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HOT WATER-SOLUBLE POLYSACCHARIDES FROM TETRASPORIC *PTEROCLADIA CAPILLACEA*

MARÍA I. ERREA and MARÍA C. MATULEWICZ*†

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

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Key Word Index—*Pterocladia capillacea*; Rhodophyta; polysaccharides; agarose; methylation analysis; ^{13}C NMR.

Abstract—Tetrasporic *Pterocladia capillacea* was sequentially extracted with water at room temperature, 50°, 70° and 90°. The polysaccharides obtained by extraction at 90° were fractionated by ion-exchange chromatography on DEAE Sephadex A-50 (Cl^-), eluting with water and solutions of increasing sodium chloride concentration; seven fractions were separated. The two major fractions, one eluted with water and the other with 0.1 M NaCl, comprised 66.9% of that recovered and were mainly agarose. Structural analysis of the fraction eluted with 0.7 M NaCl is consistent with a backbone formed mainly by alternating 3-linked β-D-galactose and 4-linked α-L-galactose residues, highly substituted with sulphate and having single stubs of xylose and galactose.

INTRODUCTION

In previous studies on agars of *Pterocladia capillacea*, the seaweeds whose sexual stages were not specified, were subjected to hot water extraction [1–3] and the isolated products purified by freeze-thawing [1, 3].

Tetrasporic *P. capillacea*, collected from the Uruguayan coast, was sequentially extracted with water at different temperatures. We report herein the characterization of the product extracted with water at 90°, after treatment of the seaweed with water at room temperature [4], 50° [5] and 70°.

RESULTS AND DISCUSSION

Tetrasporic *P. capillacea* was sequentially extracted with water at room temperature [4], 50° [5], 70° and 90°. Extraction with water at 90° led to the isolation of a product (P90) in a total yield of 35.4%. The molar ratio Gal:3,6–AnGal (1.00:0.63) (Table 1) and the sulphate content (10.0%) indicated that, in spite of the sequential extraction, agarose was not only present in this extract. Chromatography on DEAE Sephadex A-50 (Cl^-) using water and solutions of increasing sodium chloride concentration as eluents, yielded seven fractions (Table 1).

For the two major fractions, one eluted with water (Fw), the other with 0.1 M NaCl (F0.1), the molar ratio Gal:3,6–AnGal was close to that expected for agarose. For the other fractions the content of 3,6-anhydrogalactose decreased with the elution step and an increase in sulphate and xylose contents was observed (Table 1).

Fw and F0.1 were methylated according to the method of Hakomori [6, 7]. The carbohydrate compositions of both permethylated fractions were essentially the same and indicated that both were mainly constituted by agarose (Table 2). The ^{13}C NMR spectra of both fractions were in agreement with the methylation analysis showing 12 intense signals corresponding to β-D-galactose→3,6-anhydro-α-L-galactose diad [8–11]; for Fw small peaks due to the presence of floridean starch were also observed [12].

Structural analysis of F0.1 indicating that it was mainly agarose and the fact that no primary sulphate on 4-linked galactose residues was detected, were in agreement with the result that the sulphate (7.2%) present in this fraction was inorganic.

F0.7 was converted into the triethylammonium salt [7] and methylated [6, 7]. In the permethylated product, the ratio of 3-linked galactose: 4–linked galactose was 1.0:0.88 (Table 2). This molar ratio was obtained considering that 2,6-di-*O*-methylgalactose (24.9%) was derived from 3-substituted 4-linked α-L-galactose units, as was previously reported [4, 5].

The ^{13}C NMR spectrum of F0.7 was complex, probably due to an irregular substitution pattern on the galactose units, but it showed five sharp signals which were assigned to C-1 (δ 104.5), C-2 (73.9), C-3 (76.4), C-4 (70.1) and C-5 (66.0) of β-D-xylopyranosyl side-chains [13, 14]. In addition, small signals in the

*Author to whom correspondence should be addressed.

†Research Member of the National Research Council of Argentina (CONICET).

Table 1. Yields and analysis of P90 and fractions obtained by ion-exchange chromatography*

Fraction	Yield %	Gal:3,6-AnGal:sulfate molar ratio	Sugar composition				
			Gal	3,6-AnGal	Xyl	Man	Glc
			mol %				
P90	35.4†	1.0:0.63:0.33	59.4	37.2	3.3	—	tr‡
Fw	34.0 (41.6)§	1.0:1.07:0.07	40.9	43.9	—	—	15.2
F0.1	20.7 (25.3)	1.0:1.13:0.45	44.6	50.6	—	—	4.8
F0.3	4.3 (5.3)	1.0:0.40:0.85	62.1	24.6	3.4	3.9	6.0
F0.5	5.3 (6.5)	1.0:0.19:1.35	62.5	12.2	11.8	3.8	9.8
F0.7	7.3 (8.9)	1.0:0.09:1.20	69.9	6.3	11.6	4.2	8.1
F0.9	2.8 (3.5)	1.0:0.18:1.22	50.2	8.9	10.3	6.1	24.5
F4.0¶	7.3 (8.9)	1.0:0.84:0.61	52.7	44.2	—	—	3.1

*F0.3 and F0.5 contain 2-*O*-methylgalactose (2.4 and 1.8%, respectively).
†Yield given for 100 g of seaweed.
‡Percentages lower than 1.0% are considered as traces (tr).
§Figures in parentheses indicate percentages of that recovered (81.7%).
¶Obtained after treating the ion-exchanger with boiling 4.0 M NaCl.

anomeric region at δ 104.0, 102.7, 101.6, 101.4 and 98.7 were also clearly observed. The resonances at δ 102.7 and 98.7 correspond to the β-D-galactose→3,6-anhydro-α-L-galactose and/or β-D-galactose 6-sulphate→3,6-anhydro-α-L-galactose diads, while those at δ 104.0 and 101.6 may be assigned to β-D-galactose → α-L-galactose 6-sulphate diad [8–11]. The signal at δ 101.4 is possibly due to the C-1 of the α-L-galactose 3-sulphate residues [11, 15].

The structural analysis of F0.7 is, therefore, consistent with a backbone formed mainly by alternating 3-linked β-D-galactose and 4-linked α-L-galactose residues highly substituted with sulphate, and having single stubs of xylose and galactose.

In conclusion, P90 obtained after sequential extraction of the seaweed with water at different temperatures was mainly formed by agarose (*ca* 65% of the total recovered after the ion-exchange chromatography). This yield is considerably higher than that reported by Friedlander *et al.* [1] for an agar from *P. capillacea*, collected in the central Israeli Mediterranean coast, but purified by freeze-thawing; this agar was fractionated by chromatography on DEAE Sephadex A-50 giving agarose with ~45% yield of that recovered.

Table 2. Composition (mol %) of monosaccharides produced by permethylation and hydrolysis of Fw, F0.1 and F0.7*

Monosaccharide	Fw	F0.1	F0.7
2,3,4-Me_3Xyl	—	—	5.4
2,3,4,6-Me_4Gal	—	1.1	5.0
2,4,6-Me_3Gal	54.1	49.0	30.4
2,3,6-Me_3Gal	tr†	4.3	1.6
2,6-Me_2Gal	2.3	1.8	24.9
3,6-Me_2Gal	—	—	8.4
2,4-Me_2Gal	—	—	17.1
2-Me3,6-AnGal	43.6	43.8	7.1‡

*No glucose derivatives were detected in the methylation analysis of Fw.
†Percentages lower than 1.0% are considered as traces (tr).
‡Calculated from monosaccharide composition of this fraction.

EXPERIMENTAL

Materials. Tetrasporongial plants of *P. capillacea* were collected in Summer at La Paloma (Departamento de Rocha, Uruguay), dried in the open and sorted in the Museo de Ciencias Naturales Bernardino Rivadavia (Buenos Aires, Argentina). The seaweed (42 g) was washed with H_2O (1 l) containing NaOCl (1 ml of a soln with maximum available chlorine of 6%) and dried before extraction.

General. Unless otherwise stated, these are as described previously [4, 5]. Inorganic sulphate content of F0.1 was determined by the method of ref. [16] as the difference between the values obtained after and before hydrolysis of the sample.

^{13}C NMR spectra of Fw, F0.1 and F0.7 were recorded at 50 MHz with proton-decoupling at 80°, with ext. TMS as ref.; a soln of the sample (11–15 mg) in H_2O–D_2O (1:1) (0.4 ml) and a 5 mm-tube were used. Specific parameters include a pulse angle of 90°, an acquisition time of 0.37 sec, no pulse-delay and a spectral width of 11 KHz; the number of scans was 45049 for Fw, 45004 for F0.1 and 162975 for F0.7.

Extraction. Tetrasporongial plants (4.8 g) were sequentially extracted with H_2O at room temp. [4], 50° [5] and 70°. The residue was then treated with H_2O (500 ml) containing 0.5 ml of the NaOCl soln, with mechanical stirring for 2 hr at 90°. The residue was removed by hot filtration under vacuum and the supernatant dialysed, concd and freeze-dried. The extraction procedure was repeated ×9. Crude products were pooled giving P90 with a total yield of 1.7 g.

Fractionation. A jacketed column (1.5 × 44 cm) of DEAE Sephadex A-50 (Cl^-) equilibrated with H_2O and maintained at 70° was used. A soln of P90 (156.6 mg) in H_2O (10 ml) at the same temp. was adsorbed on to the top of the column. The column was first eluted with H_2O and then NaCl solns of increasing concns. Frs (1 ml) were collected and analysed for carbohydrate content [17]. When no elution was found, the concn of NaCl was increased by 0.2 M-steps. The upper limit of NaCl concn was 3.5 M. The gel was then

transferred to a beaker and treated with boiling 4.0 M NaCl (100 ml). All the products obtained, with the exception of the one eluted with H_2O, were dialysed; further concn and freeze-drying led to the isolation of the fractions shown in Table 1.

Methylation analysis. Fw (5 mg) and F0.1 (5 mg) were methylated by the Hakomori procedure as described in refs. [6, 7], but after the addition of the sodium methylsulphinylmethanide, the soln was stirred at room temp. for 30 min. Yields: Fw, 2.9 mg; F0.1, 4.4 mg. The permethylated samples were hydrolysed by the reductive hydrolysis procedure [7] and derivatized to the alditol acetates before analysis by GC and GC-MS [4].

F0.7 (2.3 mg) was converted into the triethylammonium salt [7] and methylated as described previously. Hydrolysis of the permethylated sample was carried out with 45% HCO_2H for 16 hr at 100° and the sugar mixt. was derivatized to the corresponding aldononitrile acetates [4].

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