

Carbohydrate Constituents of the Marine Algae of Sri Lanka Part II. Composition and Sequence of Uronate Residues in Alginates from some Brown Seaweeds

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Abstract: The ratio of mannuronic acid residues to guluronic acid residues (M/G ratio) of sodium alginate extracted from *Cystoseira trinodis*, *Turbinaria conoides* and *Sargassum* sp. was determined using high resolution $^1\text{H-NMR}$ spectroscopy. The intensities of the signals due to H-5 of guluronate residues and H-1 of both guluronate and mannuronate residues were used. The alginate samples were found to be rich in guluronate residues, and the polymer chains are likely to be composed of long blocks of guluronate residues, short blocks of mannuronate residues and a small proportion of blocks containing both uronide residues.

1. Introduction

Alginic acid is a mucilaginous polysaccharide which has been found in all species of brown seaweeds examined, but is not present in any other plant tissue.⁹ The polysaccharide is a linear glycuronan which consists of (1→4)-linked residues of D-mannuronic acid and L-guluronic acid arranged in a block fashion in the polymer chain. Blocks containing one type of residue (MM blocks and GG blocks) are separated by segments in which the two residues alternate (Figure 1).^{5,6,7} Physical properties of alginates depend on its uronic acid composition, i.e. the ratio of mannuronic acid residues to guluronic acid residues (M/G ratio), and also upon the relative proportion of the three types of blocks (MM, GG and MG). Both the M/G ratio and the monomer sequence distribution changes from one species of brown alga to another.

Penman and Sanderson⁸ found that $^1\text{H-NMR}$ spectroscopy could be used to distinguish between signals due to H-1 and H-5 in the guluronic acid residues and H-1 from mannuronic acid residues in homopolymeric blocks obtained by partial hydrolysis of alginates. Grasdalen *et al*² using high resolution $^1\text{H-NMR}$ spectroscopy were able to distinguish between H-5 of guluronic acid residues with a mannuronic acid neighbour (GM sequence) from those with a guluronic acid neighbour (GG sequence). In this paper we describe the results obtained using this method, in analysing alginate samples isolated from four species of brown seaweeds collected from the coastal regions of Sri Lanka.

2. Results and Discussion

The $^1\text{H-NMR}$ spectra of the partially depolymerised alginate samples were interpreted using the method described by Grasdalen *et al.*² The intensities of (i) the doublet centred at 5.1 ppm due to the H-1 of the G-residues (I_A) (see Figure 2), (ii) the singlet at 4.7 ppm due to H-1 of the M-residues and H-5 of GM residues (I_B) and (iii) the singlet at 4.5 ppm due to H-5 of GG residues (I_C) were measured. The M/G ratios as well as the doublet frequencies were calculated and are given in Table 1 for the alginates from the four species of brown algae examined by us.

The $^1\text{H-NMR}$ spectra of the samples from *Turbinaria conoides* and *Sargassum* sp. (oval) were re-run and amplified. In these two samples the intensities of the signals A, B and C were also obtained by planimetry. These values were found to be different and are considered to be more accurate than those obtained by integration. They were also found to agree with preliminary results obtained from $^{13}\text{C-NMR}$ spectroscopy^{3,4} where intensities of the signals were calculated by planimetry (see Table 1).

TABLE 1. Composition of Alginates

Seaweed		Composition		Doublet frequency			M/G ratio	
		F_M	F_G	F_{MM}	F_{MG}	F_{GM}		F_{GG}
1. <i>Cystoseira</i> <i>trinodis</i>	a	0.19	0.81	0.05	0.14	0.14	0.67	0.23
2. <i>Turbinaria</i> <i>conoides</i>	a	0.35	0.65	0.28	0.07	0.07	0.58	0.54
	b	0.24	0.76	0.13	0.11	0.11	0.64	0.32
	c	0.25	0.75	0.19	0.06	0.06	0.69	0.33
3. <i>Sargassum</i> sp. (linear)	a	0.35	0.65	0.29	0.06	0.06	0.59	0.54
4. <i>Sargassum</i> sp. (oval)	a	0.26	0.74	0.20	0.06	0.06	0.68	0.35
	b	0.33	0.67	0.25	0.08	0.08	0.59	0.49
	c	0.34	0.66	0.27	0.07	0.07	0.59	0.52

a $^1\text{H-NMR}$, int. by integration

b $^1\text{H-NMR}$, int. by planimetry

c $^{13}\text{C-NMR}$, int. by planimetry

The results indicate that all four alginate samples are rich in guluronic acid residues, and the doublet frequencies give an idea of the block character of each alginate. Therefore these four samples of sodium alginate probably contain long blocks of G, shorter blocks of M and very little alternate MG and GM blocks.

3. Methods/Experimental

Sodium alginate was isolated from four species of brown seaweeds *Cystoseira trinodis*, *Turbinaria conoides* and two unidentified species of *Sargassum* referred to as *Sargassum* sp. (linear) and *Sargassum* sp. (oval) with respect to the shape of their fronds. These seaweeds were washed, sundried and milled. Samples of seaweed (50 g) were extracted successively as follows (i) twice with 2% CaCl_2 solution (300 ml) at room temperature for 4h; (ii) twice with 2% CaCl_2 solution (300 ml) at 70° C for 4 h; (iii) four times with dil. HCl (300 ml, pH 2) at 70°C for 4h; (iv) five times with 3% Na_2CO_3 solution (300 ml) at 50° C for 4h. The combined Na_2CO_3 extract was poured with stirring into ethanol (6 l). The precipitate was filtered, dried, dissolved in water and stirred with 2% CaCl_2 solution until precipitation was complete. The calcium alginate was suspended in 0.5 M HCl, stirred occasionally for 3h and filtered. The filtrate was tested for Ca^{++} ions. The residue was washed with 0.5 M HCl until the filtrate was free of Ca^{++} ions. The alginic acid was suspended in water and titrated with 0.1M NaOH until the pH reached 7, when all the alginic acid was dissolved. The solution was dialysed for two days and then freeze dried to give a white powder. The M/G ratio and monomer sequence distribution of each sample were determined by PMR spectroscopy ($t \sim 6$ secs). The spectra were recorded at 90° C in order to increase the spectral resolution and to shift the solvent peak upfield away from the low field spectral region. ^1H -Chemical shifts were expressed in ppm downfield from the internal standard sodium 3-(trimethylsilyl) propane sulphonate. The area under each peak in the low field region was found by integration, and in two cases by planimetry.

3.1 Calculation

The M/G ratios and the doublet frequencies were calculated as follows. Quantitatively the mole fraction of G (F_G) and the doublet frequency (F_{GG}) are related to the intensities (I) of the respective lines by the following relationships.

$$F_G = \frac{I_A}{I_B + I_C}, \quad F_{GG} = \frac{I_C}{I_B + I_C}$$

The mole fraction of M is derived from the normalization condition

$$F_G + F_M = 1$$

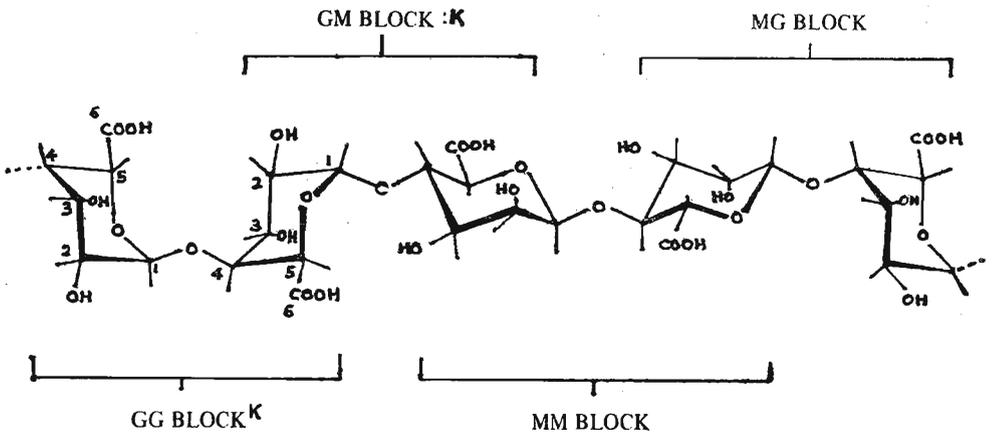


FIGURE 1 — Structure of Alginic Acid showing the block character of the Uronic Acid Residues.

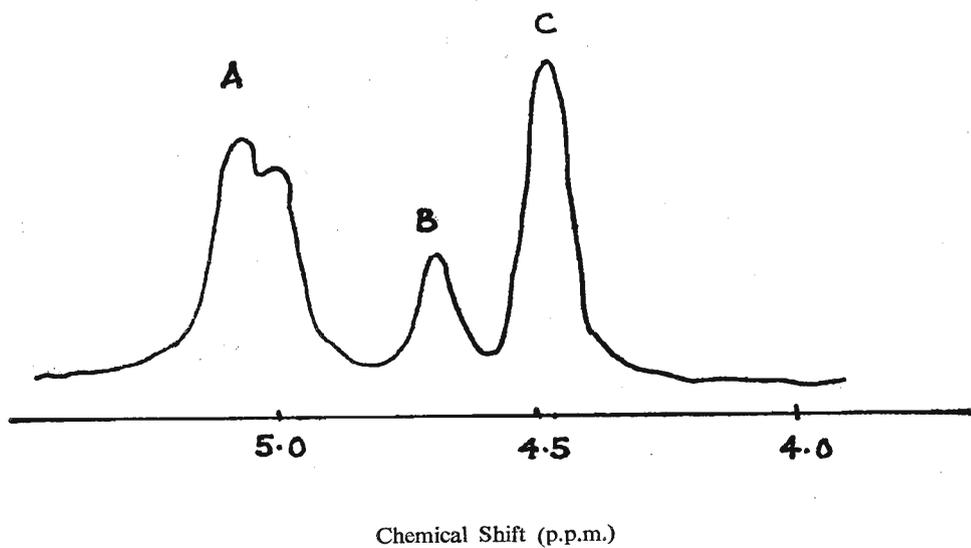


FIGURE 2 — The Low Field Region of the 99.6 MHz FT — ^1H -N.m.r. Spectrum of a Partially Depolymerized Alginate Rich in Guluronic Acid Residues.
A = H-1 of G Residues
B = H-1 of M Residues + H-5 of GM Residues
C = H-5 of GG Residues

The relationship between the doublet frequencies and the mole fractions are given by

$$F_{GG} + F_{GM} = F_G \text{ and } F_{MM} + F_{MG} = F_M$$

For long chains where the average degree of polymerization, $\bar{d}_n > 20$, corrections for the reducing end residues may be neglected so that,

$$F_{MG} = F_{GM}$$

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