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Structure of a sulfated xylogalactan from the calcareous red alga *Corallina pilulifera* P. *et* R. (Rhodophyta, Corallinaceae) [1]

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Abstract

The structure of a sulfated polysaccharide isolated from the calcareous red alga *Corallina pilulifera* was studied by methylation analysis before and after desulfation or Smith degradation, as well as by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy. The polysaccharide was shown to consist of D-galactose, L-galactose, 2-*O*-methyl-L-galactose, 3-*O*-methyl-L-galactose, 6-*O*-methyl-D-galactose, D-xylose, and sulfate in a molar ratio of 29:20:5:2:1:20:23. Its agaran-like backbone built up of alternating 3-linked β-D-galactopyranose and 4-linked α-L-galactopyranose residues bears single β-D-xylopyranosyl substituents at position 6 of β-D-galactose residues, whereas sulfate and O-methyl groups occupy positions 2 and 3 of α-L-galactose and position 6 of β-D-galactose residues. © 1997 Elsevier Science Ltd.

Keywords: Sulfated xylogalactan; Red algae; *Corallina pilulifera*; Corallinaceae; Seaweed polysaccharides

1. Introduction

Red algae belonging to different orders may contain structurally different polysaccharides [2–4]. In particular, calcareous red algae of the family Corallinaceae (order Corallinales) are the only group of Rhodophyta where alginic acids were detected [1,5,6]. It may be suggested that sulfated polysaccharides of these peculiar plants also have unusual structures, but at the beginning of our work only components of the Atlantic species *Corallina officinalis* had been studied in detail [7–9]. It was demonstrated that the alga contains a series of sulfated xylogalactans having a backbone built up of alternating 3-linked β-D-galactopyranose and 4-linked α-L-galactopyranose residues (such a structure, according to the recently proposed nomenclature of red algal galactans [10], is termed 'agaran'). This backbone bears numerous substituents masking its regular character, e.g. *O*-methyl and *O*-sulfate groups at positions 2 and 3 of L-galactose residues, as well as β-D-xylopyranosyl substituents and *O*-sulfate groups at position 6 of D-galactose residues. Our work is devoted to the structural analysis of a sulfated heteropolysaccharide isolated from one of the Pacific species of corallinean algae, *C. pilulifera* [6].

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[1] Polysaccharides of Algae. Part 50. For Part 49, see ref. [1].

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2. Results and discussion

The presence of a sulfated xylogalactan in the calcareous red alga *C. pilulifera* was detected in our previous work [6]. To isolate the polysaccharide, the algal biomass was treated with hydrochloric acid at room temperature, inorganic salts were removed by dialysis, acid polysaccharides were precipitated by the action of hexadecyltrimethylammonium bromide (Cetavlon) and transformed into water-soluble sodium salts as described earlier [6]. The resulting preparation (I) was purified on DEAE-Sephacel using aqueous sodium chloride of increasing concentration as eluant. The main part of the material was eluted with 0.4 M NaCl. Dialysis and lyophilization of this fraction gave the purified sulfated xylogalactan (Ia). It contained galactose, xylose, and sulfate in a molar ratio of about 2:1:1 together with minor amounts of 2-*O*-, 3-*O*-, and 6-*O*-methylgalactose (Table 1). According to enzymatic analysis data, galactose was a mixture of D- and L-enantiomers in nearly equimolar proportion. The D-configuration of xylose was established by GLC of the corresponding acetylated S(+)-2-octyl glycosides [11]. The L-configuration of 2-*O*- and 3-*O*-methylgalactose and the D-configuration of 6-*O*-methylgalactose were determined using NMR spectroscopy (see below).

In the course of the structural analysis of the polysaccharide several chemical modifications were employed to simplify its structure. Desulfation was performed by acid methanolysis [12]. Four treatments of preparation I with 1% methanolic HCl at 0 °C were found to diminish considerably the degree of sulfation without marked change in the proportion of galactose and xylose. Desulfated polysaccharide (II) was obtained in 48% yield.

Periodate oxidation of polysaccharide I resulted in the destruction of practically all D-xylose, whereas the ratio of D- and L-galactose in the oxidized material differed only slightly from that in the starting polysaccharide. The consumption of periodate was nearly 2 mol for every D-xylose residue present. This result was in accord with the suggestion that all the xylose residues occupy a terminal non-reducing position in the polysaccharide. The oxidized polymer was reduced with sodium borohydride and subjected to mild acid hydrolysis according to the usual Smith degradation conditions [13]. After separation of low-molecular fragments, a Smith-degraded polysaccharide (III) was obtained in 35% yield. This preparation, containing 16.7% sulfate, was desulfated by treatment with methanolic HCl, as described above. A Smith-degraded and desulfated polysaccharide (IV) containing only 0.55% sulfate was obtained in 32% yield.

Polysaccharide preparations Ia, II, III, and IV were subjected to methylation analysis. Polysaccharides were methylated with methyl iodide in the presence of sodium hydroxide in methyl sulfoxide [14], the native sulfated xylogalactan Ia being first transformed into the triethylammonium salt to enhance its solubility [15]. Other polysaccharides were readily soluble in methyl sulfoxide as sodium salts. Complete methylation was usually achieved after a single treatment with methylating reagents, since repeated methylation did not change significantly the proportion of methylated monosaccharides in hydrolyzates. Methylated polysaccharides were isolated by dialysis and lyophilization, hydrolysed, and the resulting mixtures of partially methylated monosaccharides were reduced with sodium borohydride, acetylated, and analyzed as alditol acetates by GLC–MS [16]. Comparison of the results obtained for preparations Ia–IV (Table 2) made it possible to suggest the backbone structure and the substitution pattern for these polysaccharides.

It is evident from Table 2 that polysaccharide IV contained equal amounts of 3- and 4-linked galactose, whereas the high content of terminal non-reducing galactose may be explained by a considerable degradation of the backbone during the preparation of IV from I. It was reasonable to suggest that 3-linked galactose residues belong to D-series and alternate with 4-linked L-galactose residues, as in many other

Table 1
Composition of polysaccharide preparations Ia–IV (in molar %)

Sample	D-Gal	L-Gal	D-Xyl	2-*O*-Me-Gal	3-*O*-Me-Gal	6-*O*-Me-Gal	SO_3Na
Ia	29	20	20	5	2	1	23
II	35	27	25	6	2	1	4
III	34	26	2	6	2	1	29
IV	46	37	–	11	3	2	1

Table 2
Methylation analysis of sulfated xylogalactan Ia and its derivatives II–IV (molar % of partially methylated alditol acetates)

Position of *O*-methyl groups	IV	III	II	Ia
Xylose				
2,3,4	1	2	21	26
Galactose				
2,3,4,6	14	6	4	1
2,3,6	34	29	34	26
2,4,6	34	32	14	4
2,3,4	3	1	2	–
2,6	3	5	2	4
3,6	–	12	–	8
2,3	5	1	3	1
2,4	6	12	20	30

red seaweed galactans of the agar group [2–4]; this suggestion was proved by periodate oxidation of polysaccharide II (see below). At the same time, the origin and structural significance of minor derivatives of 2,3,4-tri-*O*-methylgalactose and di-*O*-methylgalactoses were rather obscure.

Comparing the results of methylation of polysaccharides III and IV, it may be concluded that III was less degraded than IV. Sulfate groups in III are approximately equally distributed between position 6 in 3-linked galactose and position 2 in 4-linked galactose. In addition, a small part of sulfate may also occupy position 3 in 4-linked galactose. Evidently, sulfation of 4-linked galactose residues should protect them from degradation during periodate oxidation.

The partially methylated alditol acetates derived from polysaccharide II were in consistence with the conclusion about the terminal position of D-xylopyranosyl groups based upon the results of periodate oxidation and gave the evidence that these residues substitute the primary hydroxyl groups of 3-linked galactose. Comparison of methylation products of II and Ia confirmed qualitatively the position of sulfate groups determined by methylation analysis of polysaccharide III. At the same time the high 2,3,6-tri-*O*-methylgalactose content in methylated Ia did not correlate with the retention of galactose in periodate-oxidized polysaccharide I, and this fact may be probably explained by insufficient stability of sulfate groups at O-2 of 4-linked galactose residues under methylation conditions.

Additional structural information was obtained after periodate oxidation of polysaccharide II, which resulted in a practically complete destruction of xylose and a considerable increase in the D- to L-galactose ratio in the oxidized material, thus confirming

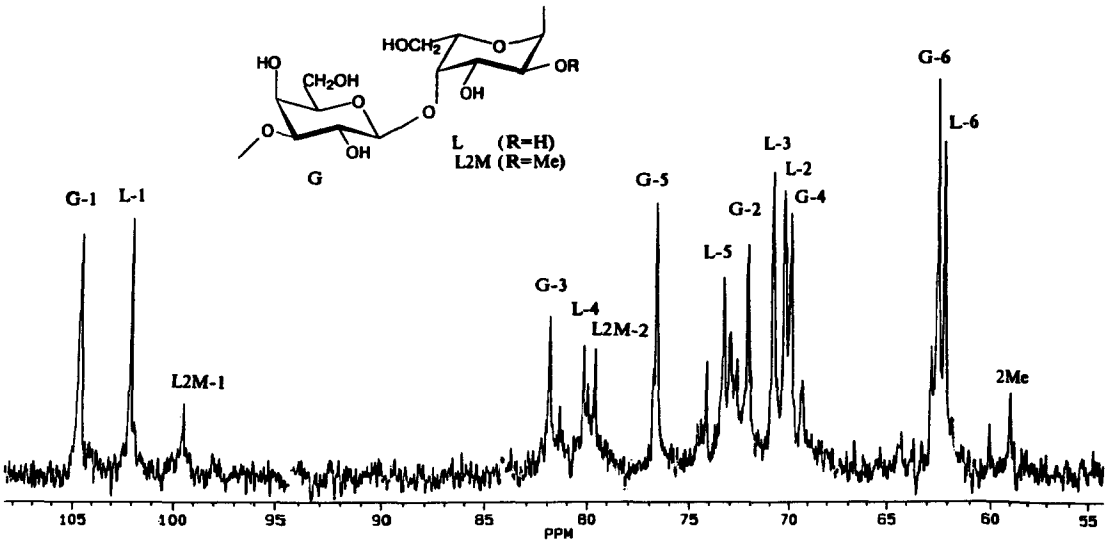

Fig. 1. ^{13}C NMR spectrum of Smith-degraded and desulfated polysaccharide IV. Notation of monosaccharide residues according to ref. [10].

Table 3
Assignments of NMR spectra of polysaccharides Ia–IV

Residue	Chemical shifts (ppm) and coupling constants (Hz) in ^{1}H NMR spectra			Chemical shifts (ppm) in ^{13}C NMR spectra	
→4)-α-L-Gal*p*-(1→	H-1	5.28	$J_{1,2}$ 3.5	C-1	101.9
	H-2	3.84	$J_{2,3}$ 9.5	C-2	70.2
	H-3	3.92	$J_{3,4}$ 4	C-3	70.7
	H-4	4.22	$J_{4,5} < 2$	C-4	80.1
	H-5	4.15		C-5	73.1
	H-6a	3.95		C-6	62.0
	H-6b	3.82			
→4)-α-L-Gal*p*-2-SO_3^--(1→	H-1	5.53	$J_{1,2}$ 3.5	C-1	100.1
	H-2	4.49	$J_{2,3}$ 9.5	C-2	77.1
	H-3	4.08	$J_{3,4}$ 4	C-3	68.1
	H-4	4.30	$J_{4,5} < 2$	C-4	79.9
	H-5	4.18		C-5	73.1
	H-6a	3.95		C-6	62.0
	H-6b	3.82			
→4)-α-L-Gal*p*-2-OMe-(1→	H-1	5.49	$J_{1,2}$ 3.5	C-1	99.4
	H-2	3.60	$J_{2,3}$ 9.5	C-2	79.5
	H-3	3.95	$J_{3,4}$ 4	C-3	70.0
	H-4	4.22	$J_{4,5} < 2$	C-4	79.9
	H-5	4.18		C-5	73.1
	H-6a	3.95		C-6	62.0
	H-6b	3.82		OMe	58.9
	OMe	3.49			
→4)-α-L-Gal*p*-3-OMe-(1→	OMe	3.44, 3.45 [a]		C-1	99.4
				C-2	70.0
				C-3	78.9
				C-4	76.7
				C-5	73.1
				C-6	62.0
				OMe	58.0
→3)-β-D-Gal*p*-(1→	H-1	4.42	$J_{1,2}$ 7.5	C-1	104.6
	H-2,3	3.75		C-2	72.0
	H-4	4.10	$J_{3,4}$ 4	C-3	81.3–82.9 [b]
	H-5	3.70	$J_{4,5} < 2$	C-4	69.8
	H-6a,b	3.85		C-5	76.5
				C-6	62.3
→3)-β-D-Gal*p*-6-OMe-(1→	OMe	3.38		C-1	104.6
				C-2	72.0
				C-3	81.3–82.9 [b]
				C-4	69.2
				C-5	74.1
				C-6	72.8
				OMe	59.9
→3)-β-D-Gal*p*-6-SO_3^--(1→	H-1	4.42	$J_{1,2}$ 7.5	C-1	104.6
	H-2,3	3.75		C-2	72.0
	H-4	4.09	$J_{3,4}$ 4	C-3	81.3–82.9 [b]
	H-5	3.93	$J_{4,5} < 2$	C-4	68.1
	H-6a,b	4.18		C-5	74.1
				C-6	68.5
→3,6)-β-D-Gal*p*-(1→				C-1	104.6
				C-2	71.9
				C-3	81.9
				C-4	~70
				C-5	75.2
				C-6	69.8

Table 3 (continued)

Residue	Chemical shifts (ppm) and coupling constants (Hz) in ^{1}H NMR spectra	Chemical shifts (ppm) in ^{13}C NMR spectra	
β-D-Xyl*p*-(1 →		C-1	104.8
		C-2	74.5
		C-3	77.1
		C-4	70.8
		C-5	66.5

[a] Two values of MeO-3 chemical shift for 3-mono- and 2,3-disubstituted residues.
[b] Depending on substitution of the residue and its neighbours.

the L-configuration of 4-linked galactopyranose. Smith degradation of II gave rise to a product, tentatively identified as β-D-galactopyranosyl-(1 → 2)-L-threitol, and this observation was consistent with the alternating sequence of 3- and 4-linked galactose residues in the polysaccharide backbone.

NMR spectroscopy was used to confirm these chemical data as well as to determine the anomeric configurations and to localize methyl groups in the polysaccharide. The anomeric region of the ^{13}C NMR spectrum of the periodate-oxidized and desulfated sample IV (Fig. 1) contained three signals of different integrated intensities. The intensity of the main signal at 104.6 ppm was approximately equal to the sum of those of the other two signals at 101.9 (more intense) and 99.4 ppm. The subspectrum in the region 58–83 ppm was typical of a polysaccharide with masked repeating structure, since it contained 10 intense peaks and several minor peaks, including signals of *O*-methyl groups at 58.9 (more intense) and 59.9 ppm. The attached proton test (APT) spectrum [17] was used to attribute the signals at 62.0 and 62.3 ppm to CH_2OH groups.

The ^{1}H NMR spectrum of IV also contained three signals as doublets in the anomeric proton region at 4.42 ppm (*J* 7.5 Hz, *β-galacto* configuration) and at 5.28 and 5.49 ppm (*J* 3.5 Hz, *α-galacto* configuration). The integrated intensity of the doublet of the β-sugar was equal to the sum of those of the α-sugars. The high-field region of the spectrum (3.38–3.49 ppm) contained three singlets belonging to OMe groups. The most intense signal of this group (about 60% of the total intensity of OMe resonances) was observed at 3.49 ppm.

The main peaks in the ^{1}H NMR spectrum of IV were assigned using 2D COSY and relay coherence transfer (COSYRCT) spectroscopy. Analysis of the chemical shifts [18] and spin–spin coupling constants [19] showed that the polysaccharide contained pyranose rings with α- and *β-galacto* configuration only.

The 2D NOE spectrum in rotating frames (ROESY [20,21]) of polysaccharide IV revealed spatial proximity of H-1 of β-galactose to H-4 of α-galactose, on the one hand, and of H-1 of α-galactose to H-3 of β-galactose, on the other hand. The absence of the correlation peak H-1(α)/H-4(β) in the coordinates of chemical shifts was a sign of different absolute configurations of α- and β-galactopyranose residues [22]. Bearing in mind the destruction of L-galactose residues by periodate, it may be concluded that polysaccharide IV contains alternating 3-linked β-D-galactose and 4-linked α-L-galactose residues, and hence, it has the structure of a partially methylated agaran. The predominant position of *O*-methylation was clear from the analysis of the ROESY spectrum, where a rather intense correlation peak OMe (3.49 ppm)/H-2 of α-galactose (3.60 ppm) was observed. The appearance of the signal at 99.4 ppm in the ^{13}C NMR spectrum of IV (upfield shift of C-1 of α-galactose from 101.9 to 99.4 ppm as the result of the β-effect of substitution) confirmed the presence of OMe groups at C-2 in part of the α-L-galactose residues.

The complete interpretation of the ^{13}C NMR spectrum of IV (Table 3) was performed using 2D ^{1}H/^{13}C correlation spectroscopy (HMQC [23]). The assignment of most of the signals coincided with the values reported for agaran in the literature [24], but the attribution of resonances to C-2 of β-D-galactose and C-3 of α-L-galactose given in [24] should be reversed, according to our data. Systematic differences in chemical shift values of about 0.7 ppm were probably the result of different recording conditions. The spectrum confirmed the structure of the main chain and the location of the *O*-methyl groups predominantly at position 2 of α-galactose (Table 3). The minor peaks of OMe groups were observed at 3.38/59.9 and 3.44,3.45/58.0 ppm for ^{1}H and ^{13}C chemical shift coordinates, respectively. The chemical shift of 59–60 ppm is characteristic of an OMe group at position 6 of a galactopyranose, and that of 58.0 ppm should belong to an OMe group at position

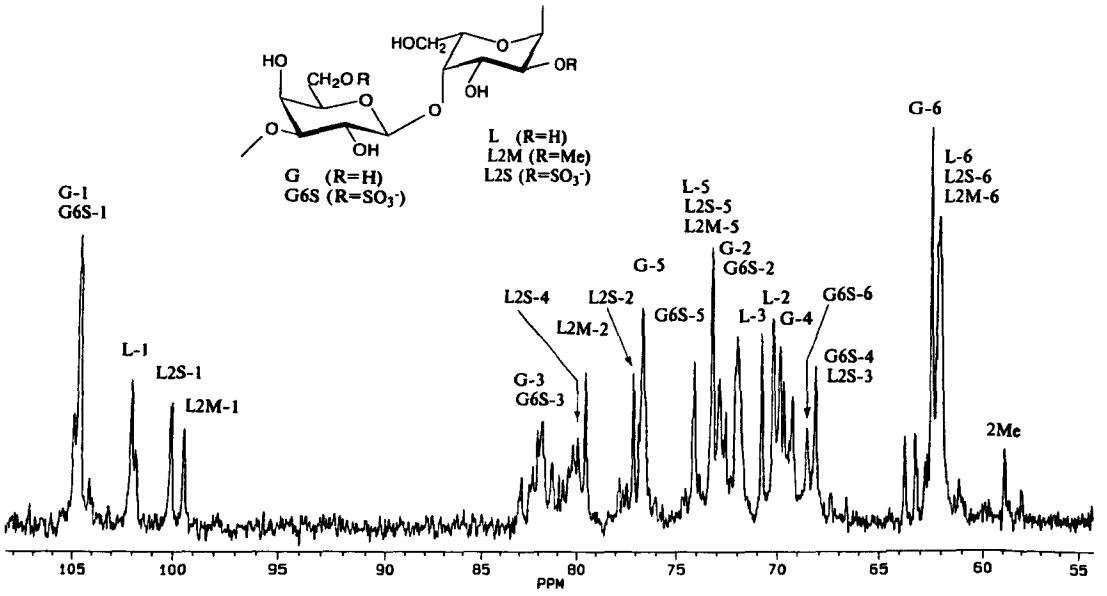

Fig. 2. ^{13}C NMR spectrum of Smith-degraded polysaccharide III.

3 [25,26]. A small peak at 74.1 ppm in the ^{13}C NMR spectrum of IV may be assigned to C-5 of 6-substituted β-galactopyranose. It is evident that some of the latter residues bear OMe group at position 6.

Taking into account all the NMR data, it may be concluded that polysaccharide IV is a partially methylated agaran having *O*-methyl groups at positions 2 (about 25%) and 3 (about 7%) of α-L-galactopyranose and at position 6 (about 7%) of β-D-galactopyranose residues.

The anomeric region of the ^{13}C NMR spectrum of sulfated polysaccharide III contained only one additional signal at 100.1 ppm, as compared with that of IV (Fig. 2). The total integrated intensity of the three α-galactose signals at 99.4, 100.1, and 101.9 ppm was nearly equal to that of β-galactose at 104.6 ppm. The APT spectrum revealed a signal of a substituted CH_2O group at 68.5 ppm. The signal at 74.1 ppm (C-5 of 6-*O*-substituted β-galactose) in the spectrum of III was much more intense than in the spectrum of IV. These observations made it possible to propose the location of sulfate groups in III at position 2 of α-L- and position 6 of β-D-galactose.

The low-field region of the ^{1}H NMR spectrum of III also contained an additional doublet at 5.53 ppm (J 3.5 Hz). In the 2D COSY spectrum this signal correlated with a doublet of doublets at 4.49 ppm. Such a low-field position of H-2 was also in agreement with the conclusion about the presence of sulfate at position 2 of α-galactopyranose.

Both ^{1}H and ^{13}C NMR spectra of polysaccharide III were completely assigned using the same procedures as for polysaccharide IV. Analysis of the whole spectra confirmed the location of sulfate groups at position 2 of α-galactose (about 35%) and at position 6 of β-galactose (about 20%). There were no signs on sulfate substitution at any other position.

It was very difficult to analyze the ^{1}H NMR spectrum of the native polysaccharide Ia due to the broadening of signals. Its ^{13}C NMR spectrum (Fig. 3) also contained rather broadened peaks of carbons belonging to galactose residues together with five very sharp and intense lines identified as subspectrum of β-xylopyranosyl groups [27]. The position of these signals and the absence of their splitting showed that all the β-xylopyranose residues are similarly attached to the backbone as single branchings devoid of additional substituents.

The ^{13}C NMR spectrum of desulfated polysaccharide II (Fig. 4) was much better resolved than the corresponding spectrum of the native polymer Ia. In the anomeric region there were three main signals at 104.8, 104.6, and 101.9 ppm belonging to β-D-xylopyranose, β-D-galactopyranose, and α-L-galactopyranose, respectively. Four additional sharp and intense signals were attributed to other carbon atoms of β-D-xylopyranose. In the APT spectrum of II, along with signals of unsubstituted hydroxymethyl groups of both galactose residues (peaks in the region 61.7–62.5 ppm, the signal of the β-D-isomer at 62.3 ppm being much less intense than that of the α-L-isomer at 62.0 ppm) and the CH_2 group of β-D-xylopyranose (66.5 ppm), an intense signal of a substituted hydroxymethyl group at 69.7 ppm was ob-

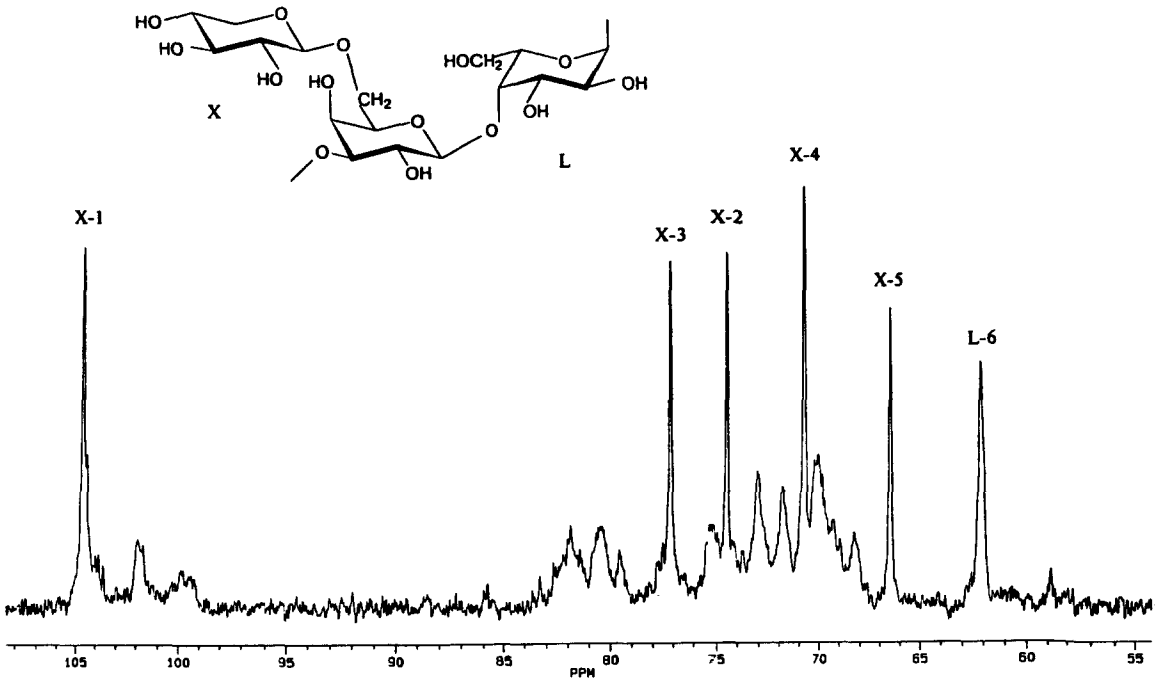

Fig. 3. ^{13}C NMR spectrum of native sulfated xylogalactan Ia.

served. This signal indicated, in accordance with the result of the methylation analysis, that β-D-xylopyranosyl groups were attached to β-D-galactopyranose residues of the backbone at position 6.

Taking into account all the results, it may be concluded that sulfated xylogalactan, isolated from the calcareous red alga *C. pilulifera*, should be regarded as a representative of the agar group polysaccharides, since its backbone is built up of alternating 3-linked β-D-galactopyranose and 4-linked α-L-galactopyranose residues. At the same time, the xylogalactan differs from typical agar-like polysaccharides, such as agarose or porphyran [2–4], in several important structural parameters. First, it does not contain 3,6-anhydro-α-L-galactopyranose and, moreover, also α-L-galactopyranose 6-sulfate, which might be used as biogenetic or chemical precursor of 3,6-anhydro-α-L-galactose. Second, the polysaccharide is characterized by a specific distribution of non-carbohydrate substituents: most of the *O*-methyl and *O*-sulfate groups occupy position 2 in α-L-galactose, some of these groups are located at position 3 of

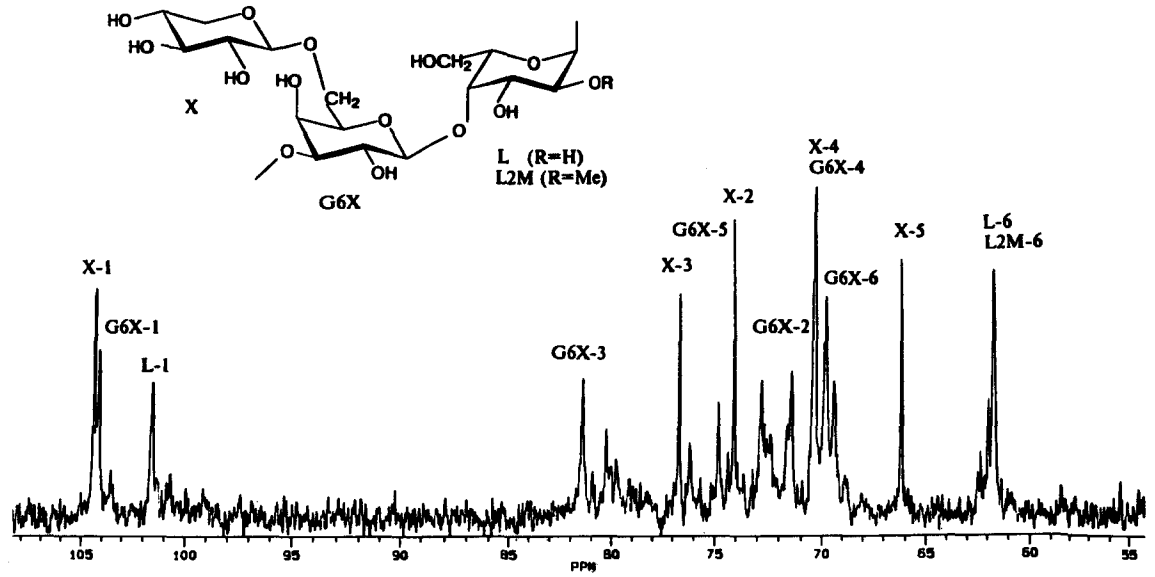

Fig. 4. ^{13}C NMR spectrum of desulfated polysaccharide II.

α-L-galactose, whereas methylation and sulfation at position 6 of β-D-galactose occur to much lesser extent. Finally, the polysaccharide contains many β-D-xylopyranosyl groups as single substituents linked at position 6 of β-D-galactose residues of the backbone. In this connection it may be pointed out that xylose was sometimes found earlier as a component of red algal galactans, but, as a rule, only in minor quantities. In addition to O-6 of β-D-galactopyranose residues of the galactan backbone [28], it was found to be also linked to O-2 of 3,6-anhydro-α-L-galactose [28], O-4 of β-D-galactose [29], and O-3 of α-L-galactose residues [30,31].

When our work was finished, a communication appeared [32] concerning the isolation and investigation of a sulfated polysaccharide from the calcareous red alga *Joculator maximus* Manza, a new representative of the Corallinaceae collected on the coast of Japan. It is interesting to compare the structure of xylogalactan from *C. pilulifera* with those of polysaccharides from *J. maximus* [32] and *C. officinalis* [8,9]. Taking into account that all three species are closely related from the taxonomic point of view, it is not surprising that sulfated polysaccharides isolated from these algae have similar structures. Their backbones are of the agaran type, contain no 3,6-anhydrogalactose, single β-D-xylopyranose substituents are located at position 6 of β-D-galactose, sulfate groups are placed at O-6 of β-D-galactose and O-2 or O-3 of α-L-galactose, and *O*-methyl groups are located mainly at position 2 of α-L-galactose residues. At the same time, there are some differences in the structures of these biopolymers: for example, four isomeric mono-*O*-methyl galactoses were detected in the polysaccharide from *C. officinalis*, only three of them in the polysaccharide from *C. pilulifera* (4-*O*-methylgalactose was absent), whereas only one isomer (2-*O*-methylgalactose) was found in the polysaccharide from *J. maximus*. Sulfated xylogalactan from *C. pilulifera* was shown to be less heterogeneous under fractionation conditions, as compared with its analogue from *C. officinalis*. Further investigations should indicate whether the presence of sulfated xylogalactans of the structure discussed here is a characteristic feature of all the algae belonging to the family Corallinaceae.

3. Experimental

General methods.—Quantitative determination of neutral monosaccharides in hydrolysates of polysaccharide samples, using GLC of acetylated aldononitriles and myo-inositol acetate as an internal standard, as well as of uronic acids by colour reaction with conc. H_2SO_4-3,5-dimethylphenol, was carried out as described earlier [6]. The concentration of D-galactose in hydrolysates was determined with D-galactose dehydrogenase using the Lactose-Galactose Determination Kit (Boehringer). Sulfate was estimated turbidimetrically after hydrolysis of polysaccharides in 1 M HCl [33]. ^1H and ^{13}C NMR spectra were recorded using Bruker AM-300 and WM-250 spectrometers and 2–5% solutions in D_2O at 50 °C with acetone (δ_H 2.225) or MeOH (δ_C 50.15) as internal references. 2D spectra were obtained using standard Bruker software for Aspect 2000 and 3000 (COSY, COSYRCT, ROESY, HMQC). Optical rotations were measured using a Jasco DIP-360 polarimeter.

Isolation and purification of sulfated xylogalactan I and Ia.—The alga *C. pilulifera* was collected from Possjet Bay of the Sea of Japan in September 1977. A crude preparation of the sulfated polysaccharide as sodium salt (I) was obtained as described earlier [6]; yield 0.87% of dry biomass; composition: galactose, 34.3%; xylose, 12.1%; SO_3Na, 9.6%; glucose, 1.6%; uronic acids, 7.4%. An aqueous solution of I (300 mg in 10 mL) was placed on a column (26 × 4 cm) containing DEAE Sephacel (Pharmacia) in the Cl^- form and eluted with water followed by NaCl solutions of increasing concentration (0.2, 0.4, 0.6, 0.8, and 1.0 M), each time up to the absence of a positive reaction of eluate for carbohydrates with phenol — conc. H_2SO_4 [34]. All the solutions obtained were dialysed and lyophilized, yields of fractions being 10, 10, 220, 20, 20, and 20 mg, respectively. The composition of the main fraction (Ia), $[\alpha]_D^{25}$ −77.2° (*c* 0.4, H_2O), eluted with 0.4 M NaCl, is given in Table 1.

Determination of the absolute configuration of xylose.—A solution of sample Ia (20 mg) in 2 M CF_3CO_2H (2 mL) was heated for 8 h at 100 °C and evaporated to dryness with EtOH. The residue was converted into acetylated S(+)-2-octyl glycosides according to ref. [11]. The mixture obtained was analyzed by GLC (Hewlett–Packard 5890A chromatograph, HP Ultra-2 capillary column, temperature programmed from 200 °C to 290 °C at a rate of 10 °C/min). Peaks of acetylated S(+)-2-octyl D-xylosides (retention times 8.1 and 8.5 min) coincided with authentic D-xylose derivatives, whereas peaks of the corresponding L-xylose derivatives (retention times 8.2 and 8.4 min) were completely absent.

Desulfation of xylogalactan I.—Sample I (250 mg) was suspended in absolute MeOH (40 mL),

cooled to 0 °C, then AcCl (1 mL) was added and the mixture was stirred for 6 h at 0 °C. The polysaccharide was separated by centrifugation, washed twice with MeOH, suspended in MeOH (40 mL) and left overnight. This treatment was repeated three more times. The washed precipitate was dried in vacuo to afford a desulfated xylogalactan (II) (160 mg), $[\alpha]_D^{25}$ −81.3° (*c* 0.4, H_2O).

Smith degradation of polysaccharides.—A solution of $NaIO_4$ (0.02 M, 40 mL) was added to a solution of sulfated xylogalactan I (100 mg) in water (40 mL), and the mixture was left in the dark for 24 h at room temperature, when the consumption of the oxidant ceased (monitored by the decrease in the optical density of the solution at 305 nm). Ethylene glycol (0.5 mL) was added to the reaction mixture, which was then dialysed, concentrated to about 20 mL, $NaBH_4$ (100 mg) was added, and the mixture was left overnight. The solution was acidified with HOAc, dialysed and lyophilized to give the oxidized and reduced polysaccharide (83 mg), containing Gal (37.9%, D:L 1.2) and Xyl (1.3%). This preparation (61.9 mg) was dissolved in 1% HOAc (10 mL), the solution was heated for 2 h at 100 °C and concentrated to dryness. A solution of the residue in water (5 mL) was placed on a column (75 × 3 cm) containing Sephadex G-15 and eluted with water. A polymeric fraction was lyophilized to afford a Smith-degraded polysaccharide III (35 mg).

Similar oxidation of desulfated xylogalactan II (40 mg) gave rise to an oxidized and reduced polysaccharide (17 mg), D-Gal:L-Gal ratio 6:1. A solution of this material in 1% HOAc (3 mL) was heated for 2 h at 100 °C, concentrated to dryness, the residue was acetylated with 1:1 pyridine–Ac_2O (1 mL) for 1 h at 100 °C and analyzed by GLC. The main component of the mixture was tentatively identified as β-D-galactopyranosyl-(1 → 2)-L-threitol acetate by comparison of its retention time and EI mass spectrum with those of authentic acetylated β-D-galactopyranosyl-(1 → 2)-D- and -L-threitol obtained by Smith degradation of desulfated galactan from *Grateloupia divaricata* [35].

Periodate oxidation and desulfation of polysaccharide I.—Oxidation of polysaccharide I (460 mg) was carried out as described above to afford an oxidized and reduced polysaccharide (380 mg). This preparation was desulfated with methanolic HCl, as above. Desulfated degraded polysaccharide IV was obtained after three treatments (52 mg), $[\alpha]_D^{25}$ −72.8° (*c* 0.8, H_2O).

Methylation of polysaccharides.—An aqueous solution of Ia (150 mg) was dialysed against aq 1% $NEt_3 \cdot HCl$ for 2 days, then against distilled water, and lyophilized to give the triethylammonium salt of Ia (140 mg), $[\alpha]_D^{25}$ −73.1° (*c* 0.4, H_2O). Other polysaccharides were methylated as sodium salts.

CH_3I (0.2 mL) and powdered NaOH (20–30 mg) were added to a solution of polysaccharide (5 mg) in Me_2SO (0.5 mL). The mixture was stirred for 1 h at room temperature, water (4 mL) was added, and the solution was dialysed and concentrated. CF_3CO_2H (2 M, 1 mL) was added to the residue, the solution was heated for 8 h at 100 °C, then co-concentrated with EtOH, and the resulting partially methylated monosaccharides were converted into alditol acetates and analyzed by GLC and GLC–MS according to conventional procedures [16].

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References

[1] A.I. Usov and M.I. Bilan, *Bioorg. Khim.*, 22 (1996) 126–133.
[2] T.J. Painter, in G.O. Aspinall (Ed.), *The Polysaccharides*, Vol. 2, Academic Press, New York, 1983, pp 195–285.
[3] J.S. Craigie, in K.M. Cole and R.G. Sheath (Eds.), *Biology of the Red Algae*, Cambridge Univ. Press, Cambridge, 1990, pp 221–257.
[4] A.I. Usov, *Food Hydrocolloids*, 6 (1992) 9–23.
[5] M. Okazaki, K. Furuya, K. Tsukayama, and K. Nisizawa, *Bot. Mar.*, 25 (1982) 123–131.
[6] A.I. Usov, M.I. Bilan, and N.G. Klochkova, *Bot. Mar.*, 38 (1995) 43–51.
[7] J.R. Turvey and P.R. Simpson, *Proc. Int. Seaweed Symp.*, 5 (1966) 323–328.
[8] M.R. Cases, C.A. Stortz, and A.S. Cerezo, *Phytochemistry*, 31 (1992) 3897–3900.
[9] M.R. Cases, C.A. Stortz, and A.S. Cerezo, *Int. J. Biol. Macromol.*, 16 (1994) 93–97.
[10] S.H. Knutsen, D. Myslabodski, B. Larsen, and A.I. Usov, *Bot. Mar.*, 37 (1994) 163–169.
[11] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[12] T.G. Kantor and M. Schubert, *J. Am. Chem. Soc.*, 72 (1956) 152–153.

[13] I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
[14] I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[15] T.T. Stevenson and R.H. Furneaux, *Carbohydr. Res.*, 210 (1991) 277–298.
[16] H. Björndal, C.G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem., Internat. Ed. Engl.*, 9 (1970) 610–619.
[17] S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, 46 (1982) 535–539.
[18] P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 188 (1989) 169–191.
[19] C. Altona and C.A.G. Haasnoot, *Org. Magn. Reson.*, 13 (1980) 417–429.
[20] D. Marion and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 113 (1983) 967–970.
[21] D.G. Davis and A. Bax, *J. Magn. Reson.*, 64 (1985) 533–535.
[22] G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1988) 59–75.
[23] A. Bax and S. Subramanian, *J. Magn. Reson.* 67 (1986) 565–569.
[24] M. Lahaye, W. Yaphe, and C. Rochas, *Carbohydr. Res.*, 143 (1985) 240–245.
[25] W. Voelter, E. Breitmaier, E.B. Rathbone, and A.M. Stephen, *Tetrahedron*, 29 (1973) 3845–3848.
[26] P. Fernandez, J. Jimenez-Barbero, and M. Martin-Lomas, *Carbohydr. Res.*, 254 (1994) 61–79.
[27] K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
[28] N.K. Kochetkov, A.I. Usov, L.I. Miroshnikova, and O.S. Chizhov, *Zh. Obshch. Khim.*, 43 (1973) 1832–1839.
[29] S. Hirase, K. Watanabe, R. Takano, and J. Tamura, *Abstr. 11th Int. Seaweed Symp.*, Inst. Oceanology, Academia Sinica, Qingdao, People's Republic of China, 1983, p 93.
[30] R.H. Furneaux and T.T. Stevenson, *Hydrobiologia*, 204–205 (1990) 615–620.
[31] A.I. Usov and M.Ya. Elashvili, *Bot. Mar.*, 34 (1991) 553–560.
[32] R. Takano, J. Hayashi, K. Hayashi, S. Hara, and S. Hirase, *Bot. Mar.*, 39 (1996) 95–102.
[33] K.S. Dodgson and R.G. Price, *Biochem. J.*, 84 (1962) 106–110.
[34] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[35] V.V. Barbakadze and A.I. Usov, *Bioorg. Khim.*, 4 (1978) 1100–1106.