 [6]; Ectocarpales—*Stilophora rhizodes* 0.44–0.47 [11], *Leathesia difformis* 0.37 [11], Desmarestiales—*Desmarestia aculeata* 0.85 [6]; and Chordariales—*Spermatochnus paradoxus* 1.3 [6] and *Chordaria flagelliformis* 0.9 [6]. There would appear to be no particular relationship between alginate structure and algal order from this data.

While significant variation has been reported for the same species at different locations, the same species, at the same location, gives alginate of the same composition in different years [11], which indicates that the composition does not happen by chance. Accordingly, it is of interest to determine why the alginate composition varies. The composition may be dependent on the amount of epimerase enzyme the plant produces (which includes cofactors, etc.), the physical conditions that determine the rate at which the enzyme operates, e.g. temperature, and the time that the alginate is in contact with the enzyme, which would be consistent with the highest contents of guluronic acid occurring in plants, where the alginates reside in regions of greatest enzymic activity, where there are lower levels of alginate per weight of plant, and with guluronic acid levels being higher in old plants. Guluronic acid contents might also depend on the use the plant makes of the alginate, which would lead to variation between different parts of the plant (i.e. the stipe has mechanical requirements different from the fronds). In this context [11], it was argued that a higher content of guluronate was required for regions of the plant requiring greater rigidity; high concentrations of guluronate are associated with brittleness.

The purpose of the present investigation was to determine whether alginate composition might be dependent on the physical nature of the plant. The choice of species was made to give a reasonably wide range of physical characteristics. Thus, the sample of *Durvillaea antarctica* used here was over 2 cm thick, consisting of a thin cuticle layer which contained all the pigments and a large alginate-rich honey-combed intermedullary tissue. D. *antarctica* is also a relatively long-lived perennial, lasting between 5 and 8 years, although the sample used here was probably young. *Lessonia* and *Ecklonia* were chosen as representing the Laminaria-type structure. *Hormosira* has a reasonably rigid structure, whereas the *Carpophyllum* and *Cystophora* samples contained a large amount of insoluble brown polymeric material. *Papenfussiella* and *Myriogloia* are mucilaginous, soft-tissued plants rich in fucoidan, while *Splachnidium rugosum* is a saccate plant, which contains a water solution of fucoidan in its central region.

RESULTS AND DISCUSSION

Alginates were extracted from whole plants that had been ion-exchanged with acid for 48 hr, to average-out the effect of plant thickness, etc. The one exception was *D. antarctica*, the plant being too large to do this conveniently in the laboratory. The M:G ratios of the resultant alginates were determined by ^{13}CNMR spectra following the method of Grasdalen *et al.* [15]. The M:G ratios could be obtained from well-resolved signals and the spectra, although from nonhydrolysed polymers, were in good accordance with those observed by Grasdalen *et al.* Although the spectral conditions used were not optimal for the carboxyl carbon atoms, an estimate was also made of the fraction of MG blocks, as opposed to MMM and GGG blocks; this estimate was in reasonable agreement with what could be deduced from the anomeric carbon atoms and from M–4 and M–5.

The range of alginates varies between 25% of the total consisting of mannuronic acid (*Myriogloeia intestinalis*) to 25% of the total consisting of guluronic acid (*D. antarctica*) (Table 1). The very high mannuronic acid content of *Durvillaea* is consistent with the concept that much of the alginate is locked away at an early stage in the central honeycomb-like medulla, where perhaps no further epimerization can take place. The very high guluronic acid contents found in the samples from the Scytosiphonales and Chordariales may be a result of the alginate always being available to enzymic activity, and to the fact that there are much lower amounts of alginate present in a given volume. Certainly, the high guluronic acid concentrations in the alginate were not there to confer rigidity. With the exception of *Splachnidium*, which has a high internal osmotic pressure, the plants with high contents of guluronic acid are rather flexible and soft. It is perhaps also worth noting that the results from members of the Chordariales chosen here, and from the *Scytosiphon* sample, give rather different results from the corresponding published figures.

It is also interesting to consider the percentage of the minor uronic acid that occurs in the alternating block, which was obtained by taking half the alternating blocks as a percentage of the total of the minor uronic acid. There are problems with this figure; very short blocks or even random fragments will distort this fraction, as it is rather prone to scatter through experimental error and

Table 1. Composition and yield of alginates from brown seaweeds

Seaweed	M:G*	%MGM†	%Minor uronic in alternating block‡	% Yield
Durvillaeales				
Durvillaea antarctica	3.0	15	28	53
Fucales				
Cystophora torulosa	0.99	26	26	14
Carpophyllum maschalocarpum	0.94	23	24	11
Hormosira banksii	1.31	30	35	22
Xiphpophora chondrophylla	1.36	25	29	24
Laminariales				
Lessonia variegata	1.95	21	31	18
Ecklonia radiata	1.60	24	31	19
Scytosiphonales				
Scytosiphon lomentaria	0.67	11	14	6
Chordariales				
Splachnidium rugosum	0.56	16	22	14
Papenfussiella lutea	0.53	13	19	7
Myriogloeia intestinalis	0.33	10	20	5

*Ratio mannuronic (M)·guluronic (G) acids.

†Percentage of uronic acids in formal alternating polymer blocks.

‡Fraction of the least common uronic acid which is committed to the alternating polymer blocks.

finally it is not correct to assume these blocks are strictly alternating [15]. However, with this kept in mind, in the alginates with very low amounts of mannuronic acid, i.e. for those in which most has been epimerized, only 20% of the remaining mannuronic acid is in the alternating segments, which implies 80% of the remaining mannuronic acid is retained in blocks of at least three units. Similarly, a little over two-thirds of the guluronic acid occurs in blocks of solely guluronic acid in alginates with a high content of mannuronic acid. When there is a clear excess of mannuronic acid, *ca* 30% of the guluronic acid ends up in the alternating block, while in the Chordariales, where there is an excess of guluronic acid, *ca* 20% of the mannuronic acid is in the alternating block. In the sample from the Scytosiphonales, the fraction is lower still (i.e., the polymer is over 70% blocks of either mannuronic or guluronic acid). There are too few data to establish whether there is any phytochemical significance in this or whether it is an accidental coincidence for these samples, but it is a point worthy of further examination.

EXPERIMENTAL

Collection of algae. Algae were collected and air-dried. Samples are lodged in the Museum of New Zealand Te Papa Tongarewa. *Myriogloeia intestinalis* (Harvey) Lindauer. WELT A20020 Collected November 1991. Princess Bay, Wellington. *Papenfussiella lutea* Kylin. WELT A20132 Collected October 1992, Eastbourne, Wellington. *Splachnidium rugosum* (Linnaeus) Greville. WELT A 20130 collected January, 1992, Mt Maunganui. *Xiphophora chondrophylla* (Turner) Montagne ex Harvey. WELT A21028 collected September, 1992 Doubtless Bay by D. Boyes. *Durvillaea antarctica* (Chamisso) Hariot. WELT A21025 collected Sept. 1993, Te Awaite, Wairarapa. *Cystophora torulosa* (R. Brown) J. Agardh. WELT A21026 collected February, 1995, Island Bay, Wellington. *Carpophyllum maschalocarpum* (Turner) Greville. WELT A21027 collected November, 1994. Princess Bay, Wellington. *Hormosira banksii* (Turner) Descaisne. WELT A21029 collected November, 1994. Princes Bay Wellington. *Lessonia variegata* J. Agardh. WELT A21023 collected October, 1994, Eastbourne. *Ecklonia radiata* (C. Agardh) J. Agardh. WELT A21024 collected October, 1994, drift, Wellington. *Scytosiphon lomentaria* (Lyngye) Link WELT A21030 collected November, 1994, Princess Bay, Wellington.

Extraction of alginates. Algal samples were immersed in 0.1 M HCl, left standing for 24 hr, the liquid removed and the process repeated. The one exception to this process was for *S. rugosum*, which was extracted in 0.1 M HCl at 70° for 4 hr, the soln decanted, the alga cut up and the process repeated. The aq. solns were kept for isolating the fucoidans (to be reported elsewhere). Algae were then washed with H_2O to remove acid, then immersed in 0.5% HCHO and stood for 20 hr. After washing free of H_2O, 50 parts by wt of the original dried alga of 2% Na_2CO_3 soln was added and the soln stood with gentle stirring overnight. The soln was strained and the seaweed re-extracted, this time with vigorous stirring in order to macerate the alga. Both Na_2CO_3 solns were diluted to a low viscosity, pressure-filtered, the solns were combined and the pH reduced to 6. The alginate was then ppted by drop-wise addition of 1 M $CaCl_2$ soln to the well-stirred soln until pptn ceased. The ppt. was dialysed for 48 hr against tap H_2O, then converted to the acid by sequential ion-exchanges with cold 0.1 M HCl over 48 hr. The ppt. was then washed with deionized H_2O and dissolved in 0.1 M Na_2CO_3 soln. This solution was then filtered to remove a slight haze and the Na alginate recovered by pption with MeOH. The residue was washed with alcohol and then dried.

^{13}C NMR. Spectra were recorded from 5% w/v solns in D_2O–H_2O (1:5) at 90° on a Bruker AC300 spectrometer (acquisition time 0.8 s; delay, 0.5 s; 90° pulse).

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