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A pyruvated carrageenan from Australian specimens of the red alga *Sarconema filiforme*[1]

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Abstract

The carrageenan from two Australian specimens of the red alga *Sarconema filiforme* was shown by a combination of compositional analyses, linkage analysis, and Fourier transform infrared and ^{13}C nuclear magnetic resonance spectroscopy to be composed predominantly of a hybrid or mixture of carrabiose 2-sulfate (the repeating unit of α-carrageenan), carrabiose 2,4′-disulfate (the repeating unit of ι-carrageenan), and the pyruvated unit 4′,6′-*O*-(1-carboxyethylidene)carrabiose 2-sulfate.

Keywords: Carrageenan; Galactans, sulfated; Gigartinales; Polysaccharides, algal; Rhodophyta; *Sarconema filiforme*; Solieriaceae; Structure determination

1. Introduction

The cosmopolitan red algal genus *Sarconema* contains two species, *Sarconema scinaioides* Børgesen and *S. filiforme* (Sonder) Kylin, both of which occur through the Indo-Pacific tropics and subtropics [2,3]. One of these species, *S. filiforme*, is distributed along the western and southern coasts of Australia, but occurs in greatest concentration in the west [3]. Thalli of *S. filiforme* grow up to 30 cm and are firm and regularly subdichotomously branched.

The sulfated extracellular polysaccharides of specimens of *S. filiforme* from Tanzania and India have been the focus of two previous studies [4,5]. In these studies, it was concluded that *S. filiforme* produced ι-carrageenan [composed of carrabiose 2,4′-disulfate units, where the term "carrabiose" refers to the repeating disaccharide 3′-linked *O*-β-D-Gal*p*-(1→4)-3,6-anhydro-α-D-Gal*p*] based on the polysaccharides' IR spectra, positive optical rotation, insolubility in KCl solution, and ability

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[1] Cell-wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta). For previous instalment, see ref. [1].

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to gel. However, the sulfate contents reported for the polysaccharides in both studies were unusually low, ca. 18.3% w/w for the Tanzanian sample [4] and 21.35% w/w for the Indian specimen [5], by comparison with ι-carrageenans from various other sources which typically contain >30% sulfate [6]. The availability of material from two Australian specimens of *S. filiforme* permitted an investigation of their sulfated polysaccharides.

2. Experimental

Algal samples.—The samples of *S. filiforme* were collected: (A) as drift from Cottesloe Beach, Western Australia on 23 May 1993 by J. Huisman (WELTA 21170); and (B) on 2 February 1994 by G. Kraft, G. Saunders, and A. Millar at 15–17 m depths using SCUBA from Roe Reef, Rottnest Is, Western Australia (MELU, K9696).

Extraction and treatment of the polysaccharides.—Sample A (5 g) was soaked in aqueous $NaHCO_3$ (0.05 M, 300 mL) for 1.5 h then heated in the same solution for 4 h at 90 °C. After this time the extract was filtered and the weed residue (which was still relatively intact) was re-extracted in the same volume of fresh solution for a further 2 h at 90 °C. The extract was again filtered and kept separately. The weed residue was then extracted further for 10 min at 120 °C. Each of the three extracts was treated with amyloglucosidase to digest starch [7], purified by filtration through a Whatman GF/D glass microfibre filter and dialysis (distilled water×3), then lyophilised to give polysaccharide samples SfA-1, SfA-2, and SfA-3, respectively. Polysaccharides were extracted from ground-up meal of sample B using hot water, clarified, and precipitated with 2-propanol as described previously [8] to give SfB. Starch was removed from the preparation by amyloglucosidase digestion [8]. The sulfated galactans in SfB were alkali-modified as described by Craigie and Leigh [9] to give SfB-AM.

Sulfate analysis.—The sulfate content of SfB-AM was determined by the method of Tabatabai [10] as modified by Craigie et al. [11].

Pyruvate analysis.—The pyruvate contents of samples SfA-1, SfA-2, and SfA-3 were determined using the 2,4-dinitrophenylhydrazine method of Nelson et al. [12]. The pyruvate content of SfB-AM was determined by the enzymatic method of Duckworth and Yaphe [13].

Constituent sugar analysis.—Constituent sugars in the various polysaccharide samples were determined quantitatively as their peracetylated alditols derived by reductive hydrolysis followed by acetylation as described by Stevenson and Furneaux [14]. The alditol acetates prepared from SfA-1, -2, and -3, and from SfB-AM were separated by gas chromatography (GC) and identified by their retention times relative to *myo*-inositol hexaacetate as described previously (refs. [7,15], respectively). Quantification relied upon molar response factors (RFs) measured directly using standards [7], although in the case of sample SfB-AM the RF of 3,6-anhydrogalactose (AnGal) was derived by analysis of commercially available κ-carrageenan from "*Eucheuma cottonii*" [presumably *Kappaphycus alvarezii* (Doty) Doty] known to contain Gal and AnGal in approximately equimolar proportions [14].

Linkage analysis.—The polysaccharide preparations were converted into their corresponding Me_2SO-soluble triethylammonium salt forms and methylated essentially by the protocol of Stevenson and Furneaux [14], except that for SfB-AM a NaOH-Me_2SO suspension was used to generate the alkoxide [16]. Permethylated alditol acetates (PMAAs) were produced in each case by reductive hydrolysis and acetylation [14]. In the case of SfA-1, -2, and -3, the PMAAs were separated by GC using a SP-2330 (Supelco, USA) capillary column with flame-ionisation detection, and identified and quantified by comparison with authentic standards [7]. In the case of SfB-AM, separation was effected by GC on a BPX70 (SGE, Australia) capillary column with detection by electron impact ionisation-mass spectrometry (MS), with the PMAAs being identified by their mass spectra and their retention times relative to *myo*-inositol hexaacetate [17]. The permethylated species from SfB-AM were quantified directly from the reconstructed ion chromatogram with the assumption that the ion intensity of each of the PMAAs was the same on a molar basis, except for the product derived from AnGal 2-sulfate, the RF of which was determined as described above. The location(s) of the naturally-occurring methyl group(s) on Gal*p* residues in SfA-3 were determined by trideuteriomethylation of the polysaccharide [14], reductive hydrolysis, acetylation, and analysis of the resulting partially trideuteriomethylated PMAAs by GC-MS according to the method of Falshaw and Furneaux [7].

Infrared spectroscopy.—A polysaccharide film for Fourier transform infrared (FTIR) spectroscopy was prepared from SfB-AM [18] and the FTIR spectrum was recorded on a Perkin–Elmer series 2000 FTIR spectrometer in transmittance mode (8 scans, collected at a resolution of 4 cm^{-1}).

^{13}C NMR spectroscopy.—The polysaccharide samples were dissolved in either 1:1 D_2O–H_2O or D_2O (3% w/v) for ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The proton-decoupled ^{13}C NMR spectra were recorded at 80 °C. For SfA-1, -2, and -3, a Bruker AC 300 spectrometer (operating at 75.5 MHz) was used with a 0.885 s acquisition time, 0.5 s delay time, and 80° pulse width. For SfB-AM, a Bruker ARX500 spectrometer (operating at 125.8 MHz) was used with a spectral width of 27.8 kHz, 45° pulse (8.0 ms), an acquisition time of 0.29 s, and a relaxation delay of 1.0 s for approximately 60,000 scans. Chemical shifts are quoted relative to internal Me_2SO at 39.6 ppm.

3. Results and discussion

Yield and constituent sugar analysis.—Sample A was sequentially extracted with dilute $NaHCO_3$ solution (twice at 90 °C, and finally at 120 °C) and the three preparations treated with amyloglucosidase to remove floridean starch. The yields of recovered material after this step were 6% for SfA-1, 7% for SfA-2, and 13% for SfA-3, respectively, from air-dried weed (total yield 26%). The yield of the crude preparation extracted from sample B with water at 95 °C was 31% w/w of the dry weight of the seaweed meal. These yield values are within the range reported previously for *S. filiforme* (25–35% w/w [4,5]). The extract from sample B was subsequently treated with amyloglucosidase and alkali-modified (SfB-AM) prior to chemical and spectroscopic analyses. The polysaccharide preparations were all rich in pyruvate: 4.0% for SfA-1, 5.8% for SfA-2, 5.7% for SfA-3, and 5.6% for SfB-AM (w/w as CH_3COCO_2H). SfB-AM was also rich in sulfate (25.0% w/w, as SO_3Na).

Constituent sugar analysis showed that the polysaccharide preparations contained mainly galactans (Table 1). The compositions of all the extracts were very similar, with the major species present being Gal (determined as 1,2,3,4,5,6-hexa-*O*-acetylgalactitol) and AnGal (as 1,2,4,5-tetra-*O*-acetyl-3,6-anhydrogalactitol). The SfA extracts contained lower levels of AnGal than SfB-AM which indicates the presence of "precursor" (4-linked Gal*p* 6-sulfate and/or Gal*p* 2,6-disulfate) residues. Such residues commonly occur in red algal polysaccharides. They are often referred to as "precursors" because they are considered the biosynthetic precursors of 4-linked AnGal*p* residues and because the same conversion, involving intramolecular displacement of the 6-sulfate group by O-3, can also be effected by hot aqueous alkali. The SfA extracts also contained small amounts of 2,4-Me_2Gal and 4-MeGal (Table 1).

Table 1
Constituent sugars of the native (SfA-1, -2, and -3) and alkali-modified (SfB-AM) polysaccharide preparations from *Sarconema filiforme*

Constituent monosaccharide[a]	Sample (mol%)			
	SfA-1	SfA-2	SfA-3	SfB-AM
AnGal	20	19	15	32
6-MeGal	3	2	1	1
2,4-Me_2Gal	1	2	3	—
4-MeGal	1	—	3	—
Gal	63	68	72	62
Xyl	4	5	3	2
Ara	2	1	—	—
Man	2	—	—	—
Glc	4	3	3	3

Constituent monosaccharides: AnGal = 3,6-anhydrogalactose; 6-MeGal = 6-*O*-methylgalactose; 2,4-Me_2Gal = 2,4-di-*O*-methylgalactose; 4-MeGal = 4-*O*-methylgalactose; Gal = galactose; Xyl = xylose; Ara = arabinose; Man = mannose; Glc = glucose.

[a] AnGal determined as 1,2,4,5-tetra-*O*-acetyl-3,6-anhydrogalactitol; Gal as galactitol hexaacetate, etc.

FTIR spectroscopy.—The FTIR spectrum of the alkali-modified polysaccharide, SfB-AM (Fig. 1)

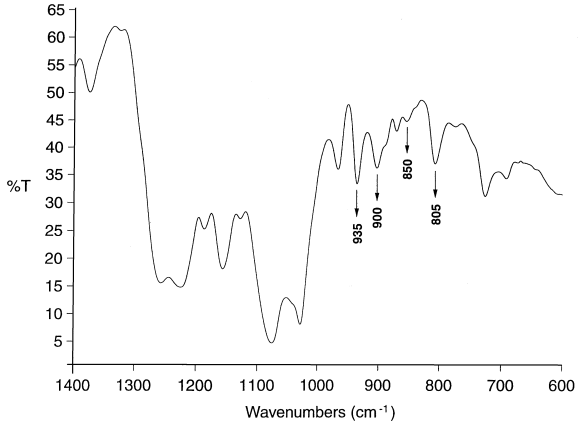

Fig. 1. FTIR spectrum of the alkali-modified polysaccharide preparation (SfB-AM) from *Sarconema filiforme*.

displayed a strong absorption band at 1240 cm^{-1} (general for sulfate ester [19]). The diagnostic region (940 to 800 cm^{-1}) of the FTIR spectrum resembled those of α-carrageenan (composed of carrabiose 2-sulfate units [20,21]) and the highly pyruvated carrageenans from *Callophycus* spp. [22]. Characteristic peaks included those at 935 cm^{-1}, which demonstrated the presence of AnGal residues, and 805 cm^{-1}, which indicated that the AnGal residues were substituted with axial sulfate ester at O-2 [19]. The band at 900 cm^{-1} suggested that the polysaccharide contained unsulfated, 3-linked Gal residues [6,18,20,23,24] and/or unsulfated, 3-linked Gal*p* residues bearing pyruvate acetal substitution [22]. A small peak was observed at 850 cm^{-1}, indicating the presence of axial sulfate ester at O-4 of some 3-linked Gal residues [19]. The intensity of this peak was considerably weaker, however, than the corresponding peak for typical ι-carrageenan. In the IR spectra of ι-carrageenan [6,19], the intensity of the 850 cm^{-1} peak is approximately equal to that of the 805 cm^{-1} peak.

Linkage analysis.—The results of the linkage analyses of the native polysaccharides SfA-1, -2, and -3, and the alkali-modified polysaccharide SfB-AM from *S. filiforme* are summarised in Table 2. For each polysaccharide preparation, the major components were 2,4-linked AnGal*p*, 3-linked Gal*p*, 3,4-linked Gal*p*, and 3,4,6-linked Gal*p*. The 2,4-linked AnGal*p* was interpreted (in conjunction with FTIR data for SfB-AM) as 4-linked AnGal*p* 2-sulfate. The 3,4-linked Gal*p* was interpreted mainly as 3-linked Gal*p* 4-sulfate and its abundance in the linkage analysis data for SfB-AM suggests that about a third of the 3-linked Gal*p* residues are sulfated at O-4. The intensity of the band at 850 cm^{-1} in the FTIR spectrum (Fig. 1), however, is less than one third that at 850 cm^{-1}. An anomalously low IR intensity at 805 cm^{-1} was also observed for the polysaccharide preparations from Burmese and Thai specimens of *Catenella nipae* Zanardini [21], which have FTIR spectra similar to that of the *S. filiforme* polysaccharide, but were shown by ^{13}C NMR and linkage analyses to contain 20–30 mol% 3-linked Gal*p* 4-sulfate [21]. Comparable observations were also made of the polysaccharide preparations from Australian *Erythroclonium* species [1]. In accord with the high pyruvate content of the *S. filiforme* preparations, the large amounts of 3,4,6-linked Gal*p* detected in the linkage analysis data were potentially derived from 3-linked Gal*p* bearing pyruvate acetal substitution in the form of 4,6-*O*-(1-carboxyethylidene)-Gal*p* [22,25]. This interpretation was confirmed by ^{13}C NMR spectroscopy (see below). Minor amounts of 4- and 3,6-linked Gal*p* and terminal Xyl*p* were also detected. The level of 2,4-linked AnGal*p* was lower for the SfA extracts compared with SfB-AM indicating the presence of precursors, consistent with constituent sugar analysis. The precursor residue of 4-linked AnGal*p* 2-sulfate is 4-linked Gal*p* 2,6-disulfate, and its presence in SfA was supported by the occurrence of 2,4,6-linked Gal*p* in the SfA extracts. The levels of 3-linked Gal*p* and 3,4,6-linked Gal*p* were lower in all the SfA extracts compared with SfB-AM and, in addition, terminal galactosyl residues and higher levels of 3,6-linked Gal*p* were found in the SfA extracts. The levels of 3,6-Gal*p* are similar to the total amounts of 2,4-Me_2Gal and 4-MeGal seen in the constituent sugar analysis data, which suggests that these methylated species may occur as terminal Gal*p* residues attached to the 6-position of 3-linked Gal*p* residues. The locations of these naturally occurring methyl groups in terminal Gal*p* residues in SfA were confirmed by trideuteriomethylation of a sample of SfA-3 prior to the preparation of alditol acetates. GC-MS then revealed the presence of terminal Gal*p* residues as the chromatographically indistinguishable 1,5-di-*O*-acetyl-4-*O*-methyl-2,3,6-tri-*O*-trideuteriomethylgalactitol

Table 2
Linkage analysis of constituent sugars of the native (SfA-1, -2, and -3) and alkali-modified (SfB-AM) polysaccharide preparations from *Sarconema filiforme*

Constituent monosaccharide[a]	Deduced linkage[b]	Sample (mol%)			
		SfA-1	SfA-2	SfA-3	SfB-AM
AnGal*p*	2,4-	28	25	22	34
Gal*p*	Terminal	1	2	2	—
	3-	11	16	15	22
	4-	2	2	2	1
	3,4-	39	27	25	19
	3,6-	1	2	5	tr[c]
	3,4,6-	14	19	19	24
	2,3,4-	—	—	1	—
	2,4,6-	4	6	7	—
	2,3,4,6-	—	—	1	—
Xyl*p*	Terminal	tr	1	1	tr

[a] AnGal*p* = 3,6-anhydrogalactopyranose; Gal*p* = galactopyranose; Xyl*p* = xylopyranose.
[b] 2,4-Linked AnGal*p* deduced from 1,2,4,5-tetra-*O*-acetyl-3,6-anhydrogalactitol, 3-linked Gal*p* deduced from 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol, etc.
[c] tr = trace (< 0.8%).

and 1,5-di-*O*-acetyl-2,4-di-*O*-methyl-3,6-di-*O*-trideuteriomethylgalactitol in the ratio 3:7. The amount of terminal-Gal*p* observed is less than expected from constituent sugar analysis, but the tetra-*O*-(trideuterio)methylated species are somewhat volatile and may be lost during preparation. The structural differences observed in the polysaccharides obtained from the two specimens of *S. filiforme* may be due to varietal, seasonal, or environmental differences between the two algal specimens. The lower levels of 3-linked Gal*p* and 3,4,6-linked Gal*p* in SfA-1 compared to SfA-2 and SfA-3 suggest that ι-carrageenan may be easier to extract than either α- or pyruvated α-carrageenans.

NMR spectroscopy.—The proton-decoupled ^{13}C NMR spectrum of the alkali-modified preparation from *S. filiforme* was obtained (Fig. 2) and the signal assignments are summarised in Table 3. The ^{13}C NMR spectrum showed that the polysaccharide preparation was a hybrid or mixture of carrabiose 2,4′-disulfate of ι-carrageenan, carrabiose 2-sulfate of α-carrageenan, and 4′,6′-*O*-(1-carboxyethylidene)carrabiose 2-sulfate (Fig. 3). The signals were assigned by comparison with reported data [21,22,26–28]. Diagnostic resonances observed for carrabiose 2,4′-disulfate were C-1 of AnGal*p* 2-sulfate at 92.2 ppm and C-4 of 3-linked Gal*p* 4-sulfate at 72.2 ppm. Diagnostic resonances for carrabiose 2-sulfate were C-1 of AnGal*p* 2-sulfate at 94.7 ppm and C-3 and C-4 of 3-linked Gal*p* at 82.1 ppm and 66.9 ppm, respectively.

Diagnostic resonances for the pyruvated disaccharide included those for the carboxyl, acetal, and methyl carbons of the pyruvate acetal substituent at 175.9 (not shown in Fig. 2), 101.7, and 25.5 ppm (not shown), respectively, C-1 of the 4-linked residue at 91.4 ppm, and C-1, C-2, C-4, C-5, and C-6 of the pyruvated, 3-linked residue at 102.0, 69.2, 67.5, 66.7, and 65.6 ppm, respectively. The ^{13}C NMR spectra of SfA-1, -2, and -3 (not shown) were less well resolved than that for SfB-AM but the same pattern of signals was observed. In addition, a weak signal at 104.9 ppm was observed in each of the SfA spectra. This signal corresponds to the presence of small amounts of precursor residues, in agreement with constituent sugar and linkage analyses, and is attributable, for example, to C-1 of 3-linked Gal*p* 4-sulfate residues which have neighbouring 4-linked Gal*p* 2,6-disulfate residues [29]. Further small signals at 103.9 and 109.2 ppm were also observed. These may correspond to C-1 of 3-linked Gal*p* or 3-linked 4,6-*O*-(1-carboxyethylidene)-Gal*p* residues adjacent to 4-linked Gal*p* 2,6-disulfate residues. The distribution of the 4-linked Gal*p* 2,6-disulfate residues in the *S. filiforme* carrageenan remains uncertain, however, because the C-1 signals of the 4-linked precursor residues themselves were not unambiguously observed and ^{13}C NMR data for the "precursor disaccharide units" of carrabiose 2-sulfate and 4′,6′-pyruvated carrabiose 2-sulfate are lacking.

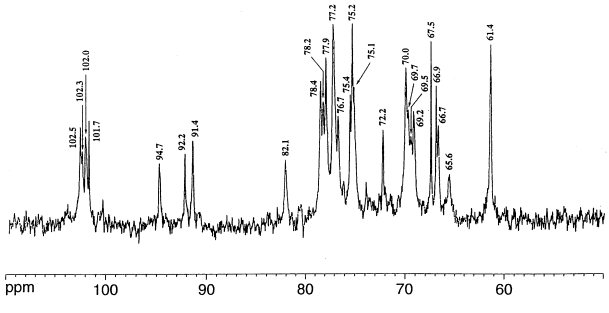

Fig. 2. Proton-decoupled ^{13}C NMR spectrum of the alkali-modified polysaccharide preparation (SfB-AM) from *Sarconema filiforme*. The resonances arising from the methyl (at 25.5 ppm) and carboxyl (at 175.9 ppm) groups of the pyruvate acetal substituent are not shown.

The ^{13}C NMR data confirmed that the 3,4-linked Gal*p* and the 3,4,6-linked Gal*p* obtained in the linkage analysis (Table 2) were essentially derived from 3-linked Gal*p* 4-sulfate and 3-linked 4,6-*O*-(1-carboxyethylidene)-Gal*p*, respectively. The relative intensities of the three C-1 signals of the D-AnGal*p* 2-sulfate residues in the SfB-AM spectrum (Fig. 2) indicated that the three corresponding repeating units occurred in approximately equal proportions in this sample. This observation is consistent with linkage analysis, which showed that 3-linked Gal*p*, 3,4-linked Gal*p*, and 3,4,6-linked Gal*p* occurred in similar proportions in SfB-AM (in the range 19–25 mol% each). By contrast, the signal at 92.2 ppm was the most intense in each of the SfA NMR spectra, although the intensity of the signals at 91.4 and 94.7 ppm, as well as that at 82.1 ppm, increased from SfA-1 to SfA-3. In agreement with the NMR data, the level of 3,4-linked Gal*p* (Table 2) was the highest of the Gal*p* residues in the SfA samples while the proportions of 3-linked Gal*p* and 3,4,6-linked Gal*p* increased from SfA-1 to SfA-3. As with the carrageenans from *Callophycus* spp. [22], both the unsubstituted, 3-linked Gal*p* and the

Table 3
Assignments of resonances[a] observed in the ^{13}C NMR spectrum of the alkali-modified polysaccharide preparation (SfB-AM) from *Sarconema filiforme*[b]

		Carbon atom								
								Pyruvate acetal		
Repeating unit	Sugar	C-1	C-2	C-3	C-4	C-5	C-6	Methyl	Acetal	Carboxyl
G4S-DA,2S	3-linked:	102.3	69.5	77.2[c]	72.2	75.1	61.4			
	4-linked:	92.2	75.2[d]	77.9	78.4	77.2[c]	70.0			
G-DA,2S	3-linked:	102.5	69.7	82.1	66.9	75.2[d]	61.4			
	4-linked:	94.7	75.4	78.2	78.4	77.2[c]	70.0			
GP-DA,2S	3-linked:	102.0	69.2	76.7	67.5	66.7	65.6	25.5	101.7	175.9
	4-linked:	91.4	75.2[d]	77.9	78.4	77.2[c]	70.0			

Repeating units: G4S-DA,2S = carrabiose 2,4′-disulfate of ι-carrageenan; G-DA,2S = carrabiose 2-sulfate of α-carrageenan; GP-DA,2S = 4′,6′-*O*-(1-carboxyethylidene)carrabiose 2-sulfate.
[a] Chemical shifts in ppm referenced to Me_2SO at 39.6 ppm.
[b] Spectrum of a 3% w/v solution in D_2O recorded at 80 °C.
[c,d] Coincident resonances.

4,6-pyruvated, 3-linked Gal*p* residues probably contributed to the intense absorption at 900 cm^{-1} in the FTIR spectrum of the alkali-modified *S. filiforme* polysaccharide (SfB-AM).

The ^{13}C NMR spectroscopy clearly demonstrated that the alkali-modified galactans from *S. filiforme* were essentially composed of repeating units containing D-AnGal*p* 2-sulfate as the 4-linked residue. The ratio of Gal:AnGal estimated in constituent sugar and linkage analyses was considerably less, however, than the 1:1 expected for such galactans (see Table 2 and discussion of results above). This is most likely due to the incomplete recovery of AnGal by reductive hydrolysis. It was shown previously for ι- and α-carrageenans, which contain AnGal*p* 2-sulfate as the major 4-linked residue, that the recovery of AnGal is typically less than that for other red algal galactans that lack sulfate ester substitution at O-2 of the AnGal residues, such as κ-carrageenan or agarose [14,21]. The occurrence of high levels of pyruvate acetal substitution in the *S. filiforme* polysaccharides are likely to further adversely affect the recovery of AnGal (for a discussion, see ref. [22]).

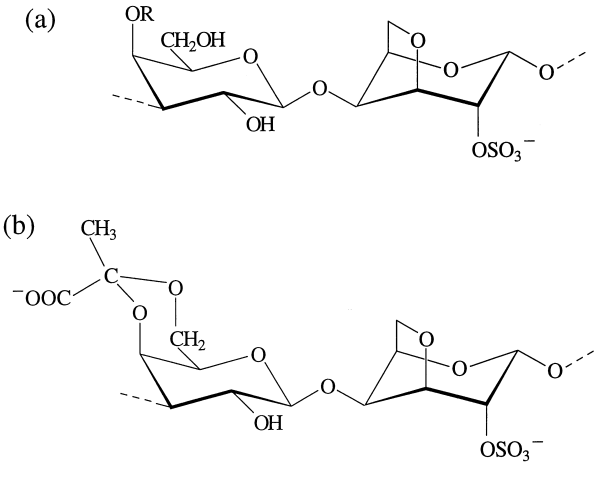

Fig. 3. Proposed repeating disaccharide units in the carrageenan from *Sarconema filiforme*: (a) = carrabiose 2-sulfate of α-carrageenan (R = H); carrabiose 2,4′-disulfate of ι-carrageenan (R = SO_3^-); (b) 4′,6′-*O*-(1-carboxyethylidene)carrabiose 2-sulfate.

4. Conclusion

The three major repeating units of the polysaccharides from two specimens of *S. filiforme* were carrabiose 2-sulfate of α-carrageenan, carrabiose 2,4′-disulfate of ι-carrageenan, and 4′,6′-*O*-(1-carboxyethylidene)carrabiose 2-sulfate (Fig. 3). The varying proportions of the three types of repeating units, and the presence of low levels of naturally methylated terminal Gal*p* residues found in one of the specimens may be due to seasonal, environmental, or varietal differences. In the light of these data, the relatively low sulfate contents reported [4,5] for the "ι-carrageenans" from the Tanzanian and Indian specimens of *S. filiforme* are possibly due to the occurrence of unsulfated, 3-linked Gal*p* residues in the polysaccharide and/or the replacement of sulfate ester on the 3-linked Gal*p* residues with pyruvate acetal substitution. The polysaccharides from the Tanzanian and Indian specimens thus deserve further examination.

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