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Agarans from the red seaweed *Polysiphonia nigrescens* (Rhodomelaceae, Ceramiales)

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Abstract—*Polysiphonia nigrescens* was sequentially extracted with water at room temperature, 70 and 90 °C. The extracts were analyzed and the major one, isolated at 70 °C, was fractionated by ion-exchange chromatography, eluting with water and solutions of increasing sodium chloride concentration; five main fractions were separated. Structural analysis, carried out by methylation analysis and NMR spectroscopy, showed that four of these were partially cyclized agarans that were highly substituted on C-6 mainly with sulfate, although methyl ether and single stubs of β-D-xylose were found in minor proportions. A fifth fraction comprising 6-sulfated agarose was also isolated. The use of 2D NMR techniques allowed us to assign the ^{1}H and ^{13}C NMR resonances of the G6S→L6S diad for the first time.

Keywords: Algal polysaccharides; Sulfated galactans; Agarans; Structural analysis; *Polysiphonia nigrescens*; Rhodomelaceae

1. Introduction

The family Rhodomelaceae comprises a wide range of algae among which the genus *Polysiphonia* is included. The chemical structures of polysaccharides of a few species of this genus have been determined: *Polysiphonia lanosa*,[1] *Polysiphonia morrowii*,[2,3] *Polysiphonia strictissima*,[4] *Polysiphonia abscissoides*[4] and *Polysiphonia atterima*.[5] With the exception of the studies reported for the polysaccharides of *P. lanosa* and *P. morrowii*, the rest were mainly carried out by ^{13}C NMR spectroscopy of the native and alkali-treated polysaccharides. These polysaccharides are sulfated galactans of the agaran type consisting of linear chains of alternating 3-linked β-D-galactopyranosyl and 4-linked α-L-galactopyranosyl units; some of the latter also occur in the 3,6-anhydro form. This regular backbone is usually masked by different O-linked groups, particularly methyl ether, sulfate ester and β-D-xylopyranosyl residues.

This paper describes the characterization of the polysaccharides of *Polysiphonia nigrescens* obtained by sequential extraction of the seaweed with water at different temperatures, the fractionation of the main extract and structural analysis of the major fractions.

2. Results and discussion

2.1. Analysis of the native polysaccharides

The seaweed was sequentially extracted with water at room temperature, 70 and 90 °C. Table 1 depicts the yield and composition of the different extracts obtained at each temperature. The highest total yield (ca. 9%) was obtained after extraction at 70 °C. The monosaccharide composition of all the extracts showed mainly the presence of galactose and 3,6-anhydrogalactose. In addition, minor quantities of 6-*O*-methylgalactose (mol %, 2–6%)

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Table 1. Yields and analysis of the products obtained by extraction with water at room temperature, 70 and 90 °C

Product	Yield[a] (%)	Molecular weight (kDa)	Sulfate (% $NaSO_3$)	Gal:AnGal:sulfate (molar ratio)	Protein %	Monosaccharide composition (mol %)						
						D-Gal	L-Gal	L-AnGal	6-Me L-Gal	3-Me Gal	Xyl	Glc
RT-1	3.6	7.0	25.0	1.0:0.3:0.7	5.5	48	26	19	2	—	3	2
RT-2	1.3	6.0	24.8	1.0:0.3:0.8	10.4	47	23	23	1	—	4	2
RT-3	0.8	6.0	22.0	1.0:0.3:0.7	18.0	48	24	21	—	—	5	2
70-1	3.1	10.6	25.4	1.0:0.6:1.0	5.0	40	15	31	1	—	10	1
70-2	5.0	25.1	25.6	1.0:0.5:1.0	8.5	41	17	31	3	—	7	1
70-2T[b]	69.2[c]	7.6	21.0	1.0:1.0:1.0	3.0	46	—	48	tr[d]	1	4	1
70-3	0.8	19.6	25.8	1.0:0.5:1.4	10.0	37	13	32	6	1	8	3
90-1	2.2.	9.0	25.0	1.0:0.6:1.3	16.5	36	13	30	6	2	11	2
90-2	1.1	7.9	25.1	1.0:0.6:1.3	22.8	40	14	29	5	2	8	2
90-3	0.8	6.1	18.3	1.0:0.4:1.2	36.8	41	16	25	4	2	8	4

[a] Yields are given per 100 g of dry seaweed.
[b] Alkaline treatment of 70-2 afforded 70-2T.
[c] Yield from alkaline treatment.
[d] Percentages lower than 1% are given as traces (tr).

and xylose (3–11%) were detected, together with small quantities of 3-*O*-methyl-/4-*O*-methyl-galactose (1–2%) and glucose (1–4%). Extracts 90-1–90-3 contained considerable amounts of protein (16.5–36.8%) and the highest number-average molecular weights were obtained for 70-2 and 70-3.

The presence of cations in 70-2 was determined by flame atomic absorption spectrophotometry, which showed an important prevalence of divalent counterions in this extract: Ca^{2+}, 0.160 equiv/100 g; Mg^{2+}, 0.044 equiv/100 g; Na^{+}, 0.021 equiv/100 g and K^{+}, 0.003 equiv/100 g. As expected, the sum of cation equivalents (0.228) is similar to the sulfate equivalents (0.248) measured for this sample, indicating that the former are counterions of the sulfate groups.

In the FTIR spectrum of 70-2 signals at 934 and 822 cm^{-1} were found; the former was attributed to the absorption of 3,6-anhydrogalactose while the latter was indicative of sulfate attached to primary hydroxyl groups. The second derivative spectrum in the region 1250–700 cm^{-1} was similar to those reported previously[6,7] for agar-type polysaccharides showing the two diagnostic bands at 787 and 720 cm^{-1}.

The mol % of D- and L-galactose and the absolute configurations of 6-*O*-methyl-D-galactose and 3,6-anhydro-L-galactose confirmed the presence of agaran structures, no evidence of DL-hybrid galactans[8] was found.

2.2. Fractionation of the major extract

The major extract 70-2 was fractionated on DEAE-Sephadex A-25 (Cl^{-}) by eluting with water and aqueous solutions of increasing sodium chloride concentration, identical chromatographic profiles were obtained in the analytical and preparative fractionations. Table 2 shows the yield and analysis of the isolated fractions. From the data in this table, it can be observed that fractionation in the 0–2.5 M NaCl range was based on the sulfate content, which increased with the molarity of the eluent; fractions with similar sulfate percentages were separated, possibly, according to their molecular weight.

When determined by GLC analysis, the 3,6-anhydrogalactose content showed no significant differences for the same elution range; however, the resorcinol–HCl method[9] suggested decreasing molar percentages with increasing sodium chloride concentration.

It is noteworthy that Fw had a significant sulfate content of 16.6%; anion-exchange chromatography of an extract of *Bostrychia montagnei* (Rhodomelaceae) yielded, after elution with water, a fraction which also contained a considerable amount of sulfate (13.0%).[10]

The composition of F2.7 was similar to that of F2.5 but its number-average molecular weight was slightly lower. The elution of this fraction with a higher sodium chloride concentration could be due to the sulfate distribution, with highly charged zones in the polysaccharide backbone. The separation of F4b was achieved by solubilization in boiling 4.0 M NaCl.

2.3. NMR spectroscopy of the fractions

The main fractions Fw, F1.5, F2.0, F2.5 and F4b were analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy. For Fw, F2.0 and F4b, 2D experiments (^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC) were also carried out.

The ^{13}C NMR spectrum of F4b showed in the anomeric region only two peaks, at 102.8 and 98.9 ppm, which together with a strong resonance at 67.9 ppm and the absence of signals in the 62–61 ppm range indicated a 6′-sulfated agarose backbone[2] (Fig. 1). This result is consistent with the solubilization of this fraction in hot aqueous solution.

The ^{13}C NMR spectra of Fw–F2.5 were more complex showing in the anomeric region the resonances of the G6S→DA diad (nomenclature of Knutsen et al.)[11]