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CARRAGEENANS FROM TETRASPORIC AND CYSTOCARPIC *CHONDRUS CANALICULATUS**

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Key Word Index—*Chondrus canaliculatus*; Rhodophyta; Gigartinaceae; carrageenans; nuclear phases.

Abstract—Hot water extraction of tetrasporangial and cystocarpic plants of *Chondrus canaliculatus* afforded carrageenan-type polysaccharides. Hydrolysis, methanolysis and fractionation with KCl analyses showed no remarkable differences between the polysaccharides from the two nuclear phases. The results obtained on fractionation are indicative of the hybrid nature of these polysaccharides.

INTRODUCTION

The soluble polysaccharide from the red seaweed *Chondrus canaliculatus* (C.Ag.) Grev (Gigartinaceae) was previously studied in this laboratory [2]. The composition of the whole polysaccharide as well as those of the fractions separated by the addition of KCl solutions of increasing concentration, agree with a carrageenan-type structure.

Several studies have pointed out that chemical differences exist among carrageenans from different stages of the life history of some carragenophytes. According to some authors [3–5] the haploid gametophytes of algae belonging to the Gigartinaceae contain predominantly κ-carrageenan and diploid tetrasporophytes contain predominantly λ-carrageenan. On the other hand, Doty and Santos [6] studied the tetrasporangial and cystocarpic thalli of six *Eucheuma* species (Solieriaceae). They found in each of the species analysed only one type of carrageenan, no matter which generation was involved. In this paper the carrageenans isolated from tetrasporangial and cystocarpic plants of *Chondrus canaliculatus* are described.

RESULTS AND DISCUSSION

Extraction of cystocarpic and tetrasporangial plants of *Chondrus canaliculatus* afforded soluble polysaccharides, hereinafter known as polysaccharides C and T. The weights and composition are given in Table 1. It can be seen that the yield and composition for both polysaccharides are quite similar. These data lie within the reported values found for carrageenans from *C. canaliculatus* [2] and from other species of Gigartinaceae [7].

Both polysaccharides contain galactose, glucose, xylose and/or 6-*O*-methylgalactose as shown by hydrolysis, followed by PC. Xylose and 6-*O*-methylgalactose showed the same behaviour in all the chromatographic systems used. The approximate molar proportions of monosaccharides in the hydrolysates of polysaccharides C and T are given in Table 1. The same monosaccharides are present in both hydrolysates. As expected, galactose is the major component, the proportion of glucose in polysaccharide C, presumably due to Floridean starch, is higher than previously reported in Gigartinaceae. 6-*O*-Methylgalactose, a common sugar in agar-type polysac-

*Part 17 in the series "Polysaccharides from Chilean Seaweeds". For Part 16 see ref. [1]. Work (by H.A.A.) done in partial fulfilment of the Degree of Master of Science, Universidad de Santiago.

Table 1. Yields and constituents of polysaccharides

	Yield (%)	3,6-AnGal (%)	SO_3Na (%)	Nitrogen (%)	Molar proportions*			
					Gal	Glu	6-*O*-MeGal	Xyl
PS C†	54	16.1	20.6	4.0	9.9	4.3	1.1	1.0
PS T	51	13.3	25.4	1.3	4.3	0.9	0.7	1.0

*Approximate relative molar proportions of the constituent monosaccharides in the hydrolysates of polysaccharides from GC analysis of the alditol acetate derivatives.

†PS C = polysaccharide C; PS T = polysaccharide T; 3,6-AnGal = 3,6-anhydrogalactose.

charides, has seldom been identified in carrageenans [1, 8, 9]. However, both polysaccharides contain similar proportions of it. It seems that 6-*O*-methylgalactose is not after all an uncommon sugar in carrageenans.

From the methanolysates of polysaccharides C and T, 3,6-anhydrogalactose dimethyl acetal and methyl α-D-galactopyranoside were identified by TLC and GC of their peracetyl derivatives.

According to Pernas *et al.* [7], the carrageenans do not consist of a mixture of κ- and λ-components, but rather of a series of polysaccharides with different chemical composition, and with different solubility in potassium chloride solutions. Fractionation of polysaccharides C and T was achieved by treatment with KCl solutions. The results are shown in Table 2. Polyacrylamide gel electrophoresis analysis showed that the whole polysaccharides and all the fractions obtained by treatment with KCl solutions were not homogeneous. The IR spectra of all of the fractions showed at 930 cm^{-1} a band corresponding to 3,6-anhydrogalactose [10]. The signals in the 850–800 cm^{-1} region are indicative of the presence of sulphate attached to different hydroxyl groups [11]. It is noteworthy that fraction 2 of polysaccharide T does not show any signal in the 850–800 cm^{-1} region, even though it contains a considerable proportion of sulphate.

Gels from both polysaccharides, which were precipitated by 62.5 mM KCl solution, were repeatedly precipitated at the same concentration until they showed homogeneity by polyacrylamide gel electrophoresis. The homogeneous fractions were permethylated, hydrolysed and converted to partially methylated alditol acetates. GC and GC-MS analysis of the resulting mixtures gave the composition shown in Table 3. The peaks corresponding to 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methylgalactitol and 1,2,3,4,5,6-hexa-*O*-acetylgalactitol could not be separated. However, since PC of the hydrolysis products of the methylated fractions failed to show the presence of galactose it can be assumed that a considerable proportion of 3-*O*-methylgalactose is present. From this interpretation the presence of galactose 2,6-disulphate residues in the polysaccharide follows.

The results of methylation analysis are similar for both fractions. It can be seen that the concentration of 2,6-di-*O*-methylgalactose is quite remarkable. Crystalline 2,6-di-*O*-methylgalactose has been obtained from methylated carrageenans [12] indicating the presence of D-galactose-4-sulphate residues linked at O-3, one of the repeating units of 'κ family' carrageenans [8].

The sulphation pattern suggests a *ν*-carrageenan structure, for the fractions precipitated by 62.5 mM KCl solutions. However, molar ratios for galactose–3,6-anhydro-galactose–sulphate, for fractions precipitated by 62.5 mM KCl from polysaccharide C (1.00:0.36:0.64) and from polysaccharide T (1.00:0.25:0.72), showed a lower proportion for sulphate as compared with an ideal *ν*-carrageenan (2.00:0.00:3.00). On the other hand, the presence of 2,4-di-*O*-methylgalactitol is rather unusual in a carrageenan, and might indicate, among other possibilities, a considerable proportion of galactose-6-sulphate units linked through O-3 in the fractions. From these results, and the 3,6-anhydrogalactose and sulphate contents, no unique carrageenan-type structure can be proposed for either fraction. It may be assumed that these homogeneous fractions are hybrid polymers of carrageenan-type components.

The remaining fractions from polysaccharide C showed

Table 2. Fractions from KCl treatment of polysaccharides

	Polysaccharide C					Polysaccharide T				
	Yield (%)	3,6-AnGal (%)	SO_3Na (%)	N (%)	IR (cm^{-1})	Yield (%)	3,6-AnGal (%)	SO_3Na (%)	N (%)	IR (cm^{-1})
Fraction 1 (insoluble at 0.0625 M)	23.0	14.54	14.37	3.50	930, 835, 820, 805	17.0	9.37	15.12	4.61	930, 825–805
Fraction 2 (insoluble at 0.125 M)	11.2	19.44	15.95	2.84	930, 850–840, 800	5.3	9.39	23.07	2.70	930
Fraction 3 (insoluble at 0.25 M)	5.2	19.45	16.42	2.63	930, 840, 800	8.2	11.28	23.05	3.57	930, 830–800
Fraction 4 (soluble at 0.25 M)	33.7	19.62	17.80	0.60	930, 845–820, 800	52.0	11.45	16.25	3.21	930, 830–800

3,6-AnGal = 3,6-anhydrogalactose.

Table 3. Methylation analysis of the fractions precipitated at 62.5 mM KCl from polysaccharides C and T

Methylated sugars* (as alditol acetates)	Mole %† I	II
2,3,4,6-Gal‡	2.8	5.2
2,4,6-Gal	2.8	—
2,6-Gal‡	41.9	37.0
2,4-Gal	23.0	16.3
6-Gal	2.4	3.3
2-Gal	2.6	5.5
3-Gal + 1,2,3,4,5,6-hexa-*O*-acetyl-Gal	24.5	32.6

*2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, etc.

†Values are corrected by use of the effective carbon-response factors given by Albersheim *et al.* [16].

‡Identified by GC-MS.

I, Fraction precipitated at 62.5 mM KCl from polysaccharide C. II, Fraction precipitated at 62.5 mM KCl from polysaccharide T.

few differences from those fractions from polysaccharide T, precipitated at the same concentration of KCl solutions. It seems that the whole polysaccharides C and T, and all the fractions are mixtures of hybrid polymers of a carrageenan-type.

These results agree with those reported recently in the literature. Thus, Bellion *et al.* [8] showed by ^{13}C NMR spectroscopy that the polysaccharide from gametophytic plants of *Chondrus crispus* is composed of κ-carrageenan (73%), ι-carrageenan (17%) and sulphated galactans (10%). Fractionation with 0.33 M KCl gave a soluble fraction (6% yield) which contains 60% of μ-carrageenan. The authors concluded that ^{13}C NMR spectroscopy gives more accurate results than IR spectroscopy. Yaphe *et al.* [13] found by enzymic and ^{13}C NMR spectral analysis that the polysaccharide from *Hypnea musciformis* is a hybrid polymer composed primarily of κ-carrageenan, with minor components of ι-carrageenan, and possibly other carrageenan-type components.

EXPERIMENTAL

Algal material. Samples of *Chondrus canaliculatus* were collected in Puerto Aldea 30°15'S 71°35'W and sorted in Laboratorio de Zoología y Biología of Pontificia Universidad Católica de Chile. Tetrasporangial and cystocarpic plants were obtained from samples collected in the months of December and April, respectively.

General methods. The general methods have been described previously [9]. The solvent systems used for PC were: (A) *n*-BuOH–EtOH–H_2O (4:1:5); (B) EtOAc–EtOH–H_2O (8:2:1); (C) *n*-BuOH–EtOH–H_2O (4:1:2) and (D) pyridine–EtOAc–H_2O (4:10:3). TLC was on precoated silica gel 60, zones were detected by charring with 25% H_2SO_4 and eluting solvents were (E) *n*-hexane–EtOAc (3:1) and (F) cyclohexane–Me_2CO (4:3). GC analysis was performed on dual 2.0 × 2.0 mm stainless steel columns packed with (G) 3% SP-2340, (H) 3% ECNSS-M and (I) OV-17 using FID. GC-MS analysis was performed on dual 1.8 × 2.0 mm glass columns packed with (H).

Extraction. The dried ground samples (200 g each) were extracted × 3 with H_2O at 80° for 1 hr and filtered through muslin. The filtrates were centrifuged and the supernatants concd to thin syrups and then poured into EtOH (3.5 vols) giving fibrous ppts.

Hydrolysis. Each of the extracts (0.100 g) was treated with 20 ml of 2 M TFA at 90° for 16 hr. The excess of acid was removed by repeated evaporations with H_2O *in vacuo*. PC (systems A, B and D) showed the presence of galactose, glucose, xylose, and/or 6-*O*-methylgalactose. Aliquots of each hydrolysate were reduced and acetylated according to the procedure reported by Wolfrom and Thompson [14]. The resulting syrups were analysed by GC (systems G and H).

Methanolysis. Two grams of each polysaccharide were treated with 20 ml of dry MeOH and 0.6 ml of AcCl according to the procedure reported by Araki and Hirase [15]. PC (system C) showed two main components in the methanolysates with the same R_f of methyl α-D-galactopyranoside and 3,6-anhydro-D-galactose dimethyl acetal. Aliquots of each methanolysate were acetylated with Ac_2O–pyridine and the resulting syrups were studied by TLC (solvents E and F) and by GC (systems H and I). Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside and 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal were identified by co-chromatography with authentic samples.

Fractionation of the polysaccharides. Solns of each polysaccharide (10 g) in H_2O were fractionated by addition of solns of increasing concn of KCl as described elsewhere [9]. Homogeneity of fractions obtained by treatment with KCl solns was checked by PAGE.

Methylation analysis. The methylation was carried out by the method of Haworth as described elsewhere [1], followed by 2.0 M TFA hydrolysis. The partially methylated sugars were analysed as the alditol peracetates by GC and in some cases by GC-MS.

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