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The structure of a galactan sulfate from the red seaweed *Bostrychia montagnei*

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Abstract

The sulfated, methylated galactan isolated from the red seaweed *Bostrychia montagnei*, showed an unusually narrow structural dispersion. This agaran has the defining linear backbone of alternating 3-linked β-D-galactopyranosyl units and 4-linked α-L-galactopyranosyl and 3,6-anhydrogalactopyranosyl residues. The D-units have C-6 methylation, C-6 single stubs of xylopyranosyl and minor to trace amounts of (possible) C-6 linked single stubs of galactopyranosyl. These units are mainly sulfated on C-4 with lesser sulfation at C-6 and minor at C-2. The L-residues are mainly methylated on C-2 of the 3,6-anhydrogalactopyranosyl and sulfated on C-3 of the L-galactopyranosyl; minor amounts of 2,3- and 3,6-disulfated and 2-*O*-methyl or 2-*O*-glycosyl 3-sulfated L-galactopyranosyl were also found. © 2002 Published by Elsevier Science Ltd.

Keywords: Sulfated galactans; Chemical structure; Methylation analysis; NMR spectroscopy; Seaweed; *Bostrychia montagnei*; Rhodomelaceae

1. Introduction

The family Rhodomelaceae includes a wide range of algae, and the polysaccharides present in several genera (*Laurencia*,[1,2] *Odonthalia*,[3] *Polysiphonia*,[4] *Rhodomela*,[5] *Chondria*,[6] *Dasyclonium*,[7] *Lenormandia*,[2] *Osmundaria*[2] and *Bryocladia*[2]) have been studied. All polysaccharides contain an agaran backbone of alternating 3-linked β-D-galactopyranosyl units with 4-linked 3,6-anhydro-α-L-galactopyranosyl and α-L-galactopyranosyl residues, with a wide range of substitution options.

Bostrychia montagnei Harvey is a red seaweed of the family Rhodomelaceae (order Ceramiales) growing in the tropical Atlantic Ocean. The isolation and preliminary analysis of the polysaccharides extracted with water at room temperature, as well as the analyses of the fractions obtained by ion-exchange chromatography from this extract, have been reported,[8] together with its inhibitory effect on herpes simplex virus replication in vitro.[9] We now report on the detailed chemical structure of a galactan sulfate extracted at room temperature from this species.

2. Experimental

Major fractions (B1, B4–B6) from the anion-exchange chromatography of the native galactan extracted at rt with water (CW)[8] were used for structural work (Table 1). The configuration of the galactosyl residues was determined as described by Cases et al.[10] and that of the 3,6-anhydro units by ^{13}C NMR spectroscopy.[11] Sulfate content was determined by the turbidimetric method of Dodgson and Price.[12]

Methylation analysis.—The polysaccharides (B1, B4–B6) were converted to their triethylammonium salts by ion-exchange with Dowex-50 resin.[13] Trideuteromethylation of the polysaccharides as their triethylammonium salts was carried out by the method of Ciucanu and Kerek.[14] The dry polysaccharides (50 mg) were dissolved in Me_2SO (3.0 mL) with stirring. Powdered NaOH (100 mg) was added, and the mixture was stirred for 30 min at rt. CD_3I (0.2 mL) was added, and

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the reaction was allowed to proceed at 25 °C for 30 min. The addition was repeated twice. Water (2 mL) was added to stop the reaction, and the solution was neutralized with AcOH, dialyzed against distilled water, and lyophilized to give the trideuteromethylated polysaccharides (yield ~85%). A second trideuteromethylation was carried out following the same procedure (yield ~90%). The desulfated fractions (see later) were also submitted to two trideuteromethylations. Fraction B4 was also methylated with CH_3I producing B4m, which was desulfated and deuteromethylated (B4md, see later). The partially methylated and trideuteromethylated alditol acetates were generated by reductive hydrolysis and acetylation,[15] analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). The relative molar GC response factors, determined by Stevenson–Furneaux,[15] were used to calculate the corresponding permethylated content. For fraction B4md partially methylated alditol acetates were also generated by hydrolysis in aqueous formic acid (45%), followed by $NaBD_4$ reduction, acetylation and GC, and GC–MS analyses.

Desulfation.—Partial solvolitic desulfation of the polysaccharides (B1, B4–B6) and of permethylated B4 (B4m) (50 mg) was carried out as reported previously,[16] at 105 °C for 4 h, using 80:10:1 Me_2SO–MeOH–pyridine to yield B1D, B4D-B6D (yield 67–78%) and desulfated B4m (60% yield). Trideuteromethylation analysis of the desulfated polysaccharides was carried out as described above.

Gas chromatography.—GC analyses were carried with an HP-5890 gas chromatograph equipped with a flame ionization detector (FID), using two different fused silica capillary columns (30 m × 0.25 mm), one coated with DB-225 (Durowax) and the other with SP-2330 (Supelco). The first column resolves all the partially methylated derivatives found in the hydrolyzates of methylated galactans except 3,6-anhydrogalactose and 4,6-di-*O*-methylgalactose, which were resolved using the second column. Chromatography was run isothermically at 210 °C. Both injector and FID temperatures were 250 °C. Nitrogen was used as carrier gas at a flow rate of 1 mL/min and a split ratio of 100:1. GC–MS analyses were performed using the same columns with a Varian 3300 chromatograph and a Finnigan Mat ITD spectrometer. Helium was used as carrier gas at 1 mL/min.

Spectroscopic methods.—Fourier-transform infrared (FTIR) spectra of KBr pellets of the polysaccharides were recorded in a Perkin–Elmer Series 2000 FTIR spectrophotometer (eight scans, at a resolution of 4 cm^{-1}) scanning between 4000 and 400 cm^{-1}. For nuclear magnetic resonance (NMR) spectroscopic analysis, the lyophilized sample was dissolved in D_2O (20–40 mg/mL). NMR spectra of solutions were recorded at 70 °C using a Bruker Avance DRX 400 NMR spectrometer. DEPT spectra were used to assign C-5 and C-6 signals of pentosyl and hexosyl units, respectively. The polysaccharide samples were analyzed by one-dimensional ^{13}C and ^{13}C DEPT (DEPT135) using a multinuclear inverse detection, 5-mm probe. Chemical shifts are expressed in ppm using acetone as internal standard at 30.2 ppm.

Table 1
Constituent monosaccharides and sulfate content of the room temperature-extracted galactan (B-CW) and of its anion-exchange chromatographic fractions (B) and yield of the corresponding desulfated derivatives (BD)

Constituent	Samples [a] (mol%)								
	B1 [b]	B1D [b]	B4	B4D	B5	B5D	B6	B6D	B-CW [b]
2-Me-L-AnGal	8.8	5.7	9.2	8.4	4.2	4.1	3.7	4.2	4.2
L-AnGal	9.0	11.9	11.6	12.7	13.0	12.7	11.5	12.0	12.4
6-Me-D-Gal	10.1	10.7	8.0	9.0	8.1	10.6	9.1	9.8	6.7
2-Me-L-Gal	1.0	1.0	0.9	0.8	1.0	0.7			0.2
D-Gal	42.5		43.8		43.2		42.2		
Gal		65.2		66.0		67.7		68.9	72.3
L-Gal	23.8		23.4		27.3		28.6		
Xyl	4.8	5.5	3.1	3.1	3.2	4.2	4.9	5.1	4.2
SO_3Na (%)	13.0	2.5	22.0	5.4	24.0	4.8	22.0	4.0	23.0
Yield [c] (%)		67.0		72.0		74.0		78.0	

[a] Samples as defined in text.
[b] Normalized to exclude glucose.
[c] Yields of desulfated fractions.

Table 2
Trideuteromethylation analysis of the native (B) and partially desulfated (BD) galactans from *B. montagnei*

Monosaccharide [a]	Deduced unit and substitution pattern	Fractions							
		B1 [b]	B1D	B4	B4D	B5	B5D	B6	B6D
2,4,6*-Gal [c]	G6M	9.7		9.4		8.4		8.9	
2,4,6-Gal [g]	G+G6M		52.4		49.2		54.0		48.5
2,4,6-Gal [c]	G	9.8		8.2		4.1		6.6	
2,4-Gal	G6S+G6R	15.9	2.4	12.4	Tr [d]	20.2		17.9	5.0
4,6-Gal	G2S	7.8	Tr	2.3		2.9		2.1	
2,3,6-Gal [e]	L	Tr	14.3	Tr	19.5	Tr	20.8	1.0	13.5
3,6-Gal	L2S+L2R	1.0	4.3	1.2	4.0	Tr	4.4	1.2	4.4
2*-AnGal [f]	LA2M	9.0		9.6		4.0		3.8	
2-AnGal [g]	LA+LA2M		14.5		14.4		10.7		10.7
2-AnGal [f]	LA	4.7		6.8		8.4		1.6	
AnGal	LA2S+LA2R	3.4	3.3	6.5	4.8	3.7	3.9	11.1	5.5
2,3,4,6-Gal	R	2.1	2.4	2.0	3.4	2.1	2.0	3.1	2.0
2,3,4-Xyl	R	5.1	5.3	2.7	2.8	3.8	3.0	3.5	2.8
6-Gal	L2,3S+L2R3S	6.0	Tr	6.2		5.2		5.2	2.1
2-Gal	L3,6S	3.0	Tr	4.1		5.2		2.8	1.1
2,6-Gal	L3S+G4S	22.5	1.1	28.6	1.9	32.0	1.2	31.2	4.4

[a] Normalised mol% of monosaccharide having methyl groups at the positions indicated.
[b] Normalized to exclude glucose.
[c] The relative proportions of the 2,4,6*-Gal and 2,4,6-Gal was determined using the fragments ions (m/z) 45, 104, 164 and 48, 107, 167, respectively.
[d] Tr = trace (<1.0%).
[e] The fractions B1D, B4D and B5D showed traces of 2*,3,6-Gal.
[f] The relative proportions of the 2*-AnGal and 2-AnGal was determined using the fragments ions (m/z) 117 and 120, respectively.
[g] Non-differenciated methoxyls.
R = glycosylation with either Xyl or Gal.
* Corresponds to natural methoxyl.

3. Results

Sugar analysis.—The polysaccharides extracted with water at 25 °C (CW) were fractionated by anion-exchange chromatography yielding galactose-rich fractions (B1–B6) and xylose-rich fractions (B7 and B8).[8] The monosaccharide composition (mol%) of the major galactan fractions, namely B1, B4–B6 are given in Table 1. All fractions showed galactose (66.3–70.8%) and 3,6-anhydrogalactose (9.0–13.0%) as the main saccharides. Naturally occurring methyl groups were present as 2-*O*-methyl 3,6-anhydro-L-galactose (3.7–9.2%), 6-*O*-methyl-D-galactose (8.0–10.1%), and 2-*O*-methyl L-galactose (0.9–1.0%). Small quantities of xylose were also present (3.1–4.9%). The ratio of D-galactose plus methylated D-galactoses to L-galactose plus anhydro-L-galactosyl residues was close to, but not exactly 1.0 (1.13–1.23, Table 1).

Desulfation.—The high yields (67–78%), as well as the compositional analysis of the desulfated derivatives (B1D, B4D–B6D), which was very similar to those of the parent fractions (B1, B4–B6), and the ^{13}C NMR spectra, suggest little degradation of the polysaccharides in spite of the high percentages of desulfation (75.5–81.8%) (Table 1).

FTIR spectroscopic analysis.—The FTIR spectra (not shown) of the native fractions showed an intense band at 1230–1260 cm^{-1} indicative of sulfate ester. The diagnostic region (940–800 cm^{-1}) contained two major absorption bands at 933 cm^{-1} and at 847 cm^{-1}, which indicates the presence of anhydrogalactopyranosyl and axial sulfate ester at O-4 of D-galactopyranosyl, respectively. Additionally a small peak was observed at 814–820 cm^{-1}, suggesting some primary sulfate ester substitution.[3]

Linkage analysis.—The native and desulfated galactans were converted into the triethylammonium-salt form and trideuteromethylated, and the partially trideuteromethylated alditol acetates, obtained by reductive hydrolysis,[15] were analyzed by GC–MS. The results of the linkage analyses are summarized in Tables 2 and 3. The trideuteromethylation allowed the type of glycosidic linkages of the naturally occurring methylated units to be determined. The values obtained

(Table 2) are similar to those of the compositional analyses (Table 1).

Trideuteromethylation (Table 2) confirmed that about 50, 40, and 25% of the 3,6-anhydrogalactosyl residues in fractions B1, B4 and B5–B6, respectively, are naturally methylated on O-2 and that some of these units are not sulfated in this position [$\sim$30% (B1 and B4), $\sim$50% (B5), and $\sim$20% (B6)]. The amounts of non-naturally methylated 2,4-linked 3,6-anhydrogalactosyl units in galactans B1, B4, and B5 are not modified after desulfation (Tables 2 and 3), indicating that this unit is glycosylated on O-2. In contrast, for fraction B6, the percentage of non-naturally methylated 2,4-linked anhydrogalactosyl units in the desulfated polymer is 50% lower (5.5%) than in the native polymer (11.1%). Thus, galactan B6 contained 5.6% of 3,6-anhydrogalactosyl units sulfated on O-2, besides 5.5% of units glycosylated O-2.

The GC–MS analysis of 2,4,6-Gal in all the native fractions showed 2,4-bis (trideuteromethylation) in percentages consistent with the content of 6-*O*-methylgalactose obtained by sugar constituent analysis (Tables 1 and 2).

2,6-Gal was the principal derivative in the trideuteromethylation analysis of the galactan fractions (Table 2). This can correspond to either 3-linked 4-sulfated galactopyranosyl units and/or 4-linked 3-sulfated galactopyranosyl units (see later). The 3-linked galactopyranosyl units in B1D, and B4D–B6D (48.5–54.0%) originated from D-galactopyranosyl (G) plus 6-*O*-methyl D-galactopyranosyl (G6M, 12.5–19.5%), D-galactopyranosyl 6-sulfate (G6S, 12.4–20.2%), D-galactopyranosyl 2-sulfate (G2S, 2.1–7.8%) and D-galactopyranosyl 4-sulfate (G4S, 11.6–18.4%) units in the corresponding native fractions (Table 2). The 4-linked galactopyranosyl units in B1D, and B4D–B6D (13.5–20.8%) originated from L-galactopyranosyl (L, 0–1.0%), L-galactopyranosyl 2-sulfate (L2S, 0–1.2%), L-galactopyranosyl 2,3-sulfate (L2,3S, 0.8–2.2%), L-galactopyranosyl 3,6-sulfate (L3,6S, 1.7–5.2%), and L-galactopyranosyl 3-sulfate (L3S, 8.6–13.6%) units in the corresponding native fractions (Table 2). All these are units that cannot be cyclized to 3,6-anhydro derivatives.

To confirm whether the 2,6-Gal and 6-Gal observed in the analysis of the native fractions were derived from 3- and/or 4-linked galactopyranosyl units, B4 was successively methylated, desulfated, and trideuteromethylated to produce B4md. The GC–MS analysis of this sample (Table 4) revealed five derivatives: 2,4,6-Gal, 2,3,6-Gal, 3,6-Gal, 2,3,4,6-Gal, and 2,3,4-Xyl. 2,4,6-Gal corresponds to 3-linked galactopyranosyl (G) units that

Table 3
Linkage analyses of the constituent sugars of the native (B) and partially desulfated (BD) galactans from *B. montagnei*

Deduced linkage and substitution [a]	Samples (mol%)							
	B1 [b]	B1D [b]	B4	B4D	B5	B5D	B6	B6D
3-Linked residues								
3-Gal	19.5	52.4	17.6	49.2	12.5	54.0	15.5	48.5
3,6-Gal	15.9	2.4	12.4	Tr [c]	20.2		17.9	5.0
2,3-Gal	7.8	Tr	2.3		2.9		2.1	
3,4-Gal	11.6		16.9		18.4		18.0	
4-Linked residues								
4-Gal	Tr	14.3	Tr	19.5	Tr	20.8	1.0	13.5
2,4-Gal	1.0	4.3	1.2	4.0	Tr	4.4	1.2	4.4
2,3,4-Gal	6.0	Tr	6.2		5.2		5.2	2.1
3,4-6-Gal	3.0	Tr	4.1		5.2		2.8	1.1
3,4-Gal	8.6		11.7		13.6		10.8	
4-AnGal	13.7	14.5	16.4	14.4	12.4	10.7	5.4	10.7
2,4-AnGal	3.4	3.3	6.5	4.8	3.7	3.9	11.1	5.5
Terminal residues								
T-Gal	2.1	2.4	2.0	3.4	2.1	2.0	3.1	2.0
T-Xyl	5.1	5.3	2.7	2.8	3.8	3.0	3.5	2.8
Undefined residues								
3,4-Gal	2.3	1.1		1.9		1.2	2.4	4.4

[a] 3,6-Gal means a 3,6-disubstituted galactopyranosyl residue; T- means a terminal sugar unit.
[b] Normalized to exclude glucose.
[c] Tr = trace (<1.0%).

Table 4
Methylation, desulfation and trideuteromethylation of B4

Derivative	Composed by	Corresponding units	% [a]	Mass fragments (m/z) [b]
2,4,6-Gal	2,4,6-MeGal	G+G6M	17.6	234, 174, 161, 129, 101, 45
	2,4-Me-6CD_3Gal	G6S	12.4	234, 174, 164, 132, 104, 48
	4,6-Me-2CD_3Gal	G2S	2.3	237, 177, 161, 129, 101, 45
	2,6-Me-4CD_3Gal	G4S	16.9 (14.9–19.1)	237, 177, 164, 132, 104, 45
2,3,6-Gal	6-Me-2,3CD_3Gal	L2,3S	2.2 (2.8–3.7)	168, 116, 108, 45
	2,6-Me-3CD_3Gal	L3S	11.7 (10.5–11.4)	165, 116, 105, 45
	2-Me-3,6CD_3Gal	L3,6S	4.1 (4.5–4.7)	165, 119, 105, 48
	3,6-Me-2CD_3Gal	L2S	1.2 (1.2–1.3)	165, 113, 102, 45
3,6-Gal	3 CD_3-6-MeGal	L3S2R	4.0 (2.5–3.4)	236, 193, 176, 133, 116, 45
2,3,4,6-Gal	2,3,4,6-MeGal	R	2.0	
2,3,4-Xyl	2,3,4-MeXyl	R	2.7	

CD_3, trideuteromethyl.

[a] In parenthesis, values calculated using the relative abundance of mass fragments [m/z (%)] of the derivatives 2,4,6-tri-*O*-methylgalactose, 2,3,6-tri-*O*-methylgalactose and 3,6-di-*O*-methylgalactose, in B4md (B4 after methylation, desulfation and trideuteromethylation).

[b] Fragments ions used to determine the relative proportions of the various partially methylated, partially trideuterated alditol acetates.

would be produced on desulfation of 3-linked β-D-galactopyranosyl 4-, 6-, and 2-sulfate present in the native sample (Tables 2 and 4). Of the 49.2% of 2,4,6-Gal in B4D (Table 2), 16.9% correspond to 3-linked β-D-galactopyranosyl 4-sulfate (G4S) units and none from 3-linked β-D-galactopyranosyl 2,4-disulfate (G2,4S) units. 2,3,6-Gal corresponds to 4-linked α-L-galactopyranosyl units that would be produced on desulfation of 4-linked β-D-galactopyranosyl 2,3-, 3,6-, 2-, and 3-sulfate present in the native sample (Table 3). Of the 19.5% of 2,3,6-Gal in B4D (Table 2), 11.7% corresponds to 4-linked α-L-galactopyranosyl 3-sulfate units and 2.2% corresponds to 4-linked α-L-galactopyranosyl 2,3-disulfate units (Table 4). The remainder corresponds to 4-linked α-L-galactopyranosyl 2-sulfate units (1.2%) and 4-linked α-L-galactopyranosyl 3,6-disulfate units (4.1%). 3,6-Gal (4.0%) was also observed. This corresponds to 4-linked α-L-galactopyranosyl 3-sulfate units that are 2-*O*-glycosylated.

The value of 2,4-Gal increased on desulfated fractions and is attributed to the desulfation of 2-*O*-methyl or 2-*O*-glycosyl 4-linked galactopyranosyl 3-sulfate units. The difference between the value of 2,3,4-Gal in the native fraction and the value of 2,4-Gal in the desulfated fraction corresponds to 4-linked galactopyranosyl 2,3-disulfate units (Tables 3 and 4).

NMR spectroscopy.—The ^{13}C NMR spectra of the fractions B1 and B4–B6 were nearly identical, as well as those of the desulfated derivatives. Fig. 1 shows a partial spectra of B4 (a, b) and its desulfated derivative (c). The ^{13}C NMR spectrum of the desulfated galactan contains an anomeric signal at 103.9 ppm (minor) corresponding to β-D-xylopyranosyl single stubs linked to a position of high mobility, possibly C-6 of β-D-galactopyranosyl.[17] Other signals correspond to C-2–C-5 at 72.8, 75.7, 69.4, and 65.2 ppm, respectively. A major anomeric signal (103.3–103.4 ppm) corresponding to β-D-galactopyranosyl linked to α-L-galactopyranosyl[18] (major, 100.5 ppm) or 2-*O*-methyl or 2-*O*-glycosyl α-L-galactopyranosyl (minor) units at 99.5 ppm were also present. The anomeric signal at 102.1–102.3 ppm (major) corresponds to β-D-galactopyranosyl and/or its 6-*O*-methyl derivative linked to 3,6-anhydro-α-L-galactopyranosyl and/or its 2-*O*-methyl derivative (97.9 ppm, major and 98.3 ppm, minor, respectively).[19]

The ^{13}C NMR spectrum of the galactan had characteristic signals of sulfated carbons. This substituent causes a downfield (by 6–10 ppm) and an upfield shift (by 1–1.25 ppm) of the α- and β-carbons, respectively.[19–23] It showed signals at: (a) 102.1 (major), 79.7 and 76.4 ppm corresponding to C-1, C-3, and C-4 of β-D-galactopyranosyl 4-sulfate,[19] and 72.5 (not labeled) and 66.6 ppm corresponding to C-5 and C-6 of a β-D-galactopyranosyl 6-sulfate, both linked to 3,6-anhydro-α-L-galactopyranosyl[24] (96.3 and 97.9 ppm, respectively); and (b) 100.4, 66.8, 78.1, 77.3, and 70.6 ppm, corresponding to C-1–C-5 of an α-L-galactopyranosyl 3-sulfate[23] linked to β-D-galactopyranosyl (103.9 ppm). The assignment of this sulfated unit was based on chemical shifts for methyl α-D-galactopyranoside 3-sulfate,[23] considering the C-4 α-downfield shift

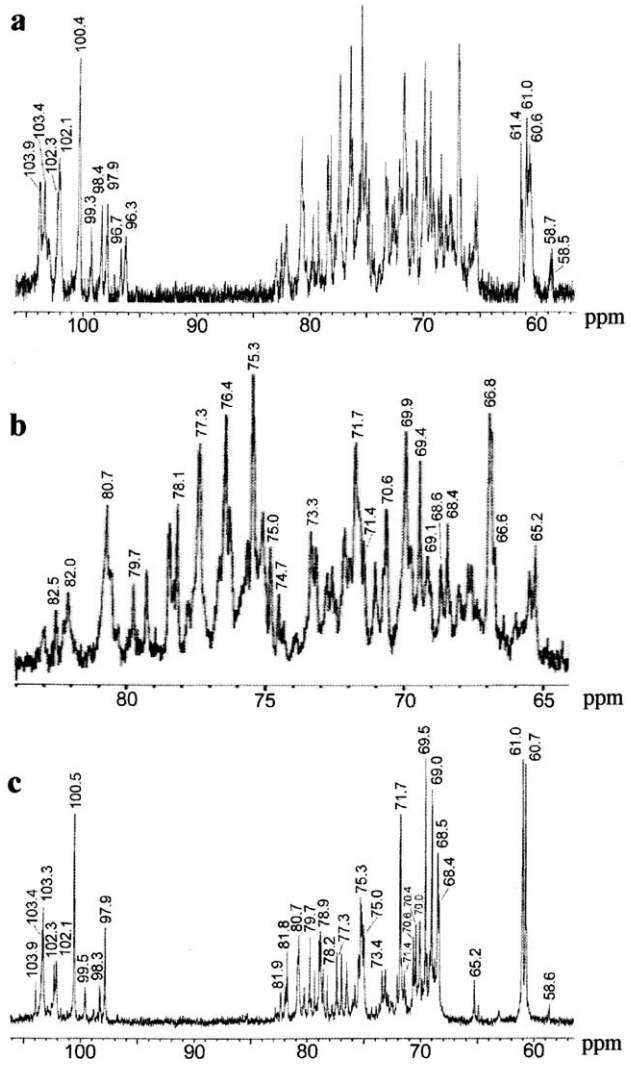


Fig. 1. ^{13}C NMR spectra of (a) fraction B4; (b) enlarged 83–65 ppm region of the spectrum; and (c) desulfated B4 (B4D).

by 8.5 ppm as expected due to glycosidic substitution of this carbon. After desulfation, the C-3 and C-4 signals shifted upfield and downfield by 7.5 and 1.6 ppm, respectively, in accordance with C-3 sulfation and the C-4 glycosidic linkage. All the resonances assigned for other carbons of the above-mentioned units are shown in Table 5.

4. Discussion

All the representatives of the Ceramiales studied contained agarans with a wide range of substituents occurring in a variety of positions, possibly with more than one substituent per saccharide unit and with rather high 3,6-anhydrogalactopyranosyl levels [*Neoptilota asplenioides*, Ceramiaceae or *Neorhodomela*, *Odonthalia*, *Pterosiphonia*, *Rhodomela*,[25] *Acanthophora spicifera*,[26] *Bostrychia moritziana*,[27] Rhodomelaceae].

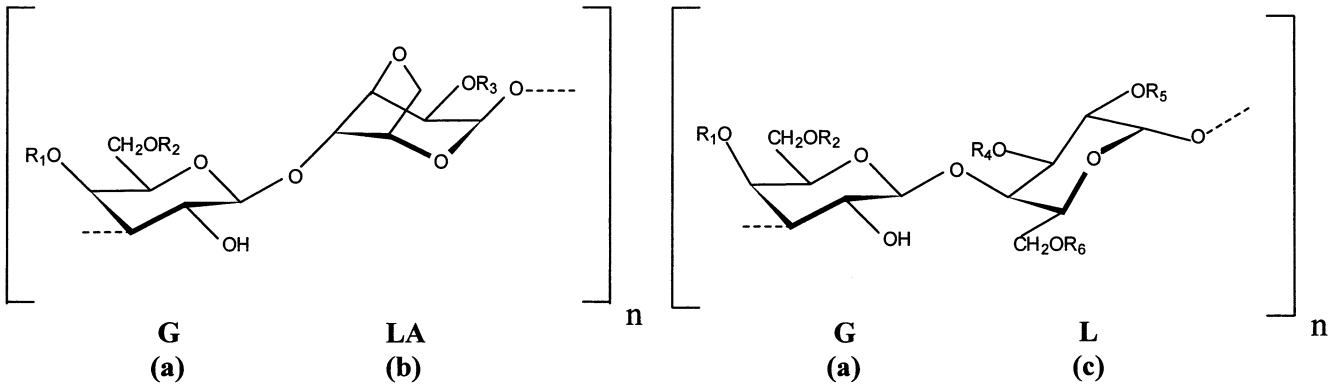
The water-extractable polysaccharides from the red seaweed, *B. montagnei*, are predominantly sulfated agarans of low molecular weights, together with lesser amounts of xylans and/or xylogalactans, also of low molecular weight.[8] The Rhodymeniales were assumed to contain xylans by the occurrence of this polysaccharide in both *Rhodymenia palmata*[28] and *R. stenogona*.[29] However, both seaweeds were later reclassified to the order Palmariales.[30] The agarans of *B. montagnei* do not show gelling properties in agreement with other agarans from species of the same family (Rhodomelaceae), namely: *Chondria decipiens*[31] *Lenormandia chauvinii*,[2] *L. angustifolia*,[2] *Bryocladia ericoides*,[2] *Osmundaria colensoi*,[2] *Laurencia thyrsifera*,[2] and *Cladhymenia oblongifolia*.[32]

The agarans from *B. montagnei* have the defining linear backbone of alternating 3-linked β-D-galactopy-

Table 5
Assignments of chemical shifts in the ^{13}C NMR spectra of the galactans B1, B4–B6

	3-linked β-D-							4-linked α-L-					
	C-1	C-2	C-3	C-4	C-5	C-6		C-1	C-2	C-3	C-4	C-5	C-6
Gal [a]	102.1	69.9	82.0	68.4	75.0	61.0	AnGal	97.9	69.4	79.7	77.3	75.3	69.1
Gal 4S [a]	102.1	70.6	79.7	76.4	74.7	61.0	AnGal	96.3	69.4	79.7	77.3	75.3	69.1
Gal 6S [b]	102.1	69.9	81.8	67.9	72.5	66.6	AnGal	97.9	69.4	79.7	77.3	75.3	69.1
6Me Gal [a]	102.1	69.9	81.8	68.6	73.3	71.4	AnGal	97.9	69.4	79.7	77.3	75.3	69.1
Gal [a]	102.3	69.9	82.5	68.4	75.0	61.0	2Me AnGal	98.4	78.5	78.1	77.3	75.3	69.1
Gal [c]	103.9						Gal 3S [c]	100.4	66.8	78.1	77.3	70.6	60.7
Gal [d]	103.3	69.5	80.7	68.5	75.2	61.0	Gal	100.5	69.0	70.6	78.9	71.7	60.7

[a] In accordance with Ref. 19.
[b] In accordance with Ref. 24.
[c] In accordance with Ref. 23.
[d] In accordance with Ref. 18.



(a)			(b)		(c)			
R_1= SO_3^-	R_2= H	(11.6 – 18.4 %)	R_3= CH_3	(3.8 – 9.6 %)	R_4= SO_3^-	R_5= H	R_6= H	(8.6 – 13.6 %)
R_1= H	R_2= SO_3^-	(12.4 – 20.2 %)	R_3= H	(1.6 – 8.4 %)	R_4= SO_3^-	R_5= H	R_6= SO_3^-	(2.8 – 5.2 %)
R_1= H	R_2= CH_3	(8.4 – 9.7%)	R_3= SO_3^-	(3.3 – 5.5 %)	R_4= SO_3^-	R_5= glycosyl or CH_3	R_6= H	(3.2 – 4.4 %)
R_1= H	R_2= H	(4.1 – 9.8%)	R_3= glycosyl	(5.5%, B6 only)				
R_1= H	R_2= glycosyl	(2.4 – 5.0%)						

Fig. 2. Principal structures for the galactan from *B. montagnei*. In parenthesis, percentages of the structures of fractions B1, B4–B6.

ranosyl and 4-linked α-L-galactopyranosyl and 3,6-anhydro-α-L-galactopyranosyl residues. The D/L-galactose ratio is not exactly 1.0 but 1.13–1.23. This departure of the ideal ratio might be due, as it was supposed in other cases,[33] to experimental error, but it is noteworthy that a reinvestigation of a fraction of the extract from the red seaweed *Rhodomela larix* (Ceramiales) previously considered as an agaran indicated the occurrence of minor amounts of the carrageenan-type backbone in addition to the agaran-type backbone.[34] Fractionation of the agaran and compositional and structural study of the fractions and of its desulfated derivatives indicated that they were parts of a sulfated, methylated agaran with an unusually narrow structural dispersion (Fig. 2). The 3-/4-linked units ratio is higher than 1.0 (1.1–1.4) (Table 3). This may be due to the use of a set of predefined response factors to calculate the normalized mol% of the various partially methylated alditol acetates.[35] The 3-linked β-D-galactopyranosyl units in *B. montagnei* have minor quantities of C-6 methylation (8.0–10.0%) (Table 1) and minor to trace amounts of single stubs of galactopyranosyl (2.0–3.4%) (Table 2). 6-*O*-Methyl-β-D-galactopyranosyl units are also found in significant amounts in the *Laurencia thyrsifera* and *Lenormandia angustifolia* agarans.[2] In *B. montagnei*, the 3-linked β-D-galactopyranosyl units carry also partial sulfation on C-2 (2.1–7.7%), C-4 (11.6–18.4%), and C-6 (12.4–20.2%) (Table 3). The sulfate is similarly distributed on the 3-linked β-D-galactopyranosyl unit, of the agaran from *Ceramium rubrum*, i.e., mainly at position-6 but with smaller amounts at positions-4 and -2.[36]

Approximately 40.1–49.7% of the 4-linked units are 3,6-anhydrogalactopyranosyl units which maybe unsubstituted, methylated, xylosylated or, in the case of B6, sulfated. 2-*O*-Methyl-3,6-anhydro-α-L-galactopyranosyl units were previously found in *Osmundaria colensoi*.[2] The remaining 4-linked units were also of the L configuration with complete sulfation on C-3 in the native polysaccharide, with minor amounts of additional 2- and 6-sulfation (0.8–2.2% and 2.8–5.2%, respectively) and 2-methyl ether or xylopyranosyl substitution (3.2–4.4%). It is noteworthy that these sulfated derivatives are not "precursor" units as they cannot be cyclized to 3,6-anhydro derivatives. Thus, the molar ratio L-galactopyranosyl/3,6-anhydro-L-galactopyranosyl is established and cannot be altered by metabolic needs.

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