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Concurrence of agaroid and carrageenan chains in funoran from the red seaweed *Gloiopeltis furcata* Post. et Ruprecht (Cryptonemiales, Rhodophyta)

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Funoran extracted from the red seaweed *Gloiopeltis furcata* Post. et Ruprecht was fractionated into four polysaccharide fractions in terms of differences in solubility of their cetylpyridinium salt in potassium chloride solution. Besides the main fraction constructed by an agarose sulphate structure with negative optical rotation, minor polysaccharide fractions with positive optical rotation were obtained. One of the minor polysaccharide fractions contained D- and L-galactose (Gal), 6-O-methyl-D-Gal, 3,6-anhydro-L-Gal and sulphate. Partial hydrolysis and partial methanolysis studies of this fraction led to identification of oligosaccharides attributable to both agaroid and carrageenan backbones, i.e. [→ 3)D-Gal(1 → 4)L-Gal(1 →], [→ 3)D-Gal(1 → 4)3,6-anhydro-L-Gal(1 →], [→ 3)D-Gal(1 → 4)D-Gal(1 →] and [→ 3)6-*O*-methyl-D-Gal(1 → 4)D-Gal(1 →]. Methylation analysis and alkaline treatment study of this fraction revealed that the sulphate groups were located at O-6 of all the (1 → 4)-linked L- and a part of the (1 → 4)-linked D-Gal residues, O-6 of a part of the (1 → 3)-linked D-Gal residue, and both O-2 and O-4 of the (1 → 3)-linked 6-O-methyl-D-Gal residue and the other (1 → 3)-linked D-Gal residue. © 1998 Elsevier Science Limited. All rights reserved.

INTRODUCTION

Galactan derivatives that commonly occur in extracellular matrices of red seaweeds consist of a backbone with a regularly alternating repeat of a (1 → 4)-linked α-galactopyranose residue (or its 3,6-anhydride) and a (1 → 3)linked β-galactopyranose residue (Painter, 1983). The latter residue always occurs as the D-enantiomer. In contrast, the former may occur as either D- or L-form, on which basis the red algal galactans are classified into a carrageenan or an a agaroid, respectively. Although the agaroid and the carrageenan are obtained from the algae termed 'agarophyte' and 'carrageenophyte', respectively, both agaroid and carrageenan backbones have been found simultaneously in several red algae (Nunn *et al.*, 1971; Usov and Barbakadze, 1978; Takano *et al.*, 1994).

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Funoran extracted from the edible red algae of *Gloiopeltis* is employed for a variety of traditional Japanese and Chinese industries and arts owing to its excellent sizing and adhesive properties (Schachat and Glicksman, 1959). The main component of the funoran has been indicated to be a sulphated agaroid by early studies for *Gloiopeltis furcata* Post. et Ruprecht and *G. cervicornis* (Sur.) Schm. (Hirase *et al.*, 1958; Lawson *et al.*, 1973; Penman and Rees, 1973). The funorans from *G. furcata* (Hirase and Watanabe, 1971) and *G. complanata* Yamada (Takano *et al.*, 1995) have been fractionated into several sulphated polysaccharide fractions in terms of differences in solubility of their cetylpyridinium salt in potassium chloride solution. These fractionation studies have led to isolation and identification of the main polysaccharides ideally constructed by a regular repeat of [→ 3)β-D-Gal-6-SO3-(1 → 4)3,6-anhydro-L-Gal(1 →], i.e. an agarose 6-sulphate structure. However, the other minor polysaccharide fractions have appeared to

form aqueous solution with rather largely positive optical rotation contrary to the agaroid. We now describe the further studies on one of the minor sulphated polysaccharide fractions from the *G. furcata* funoran.

MATERIALS AND METHODS

Materials

Gloiopeltis furcata Post. et Ruprecht (Rhodophyta, Endocladiaceae) was harvested in an intertidal marsh at the Shitaru coast neighbouring the city area of Shimoda, Shizuoka prefecture, Japan. The hand-sorted algae were washed with fresh water and air-dried prior to extraction. Derivatives of 4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-L-galactose (agarobiose), 4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-D-galactose (carrabiose) and 4-*O*-(6-*O*-methyl-β-D-galactopyranosyl)-3,6-anhydro-D-galactose (6-methyl-carrabiose) were prepared by partial hydrolysis or partial methanolysis of commercial agar, commercial κ-carrageenan and a polysaccharide from the red alga *Melistotheca papulosa* J. Ag. (Hirase *et al.*, 1976), respectively. Other authentic disaccharides and trisaccharides were prepared from a polysaccharide of the red alga *Lomentaria catenata* Harvey, as described previously (Takano *et al.*, 1994).

General methods

Colorimetric determination of galactose and 3,6-anhydrogalactose was carried out as described by Yaphe (1960). Methods for sulphate analysis, gas–liquid chromatography (GLC) using a chromatograph GC-8A (Shimadzu Corp.), combined GLC and mass spectrometry (GC/MS) using a spectrometer GC-QP1000 (Shimadzu Corp.), ion-exchange chromatography of sugar–borate complex and gel-filtration were previously described (Takano *et al.*, 1994). High-performance liquid chromatography (HPLC) was carried out using a chromatograph LC-5A (Shimadzu Corp.) equipped with a refractive index detector RID-2A (Shimadzu Corp.). Optical rotation was measured with a digital polarimeter PM-101 (Union Co.); operating temperature, 20°C; cell path length, 10 mm. Infrared (IR) spectrum was recorded using a spectrometer model 215 (Hitachi Co.) by potassium bromide disc method (1 mg sample/200 mg KBr).

Extraction and fractionation of funoran

A 30 g portion of the dry algae was macerated in 1500 ml of water for 1 h, homogenized with a blender, and then extracted twice for 2 h at 100°C. After centrifugation, the combined extract was mixed with 640 ml of 10% cetylpyridinium chloride solution. The resulting precipitate of the cetylpyridinium salt of a sulphated polysaccharide was washed successively with water, methanol and acetone. The dried cetylpyridinium salt (20.7 g) was extracted with 800 ml of 1 M potassium chloride at room temperature for 2 h. After centrifugation, the extract was concentrated *in vacuo* and poured into 4 vol. of ethanol. The resulting precipitate was washed with ethanol and acetone, dried and redissolved in water. The solution was dialysed against water, concentrated and poured into 4 vol. of ethanol. Then the precipitate obtained was washed with ethanol and acetone to afford potassium salt of a sulphated polysaccharide fraction designated PS1 (0.63 g). The cetylpyridinium salt insoluble in 1 M potassium chloride was successively extracted with 2 and 4 M potassium chloride in the similar manner to PS1 to afford potassium salts of sulphated polysaccharide fractions PS2 (0.48 g) and PS3 (1.1 g), respectively. The remaining cetylpyridinium salt was further extracted with 4 M potassium chloride at 100°C for 2 h. The resulting solution was immediately poured into 4 vol. of ethanol and treated similarly to afford a sulphated polysaccharide fraction PS4 (13.7 g).

Composition analyses

A sample was hydrolysed by 'double hydrolysis method' as described by Stevenson and Furneaux (1991), and the hydrolysate was analysed with GLC after derivatization into an alditol acetate mixture. The absolute configuration and the D/L ratio of the component sugars were determined as described elsewhere (Takano *et al.*, 1993), except that those of 3,6-anhydrogalactose were determined by isolation and identification of 4-*O*-β-D-galactopyranosyl-3,6-anhydro-D-galactose (carrabiose) and/or 4-*O*-β-D-galactopyranosyl-3,6-anhydro-L-galactose (agarobiose) dimethylacetals after partial methanolysis as described later.

Partial hydrolysis of PS3 and identification of the oligosaccharides

Partial hydrolysis and the isolation of the neutral hydrolysate were carried out as described elsewhere (Takano *et al.*, 1994). A 500 mg portion of PS3 was partially hydrolysed at 100°C for 3 h with 0.1 N sulphuric acid, and the solution was neutralized with barium carbonate. After removal of the resulting precipitate of barium sulphate and excess barium carbonate by filtration, the filtrate was applied to serially connected columns of Amberite IR-120 (H^+ form, 2 × 20 cm), Dowex 1 × 4 (sulphate form, 2 × 20 cm) and Amberite IR-45 (OH^- form, 2 × 20 cm) to remove acidic hydrolysis products. The neutral hydrolysate (35 mg) eluted with deionized water was gel-filtered with a Bio-Gel P-2 column (400 mesh, 2.2 × 100 cm) calibrated with the authentic mono- and oligosaccharides. The fractions corresponding to the disaccharide and the trisaccharide were collected and designated DP2 (8.6 mg) and DP3 (3.7 mg), respectively. Both fractions were subjected to semipreparative size-exclusion chromatography using an SCR-101N column (7.5 mm × 25 cm, Shimadzu Corp.) on HPLC. The column was eluted with water at 40°C at a flow rate of 0.5 ml/min, to afford subfractions DP2a

(7.0 mg) and DP2b (1.4 mg) from DP2, and DP3a (1.1 mg) and DP3b (2.0 mg) from DP3. The subfractions DP2a and DP3a were further analysed with ion-exchange chromatography as sugar–borate complex to identify disaccharides and trisaccharides based on their elution time and co-elution with the authentic oligosaccharides.

Structural determination of the subfractions DP2b and DP3b

The subfraction DP2b was identified as 6-*O*-methyl-D-galactose on the basis of the results obtained from GLC and GC/MS analyses after conversion into the pertrimethylsilyl ether and the alditol acetate. As for DP3b, a portion of this subfraction was hydrolysed at 100°C for 16 h with 1 N sulphuric acid and then the hydrolysate was analysed with GLC after derivatization into pertrimethylsilyl ether and alditol acetate to detect D-galactose and 6-*O*-methyl-D-galactose (1:1.0 in molar ratio from GLC detector response). The absolute configuration was assigned as described elsewhere (Takano *et al.*, 1993). Another portion of DP3b was reduced at room temperature for 2 h with potassium borohydride. After addition of Amberlite IR-120 (H + form), the solution was evaporated to dryness and the resulting boric acid was removed by co-distillation with methanol. The syrup obtained was hydrolysed and analysed with GLC after trimethylsilylation to detect galactitol and 6-*O*-methyl-D-galactose (1:1.1). Yet another portion of DP3b was methylated by the method of Hakomori (1964), and the methylated product was similarly hydrolysed and analysed with GLC as partially methylated alditol acetate to detect 2,3,4,6-tetra-*O*-methylgalactose and 2,3,6-tri-*O*-methylgalactose derivatives (1:0.9). The subfraction DP3b was thus identified as 4-*O*-(6-*O*-methyl-D-galactopyranosyl)-D-galactose.

Alkaline treatment of the PS3

A 200 mg portion of PS3 was dissolved in water (50 ml), potassium borohydride (0.1 g) was added, and the solution was kept at room temperature for 18 h. To the solution were added 2 N potassium hydroxide (50 ml) and potassium borohydride (0.3 g). The mixture was heated at 80°C for 4 h and then neutralized with acetic acid. The reaction mixture was dialysed against water and lyophilized to give the alkali-treated PS3 termed PS3A (191 mg).

Partial methanolysis of PS3 and PS3A

Each specimen was methanolysed at 70°C for 2 h with 0.1 N methanolic hydrogenchloride. After neutralization with silver carbonate and removal of the resulting precipitate of silver chloride and excess silver carbonate by filtration, the methanolysate was analysed by a TSK-Gel Amide 80 (4.6 mm × 25 cm, Tosoh Corp.) on HPLC. The column was eluted with 86% acetonitrile at 25°C at a flow rate of 1 ml/min.

Methylation analysis of PS3 and PS3A

Methylation was carried out by the method of Isogai *et al.* (1985) and Ciucanu and Kerek (1984). After two methylation stages, the methylated product was hydrolysed by the double hydrolysis method (Stevenson and Furneaux, 1991), and then the hydrolysate was analysed with GLC after derivatization into a partially methylated alditol acetate mixture. As for D/L assignment of the partially methylated galactose residues, another portion of the methylated polysaccharide was hydrolysed, and the hydrolysate was derivatized into a trimethylsilylated L-2-octyl glycoside mixture, which was analysed by GC/MS as described elsewhere (Takano *et al.*, 1993). Since all the 3,6-anhydrogalactose residue in PS3 was detected as 2-*O*-methyl-3,6-anhydrogalactose after the methylation, D/L ratio of 2-*O*-methyl-3,6-anhydrogalactose was estimated from that of 3,6-anhydrogalactose in native PS3.

^{13}C-NMR spectrum

Proton-decoupled ^{13}C-NMR spectrum of 5% D_2O solution of PS3A at 50.3 MHz was recorded using a Varian XL-200 spectrometer operated at 80°C. Chemical shift values were measured relative to internal methanol (49.3 ppm).

RESULTS AND DISCUSSION

A funoran from *Gloiopeltis furcata* was extracted and fractionated in a similar manner to the previous study (Hirase and Watanabe, 1971). From the hot water extract of the algae, a sulphated polysaccharide was isolated as an insoluble cetylpyridinium salt. Stepwise extraction of the salt with 1, 2 and 4 M potassium chloride at room temperature afforded potassium salts of sulphated polysaccharide fractions, termed PS1, PS2 and PS3, respectively. The remaining cetylpyridinium salt was further extracted with 4 M potassium chloride at 100°C to recover a potassium salt of a sulphated polysaccharide fraction PS4. In agreement with the results of the previous study (Hirase and Watanabe, 1971), the major part (86%) of the funoran was recovered as the fraction PS4 with negative specific optical rotation; $[\alpha]_D^{20}$, −23°(*c* 0.5). This fraction was not further investigated, because the corresponding fraction has been already determined to be an almost ideal 6-sulphated agarose in the cases of the funorans from *G. furcata* (Hirase and Watanabe, 1971) and *G. complanata* (Takano *et al.*, 1995). In contrast to the main fraction PS4, the other minor polysaccharide fractions, PS1 (4% of total funoran), PS2 (3%) and PS3 (7%), exhibited positive or only slightly negative specific optical rotation (+13°, −7° and +23°, respectively) suggesting occurrence of polysaccharide other than the sulphated agaroid. In order to obtain structural information on polysaccharide within these minor fractions, the relatively abundant PS3 of relatively high positive optical rotation was further investigated.

Table 1. Compositions[a] of PS3 and PS3A from *Gloiopeltis furcata*

	PS3	PS3A
D-Gal	30.1 (100)	30.7 (100)
6-*O*-Methyl-D-Gal	2.3 (7)	2.9 (8)
3,6-Anhydro-D-Gal	0 (0)	6.1 (22)
L-Gal	5.4 (18)	0 (0)
3,6-Anhydro-L-Gal	2.7 (10)	10.0 (36)
Sulphate (as $-SO_3K$)	39.7 (179)	34.9 (155)

[a]Expressed as wt.%. The calculated values for the sugars are based on molecular weight of 'anhydro sugar unit'. The numbers in the parentheses express molar ratio (the value for D-Gal is taken as 100).

In agreement with the positive optical rotation, the total amount of D-galactose and its 6-*O*-methyl ether in PS3 exceeds that of L-galactose and 3,6-anhydro-L-galactose (Table 1). In addition, a considerably high sulphate content distinguished this fraction from the main funoran fraction so far reported (Hirase and Watanabe, 1971; Takano *et al.*, 1995).

The polysaccharide fraction PS3 was partially hydrolysed as previously described (Takano *et al.*, 1994). The neutral hydrolysate thereby obtained was gel-filtered with a Bio-Gel P-2 column. The resulting fractions corresponding to the disaccharide (DP2) and the trisaccharide (DP3) were semipreparatively chromatographed further on HPLC based on size-exclusion mode to obtain subfractions, DP2a, DP2b, DP3a and DP3b. By the subsequent ion-exchange chromatography of the subfractions DP2a and DP3a as sugar–borate complexes, the disaccharides 1, 2, 3, 4 and 5 and the trisaccharides 7, 8, 9 and 10 listed in Table 2 were identified on the basis of the elution times and coelution with the authentic oligosaccharides. The subfractions DP2b and DP3b were identified as 6-*O*-methyl-D-galactose and the disaccharide 6, respectively (for details, see Section 2). It is reasonable that the methoxyl group-containing monosaccharide and disaccharide were identified from the subtractions DP2b and DP3b, respectively, because methylated monosaccharides behave like higher oligosaccharides on gel filtration depending on the degree of methylation when Bio-Gel is employed as the matrix (Grellert and Ballou, 1973). Since the series of oligosaccharides attributable to both an agaroid backbone (1, 3, 5, 7 and 9) and a carrageenan backbone (2, 4, 6, 8 and 10) were obtained, PS3 is most likely to have contained both structural moieties.

Table 2. Disaccharides and trisaccharides isolated from partial hydrolysis product of PS3

Product identified	
1	β-D-Gal(1 → 4)L-Gal
2	β-D-Gal(1 → 4)D-Gal
3	α-L-Gal(1 → 3)D-Gal
4	α-D-Gal(1 → 3)D-Gal
5	β-D-Gal(1 → 4)3,6-anhydro-L-Gal
6	6-*O*-Methyl-D-Gal(1 → 3)D-Gal
7	α-L-Gal(1 → 3)β-D-Gal(1 → 4)L-Gal
8	α-D-Gal(1 → 3)β-D-Gal(1 → 4)D-Gal
9	β-D-Gal(1 → 4)α-L-Gal(1 → 3)D-Gal
10	β-D-Gal(1 → 4)α-D-Gal(1 → 3)D-Gal

In red algal galactan sulphates, the (1 → 4)-linked galactose residue carrying a sulphate group at O-6 is the biological precursor of its 3,6-anhydride (Rees, 1961a; Rees and Conway, 1962), and the similar conversion into the 3,6-anhydride occurs artificially under alkaline conditions (Rees, 1961b). The polysaccharide fraction PS3 was treated with alkali to afford a product termed PS3A. The treatment caused an increase in the 3,6-anhydrogalactose content, disappearance of the L-galactose residue and decrease in the sulphate content (Table 1). On partial methanolysis, PS3A afforded dimethylacetals of 4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-L-galactose (agarobiose), 4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-D-galactose (carrabiose) and 4-*O*-(6-*O*-methyl-β-D-galactopyranosyl)-3,6-anhydro-D-galactose (6-methylcarrabiose), while PS3 afforded only agarobiose dimethylacetal as disaccharide. The results indicates that all the L-galactose residues and the some D-galactose residues in PS3 are (1 → 4)-linked and carry the sulphate groups at O-6 to construct sulphated agaroid and carrageenan chains.

Location of the other sulphate groups can be determined by methylation studies. However, a problem is that the methylation is carried out under strong alkaline conditions, which may cause non-quantitative structural changes to yield a partially alkali-treated and methylated polysaccharide. The methylation analysis of PS3, thus, led to identification of larger amount of 2-*O*-methyl-3,6-anhydrogalactose than expected from the composition (Table 3). To obtain a quantitative result, PS3A was also subjected to the methylation analysis (Table 3). The resulting 6-*O*-methyl-D-galactose was interpreted to have arisen from a (1 → 3)-linked 2,4-disulphated galactose (and/or its 6-methyl ether) residue. This was confirmed by IR spectra of PS3 and PS3A, where a band at 850 cm^{-1} attributable to a sulphate group located at axial O-4 was observed besides a

Table 3. Methylation analysis of PS3 and PS3A

Product	Molar ratio	
	PS3	PS3A
2-Me-3,6-anhydro-L-Gal	32[a]	39[b]
2-Me-3,6-anhydro-D-Gal		26[b]
2,3,6-Me3-D-Gal	21	19
2,3-Me2-D-Gal	53[c]	0
2,3-Me2-L-Gal		0
2,4-Me2-Gal	47	44
6-Me-D-Gal	53	56

Values were calculated from peak area of GLC as partially methylated alditol acetates. Total value for 2,4-Me2-D-Gal and 6-Me-D-Gal attributable to (1 → 3)-linked residues is taken as 100.

[a]D/L configuration was not determined.

[b]D/L ratio was estimated from compostion of PS3A before methylation.

[c]D/L ratio was not determined, but detected as D/L admixture.

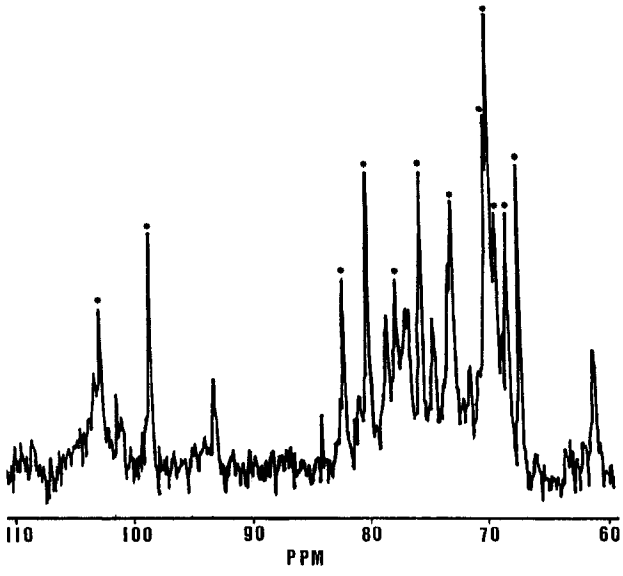

Fig. 1. Proton decoupled ^{13}C-NMR spectrum of PS3A. Peaks marked with * are attributed to signals from agarose 6-sulphate moiety (see text).

broad band around 820 cm^{-1} attributable to that located at an equatorial or a primary hydroxyl group. Alternatively, 6-*O*-methylgalactose might be attributed to a (1 → 4)-linked 2,3-disulphated residue. Based on this interpretation, however, the sum of methylated sugars to be attributed to (1 → 4)-linked residues exceeds the sum of those attributed to (1 → 3)-linked residues. This disagrees with the general structure of red algal galactans consisting of equimolar (1 → 4)- and (1 → 3)-linked residues. Moreover, since the (1 → 4)-linked 2,3-disulphated galactose residue should change into 2-sulphated 3,6-anhydrogalactose residue after the alkaline treatment, the sulphation at such positions should not cause formation of 6-*O*-methylgalactose on methylation. Other parts of the (1 → 3)-linked D-galactose residues are likely to have carried sulphate groups at O-6, since 2,4-di-*O*-methyl-D-galactose was identified. Some 6-*O*-monosulphated (1 → 4)-linked residues are likely constituent of PS3, because 2,3-dimethyl-D- and L-galactose derivatives were detected in the analysis of the intact PS3 as well as 3,6-anhydro-2-*O*-methyl-D- and -L-galactose derivatives in the analysis of PS3A. In addition, 2,3,6-tri-*O*-methyl-D-galactose is likely to have arisen from (1 → 4)-linked D-galactose residues bearing no substituent.

Although PS3 did not afford a good ^{13}C-NMR spectrum, PS3A gave a relatively well-resolved spectrum (Fig. 1). As shown in Table 4, the chemical shift values of the 12 peaks (* in Fig. 1) were identical to those for the signals from the main polysaccharide fraction of a funoran consisting of an agarose 6-sulphate structure (Takano *et al.*, 1995).

Accordingly, a part of PS3 is likely to have consisted of 6-sulphated agarose structure, and consequently, the (1 → 3)-linked D-galactose 6-sulphate residue would be a constituent of the agaroid chain in PS3. The other signals are likely to have arisen from the carrageenan moiety as assigned in Table 4 on the basis of the results from the so far reported chemical shift data for κ-carrageenan (Usov *et al.*, 1980). Owing to the effect of the sulphate group at O-2, the signal from C-2 of (1 → 3)-linked β-D-galactose 2,4-disulphate residue appeared at lower field in comparison with (1 → 3)-linked β-D-galactose 4-sulphate residue of κ-carrageenan, whereas the signals from C-1 and C-3 exhibited upfield shift. The position of C-1 resonance of 3,6-anhydro-α-D-galactose residue of PS3 (92.97 ppm) was lower than that of κ-carrageenan (95.1 ppm, Usov *et al.*, 1980). This anomalous upfield shift would be owing to the adjacent 2,4-disulphated D-galactose residue. In addition, the signal at 71.41 ppm would arise from C-6 of 6-*O*-methylated, 2,4-disulphated D-galactose residue, when considering the position of C-6 resonance of 6-*O*-methylated ι-carrabiose repeating unit (71.9 ppm, Chiovitti *et al.*, 1996).

Based on the previous evidence, the suggested disaccharide structure involved within PS3 is illustrated in Fig. 2. Concurrence of the agaroid chain and the carrageenan chain has been already reported in several red algal polysaccharides (Nunn *et al.*, 1971; Usov *et al.*, 1975; Usov and Barbakadze, 1978; Whyte *et al.*, 1985; Takano *et al.*, 1994). In the sense that the main polysaccharides of the known funorans are virtually sulphated agarose, there has been a reason why the algae of *Gloiopeltis* are classified into the 'agarophytes'. However, it would now become a delicate question whether the alga *G. furcata* is classified

Table 4. ^{13}C-NMR chemical shift values and peak assignments of PS3A and other polysaccharides

	C1	C2	C3	C4	C5	C6
→ 3)β-D-Gal-6-SO_3^- (1 → (agarose sulphate moiety in PS3A)	102.39	69.90	82.01	68.20	72.80	67.21
→ 3)β-D -Gal-6-SO_3^- (1 → (aggrose sulphate from *Gloiopeltis complanata* funoran)[a]	102.35	69.91	81.99	68.21	72.80	67.22
→ 4)3,6-anhydro-α-L-Gal(1 → (agarose sulphate moiety in PS3A)	98.18	69.63	79.97	77.59	75.51	69.21
→ 4)3,6-anhydro-α-L-Gal(1 → (agarose sulphate from *Gloiopeitis complanata* funoran)[a]	98.16	69.64	79.96	77.58	75.51	69.23
→ 3)β-D-Gal-2,4-SO_3^- (1 → (carrageenan moiety in PS3A)	100.78	76.54	78.36	73.08	74.49	61.13
→ 3)β-D-Gal-4-SO_3^- (1 → (κ-carrageenan)[b]	102.5	69.9	78.8	74.0	74.8	61.3
→ 4)3,6-anhydro-α-D-Gal(1 → (carrageenan moiety in PS3A)	92.97	(69.92)[c]	78.42	(79.97)[c]	76.76	68.73
→ 4)3,6-anhydro-α-D-Gal(1 → (κ-carrageenan)[b]	95.1	69.7	78.3	79.1	76.8	69.4

[a]Chemical shift values and assignment based on Takano et al. (1995).
[b]Chemical shift values and assignment based on Usov et al. (1980).
[c]Overlapped with other signal.

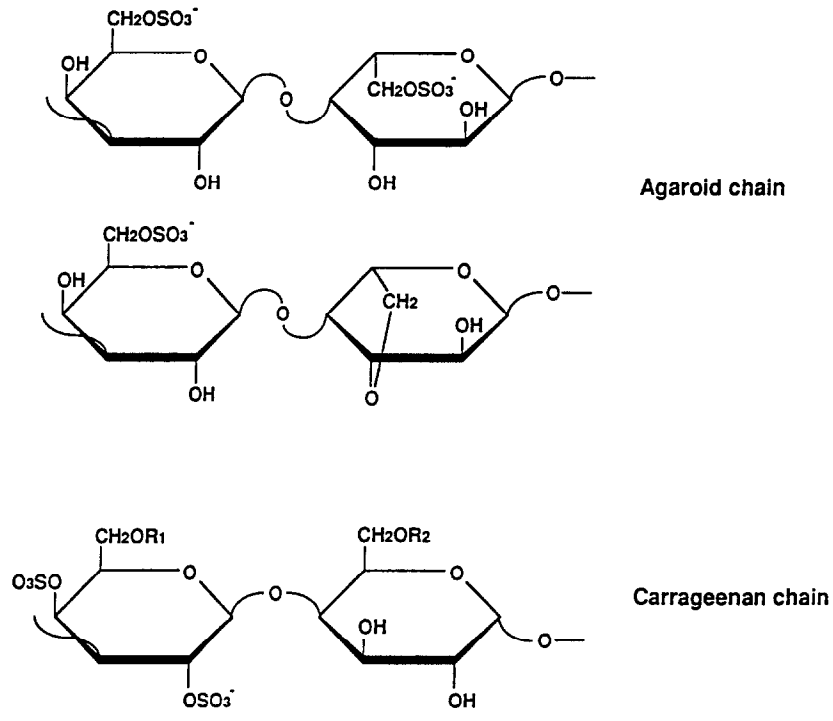

Fig. 2. Proposed structures of agaroid chain and carrageenan chain in PS3. R_1 = H, CH_3; R_2 = SO_3^-, H.

into the agarophyte or the carrageenophyte, since both agaroid chain and carrageenan chain occur simultaneously in PS3. The similar suspicion may be also aroused against the closely related red alga *G. complanata*, from which a polysaccharide fraction with highly positive optical rotation has been isolated (Takano *et al.*, 1995).

The family Endocladiaceae including *G. furcata* is a member of the former order Cryptonemiales, which was proposed to be merged with the Gigartinales into the newly defined single order Gigartinales because of similarities in several taxonomical characteristics (Kraft and Robins, 1985). However, this merger may cause controversy (Chopin *et al.*, 1994). According to a phylogeny based on sequence analyses of ribulose-1,5-biphosphate carboxylase gene (Freshwater *et al.*, 1994), the merged Gigartinales have appeared polyphyletic. In this phylogeny, some traditional gigartinalen families including carrageenophytes such as the Gigartinaceae, the Petrocelidaceae, the Phyllophoraceae, the Solieriaceae and the Furcellariaceae make a distinct group, while traditional cryptonemialen families, the Halymeniaceae and the Endocladiaceae, are closely related to the orders Rhodymeniales and Bonnemaisoniales, respectively. The phylogenic tree agrees neither with the traditional classification nor with the revised one. With regard to classification of the phycocolloids, algae of *Grateloupia* belonging to the Halymeniaceae (Usov *et al.*, 1975; Usov and Barbakadze, 1978) and *Lomentaria catenata* Harvey belonging to the Rhodymeniales (Takano *et al.*, 1994) produce agaroid–carrageenan hybrids containing no or only small amount of 3,6-anhydrogalactose. As for the Endocladiaceae, agaroid-carrageenan hybrids containing relatively large amounts of the 3,6-anhydride were isolated from *Endocladia muricata* (Post. et Ruprecht) J.G. Ag. (Whyte *et al.*, 1985) and *Gloiopeltis furcata* in the present report. These results for the phycocolloid structure may agree with the observation from the gene phylogeny. Extensive structural studies of red algal polysaccharides may complement the traditional taxonomy, and may also construct molecular bases for physico-chemical and biological properties.

REFERENCES

Chiovitti, A., Liao, M.-L., Kraft, G. T., Munro, S. L. A., Craik, D. J., Bacic, A. (1996) Cell wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): highly methylated carrageenans from the genus *Rhabdonia Bot. Mar.*, **39**, 47–59.

Chopin, T., Hanisak, M. D., Craigie, J. S. (1994) Carrageenans from *Kallymenia westii* (Rhodophyta) with a review of phycocolloids produced by the Cryptonemiales *Bot. Mar.*, **37**, 433–444.

Ciucanu, I., Kerek, F. (1984) A simple and rapid method for the permethylation of carbohydrates *Carbohydr. Res.*, **131**, 209–217.

Freshwater, D. W., Fredericq, S., Butler, B. S., Hommersand, M. M., Chase, M. W. (1994) A gene phylogeny of the red algae (Rhodophyta) based on plastid rbcL *Proc. Natl Acad. Sci. USA*, **91**, 7281–7285.

Grellert, E., Ballou, E. (1973) Separation of methyl ethers of sugars by gel filtration *Carbohydr. Res.*, **30**, 218–219.

Hakomori, S. (1964) Rapid permethylation of glycolipids and polysaccharides, catalysed by methylsulfinyl carbanion in dimethyl sulfoxide *J. Biochem. (Tokyo)*, **55**, 205–208.

Hirase, S., Araki, C., Ito, T. (1958) Isolation of agarobiose derivative from the mucilage of *Gloiopeltis furcata Bull. Chem. Soc. Jpn*, **31**, 428–431.

Hirase, S., Watanabe, K. (1971) Fractionation and structural investigation of funoran *Proc. Int. Seaweed Symp.*, **7**, 451–454.

Hirase, S., Watanabe, K., Irei, T. (1976). The structure of a sulfated polysaccharide from the red seaweed *Melistotheca papulosa*. In *Abstr. 8th Int. Symp. Carbohydr. Chem.*, p. 56.

Isogai, A., Ishizu, A., Nakano, J., Eda, S., Kato, K. (1985) A new facile methylation method for cell-wall polysaccharides *Carbohydr. Res.*, **138**, 99–108.

Kraft, G. T., Robins, P. (1985) Is the order Cryptonemiales defensible? *Phycologia*, **24**, 67–77.

Lawson, C. J., Rees, D. A., Stancioff, D. J., Stanley, N. F. (1973). Carrageenans. Part VIII. Repeating structures of galactan sulphates from *Furcellaria fastigia, Gigartina canaliculata, Gigartina chamissoi, Eucheuma spinosum, Eucheuma isiforme, Eucheuma uncinatum, Aghardihiella tenera, Pachymenia hymantophora*, and *Gloiopeltis cervicornis*. *J. Chem. Soc. (Perkin I)*, 2177–2182.

Nunn, J. R., Parolis, H., Russell, I. (1971) Sulphated polysaccharides of the *Solieriaceae* family. Part I. A polysaccharide from *Anatheca dentata Carbohydr. Res.*, **20**, 205–215.

Painter, T. J. (1983). Algal polysaccharides. In G. O. Aspinall (Ed.), *The Polysaccharides*, Vol. 2 (pp. 195–285). New York: Academic Press.

Penman, A., Rees, D. A. (1973). Carrageenans. Part IX. Methylation analysis of galactan sulfates from *Furcellaria fastigiata, Gigartina canaliculata, Gigartina chamissoi, Gigartina atropurpurea, Ahnfeltia durvellaei, Gymnogungrus furcellatus, Eucheuma isiforme, Eucheuma uncinatum, Aghardhiella tenera, Pachymenia hymantophora* and *Gloiopeltis cervicomis*. Structure of ξ-carrageenan. *J. Chem. Soc. (Perkin I)*, 2182–2187.

Rees, D. A. (1961) Enzymic synthesis of the 3,6-anhydro-L-galactose within porphyran from L-galactose-6-sulfate units *Biochem. J.*, **81**, 347–352.

Rees, D. A. (1961b). Estimation of the relative amounts of isomeric sulphate esters in sulphated polysaccharides. *J. Chem. Soc.*, 5168–5171.

Rees, D. A., Conway, E. (1962) The structure and biosynthesis of porphyran: a comparison of some samples *Biochem. J.*, **84**, 411–416.

Schachat, R. E., Glicksman, M. (1959). Some lesser known seaweed extracts. In R. L. Whistler (Ed.), *Industrial Gums* (pp. 135–191). New York: Academic Press.

Stevenson, T. T., Furneaux, R. H. (1991) Chemical methods for the analysis of sulphated galactans from red algae *Carbohydr. Res.*, **210**, 277–298.

Takano, R. K., Hayashi, K., Hara, S., Hirase, S. (1993) Assignment of the absolute configuration of partially methylated galactoses by combined gas–liquid chromatography–mass spectrometry *Biosci. Biotech. Biochem.*, **57**, 1195–1197.

Takano, R., Nose, Y., Hayashi, K., Hara, S., Hirase, S. (1994) Agarose–carrageenan hybrid polysaccharides from *Lomentaria catenata Phytochemistry*, **37**, 1615–1619.

Takano, R., Hayashi, K., Hara, S., Hirase, S. (1995) Funoran from the red seaweed, *Gloiopeltis complanata*: polysaccharides with sulfated agarose structure and its precursor structure *Carbohydr. Polym.*, **27**, 305–311.

Usov, A. I., Barbakadze, V. V. (1978) Polysaccharides of algae. XXVII. Partial acetolysis of the sulfated galactan from the red seaweed *Grateloupia divaricata* Okam *Bioorg. Khim.*, **4**, 1107–1115.

Usov, A. I., Miroshinikova, L. I., Barbakadze, V. V. (1975) Polysaccharide of algae. XVII. Water-soluble polysaccharide of the red algae *Grateloupia divaricata* Okamura and *Grateloupia turuturu* Yamada *Zh. Obsh. Khim.*, **45**, 1618–1624. English translation, 1583–1587.

Usov, A. I., Yarotsky, S. V., Shashkov, A. S. (1980) ^{13}C-NMR spectroscopy of red algal galactans *Biopolymers*, **19**, 977–990.

Whyte, J. N. C., Hosford, S. P. C., Englar, J. R. (1985) Assignment of agar or carrageenan structures to red algal polysaccharides *Carbohydr. Res.*, **140**, 336–341.

Yaphe, W. (1960) Colorimetric determination of 3,6-anhydrogalactose and galactose in marine algal polysaccharides *Analyt. Chem.*, **32**, 1327–1330.