Carrageenans biosynthesized by gametophytic and tetrasporic plants of seaweeds belonging to the Gigartinaceae and Phyllophoraceae are different: gametophytes produce carrageenans of the kappa family, whereas lambda-carrageenans are extracted from tetrasporophytes. For Gigartina skottsbergii Setchell and Gardner and Gymnogongrus torulosus Hooker et Harvey, mature cystocarps were isolated and carrageenans were extracted. Structural determination by methylation analysis, Fourier transform infrared spectroscopy, and 13C-NMR spectroscopy showed that they were kappa/iota-carrageenans. For the extract obtained from cystocarps of Gigartina skottsbergii with water at room temperature, the ratio kappa:iota was 1:0.30 and at 90°C was 1:0.43; significant amounts of precursors were also present. The extract obtained from cystocarps of Gymnogongrus torulosus at 90°C showed prevalence of iota-carrageenans (ratio kappa:iota 1:1.21). These extracts are similar to the polysaccharides produced by gametophytes of these seaweeds. For Gigartina skottsbergii, it was possible to separate the pericarpic tissue from the carpogonial plant. Thus, they were extracted separately, and the carrageenans isolated were studied as described before, obtaining similar conclusions. These results clearly show that whereas the carposporophytes are located inside the cystocarp, they produce carrageenans of the kappa family despite of being diploid cells.

Key index words: carposporophyte; carrageenan structure; cystocarps; gametophyte; Gigartina skottsbergii (Gigartinaceae); Gymnogongrus torulosus (Phyllophoraceae); kappa/iota-carrageenan; lambda-carrageenan

Abbreviations: FT-IR, Fourier transform infrared; GC, gas–liquid chromatography; GC-MS, gas–liquid chromatography mass spectrometry

Carrageenans are sulfated galactans extracted from red seaweeds of certain families of the order Gigartinales. They are unbranched polysaccharides comprised of alternating 3-linked β-d-galactose and 4-linked α-d-galactose or 3,6-anhydro-α-d-galactose units, which are usually sulfated at specific positions. Carrageenans have been classified in different families according to sulfation on the 3-linked β-d-galactose units (Percival 1978, McCandless and Craige 1979, Greer and Yaphe 1984).

The life cycle of the Florideophyceae consists of three phases: a haploid sexual phase (the gametophyte), a parasitic diploid phase that develops directly on the female thallus (the carposporophyte), and a free living diploid phase (the tetrasporophyte). In some cases, including the seaweeds studied here, the carposporophyte is surrounded and protected by gametophytic tissue (pericarp) and the whole structure is called the cystocarp. The cystocarpic plant comprises the female gametophyte and the developed cystocarp.

It has been found that in seaweeds belonging to the Gigartinaceae and Phyllophoraceae, gametophytes and tetrasporophytes biosynthesize different carrageenans (Chen et al. 1973, McCandless et al. 1973, 1982, 1983, Pickmere et al. 1973, Waaland 1975, Craigie 1990). Carrageenans extracted from gametophytes belong to the kappa family (the 3-linked β-d-galactose units are sulfated on C-4), whereas those obtained from tetrasporophytes are mainly lambda-carrageenans (the 3-linked β-d-galactose units are sulfated on C-2) (Matulewicz et al. 1989, Storz and Cerezo 1993). It has been reported (Gordon-Mills and McCandless 1975), on the basis of results from histochemical techniques, that the carposporophytes biosynthesized lambda-carrageenans, in agreement with the diploid character of the phase. This difference between carrageenans biosynthesized by the alternating life stages has not been observed in seaweeds from other families (DiNinno and McCandless 1978, Bert et al. 1989).

The different chemical structure of these two families of carrageenans gives rise to different rheological properties: carrageenans of the kappa family form gels at low concentrations of potassium chloride or can be converted into gelling carrageenans by treatment with alkali, whereas lambda-carrageenans do not gel at low concentrations of potassium chloride even after alkaline treatment but give viscous solutions. As a consequence, they have different industrial applications (Glicksman 1983) and, possibly, different biological functions. The aim of this article is to determine unequivocally the type of carrageenans biosynthesized by the carposporophytes of Gigartina skottsbergii Setchell and Gardner and Gymnogongrus torulosus.
extraction (5 mL) was carried out (Fig. 2, HY).

80%, heating at 100°C for 1 h, and further dilution to 2 M to account conditions used to obtain the extracts from the female gametophytic samples in which the lambda structures had been previously detected (Ciancia et al. 1993b, 1993c) were isolated. Proton decoupled 125-MHz 1H-NMR spectra were recorded on a Bruker AM500 (Bruker Instruments, Billerica, MA, USA) at room temperature, with external reference of tetramethylsilane. The parameters were as follows: pulse angle 51.4 degrees, acquisition time 0.56 s, relaxation delay 0.6 s, spectral width 29.4 KHz, and scans 19,000–34,000. Chemical shifts were referenced to internal acetone (δ 21.82 and 31.1).

RESULTS

Thalli of Gigartina skottsbergii present numerous cystocarps as papillae jutting out from the surface of the female gametophyte. Figure 1A shows a general aspect of the papillae with cystocarpic structures; Figure 1B shows pericarpic tissue and the inner carposporophyte, mainly composed of carposporangia; and Figure 1C shows the carpospororangia in detail. The cystocarps were isolated by excising them by hand and then milling and extracting them with water at room temperature to give extract RI. The residue was further extracted at 90°C to give extract HI (Fig. 2).

The general appearance of the branched thalli of Gymnogongrus torulosus is presented in Figure 1E. Cystocarps are developed on terminal branches (Fig. 1F), with small colored carposporangia (Fig. 1G). Extract HY was obtained from the cystocarps of Gymnogongrus torulosus by extraction at 90°C (Fig. 2). Extraction conditions for each seaweed were chosen, taking into account conditions used to obtain the extracts from gametophytic samples in which the lambda structures had been previously detected (Giancia et al. 1993b, 1997, Estevez et al. 2001).

Yields and analyses of the extracts are shown in Table 1. For RI and HY, galactose was the major sugar component, but the percentages of 3,6-anhydrogalactose were also important. For HI, the initial analysis showed that the major sugar component was glucose (55.1%), although important percentages of galactose (30.4%) and 3,6-anhydrogalactose (9.6%) and minor quantities...
of 3-O-methyl- and 6-O-methyl-galactose and xylose were also detected. The high percentage of glucose arose mainly from floridian starch, as confirmed by methylation analysis of HI (17.7% of 2,3,6-tri-O-methylglucose, see later). Thus, the sample was treated with α-amylase and the percentage of glucose fell to 21.3%. Small amounts of l-galactose were detected by enantiomeric analysis of RI, HI, and HY (Cases et al. 1995), suggesting the presence of agarans and/or η/θ-hybrid chains, as had been previously found in the system of polysaccharides synthesized by cystocarpic plants (Ciância et al. 1993a, 1997, Estevez et al. 2001) of these seaweeds.

FT-IR spectra of extracts RI and HY (Fig. 3) showed absorptions at 932 cm\(^{-1}\) corresponding to the 3,6-
anhydro ring (Stancioff and Stanley, 1969) and at 851 cm\(^{-1}\) and 807 cm\(^{-1}\), due to the axial sulfate group in the 3-linked \(\beta\)-d-galactose 4-sulfate units and to the axial sulfate group in the 4-linked 3,6-anhydro-\(\alpha\)-d-galactose 2-sulfate residues, respectively. The last signal was more important in the spectrum of HY. No absorption around 830–820 cm\(^{-1}\) (C2-equatorial sulfate group and primary sulfate group) was observed (Rees 1961).

Methylation analyses of RI, HI, and HY showed they are kappa/iota-carrageenans (Table 2). RI has a ratio kappa:iota 1:0.30, and only trace amounts of 4,6-di-\(\text{O}-\text{methylgalactose}\) (that would correspond to \(\beta\)-d-galactose 2-sulfate units in a lambda structure; see Discussion) were detected between the partially methylated monosaccharides. The only unusual methylated unit detected was 6-\(\text{O}-\text{methylgalactose}\) (3.4%), which would correspond to disubstituted galactose units. The presence of this sugar is the only significant difference between these results and those obtained for the room temperature extract from cystocarpic plants of \textit{Gigartina skottsbergii} (Fig. 1C) (Matulewicz et al. 1989).

Methylation analysis of HI before and after treatment with \(\alpha\)-amylase gave identical results (with previous deduction of the amount of 2,3,6-tri-\(\text{O}-\text{methylglucose}\) in the former case). Although HI contains small quantities of different methylated monosaccharides, indicating a higher complexity of this sample, it is also basically a kappa/iota-carrageenan (ratio kappa:iota 1:0.43). In this sample, 1.5% of 4,6-di-\(\text{O}-\text{methylgalactose}\) was detected, indicating the presence of small quantities of lambda structure.

HY is also a kappa/iota-carrageenan but with prevalence of the iota structure (ratio kappa:iota 1:1.21), as was determined previously for carrageenans extracted from gametophytes of \textit{Gymnogongrus torulosus} (C1) (Estevez et al. 2001).

![Fig. 2. Extraction procedure for the polysaccharides.](image)

![Fig. 3. FT-IR spectra RI, RIp, Rlc, and HY. Arrows indicate the mayor bands at 932 cm\(^{-1}\), 851 cm\(^{-1}\), and 807 cm\(^{-1}\), typical of kappa/iota-carrageenans.](image)
Table 2. Composition of partially methylated monosaccharides produced by permethylation and hydrolysis of the carrageenans obtained from Gigartina skottsbergii (IC, RI, and HI) and Gymnogongrus torulosus (C1 and HY).

| Monosaccharide | IC | RI | RIp | RIt | HI | HIc | HIc | C1 | HY |
|----------------|----|----|-----|-----|----|-----|-----|----|----|---|
| 2,3,6-Gal      | tr | tr | 1.7 | 1.6 | 1.0 | tr  | 1.1 | 1.6 | tr |   |
| 2,4,6-Gal      | 22 | 1.6 | 7.4 | 7.5 | 3.5 | 3.5 | 6.6 | 3.0 | 2.5 |
| 2,6-Gal        | 40.9 | 52.2 | 46.6 | 41.8 | 47.1 | 48.9 | 45.3 | 55.7 | 40.3 |
| 4,6-Gal        | 2.0 | tr  | tr  | 1.2 | 1.5 | n.d.| 2.9 | tr  | tr |
| 2,4-Gal        | —  | 2.9 | 2.7 | 2.6 | 1.0 | tr  | tr  | tr  | tr |
| 6-Gal          | tr  | 3.4 | 2.3 | 2.2 | 1.2 | tr  | 1.6 | tr  | —  |
| 3-Gal          | 2.6 | 1.4 | 4.3 | 4.2 | 4.0 | 1.6 | 3.3 | tr  | tr |
| 2-Gal          | 2.2 | tr  | —  | 2.8 | 2.3 | 1.1 | tr  | tr  | 1.8 |
| 2-AnGal        | 37.0 | 31.9 | 28.0 | 29.3 | 25.7 | 30.0 | 28.8 | 17.9 | 25.1 |
| 3-AnGal        | 13.1 | 9.5  | 6.8  | 6.7  | 11.1 | 13.9 | 10.4 | 21.8 | 30.3 |

* Mol% of monosaccharide having methyl groups at the positions indicated.

![Fig. 4. 13C-NMR spectra of (A) RI and (B) Rlc.](image-url)

The 13C-NMR spectrum of RI (Fig. 4) is in agreement with results obtained from methylation analysis. Signals corresponding to a kappa structure are the most important (Usov and Shashkov 1985), although those corresponding to iota (Usov and Shashkov 1985) and nu diads are also obvious, the last structure being the only precursor detected by this method (Ciancia et al. 1993b). The absence of lambda-carrageenans was inferred by the lack of signals in the ranges 105.0–103.2 and 67.0–62.5 ppm (signals at 103.9 and 64.5 ppm were also detected).

The FT-IR spectra of RLc and RIp were similar to that of RI (Fig. 2). Methylation analyses of RLc and RIp (Table 2) also gave results similar to those obtained from RI. The same conclusion was drawn from HIp, HIc, and HI, where only minor differences were observed.

The only difference between the 13C-NMR spectra of RI (see above) and RLc (Fig. 4) is the presence in the anomeric region of the signal at 100.8 ppm, corresponding to C-1 of an a-(1→4)-glucan (floridian starch); the other signals corresponding to this structure are also present, partially superimposed on those of carrageenans (Seymour et al. 1976).

Analysis of the residue obtained after extraction of the carposporophytes of Gigartina skottsbergii with hot water (residue after extraction of HIc) indicated that the total carbohydrate content was 55%. The major sugar constituent was glucose, with minor amounts of galactose, 3,6-anhydrogalactose, and mannose, suggesting that the remaining carrageenans belong also to the kappa family, as has been shown for the skeletal cell wall of cystocarpic Sarothalia crispatula (Bory) Leister (formerly Iridaea undulosa) (Flores et al. 1997). For Gymnogongrus torulosus, it was not possible to separate the inner carposporophyte from the pericarpic tissue due to their spatial arrangement (Fig. 1F) and the small size of the carposporophytic tissue inside the cystocarp (Fig. 1G).

**Discussion**

The cystocarps of the Florideophyceae comprise three compartments: the outer photosynthetic and nonphotosynthetic tissues (haploid pericarpic tissues) that, respectively, produce and process and store the metabolites of photosynthesis and the parasitic developing carposporophyte (diploid tissue). Organic molecules are produced in the pericarp, transported and

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used in the development of the parasitic carposporophyte (Homersand and Fredericq 1990).

The isolation, chemical, and spectroscopic characterization of carrageenans extracted at room temperature from the pericarp and the carposporophyte of *Gigartina skottsbergii* showed that they were kappa/iota-carrageenans with similar kappa:iota ratios (1:0.24 and 1:0.23, respectively). These determinations also indicated the presence of lesser amounts of agarans and/or D,L-galactan hybrids. Similar determinations carried out on the hot water extracts indicated kappa:iota-carrageenan ratios with higher iota content (1:0.46 and 1:0.36, for the pericarp and carposporophyte, respectively) and small but significant amounts of 6-O-methylgalactose and xylose. The cystocarp of *Gymnogongrus torulosus* also contained a kappa/iota-carrageenan with similar amounts of both structures (kappa:iota ratio 1:1.21). No evidence of lambda-carrageenan and/or lambda structure were found in the pericarps of *Gigartina skottsbergii* or in the cystocarps of *Gymnogongrus torulosus*. The term lambda structure is not used as synonym of lambda-carrageenan but naming the structural unit of this product. This structural unit can be found as constituent of the lambda-carrageenan or, when in small or trace amounts, interspersed in a backbone of another type of carrageenan. Small amounts of lambda structures were only found in the carposporophytes of *Gigartina skottsbergii*. Analyses of the residue after extraction of the carposporophytes of *Gigartina skottsbergii* with hot water suggests that the small amounts of nonextracted carrageenans were also of the kappa type and similar to those produced by cystocarpic plants of *Gigartina skottsbergii* (Matulewicz et al. 1989).

Gordon-Mills and McCandless (1975) reported that the cell wall of carposporophytic tissues of *Chondrus crispus* Stackhouse stained with anti-lambda polyclonal antibodies but not with anti-kappa ones. It was then deduced that the cells of the carposporophytes produced lambda-carrageenan. Nevertheless, in the same article it was also reported that the outer cell walls of the tetrascorpians (carposporophytic tissue) showed strong fluorescence when treated with both anti-kappa and anti-lambda antibodies, suggesting a lack of specificity of the antibodies used.

Lestang Bremond et al. (1987) reported 4.8% of lambda-carrageenan in carrageenans extracted from gametophytes of *Chondrus crispus*, but the spectrum presented was not the one corresponding to this product (Falshaw and Furneaux 1994, Stortz et al. 1994). On the basis of this circumstantial evidence, it was sometimes erroneously supposed that the carposporophyte produced lambda-type carrageenans. Recently, small amounts of lambda structure were found in the system of carrageenans extracted from cystocarpic plants of *Gigartina skottsbergii* and *Gymnogongrus torulosus* (Ciancia et al. 1993b, 1997, Estevez et al. 2001). These results, together with those obtained from seaweeds in which both phases produce only kappa/iota-carrageenans (DiNinno and McCandless 1978, Bert et al. 1989), indicated that the type of carrageenan biosynthesized does not depend on the cell ploidy.

Santelices et al. (1999) studied the development of the carpospores in the coalescent process of some red seaweeds, belonging to the Gigartinaceae and Phyllophoraceae in culture. When the naked carpospores were released from the cystocarp, they settled on the substratum and developed at first mitosis a new cell wall. The spore cell wall had two well-defined components: a thin inner layer surrounding each of the daughter cells and a less defined outer layer surrounding the entire sporeling. This would be the stage where the cells start producing lambda-carrageenans.

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