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λ-CARRAGEENAN IN THE GAMETOPHYTES OF *CHONDRUS CRISPUS*

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Key Word Index—*Chondrus crispus*; Gigartinale; gametophytes; cell-wall polysaccharides; λ-carrageenan; characterization.

Abstract—λ-Carrageenan, which was believed to be produced by the sporophytes only, has been extracted and characterized from gametophytes of *Chondrus crispus*. Roughly 10% is found in the cell walls. A decalcification step is required to dissociate λ- and κ-carrageenans.

INTRODUCTION

At an international seaweed symposium [1], we announced that we had found a turnover of sulphates in the red seaweed *Chondrus crispus* comparable to that we had discovered in the brown seaweed *Pelvetia* [2]. It occurred on the λ-carrageenan of the red seaweed as opposed to the fucoidan of the brown seaweed. [1, 2]. These results suggested that the turnover was part of some fundamental process which we suggested was the regulation of the penetration, across the cell walls, of the surrounding Na^+ ions.

An important objection to this proposal arose from the work of McCandless *et al.* [3–6] who reported that Gigartinale gametophytes did not contain λ-carrageenan. Thus the physiological function and the mechanism appeared doubtful.

In the present paper we provide unequivocal evidence that λ-carrageenan constitutes some 10% of the total polysaccharides extracted from decalcified cell wall preparations of gametophytes of *Chondrus crispus*.

RESULTS AND DISCUSSION

Extraction of λ-carrageenan

To prevent any doubt about the conclusions of our experiments, we chose well fructified thalluses. Each thallus was examined under a microscope and all atypical ones were discarded. That only gametophytes were collected for this study was verified by two well-known algologists.

The sporophytes were collected as a source of authentic λ-carrageenan.

Extraction and purification of the λ-carrageenan from gametophytes or sporophytes followed the Craigie technique [5], except that the pre-treatment with boiling 80% ethanol was increased (1 hr in a BBS extractor) and calcium was removed before the extraction. This meant that we were able to use a smaller volume of 0.5 M $NaHCO_3$ and then to separate the λ-carrageenan from the mixture of carrageenans by solubilizing it in 0.3 M KCl, precipitating it with iso-propanol and washing the precipitate with 3 M KCl. The 0.3 M KCl left insoluble, mainly κ-carrageenan. The 3 M KCl solubilized small molecules of carrageenans which we could not identify with certainty. The fraction which was insoluble in 3 M KCl, freed of salts, was a typical λ-carrageenan, only slightly contaminated by proteins which were eliminated by drying, redissolving the sample in water and filtration.

Characterization of the λ-carrageenan from the gametophytes

1. Its aqueous solutions ($c = 2\%$) did not gel and were highly viscous from $c = 1\%$.

2. It was composed of sulphate and galactose in a mole ratio (1.5:1) similar to that found in the reference material extracted from the sporophytes. It contained (w/w) 59% galactose and 48% $-OSO_3$ (measured at pH 7 as well at pH 1.5, which excluded residual contamination by proteins). It contained no 3,6-anhydrogalactose: the faint absorbance given by the resorcinol technique being assigned to the galactose itself and possibly to traces of κ-carrageenan.

3. After alkaline modification [5] followed by a long dialysis period and drying under vacuum ($+ P_2O_5$) it contained per 100 mg of dried material: galactose 37 mg; 3,6-anhydrogalactose 27 mg and $-OSO_3$ 39 mg. These proportions indicated a classical yield for the elimination (81%) reaction; this was confirmed by its weighed yield: 82 mg of polysaccharide from 100 mg of the carrageenan.

4. Its IR spectra obtained before and after alkaline modification were identical to those of the authentic λ-carrageenan (Fig. 1). The ester sulphates gave rise to a strong band at 1240 cm^{-1}. The strong band at 830 cm^{-1} (C_2 equatorial–OSO_3) in the starting product was reduced and two new peaks appeared at 805 cm^{-1} ($-OSO_3$ becoming axial with the production of 3,6-anhydrogalactose) and 930 cm^{-1} (arising from cyclization of the 3,6-anhydrogalactose) on treatment with alkali.

5. Its ^{13}C NMR spectrum fully confirmed these results (Fig. 2). The observed signals being quite different from those which would arise from a μ- or a κ-carrageenan. Moreover, the chemical shifts were identical after alkaline modification (Table 1) for the gametophyte and the sporophyte extracts.

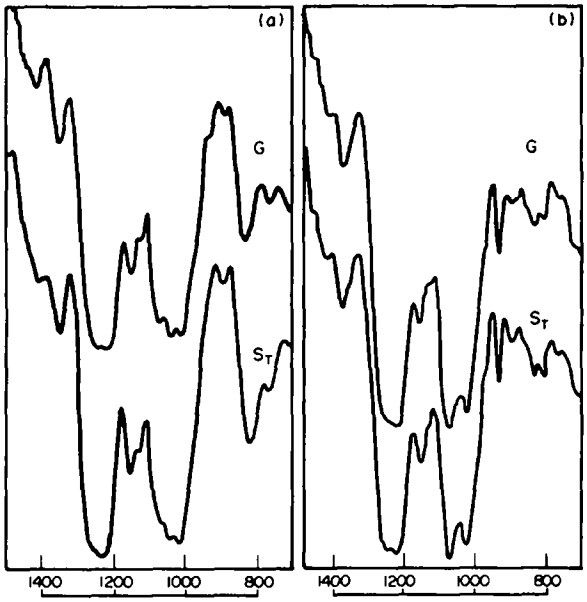

Fig. 1. IR spectra of λ-carrageenans. G, from *C. crispus* gametophytes; S_T, from *C. crispus* sporophytes; A, before the alkaline modification; B, after the alkaline modification.

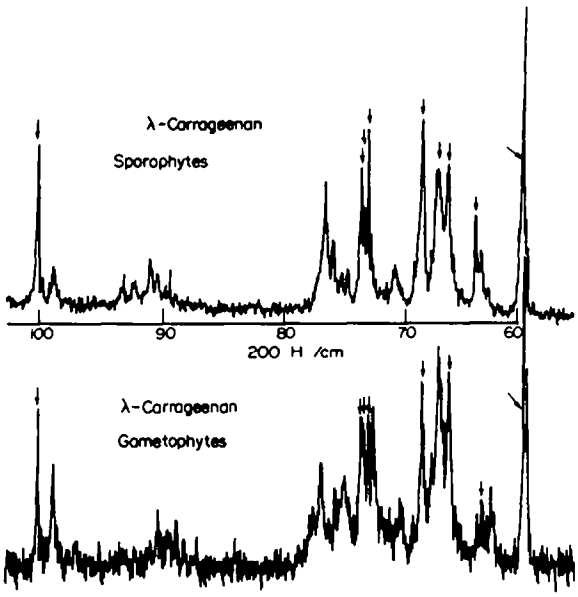

Fig. 2. ^{13}C NMR spectra of λ-carrageenans from *C. crispus*.

CONCLUSION

This study removes any doubt as to the presence of λ-carrageenan in the gametophytes of *Chondrus crispus* Stack., the classical type of the Gigartinales.

The studies of McCandless and coworkers relied mainly upon a technique of extraction insufficiently developed. The immunological technique they used was a good one for the general survey of the composition of the red algae cell walls. Unfortunately it was not sufficiently accurate to detect a minor component (*ca* 10%) the active groups of which are probably masked due to cross-linking by Ca^{2+}. Indeed, it appears likely that the unknown polysaccharide described by McCandless [3] is in fact a stable association of λ- and κ-carrageenans, maintained by Ca^{2+}. So the discovery of the λ-carrageenan seems to arise mostly from the modifications we made to the Craigie technique of extraction.

The main objection to the existence of turnover of a sulphates on the λ-carrageenan of Gigartinales has now been removed. Indeed we will soon present direct evidence for the operation of such a mechanism in the gametophytes of *C. crispus*.

EXPERIMENTAL

Decalcification. The Me_2CO and EtOH extracted powder (9 g) was shaken in 30 ml 80% EtOH, 0.5 M HCl (5 min at 4°), quickly filtered (sintered glass no. 4) and abundantly washed at 4° with 80% EtOH. The treatment was then repeated before drying with EtOH and Et_2O. The dried material was immediately treated with 0.5 M $NaHCO_3$ (1.5 l.; 1.5 hr at 75–80°; strong agitation) and then centrifuged at 30° (28 000 *g* for 1 hr). The perfectly clear supernatant was flocculated by cetavlon, which was then eliminated [5] to yield 2.95 g purified carrageenans.

Separation of the λ-carrageenan. The mixture of carrageens (2.95 g) was finely ground in 0.3 M KCl (250 ml; 2 hr at 20°) and the mixture centrifuged for 30 min at 10° (28 000 *g*). The supernatant was dialysed (12 000 μm pore size) for 24 hr against running water, then distilled water, concd to 150 ml, flocculated in 650 ml EtOH containing 10% isoPrOH and dried (EtOH, Et_2O) to give 1.75 g material which was leached with 3 M KCl (25 min; 0°). The soluble part was discarded after a centrifugation at 0° while the insoluble part was dispersed in water (30 ml) and dialysed until free of KCl. After concn and drying at 20° under red. pres. 0.43 g λ-carrageenan was obtained, the purification of which was achieved by redissolving and filtration (pore size: 0.2 μm).

Table 1. Chemical shifts of the alkali-modified λ-carrageenan extracted from *C. crispus*

	δ							
Sporophytes	76.25	75.36	73.36	68.3	67.84	66.11	65.05	59.53
Gametophytes	76.2	75.3	73.2	68.3	67.9	66.1	65.0	59.5

Chemical analysis. Galactose was measured with orcinol [7], taking into account, the interference due to 3,6-anhydrogalactose produced on treatment of the λ-carrageenan with alkali. 27 μg of 3,6-anhydrogalactose (from an authentic Me-3,6-anhydrogalactoside) gave the same absorbance as 22 μg galactose. This amount was deducted from the value for galactose to give the 37 mg quoted in the results.

3,6-Anhydrogalactose was estimated with resorcinol [8]. The 1% interference of galactose was enough to produce a faint colouration (equivalent to 1% 3,6-anhydrogalactose) similar to that obtained with the purified λ-carrageenan.

The ester sulphates were estimated with CPC [9] at pH 1.5 and at pH 7.

Spectroscopy. IR: thin films on Teflon discs; ^{13}C NMR: 63 MHz, spectral widths normally 11 000–15 000 Hz, 90 000–250 000 scans accumulated depending on the nature of the sample, D_2O (2% w/v solns), 320–340 K. The shifts were referred to external DMSO-d_6 contained in a capillary tube in a

10 mm o.d. sample tube and then converted to the ^{13}C scale in ppm relative to TMS using a conversion factor of +39.44 ppm.

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