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Preliminary communication

^{13}C-N.m.r. spectroscopic analysis of agar, κ-carrageenan and ι-carrageenan*

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Agar, κ-carrageenan, and ι-carrageenan are important, red-seaweed polysaccharides having similar structural features; they contain alternating, 3-*O*-linked β-D-galactopyranosyl residues and 4-*O*-linked 3,6-anhydro-α-galactopyranosyl residues that may have either the D (in carrageenan) or L (in agar) configuration[1]. There are variations with respect to the presence and location of sulfate groups (see Fig. 1). Recently, we have shown that the oligosaccharides obtained by the enzymic hydrolysis of these polysaccharides may be characterized by ^{13}C-n.m.r. spectroscopy[2,3]. In this Communication, we report that ^{13}C-n.m.r. spectroscopy also offers a novel method for characterizing the undegraded agar, κ-carrageenan, and ι-carrageenan types of polysaccharides. This finding is useful in relation to studies on chemotaxonomy and commercial utilization of marine algae, as relatively simple and rapid physical methods of structural analysis are required for these purposes.

Agar, κ-carrageenan, and ι-carrageenan afford well-resolved ^{13}C-n.m.r. spectra** having characteristic signals in the anomeric region (see Fig. 1). The peak assignments are based on the chemical shifts of oligosaccharides and model, monomeric compounds[2,3]. In each case, the peak at 103.2–103.6 p.p.m. can be attributed to C-1 of the D-galactose or D-galactose 4-sulfate residue. In agar, the signal at 99.2 p.p.m. is due to C-1 of the 3,6-anhydro-α-L-galactose residue. κ-Carrageenan produced a signal at 96.2 p.p.m., and ι-carrageenan at 93.1 p.p.m., assigned to C-1 of 3,6-anhydro-α-D-galactose and 3,6-anhydro-α-D-galactose 2-sulfate residues, respectively.

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**Proton-decoupled, ^{13}C-n.m.r. spectra were recorded with a Bruker WH-90 FT spectrometer at 22.63 MHz. A sweep width of 1.5 kHz was used, with a pulse angle of 90° (18.5 μsec) and a repetition time of 0.682 sec. Samples were examined as solutions (30–50 mg/mL) in D_2O at 95°. Chemical shifts (p.p.m.) were measured relative to internal methanol, and converted to values relative to external tetramethylsilane.

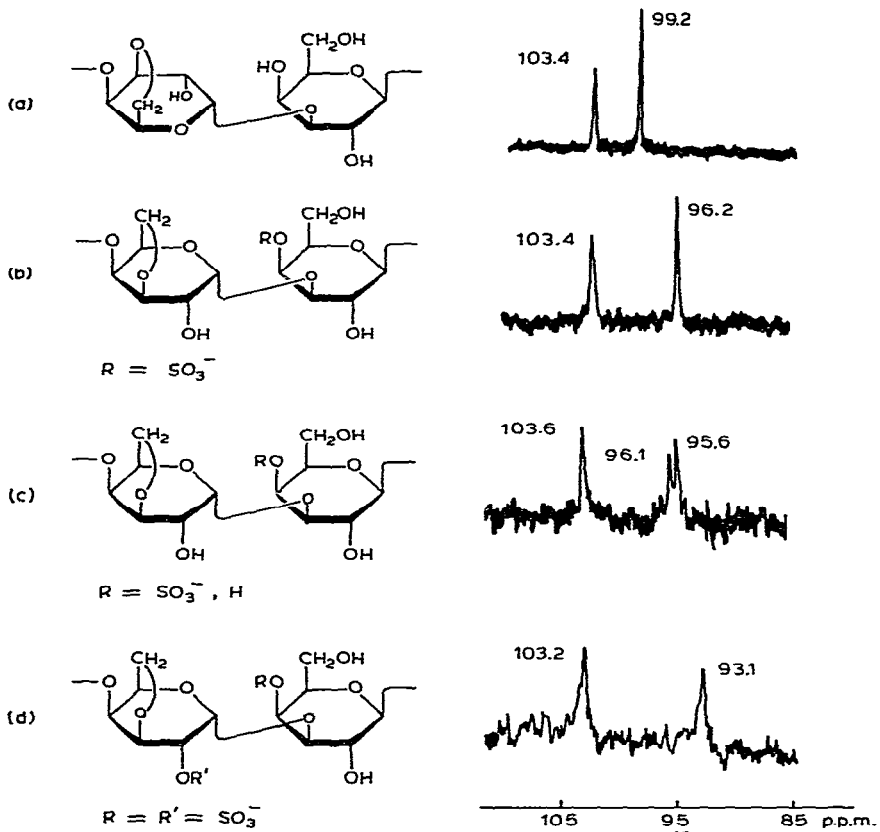

Fig. 1. Main structural feature (disaccharide repeating-unit), and ^{13}C-n.m.r. spectrum in the anomeric region: (a) agar (*Ahnfeltia plicata*); (b) κ-carrageenan (*Eucheuma striatum*); (c) partially desulfated κ-carrageenan (*Eucheuma striatum*); and (d) ι-carrageenan (*Eucheuma spinosum*). Samples of carrageenans as sodium salts.

The presence of more than one disaccharide repeating unit in modified carrageenan is also indicated by the anomeric signals in its ^{13}C-n.m.r. spectrum. For example, the anomeric region of the spectrum of κ-carrageenan partially desulfated by a solvolytic process[4] consists of three peaks [see Fig. 1(c)]. The signals at 103.6 and 96.1 p.p.m. closely correspond to those from the neocarrabiose sulfate repeating-structure shown in Fig. 1(b). The additional signal at 95.6 p.p.m. is thus ascribable to C-1 of the 3,6-anhydro-α-D-galactose residue linked to the unsulfated D-galactose residue. In another instance, a preparation of alkali-modified carrageenan obtained from the marine alga *Ahnfeltia concinna*[5] produced anomeric signals at 103.4, 96.4, and 93.1 p.p.m., indicating the presence of κ and ι types of structures, in agreement with the evidence provided by enzymic studies[6].

The results of this investigation show that ^{13}C-n.m.r. spectroscopy can conveniently be applied for the classification of red algae with respect to their polysaccharide composition.

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