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Structural analysis of *Gigartina pistillata* carrageenans (Gigartinaceae, Rhodophyta)

Abdellah Amimi,[a] Aziza Mouradi,[a,*] Thierry Givernaud,[b] Nadia Chiadmi,[a] Marc Lahaye[c]

[a]*Laboratoire de Biochimie et de Biotechnologies Marines, Département de Biologie, Faculté des Sciences, Université Ibn Tofail, B.P. 133, Kenitra 1400, Morocco*

[b]*SETEXAM, B.P. 210, Kenitra 1400, Morocco*

[c]*Unité de Recherche sur les Polysaccharides, leurs Organisations et Interactions, INRA, Rue de la Géraudière, B.P. 71627, F-44316 Nantes, France*

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Abstract

The chemical structure of carrageenans produced by the gametophytic and tetrasporophytic life cycle phases of *Gigartina pistillata* has been determined by permethylation analysis, IR and ^{13}C NMR spectroscopies. The chemistry of the galactans varies according to the biological phases of the plant, the gametophytic alga produces heterogeneous κ–ι type carrageenan containing minor amounts of ν-carrabiose. The tetrasporophytic alga synthesizes a complex sulfated galactan composed of λ-, ξ-, π-carrabioses and sulfated carrabioses containing 3-linked galactopyranose 2,6-disulfate. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Gigartina pistillata*; Rhodophyta; Carrageenan; ^{13}C NMR

1. Introduction

Carrageenans are cell wall polysaccharides found in red seaweeds and particularly in members belonging to the genus *Gigartina*.[1] They are linear sulfated galactans based on the repetition of disaccharide sequences named carrabioses which contain 3-linked β-D-galactopyranose (**G**) and 4-linked α-D-galactopyranose (**D**) residues. The latter can also occur as 3,6-anhydrogalactose (**DA**) arising from the enzymatic or chemical desulfation and cyclization of 4-linked galactose 6-sulfate (**D6S**).[2,3] The hydroxyl groups on the carrabioses can be further substituted by sulfate esters, pyruvate ketal, branched glycosides, or occasionally by methyl ethers.[4,5] The proportion and the location of the sulfate ester groups and the presence or absence of 3,6-anhydrogalactose differentiate the various types of carrageenans which are distinguished by Greek letters in the nomenclature[4,6,7] (Table 1). The different sugar residues and the types and positions of substituting groups in carrageenans are referred to in this report as letters and numbers following the algal galactan nomenclature proposed by Knutsen et al.[4]

Native carrageenans always present complex hybrid structures and are generally a mixture of galactans composed of different carrabiose types, which proportions and structures vary with species, ecophysiological and developmental conditions.[1] In particular, the

* Corresponding author.
E-mail address: lahaye@nantes.inra.fr (M. Lahaye).

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reproductive cycle stage of gigartinacean species profoundly affects carrageenans structure. Gametophytic plants produce κ/ι-type carrageenans whereas tetrasporophytic ones produce λ-type carrageenans.[1,8]

Besides helping as criteria to complement the systematic classification of algae,[1,9,10] the various carrageenans are at the basis of a wide range of functional properties in aqueous solutions used in numerous food and non-food applications.[11] Three types of carrageenan are commercially available: the gel forming κ- and ι-carrageenans and the thickening λ-type carrageenans. The continuous increase in demand and the development of new applications for these galactans prompted us to develop a research program aimed at finding new carrageenan sources. This program includes the screening of red seaweeds belonging to the Gigartinales since members of this order are commonly found on the coasts of Morocco and are already being exported as mixtures of species for industrial carrageenan production. The objective of the present study is to determine the fine chemical structure of the galactans produced by *Gigartina pistillata* (Gmelin) Stakhouse, which is one of the main species available in Morocco. A Portuguese sample of this species has previously been reported to contain κ- and λ-type carrageenans.[12]

Table 1
Disaccharide repeating structures of carrageenans [a]

(1→3)-linked [b]	(1→4)-linked [b]	Greek symbol	
G4S [c]	**DA**	κ (kappa)	Kappa-family
G4S	**DA2S**	ι (iota)	
G4S	**D6S**	μ (mu)	
G4S	**D2S,6S**	ν (nu)	
G4S	**D2S**	ο (omicron)	
G	**DA**	β (beta)	Beta-family
G	**D6S**	γ (gama)	
G6S	**DA**	ω (Omega)	
G6S	**D6S**	ψ (psi)	
G	**D2S,6S**	δ(delta)	Lambda-family
G	**DA2S**	α (alpha)	
G2S	**D2S,6S**	λ (lambda)	
G2S	**DA2S**	θ (theta)	
G2S	**D2S**	ξ (xi)	
GP,2S	**D2S**	π (pi)	

[a] Adapted from Refs. 1 and 4.
[b] Refer to (1→3)- and (1→4)-linked galactose residues.
[c] Letters: **G** β-D-galactopyranosyl; **D** α-D-galactopyranosyl; **DA** 3,6-anhydro-α-D-galactopyranosyl; **S** sulfate; **P** pyruvate acetal; numbers correspond to the carbon atom number on which the substitution is found.

2. Experimental

Algae.—*G. pistillata* (Gmelin) Stackhouse thalli were collected on the 'Plage des Nations', 20 km north of Rabat (Morocco), and cleaned of epiphytes. The reproductive cycle of the algae giving rise to isomorphous plants, tetrasporophytic and gametophytic thalli separation was based on thalli fertility (presence of carpospores or tetraspores). The two algal lots were then briefly rinsed with tap water, blotted, weighed, dried for 16 h at 60 °C and ground to a powder.

Galactans extraction.—Thalli powder (10 g) were extracted with deionized-water (500 mL) under agitation for 12 h at rt. The suspension was passed through diatomaceous earth, concentrated by rotatory-evaporation and the polysaccharides precipitated in 2.5 vol isopropanol to yield fractions GTA and TTA from gametophytes and tetrasporophytes, respectively. The algal residues scraped from diatomaceous earth were re-suspended in deionized water (1 L) and heated for 5 h at 95 °C. The aqueous phase was clarified by passing through a nylon cloth and then through diatomaceous earth before concentration by rotatory-evaporation. The material precipitated in 2.5 vol of isopropanol was dried at 60 °C to yield Fractions G1 and T1 for the gametophyte and tetrasporophyte, respectively. These extractions were performed in triplicate.

Alkali modification of the different extracts was carried out according to Craigie and Leigh.[3] The resulting fractions are referred to as GTA.tr, TTA.tr, G1.tr, and T1.tr from GTA, TTA, G1 and T1, respectively.

Chemical analyses.—Sugars in the different fractions were identified and quantified according to Stevenson and Furneaux.[13] This method combines the trifluoroacetic acid degradation of the polysaccharides and the conversion of the released monomers by 4-methylmorpholine-borane into alditols. The

Table 2
Chemical composition of native and alkali-treated (tr) water soluble extracts at room temperature (GTA, TTA) and 95 °C (G1, T1) from gametophytic and tetrasporophytic *G. pistillata*. ($\pm$ SD, $n = 3$)

	Gametophyte				Tetrasporophyte			
	GTA	GTA.tr	G1	G1.tr	TTA	TTA.tr	T1	T1.tr
Total sugars [a]	34.3 (0.4)	44.4 (1.2)	50.5 (0.4)	51.1 (0.5)	29.6 (0.6)	38.4 (0.2)	34.7 (1.3)	40.2 (0.6)
Gal [b]	61.4 (1.8)	44.8 (1.4)	54.3 (1.9)	45.9 (0.7)	90.2 (0.1)	76.7 (1.6)	93.8 (0.2)	80.7 (0.1)
AG [b]	36.6 (1.5)	52.9 (1.5)	44.6 (1.8)	53.0 (1.2)	1.6 (0.1)	15.0 (1.7)	0.6 (0.2)	14.3 (0.1)
Glc	1.0 (0.1)	1.2 (0.1)	0.6 (0.1)	0.5 (0.1)	6.0 (0.2)	6.0 (0.0)	4.8 (0.2)	4.3 (0.1)
Xyl [b]	1.0 (0.3)	1.1 (0.1)	0.5 (0.1)	0.6 (0.5)	2.2 (0.1)	2.3 (0.0)	0.8 (0.0)	0.7 (0.0)
Sulfate [a]	25.7 (0.4)	23.2 (0.7)	22.8 (0.3)	22.2 (0.3)	32.7 (0.5)	31.5 (0.2)	31.9 (0.6)	31.1 (0.4)

[a] % of fraction dry weight.
[b] mol%, Gal, galactose, AG, 3,6-anhydrogalactose, Glc, glucose, Xyl, xylose.

latter are then acetylated prior to GC analysis on a Perkin–Elmer Autosystem chromatograph equipped with a DB 225 capillary column (J&W Scientific). The sugar derivatives are eluted at 205 °C with hydrogen and quantified using specific weight-response factors relative to myo-inositol used as an internal standard.

Sulfate content was determined by HPAEC on a Ionpac column (Dionex, Sunnyvale, CA, USA) after 2 M TFA hydrolysis (120 °C, 2 h) according to Ref. 14.

Methylation of the triethylammonium salts of the sulfated polysaccharides (4 mg) followed the method of Blakeney and Stones[15]. The partially methylated alditol acetate sugar derivatives (PMAA) were obtained after reductive hydrolysis and acetylation.[13] In some cases, derivatives deuterated at C-1 were prepared according to Englyst and Cummings[16] The PMAA were identified and quantified by GC–MS using a DB 225 capillary column connected to a Delsi–Nermag R10-10C equipment. The PMAA were eluted with hydrogen and a temperature gradient (165 °C for 10 min, then 5 °C/min up to 210 °C). They were identified by their retention time relative to inositol used as an internal reference and from the EIMS fragments obtained.[17]

All the above chemical analyses were performed in triplicate.

Infrared and ^{13}C NMR analysis.—Infrared spectra were collected on a Bruker IFS-25 instrument on polysaccharide films obtained by evaporating aqueous solutions (5 mg/mL) in polyethylene caps.

^{13}C NMR spectra were obtained at 90 °C on 3% polysaccharide solutions in 1:1 D_2O–water using a Bruker ARX 400 instrument. Chemical shifts were calculated in part-per-million (ppm) from the Me_2SO line at 39.6 ppm as an internal reference.

3. Results and discussion

Polysaccharides from the gametophyte.—The yield of polysaccharides extracted by deionized water at room temperature (GTA) is low ($8.2 \pm 0.5\%$). Most of the polysaccharides (G1) were extracted at 95 °C ($30.5 \pm 0.7\%$). Using the alkali-modified extraction about 30% of the weight of the two fractions were lost. Such losses can be attributed to the removal of sulfate as well as of other components initially present in the extracts, such as proteins and/or other salts.

The chemical composition of the native and alkali-treated extracts are reported in Table 2. The native extracts are mainly composed of galactose and 3,6-anhydrogalactose. The sulfate content of 23–26% is in agreement with that of carrageenans belonging to the κ-family.[3,12,18] The chemical composition of the alkali-treated fractions shows a lower sulfate content and a decrease in galactose to the benefit of 3,6-anhydrogalactose. These data are indicative of the presence of some 4-linked

galactose 6-sulfate in the native samples which were converted to anhydrogalactose upon alkali-treatment.

The infrared spectra of the GTA and G1 (Fig. 1(A)) showed a strong absorbance at 1240 cm^{-1} for sulfate and three other bands characteristic of ι-carrageenan or of a mixture/hybrid of κ/ι-carrageenan at 802 cm^{-1} (sulfate esters of **DA2S**), at 845 cm^{-1} (ester sulfate in **G4S**) and 930 cm^{-1} (anhydride bridge in **DA**/**DA2S** and ester sulfate on **G4S**).[12,19–21] The two alkali-treated samples (GTA.tr and G1.tr) had IR spectra similar to that of the native samples (Fig. 1(A)). An increase in the relative intensity for the absorbance at 930 cm^{-1} was also observed and agrees with the increase in 3,6-anhydrogalactose content measured by GC.

The identification and the proportions of the partially methylated alditol acetates obtained from the different extracts after permethylation and GC–MS analysis are given in Table 3. Methylated sugars attributed to **G4S**, **D2S**,**6S**, **DA2S** and **DA** are common to all the extracts but in different amounts. In particular, the proportion of **D2S**,**6S** is less in G1 than in GTA while that of **DA** is higher. After alkali-treatment, the proportion of **D2**,**6S** de-

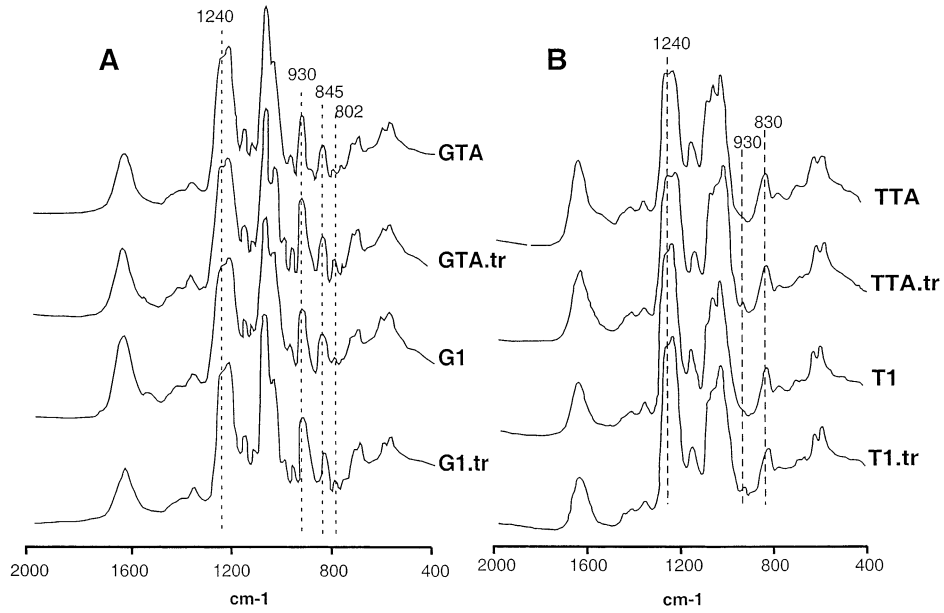

Fig. 1. IR spectra of: (A) native and alkali-treated water extracts from gametophytic *G. pistillata*; and (B) native and alkali-treated water extracts from tetrasporophytic *G. pistillata*.

Table 3
Partially methylated alditol acetates (PMAA), corresponding structure and molar proportion obtained after permethylation, hydrolysis and derivation of native and alkali-treated galactan extracts (GTA, G1 and GTA.tr, G1.tr) from gametophytic *G. pistillata* (± SD, $n = 3$)

PMAA	Structures	GTA	GTA.tr	G1	G1.tr
3-linked galactose					
2,6-Gal [a]	**G4S** [b]	54.0 (0.1)	51.2 (0.7)	52.4 (0.4)	52.5 (0.7)
6-Gal	**G2S**,**4S**	1.2 (0.0)	0.0	0.7 (0.1)	0.8 (0.3)
4-linked galactose					
3-Gal	**D2S**,**6S**	10.7 (0.1)	0.9 (0.1)	6.6 (1.8)	0.0
3,6-Gal	**DA2S**	18.4 (0.3)	29.7 (0.4)	16.8 (0.6)	22.6 (0.4)
2-AG	**DA**	15.7 (0.2)	18.2 (0.3)	23.5 (0.3)	24.1 (0.8)

[a] 2,6-Gal: 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-galactitol, 2-AG: 1,5-di-*O*-acetyl-2-*O*-methyl-3,6-anhydrogalactitol…
[b] Nomenclature following Ref. 4.

creases notably to the benefit of **DA2S**. The weak increase in **DA** on alkali-treatment of the fractions would indicate the low proportion of μ-carrabiose (**G4S-D6S**), the biological precursor of κ-carrabiose (**G4S-DA**),[22] although the corresponding methylated galactose for **D6S** residue was not observed in the native fractions. The data obtained from IR spectroscopy and permethylation analysis suggest the presence of the following structures in these galactans: κ-carrabiose [**G4S-DA**], ι-carrabiose [**G4S-DA2S**], ν-carrabiose [**G4S-D2S,6S**], the biological precursor repeating unit of ι-carrabiose.[22] This interpretation assumes a perfect alternation between 3- (**G**) and 4-linked sugars (**D**) in these galactans, which is supported by the quasi-equimolar proportions of **G4S** and **DA2S + DA** for GTA.tr and G1.tr, and an α configuration of **D** sugars and a β configuration for **G** sugars. The presence of this structure is confirmed by the disappearance of **D2S,6S** and the increase in **DA2S** after alkali-modification of the two fractions (Table 3).

On the basis of the **D2S,6S** proportion, GTA is about twice as rich in ν-carrabiose than G1. This enrichment in the room temperature water-extract agrees with observations reported in the literature.[22] According to the proportions of **DA2S** and **DA** in alkali-treated extracts, G1 is composed of about the same amount of κ- and ι-carrabioses repeating units whereas, GTA is about 1.5 times richer in ι-carrabiose. The presence of these repeating units within the same galactan or in different galactan chains remains to be established. Nevertheless, the differential distribution of these two repeating structures in the gametophyte plant is consistent with the heterogeneous distribution of repeating disaccharides and the complex structure of carrageenan extracts.[1] The biogenic origin of the ι-carrabiose may be related to the presence of ν-carrabiose but that of κ-carrabiose remains ambiguous because its proposed biological precursor, μ-carrabiose (**G4S-D6S**), is absent from the two extracts. However, the conversion of some μ-carrabiose to κ-carrabiose during the methylation reaction cannot be excluded. Other ambiguous situations have been reported in the literature. Zinoun et al.[23] reported that *Gigartina teedii*, which produces a hybrid κ/ι-carrageenan, contains only ν-carrabiose as the biological precursor. The authors suggested that ι-carrabiose is synthesized first from ν-carrabiose and then part of it is desulfated to give κ-carrabiose. Bellion et al.[22] reported the presence of ν-carrabiose in *Eucheuma cottonii* κ-carrageenan. In this case, the authors proposed that κ-carrabiose is directly produced by a combined elimination of the two sulfate groups and the cyclization of the **D** sugars in **D2S,6S**. In any case, these data need further work to clarify the biosynthetic steps of carrageenans.

Thus, the water-soluble polysaccharides from *G. pistillata* gametophyte are essentially composed of heterogeneous carrageenan with a complex hybrid structure involving various proportions of κ-, ι- and ν-carrabioses.

Polysaccharides from the tetrasporophyte.— The yield of polysaccharides extracted with deionized water at room temperature from *G. pistillata* is low ($12.8 \pm 0.3\%$). Extraction of the algal residues in water at 95 °C allowed the recovery of 26.9% ($\pm 1.4\%$) of material based on the algal dry weight. Alkali-treatment of these extracts led to weight losses (31%) close to those observed for the gametophyte extracts.

The chemical composition of the two tetrasporophyte extracts reported in Table 2 shows that galactose is the main sugar and that sulfate groups amount from 32 to 33% of the fractions dry weight. These data agree with published values for λ-type carrageenans.[3,12,18] After alkaline extraction, the galactose content decreased to the benefit of anhydrogalactose and the sulfate content decreased by about 1% dry weight. A small amount of glucose was also measured in both native and alkali-treated fractions and reflects floridean starch contamination.

The infrared spectra of the native fractions (Fig. 1(B)) showed in particular an intense absorbance at 1240 cm^{-1} for sulfate esters and a large one at 830 cm^{-1} attributed to a sulfate ester in equatorial position at C-2 of 3- or 4-linked galactose residues.[21,24] The width of this absorbance probably reflects the contribution of sulfate ester groups at C-6 of galactose which is associated with an absorbance at

Table 4
Partially methylated alditol acetates (PMAA), corresponding structure and molar proportion obtained after permethylation, hydrolysis and derivation of native and alkali-treated galactan extracts (TTA, T1 and TTA.tr, T1.tr) from tetrasporophytic *G. pistillata* (± SD, $n = 3$)

PMAA	Structures	TTA	TTA.tr	T1	T1.tr
3-linked galactose					
4,6-Gal [a]	**G2S** [b]	9.9 (0.3)	8.9 (0.2)	10.4 (0.2)	10.2 (0.1)
Gal	**GP,2S**	5.1 (0.3)	9.1 (0.3)	11.0 (0.3)	5.1 (1.7)
6-Gal	**G2S,4S**	6.8 (0.2)	6.4 (0.3)	6.4 (0.1)	5.4 (0.2)
4-linked galactose					
3,6-Gal	**D2S**	23.2 (1.5)	21.7 (0.3)	24.3 (0.2)	27.4 (0.3)
AG	**DA2S**	5.6 (0.2)	18.2 (0.2)	6.5 (0.3)	14.2 (0.0)
2-AG	**DA**	0.0	2.6 (0.3)	0.0	1.7 (0.1)
Ambiguous linkages					
3/4-Gal	**G2S,6S/D2S,6S**	44.3 (0.2)	30.4 (0.3)	39.5 (0.5)	34.0 (0.0)
Other constituents					
2,3,6-Glc		5.1 (0.2)	2.7 (0.1)	2.0 (0.2)	2.0 (0.2)

[a] 4,6-Gal: 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl-galactitol, Gal: 1,2,3,4,5,6-hexa-*O*-acetyl galactitol, AG: 1,2,5-tri-*O*-acetyl-3,6-anhydrogalactitol, 2-AG: 1,5-di-*O*-acetyl-2-*O*-methyl-3,6-anhydrogalactitol…
[b] Nomenclature following Ref. 4.

about 820 cm^{-1}.[3] The IR spectra of the alkali-treated extracts (TTA.tr and T1.tr) are not markedly different from that of the native fractions except for the small shoulder at 930 cm^{-1} attributed to 3,6-anhydrogalactose. This absorbance is absent on the spectra of the native samples and is consistent with the GC content determination of this sugar. This absorbance has already been noted on the IR spectra of alkali-treated extracts from several *Gigartina* tetrasporophytes.[25,26]

The results of the permethylation and GC–MS analyses of the PMAA from these native and alkali-treated extracts are reported in Table 4. The various PMAA identified indicate complex and heterogeneous structures. According to these results, about 20% of the Fractions TTA and T1 are based on a typical λ-type carrabiose structure with **G2S** and **D2S,6S** units, the latter being converted to **DA2S** upon alkali-treatment. Other galactose derivatives for carrageenans were observed in the *G. pistillata* tetrasporophyte galactans. Part of the 3/4-*O*-methyl-galactose content is attributable to alkali-resistant **G2S,6S** since after deuteration and GC–MS analysis of the permethylated Fraction T1.tr. (Fig. 2), 4-*O*-methyl galactose was identified (m/z:129, 189, 202, 262).[27] The 3-*O*-methyl-galactose content (m/z 130, 190, 201, 261)[27] was attributed to **D2S,6S**. Based on the ion peak intensity at m/z 261 and 262, the relative proportion of **G2S,6S**:**D2S,6S** is 73:27. Assuming an equimolar proportion of 3- and 4-linked residues, the proportions of **G2S,6S**:**D2S,6S** in TTA.tr is roughly 4:1 compared to about 83:17 for T1.tr estimated by the same approach. The **G2S,6S** unit has already been observed only in low amounts in some galactan extracts[13,25,28,29] but this sugar represents about half of the disaccharide repeating units of tetrasporic *G. pistillata* of TTA.tr and T1.tr fractions. The small proportions of the 6-*O*-methyl-galactose in the native and alkali-treated extracts was attributed to **G2S,4S** and not to **D2S,3S**. This is based on the assumptions that a 4-linked galactose 3-sulfate unit does not occur in carrageenans[29] and on the equimolar proportion of 3- and 4-linked galactose residues. Non-methylated galactose was attributed to 4,6-*O*-(1-carboxyethylidene)-galactopyranosyl 2-sulfate (**GP,2S**). Although this sugar can arise from under-methylation,[8,26] a ^{13}C NMR signal for the methyl pyruvate carbon has been observed (see below). A similar structure has already been described in π-carrageenan.[10,13,30] These galactans differ with the λ-carrageenan isolated

from *Chondrus crispus* in which 30% of the **G** units are not sulfated (δ-carrageenan, **G-D2S,6S**).[31] No **G** unit was detected in TTA and T1 and this absence is common to other galactans of other tetrasporic *Gigartina*: *G. decipiens*,[28] *G. clavifera* and *G. alveata*.[25] Alkali-treatment, modified the proportions of sugars attributed to **GP,2S**, **DA2S** and **G2S,6S/D2S,6S** (Table 3). The increase in TTA.tr and decrease in T1.tr of **GP,2S** (Table 3) may reflect an under-methylation in the case of TTA.tr and the loss of pyruvate during alkali-treatment of T1. The increase of **DA2S** observed in the alkali-treated samples is in agreement with the increase in the 3,6-anhydrogalactose measured by GC and with the appearance of the shoulder at 930 cm^{-1} on the IR spectra. This increase occurs jointly with the decrease of 3/4-*O*-methyl-galactose and confirms that part of this arises from **D2S,6S**. The most important decrease in 3/4-*O*-methyl-galactose was observed after alkali-treatment of the TTA extract and indicates that the initial fraction was rich in **D2S,6S**. The presence of a small portion of **D2S,6S** in both TTA.tr and T1.tr indicates that the alkali-conversion of this unit to **DA2S** was incomplete in agreement with the known relative stability of 4-linked galactose 2,6-disulfate to alkali desulfation and cyclization.[32] The appearance of a small proportion of **DA** after alkali treatment remains unexplained since the 2,3-di-*O*-methyl-galactose content attributable to **D6S** in the native extracts is absent. The chemical reaction, by removing other components in the extracts, such as proteins, may have contributed to reveal the presence of minor sugars that could not be properly permethylated in the native extracts.

Thus, assuming that the 3-linked galactose and derivatives are in β-anomeric configuration and alternate with 4-linked galactose and derivatives in α-anomeric configuration, it appears that the galactans from tetrasporic *G. pistillata* are carrageenans with complex repeating structures. Considering that four 3-linked and four 4-linked galactose derivatives have been identified, we can suggest the existence of 16 different carrabioses structures of which the most probable ones are: ξ-carrabiose [**G2S-D2S**], θ-carrabiose [**G2S-DA2S**], λ-carrabiose [**G2S-D2S,6S**], π-carrabiose: [**GP,2S-D2S**] which have all been reported in λ-type carrageenans.[25,28,30] Unusual structures, such as: [→3)-β-D-galactopyranosyl 2,6-disulfate-(1→4)-α-D-galactopyranosyl 2-sulfate-(1→] [**G2S,6S-D2S**], [→3)-β-D-galactopyranosyl 2,6-disulfate-(1→4)-3,6-anhydro-α-D-galactopyranosyl 2-sulfate-(1→] [**G2S, 6S-DA2S**], [→3)-β-D-galactopyranosyl 2,6-disulfate-(1→4)-α-D-galactopyranosyl 2,6-disulfate-(1→] [**G2S,6S-D2S,6S**] can be also be proposed but remain to be clearly established.

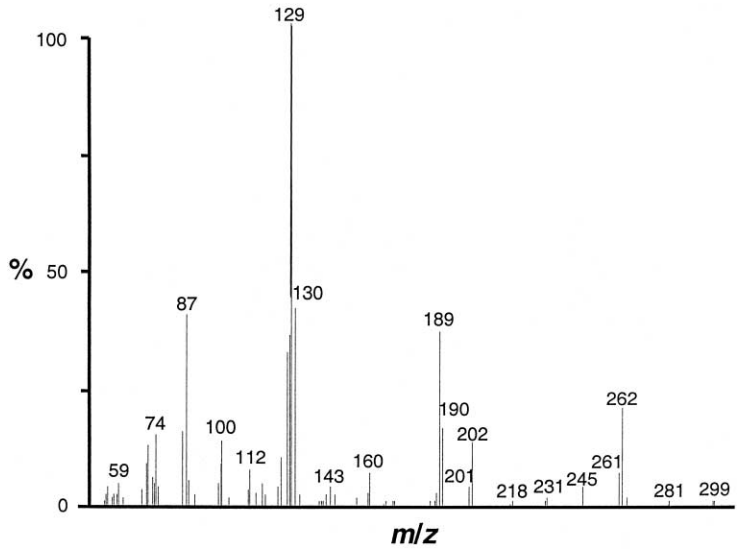

Fig. 2. EIMS spectrum of the GC co-eluting 1,2,4/3,5,6-tetra-*O*-acetyl-3/4-*O*-methyl-galactitol recovered after acid hydrolysis, reduction with $NaBD_4$ and acetylation from the permethylated tetrasporophytic *G. pistillata* alkali-treated extract (T1.tr).

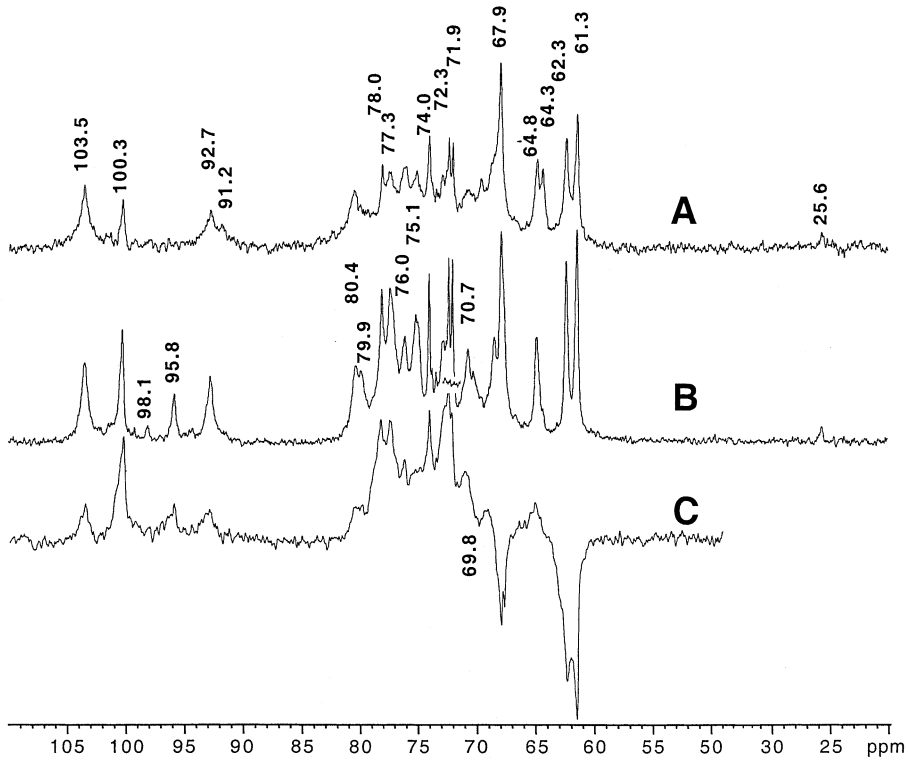

Fig. 3. ^{13}C NMR spectra of native (A) and alkali-treated (B) galactan fraction T1 and T1.tr, respectively, and (C) the DEPT 135 spectrum of T1.tr from tetrasporophytic *G. pistillata*. (A) 100,000; (B) 81,719; (C) 30,570 scans.

In order to more precisely define the chemical structure of these galactans, the ^{13}C NMR spectra of T1 and T1.tr were measured (Fig. 3(A, B), respectively). Considering the possible carrabiose repeating units suggested above, diagnostic signals for ξ-, λ-, and θ-carrabioses available in the literature[25,28] were searched for in these complex spectra. Three major resonances at 103.4, 64.8 and 92.7 ppm corresponding to G_{2S}-1, G_{2S}-4 and D_{2S}-1, of ξ-carrabiose, respectively, are observed in the spectrum of T1 and T1.tr. The spectrum of T1 shows signals corresponding to λ-carrabiose (103.4: G_{2S}-1, 77.3: G_{2S}-2, wide resonance centered at 76.0: G_{2S}-3, 64.3: G_{2S}-4, 74.0: G_{2S}-5, 61.3: G_{2S}-6, 91.2: $D_{2S,6S}$-1, 75.0: $D_{2S,6S}$-2, 69.6: $D_{2S,6S}$-3, 80.4 ppm: $D_{2S,6S}$-4 and shoulders at 68.7–68.5 ppm on the 67.9 ppm signal attributed to: $D_{2S,6S}$-5 and $D_{2S,6S}$-6). In both native and alkali-treated galactan extracts, fine resonances at 100.3, 72.3, 73.9, 78.0, 71.9, 61.3 ppm fit with C-1–C-6 signals for starch[33] and correspond to the small amount of 4-linked glucose measured (Tables 2 and 4). Several signals appeared or increased in intensity in the spectrum of the alkali-treated sample particularly at 68.4, 70.3, 70. 7, 75.1, 77.3, 79.9, 95.8 and 98.1 ppm. The disappearance of typical signals for λ-carrabiose at 91.2, 69.6 and 64.3 ppm on the spectrum of the alkali-treated fraction (T1.tr) is balanced by the appearance of diagnostic signals for θ-carrabiose (G_{2S}-1 at 100.3, $DA_{2S,6S}$-1 at 95.8, $DA_{2S,6S}$-2 at 75.1, $DA_{2S,6S}$-4 at 79. 9, $DA_{2S,6S}$-6 at 70.3 ppm[28] with a downfield shift of about 0.2–0.4 ppm). The DEPT135 spectrum (Fig. 3(C)) of T1.tr shows inverted signals at 61.4, 62.3, 67.6 and 67.9 ppm indicating the presence of at least four different methylene carbons in this carrageenan fraction. Based on the methylation analysis and published chemical shifts,[28] the following attributions are proposed: D_{2S}-6 and starch C-6 at 61.4 ppm, G_{2S}-6 at 62.3 ppm, $G_{2S,6S}$-6 at 67.9 and/or 67.6 ppm. A signal at around 69.8 ppm for DA_{2S}-6 was expected but not observed probably because it is in the noise level. The two different signals for $G_{2S,6S}$-6 can be interpreted as resulting from the different environments, such as **D2S** or/and **D** units, and/or reflect the minor amount of $D_{2S,6S}$-6. A small resonance at 25.6 ppm was observed in the spectrum of

T1 and T1.tr which can be attributed to the carbon of the pyruvate methyl group.[34,35] The presence of such substituting group is in agreement with the permethylation data.

4. Conclusion

The present study confirms that the different reproductive phases of members of the Gigartinaceae family produce different water-soluble cell-wall galactans. The gametophytic phase of *G. pistillata* synthesizes complex κ/ι-type carrageenans with varying proportions of ν-carrabiose that questions the biogenic pathway of κ-carrabiose formation. The tetrasporophytic plant synthesizes complex galactans with repeating structures corresponding to ξ-carrabiose, π-carrabiose and for about 20% of the extracts, λ-carrabiose, which after alkali-treatment converts to θ-carrabiose. These galactans are unusually rich in **G2S,6S** containing carrabioses amounting for about half of the extracts.

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