

## POLYSACCHARIDE FROM *SARCONEMA FILIFORME*, AN INDIAN MARINE ALGA

R. G. PAREKH, Y. A. DOSHI, A. V. RAO and V. D. CHAUHAN

Central Salt and Marine Chemicals Research Institute., Bhavnagar 364 002, India

(Revised received 22 June 1987)

**Key Word Index**—*Sarconema filiforme*; Solieriaceae; marine alga; carrageenan and agar; phycocolloid.

**Abstract**—Polysaccharide extracted from the marine alga *Sarconema filiforme*, with sodium, hydrogen carbonate at 90° accounted for 25% of the weight of the purified algal material. It was characterized as iota-carrageenan by IR spectral and physico-chemical analysis.

### INTRODUCTION

Some of the red seaweeds of the order Gigartinales contain considerable amount of polysaccharides [1]. *Sarconema filiforme* although reported to contain an agaroid [2], is systematically very closely related to *Eucheuma*, both being members of the Solieriaceae [3]. As *Eucheuma* is a confirmed carrageenophyte [4-6], it might be assumed that *Sarconema* would probably be a carrageenan producer rather than an agarophyte. As far as we are aware, this algal species from India has not been investigated chemically and accordingly this paper presents the characterization of the polysaccharide as *i*-carrageenan.

### RESULTS AND DISCUSSION

The water soluble polysaccharide, ca 25% of the algal dry weight was isolated as a white powder and comprised monosaccharides, e.g. D-galactose, 3,6-anhydrogalactose, sulphate and had an  $(\alpha)_D^{25}$  34.7°. The positive optical rotation, positive methylene blue test, high sulphate content and the capacity to agglomerate casein supported the carrageenan type nature of the phycocolloid.

IR spectroscopy has been effectively used to characterise algal polysaccharides [7, 8]. It is quite evident (Table 1) that the absorption bands of the polysaccharide from *Sarconema* are different from those of agar. In the IR spectra, the polysaccharide exhibits characteristic absorptions at 1240 cm<sup>-1</sup> confirming the presence of sulphated polysaccharides, 930 cm<sup>-1</sup> typical of the presence of 3,6-anhydrogalactose and 840-850 cm<sup>-1</sup> due to 1,3-linked galactose 4-sulphate [4, 7, 8]. Besides the above, a very strong absorption was also observed at 805 cm<sup>-1</sup> indicating the presence of 1,4-linked galactose 2-sulphate [4]. Kappa carrageenan is characterized by absorptions at 930 cm<sup>-1</sup> and 845-850 cm<sup>-1</sup> whereas the iota carrageenan, apart from exhibiting these absorptions, strongly absorbs at 805 cm<sup>-1</sup> with a magnitude equal to that at 840 cm<sup>-1</sup>. The differentiation of *k*- and *i*-carrageenan is based on the relative intensities of the absorptions at 805 cm<sup>-1</sup> and 845-850 cm<sup>-1</sup>, respectively [9]. From the foregoing information, the insolubility of the polysaccharide in 3M KCl solution and the data presented in

Table 1, it can be well ascertained that the polysaccharide from *Sarconema* is iota-carrageenan and not agar. This information adds clarification to the phylogenetic affinity of the seaweed, *Eucheuma*.

Table 1. Content and properties of polysaccharide from *Sarconema filiforme*

Yield (%)	25.00
Total ash (%)	15.40
Total sulphate (%)	21.35
Galactose (%)	34.56
3,6-anhydrogalactose (%)	16.74
Aqueous gel strength (1 g in 1% KCl soln.)	400 g/cm <sup>2</sup>
Appearance	White
Optical rotation (0.25% in water)	Positive $[\alpha]_D^{25}$ 34.7°
Solubility in KCl soln. (3M KCl)	Insoluble. No precipitation with <i>iso</i> -PrOH from the filtrate.
Methylene blue test	Positive. The phycocolloid precipitates Methylene Blue.
Solubility in H <sub>2</sub> O	Dissolves slowly in cold H <sub>2</sub> O but completely soluble above 70°.
Solubility in milk	Insoluble in cold milk but soluble in hot milk.
Milk reactivity at 25° (0.154% polysaccharide in homogenized milk)	60 g/cm <sup>2</sup>
IR spectra	Intensity of absorption bands (cm <sup>-1</sup> )
	1240    930    845    805
Difco-Bacto Agar (0140-01)	W    S    —    —
$\kappa$ -carrageenan (C-1263)	Vs    S    S    —
$\lambda$ -carrageenan (C-3889)	Vs    W    W    —
<i>i</i> -carrageenan (C-4014)	VS    S    S    VS
<i>Sarconema</i> polysaccharide	VS    S    S    VS

## EXPERIMENTAL

*S. filiformis* was collected during February from the low water mark at Okha (22° 18' N, 69° 05' E) on the west coast of India. The plants after collection were washed thoroughly in sea-water to remove adhering impurities, followed by rinsing with H<sub>2</sub>O drying at 60°, followed by grinding. Carrageenan was extracted by the method of ref. [10].

Dry depigmented algal powder was initially extracted with 0.5 M NaHCO<sub>3</sub> soln at 90° for 2 hr. The hot viscous soln was pressure filtered and the residues reexd as before for another 30 min. Both filtrates were combined and filtered through glass fibre filter paper (Whatman GF/C). The phycocolloid was pptd with cetyl trimethylammoniumbromide. The pptd material was washed with H<sub>2</sub>O. with a satd soln of NaOAC in EtOH, with EtOH followed by Et<sub>2</sub>O and subsequently dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>.

Fractionation of the polysaccharide was carried out with KCl as described in ref. [4]. The samples were alkali modified using the procedure of ref. [11] with NaBH<sub>4</sub>. Aq. gel strength was determined with 1% polysaccharide in 1% KCl soln. Sulphate was estimated gravimetrically after the hydrolysis of polysaccharide with NHC1 for 2 hr at 105° in sealed tubes. The 3,6-AG content was obtained using the modified resorcinol method of ref. [12], that of galactose by PC [13]. Films for IR studies were prepared by evapg a 0.2% soln of the polysaccharide over Hg [14]. Difco-Bacto agar (0140-01, Difco Labs, USA)  $\kappa$ -carrageenan (No. C-1263),  $\lambda$ -carrageenan (c-3889) and *i*-carrageenan (C-4014) were procured from Sigma. The milk reactivity was determined by a standard procedure [15], the Methylene Blue test using a 1% soln of the polysaccharide in H<sub>2</sub>O. OR was measured at *c* 0.25 in H<sub>2</sub>O. KCl solubility was determined by dissolving the powdered polysaccharide at 0.1% by warming to 70° in 3M KCl soln, cooling to 25° and filtering. Observations for the dissolution of polysaccharide in KCl soln were then made. To the filtrate, 2.5 vols of *iso*-PrOH was added to detect the repptn of the algal polysaccharide.

*Acknowledgement*—We gratefully acknowledge Prof. M. M. Taqui Khan, Director for the use of facilities and his encouragement.

## REFERENCES

1. Percival, E. and McDowell, R. H. (1967) in *Chemistry and Enzymology of Marine Algal Polysaccharides*, p. 137. Academic Press, London.
2. Levring, T., Hoppe, H. A. and Schmid, O. J. (1969) in *Marine Algae. A survey of Research and Utilization*, p. 283. Cram De Gruyter, Hamburg.
3. Papenfuss, G. F. and Edelstein, T. (1974) *Phycologia* **13**, 31.
4. Stancioff, D. J. and Stanley, N. F. (1969) *Proc. Intl. Seaweed Symp.* **6**, 595.
5. Anderson, N. S., Dolan, T. C. S. and Rees, D. A. (1973) *J. Chem. Soc. Perkin. 1*, 2173.
6. Santos, G. A. and Doty, M. S. (1979) *Proc. Intl. Seaweed Symp.* **9**, 361.
7. Anderson, N. S., Dolan, T. C. S., Penman, A., Rees, D. A., Muller, G. P., Stancioff, D. J. and Stanley, N. F. (1968) *J. Chem. Sci. C*, 602.
8. Rochas, C., Lahaye, M. and Yaphe, W. (1986) *Bot. Mar.* **29**, 335.
9. McCandless, E. L., West, J. A. and Guiry, M. D. (1982) *Biochem. Syst. Biol.* **10**, 275.
10. Craigie, J. S. and Leigh, C. (1978) in *Handbook of Phycological Methods, Physiological and Biochemical Methods* (Hellebust, J. A. and Craigie, J. S., eds) p. 109. Cambridge University Press, Cambridge.
11. Rees, D. A. (1963) *J. Chem. Soc.* 5168.
12. Yaphe, W. and Arsenault, G. P. (1965) *Anal. Biochem.* **13**, 143.
13. Wilson, C. M. (1959) *Anal. Chem.* **31**, 1193.
14. Santos, G. A. and Doty, M. S. (1975) *J. Pharm. Sci.* **64**, 1704.
15. Strong, C. H. G. (1975) U. S. Patent No 3, 907, 770.