Phytochemistry, 1973, Vol. 12, pp. 2441 to 2444. Pergamon Press. Printed in England.

COMPOSITION OF *GIGARTINA* CARRAGEENAN IN RELATION TO SPOROPHYTE AND GAMETOPHYTE STAGES OF THE LIFE CYCLE

STUART EDWARD PICKMERE, MURRAY JOHN PARSONS* and RAYMOND WELLESLEY BAILEY
Applied Biochemistry Division, D.S.I.R., Private Bag, Palmerston North, New Zealand

(*Received* 12 *March* 1973. *Accepted* 2 *May* 1973)

Key Word Index—*Gigartina*; Rhodophyta; algae; carrageenan; life cycle.

Abstract—Total carrageenan levels (55–88% of plant dry weight) of four *Gigartina* species showed little variation between male, female and tetrasporic plants. However whereas male and female gametophyte plants gave carrageenans with K: λ ratios usually ranging from 1·0 to 4·0, with one species in the range 0·3–0·8, tetrasporophyte carrageenans gave very low K: λ ratios, 0·02–0·1, indicative of a virtual absence of K-carrageenan from plants of this stage of the life cycle.

INTRODUCTION

THE POLYSACCHARIDE carrageenan, extracted by hot dilute salt solution from several red seaweeds (*Rhodophyta*), e.g. *Chondrus* and *Gigartina* species, has been shown to contain two major components; K- and λ-carrageenan.[1] The structures of these polymers are now fairly well established[1] with both of them composed of galactose, 3–6 anhydro galactose and sulphated galactose joined through 1–3 and 1–4 glycosidic links. K-Carrageenan differs from λ-carrageenan in its solubility in salt solutions (see below) and in being richer in 3–6 anhydro galactose and much lower in sulphate groups. Several studies[2,3] have pointed to considerable variation in the relative proportion of K- and λ-carrageenan in *Chondrus* and *Gigartina* species with the suggestion that such variations may be due to seasonal effects or be related to species. In these studies plant material of unspecified life cycle stage has been used. In most red seaweeds, however, three different forms occur within a single species; the male plant and the female plant (the gametophytes) produce reproductive cells (gametes) which fuse, and from the spores that develop as a result of this fusion, an asexual plant (the tetrasporophyte) arises. The tetrasporophyte produces spores that give rise to male and female plants. In each of the 4 species of *Gigartina* studied here, the two gametophytes and the tetrasporophyte are of similar size and shape. They are often found together and are only easily distinguishable when fertile.

In the course of a survey of carrageenan in *Gigartina* species in New Zealand, male and female gametophytes and tetrasporophyte were examined separately and their total carrageenans fractionated into K- and λ-carrageenan. The present paper reports the striking difference in the level of K-carrageenan in one life cycle form, the tetrasporophyte, compared to the other form. It is suggested that this difference is relevant to any theory of carrageenan biosynthesis.

* Present address: Botany Division, D.S.I.R., Private Bag, Christchurch, New Zealand.

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RESULTS AND DISCUSSION

For the purpose of the present study *total carrageenan* is defined as the total polymer extracted with hot dilute (0·04 M) salt solution from the dried seaweed. *K-carrageenan* is defined as that part of the total carrageenan which is precipitated from dilute salt solution when it is made 0·25 M with KCl and *λ-carrageenan* as the polymer remaining in solution. The polymers as isolated were not further purified or analysed chemically at this stage.

Total Carrageenan Content of Gigartina *Species*

Amounts of crude total carrageenan extracted from the various samples of the four species of *Gigartina* so far studied are given in Table 1. There are no marked differences in total carrageenan levels between the gametophyte and sporophyte generations, except in the two samples of *G. decipiens*.

TABLE 1. TOTAL CARRAGEENAN CONTENT OF *Gigartina* SPECIES

Species	Sampling* and date†	Herbarium Nos. (CHR)	Total carrageenan as % of plant dry wt. Female gametophyte	Male gametophyte	Tetra-sporophyte
G. decipiens	A 12-7-72	237527 237528 237529	61·6	59·7	39·2
	B 25-10-72	237544 237545 237546	72·9	76·1	45·8
G. angulata	C 30-11-71	237515 237516 237517	88·0	72·3	73·0
	D 3-12-71	237512 237514	77·5	—	78·6
	E 25-10-72	237541 237542 237543	62·8	67·7	61·2
G. atropurpurea	F 12-7-72	237519 237520 237521 237522	61·0	59·4	60·6‡
	G 25-10-72	237532 237534 237535 237536	55·8	—	54·0‡
G. lanceata§	H 8-9-71	237508 237618	70·8	—	69·0

* For sources of samples see Experimental.
† July = winter; Sept., Oct. = spring; Nov., Dec. = summer.
‡ Means from 2 and 3 separate samples respectively.
§ Samples preserved in formalin (10%)-sea water for 2 months before air drying.

K:λ Composition of Gigartina *Carrageenans*

When the total carrageenans from the *Gigartina* samples of Table 1 were fractionated the K-carrageenan: λ-carrageenan ratios listed in Table 2 were obtained.

Gametophyte carrageenans show a wide variation in K:λ ratios (0·28–4·8) with a value generally above 1 and with some of the variation, e.g. in *G. atropurpurea*, being related possibly to species differences. The most striking fact about the K:λ ratios concerns, however, the tetrasporophyte carrageenans. In these plants the K:λ ratio is consistently low, below 0·23 and in most cases very low indeed (0·02–0·03). In fact these results suggest a virtual absence of K-carrageenan from the tetrasporophyte although these plants contain typical (50–60% of plant dry weight) levels of total carrageenan (Table 1).

TABLE 2. K:λ-CARRAGEENAN COMPOSITION OF *Gigartina* CARRAGEENANS

Species	Sampling*	K:λ Ratios in Female gametophyte	Male gametophyte	Tetrasporophyte
G. decipiens	A	4·3	4·8	0·12
	B	3·08	1·7	0·03
G. angulata	C	4·0	3·8	0·13
	D	3·6	—	0·23
	E	1·6	1·36	0·07
G. atropurpurea	F	0·28	0·79	0·02, 0·03†
	G	0·29	—	0·03, 0·06 0·08†
G. lanceata	H	2·4	—	0·03

* See Table 1 for details of samples.
† Results from 2 and 3 separate samples.

Variations in K:λ ratios in carrageenans extracted principally from North Atlantic seaweeds (N. American and N.W. European coasts) have been recorded in *Chondrus crispus* (K:λ, 0·7–4·2) and *Gigartina* species (K:λ, 0·15–3·5) by Black *et al.*[3] and ascribed generally to seasonal effects. The present results suggest that some of this variation may be due to the presence of different amounts of gametophytes and tetrasporophytes in red seaweed samples if tetrasporic plants are present. These effects of different plant forms on sample content and composition should be no problem in the commercially important brown seaweeds, as in these plants the sporophyte is by far the dominant plant. Studies on variations in carrageenan composition in relation to growth, and possibly on other red seaweed polysaccharides such as agar, should therefore take careful account of the individual plants in the life cycle. The proportion of tetrasporic plants in the seaweed samples may also be important in relation to physical properties such as gel strength of carrageenan in so far as K-carrageenan contributes to these properties.

Differences in the K-carrageenan content in relation to the stages of the life cycle may also help in studying aspects of the biosynthesis of these polysaccharides such as their possible interconversion. Thus in another red seaweed, *Porphyra umbilicalis*, containing a sulphated galactan (porphyran), an enzyme has been demonstrated[4] which can remove sulphate from 2–6 galactose disulphate units and form 3–6 anhydro galactose sulphate. By analogy[2] it has been suggested that the action of such an enzyme could be responsible for

[4] REES, D. A. (1961) *Biochem. J.* **81**, 347.

the conversion of λ-carrageenan into K-carrageenan. If this interconversion is involved then it seems likely that the tetrasporic plant either lacks this enzyme almost completely, or the λ-carrageenan is in some way immune to its action.

EXPERIMENTAL

Seaweed samples. Bulk samples of each species (1–2 kg) were collected during spring, summer and winter of 1971–72 from intertidal rocks at Oaro, near Kaikoura, South Island, except for *G. angulata* samples C and D which were from Ringa Ringa and Half Moon Bay, Stewart Island and *G. lanceata* sample H from Shag Point, Otago, South Island. Repeat samples were collected from the same areas. Separate sub-samples (25–50 g fr. wt) of male, female and tetrasporic plants were sorted and air dried without preliminary washing as soon as possible. Species were identified from Laing and Gourlay[5,6] and Laing's herbarium specimens; voucher specimens of present material are lodged at Botany Division, DSIR Herbarium (CHR). Air dried samples were ground in a Wiley mill (1 mm sieve) and stored at room temp. in sealed bottles. Analyses were done within 2–6 weeks of collection.

Carrageenan extraction and fractionation. Both extraction and fractionation of the carrageenan were based on the methods used by Black *et al.*[3] Ground seaweed (2 g) was heated at 90° in 0·04 M NaCl (200 ml) for 2 hr; the sample being homogenized in a blendor after 30 min extraction. After centrifuging (27 000 *g* for 15 min) the residue was re-extracted in the same way. Combined supernatants were poured into EtOH (3 vol.), left overnight at 2° and precipitated *total carrageenan* harvested by centrifuging (15 000 *g* for 20 min). The total carrageenan was dispersed in H_2O (100–200 ml) dialysed overnight successively against 0·1 M NaCl and tap water, and freeze-dried.

Total carrageenan (0·4 g) dissolved in 0·1 M NaCl (200 ml) was made 0·25 M with 1 M KCl (70 ml)[7] and left at 2° for 2 to 3 hr. The precipitate was harvested by centrifuging (27 000 *g* for 15 min), washed by re-suspending in 0·25 M KCl and recentrifuging, dispersed in H_2O, dialysed successively against 0·5 M NaCl and tap water and freeze-dried to give *K-carrageenan.* The combined supernatants from the precipitation and washing of the K-carrageenan, made to 0·5 M NaCl were also dialysed successively against 0·5 M NaCl and H_2O, concentrated on a rotary evaporator (below 40°), added to EtOH (3 vol.) and left overnight at 2°. The precipitate was harvested by centrifuging, dissolved in H_2O and freeze-dried to give *λ-carrageenan.*

Polysaccharide fractions were not further purified. Moistures were measured in seaweed and polysaccharide samples by heating overnight at 110° in a forced draught oven and all results calculated on an oven-dry basis.

Note added in proof. While this paper was in press we learnt that MCCANDLESS, CRAIGIE and WALTER [(1973) *Planta*, in press] have observed a similar difference between the gametophyte and tetrasporophyte carrageenans of *Chondrus crispus.* It seems therefore, that this difference may be common to carrageenan-producing Rhodophyta.

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