ELSEVIER

Carbohydrate Research 304 (1997) 53–60

CARBOHYDRATE RESEARCH

The system of sulfated α-(1 → 3)-linked D-mannans from the red seaweed *Nothogenia fastigiata*: Structures, antiherpetic and anticoagulant properties

Adriana A. Kolender [a], Carlos A. Pujol [b], Elsa B. Damonte [b,1], María C. Matulewicz [a,1], Alberto S. Cerezo [a,*,1]

[a] *Departamento de Química Orgánica (CIHIDECAR-CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina*

[b] *Laboratorio de Virología, Departamento de Química Biologica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina*

Received 6 August 1996; accepted 30 June 1997

Abstract

Structural analysis of two xylomannans extracted from *Nothogenia fastigiata* was carried out. The results are consistent with the general pattern previously reported for other *xylo*-mannans of the same system, α-(1 → 3)-linked D-mannans 2- and 6-sulfated and having single stubs of β-(1 → 2)-linked D-xylose, but one of the new samples contains a significant amount of 2,6-disulfated units. Both xylomannans studied are obtained as complexes with a β-D-(1 → 3)-, α-L-(1 → 4)-galactan and a β-D-(1 → 3)-, β-D-(1 → 4)-'mixed linkage' xylan co-existing in the seaweed, a fact that limits the accuracy of the data determined. The structures of the galactan and the xylan are similar to those previously informed for this seaweed. The antiviral activity against four different herpes simplex viral strains and the anticoagulant properties of all the *xylo*-mannans of the system are reported. © 1997 Elsevier Science Ltd.

Keywords: α-(1 → 3)-Linked mannans; α-(1 → 3)-Linked mannans, sulfated; Xylomannans; Xylomannans, sulfated; Antiherpetic activity

* Corresponding author.

[1] Research member of the National Research Council of Argentina (CONICET).

PII S0008-6215(97)00201-2

1. Introduction

The red seaweed *Nothogenia fastigiata* (Bory) Parkinson [1] synthesizes a complex system of polysaccharides comprising neutral xylans of the β-D-(1 → 3)-, β-D-(1 → 4)-'mixed linkage' type [2–4], sulfated *xylo*-galactans of the agaroid type [5,6], and a family of sulfated *xylo*-mannans [7,8]. The sulfated polysaccharides were precipitated with Cetrimide, and the insoluble complexes were subjected to fractional solubilization in solutions of increasing sodium chloride concentration [7]. Two sulfated xylogalactan fractions were isolated in association with xylans and xylomannan sulfates; these complexes showed compositional dispersion and important molecular associations [5,6]. The same procedure yielded five *xylo*-mannan fractions with erratic solubility behaviour [7], three of which were structurally studied [7,8].

Herein we report the structures of the two remaining xylomannan sulfates, together with the antiviral activity against four different herpes simplex virus strains and the anticoagulant properties of the five *xylo*-mannans.

2. Experimental

Isolation and fractionation of the sulfated polysaccha-rides.—A sample of *Nothogenia fastigiata*, previously collected [7], was extracted with boiling water, and the water-soluble polysaccharides were fractionated by precipitation with Cetrimide and fractional solubilization in solutions of increasing NaCl concn as described previously [7,8]. More homogeneous samples were obtained from fractions 2 and 4 by redissolution in 1.0 and 2.0 M NaCl, respectively; these solutions were centrifuged, extracted with 1-pentanol, dialyzed (molecular weight cutoff of 3500), concentrated, and freeze-dried, yielding fractions 2′ and 4′.

General methods.—Hydrolyses of polysaccharides were carried out with 2 M CF_3COOH for 90 min at 121 °C. The sugar mixtures were derivatized to the alditol acetates and aldononitrile acetates, as previously described [8], for analysis by GLC and GLC–MS. Sulfate (expressed as $NaSO_3$) was analyzed by the turbidimetric method of Dodgson and Pryce [9]. Molecular weights were determined by the colorimetric method of Park and Johnson [10]. Nitrogen was determined by the method of Dumas and Pregl [11], and the protein content was calculated by multiplying the nitrogen content by 6.25.

GLC was carried out using a fused-silica capillary column (0.25 mm i.d. × 30 m) WCOT-coated with a 0.20-μm film of SP-2230. Chromatography was performed (a) isothermally at 220 °C for the alditol acetates; (b) isothermally at 210 °C for the methylated alditol acetates and methylated aldononitrile acetates, and (c) programmed from 180 to 200 °C at 2 °C min^{-1}, 2-min hold, then from 200 to 230 °C at 4 °C min^{-1}, and 30-min hold, for methylated alditol acetates and methylated aldononitrile acetates. N_2 was used as carrier at a flow rate of 1 mL min^{-1}, and the split ratio was 100:1. The injector and detector temperature was 240 °C.

Conversion of GLC peak areas to molar basis was calculated for the alditol acetates according to the weight-response theory [12].

GLC–MS of the methylated alditol acetates and methylated aldononitrile acetates was carried out as previously described [8].

The 500-MHz ^1H NMR spectra were recorded at room temperature, in D_2O solutions, with internal reference to sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS). The parameters were as follows: pulse angle, 90°; acquisition time, 4.391 s; without relaxation delay; spectral width, 7.5 KHz; and scans, 120.

The 50-MHz ^{13}C NMR ^1H-decoupled spectra were recorded at room temperature and as described previously [8] on a Bruker AC 200 spectrometer (number of scans 591, 501–603, 299 for fractions 4′ and 2′, respectively).

The Fourier-transform infrared spectra were recorded with a 510P Nicolet FTIR spectrophotometer, using a KBr pellet, at 4000–250 cm^{-1}, 32–64 scans were taken with a resolution of 2–4 cm^{-1} [8]. Derivation was performed using the Omnic software package incorporated into the hardware of the instrument.

Desulfation of xylomannans 2′ and 4′.—Fractions 2′ (21.4 mg) and fraction 4′ (90.1 mg) were desulfated in 0.1 M methanolic HCl for 72 h, as described previously [7]; dialyses were carried out with tubing of molecular weight cutoff of 1,000. Yields: fraction 2′, 14.3 mg; fraction 4′, 52.8 mg. Sulfate contents: desulfated fraction 2′, 4.4%; desulfated fraction 4′, 5.8%.

Methylation analysis.—Fraction 2′ (20.5 mg), desulfated fraction 2′ (5.2 mg), and fraction 4′ (20.3 mg) were methylated by the Haworth method [aq NaOH–$(CH_3O)_2SO_2$] as described by Cerezo [13] and further fully methylated by the Hakomori procedure (sodium methylsulfinylmethanide–iodomethane

in Me_2SO) [14]; desulfated fraction 4′ (9.4 mg) was methylated by the Hakomori procedure. The methylated derivatives were recovered by dialysis (molecular weight cutoff 3500 for fractions 2′ and 4′ and 1000 for the desulfated derivatives) and freeze-drying. Yields: fraction 2′, 19.0 mg; desulfated fraction 2′, 6.3 mg; fraction 4′, 17.0 mg; desulfated fraction 4′, 16.8 mg (hygroscopic).

Antiviral activity.—The origin of the viruses [herpes simplex type 1 (HSV-1) strain F, herpes simplex type 2 (HSV-2) strain G, and the thymidine kinase-deficient (TK^-) HSV-1 strains B2006 and Field] has been previously described [15]. All virus strains were propagated and titrated on Vero cells. Antiviral activity was evaluated by a plaque-reduction assay. Vero cell monolayers grown in 24-well plates were infected with 80 PFU/well in the absence or presence of varying concentrations of the polysaccharides, and then overlaid with culture medium containing the corresponding dose of the compound. After 2 days of incubation, plaques were counted. The cytotoxicity of the polysaccharides was evaluated in parallel with the antiviral activity by determining viability of mock-infected cells by the trypan blue exclusion method. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced the number of viable cells by 50%. The 50% inhibitory concentration (IC_{50}) was calculated as the compound concentration required to reduce virus plaques by 50%. The data shown in Table 5 are the mean values of two experiments.

Anticoagulant activity.—The thrombin time (TT) was measured using human plasma and various concentrations of the *xylo*-mannans. Human plasma (170 μL) and test solution (30 μL) were mixed and the mixture was incubated at 37 °C for 2 min. Bovine thrombin (Sigma Chemical Co., 7.5 U mL^{-1}, 100 μL) was added to the mixture and the time to clot formation was recorded.

3. Results

Fractions of the *xylo*-mannan sulfate system were newly prepared according to the procedure previously reported [7]. Data from the new fractionation, which showed the same pattern as the previous one [7], are given in Table 1. Fractions 2 and 4 were purified, as already reported for fractions 5 and 6 [8] (see Experimental), yielding fractions 2′ and 4′, respectively. The sugar compositions of the starting and purified products were similar, in spite of the incomplete recovery of material obtained in fraction 2′ (69.7%) and fraction 4′ (59.9%). It is noteworthy that, after purification, galactose was maintained in both fractions. Table 1 suggests that the solubilization of the Cetrimide salts of the sulfated *xylo*-mannans in sodium chloride solutions depends mainly on the

Table 1
Composition and molecular weight of the sulfated *xylo*-mannans [a]

Xylo-mannan	Range of redissolution (M NaCl)	Yield (%)	Sugar composition (mol%)			Sulfate (% $NaSO_3$)	Xyl:S:Man [b] (molar ratio)	Protein (%)	Molecular weight
			Mannose	Xylose	Galactose				
2	0.5–1.0	5.8 [c]	60.8	23.4	15.8	n.d. [d]		n.d.	11,000
2′	0.5–1.0	69.7 [e]	62.3	21.5	16.2	17.6 (~ 14) [f]	34:59:100	10.1	12,000
3	1.0–1.5	10.8	73.8	20.4	5.8	26.1 (~ 29)	28:84:100	10.6	39,100
4	1.5–2.0	8.7	78.4	17.5	4.1	n.d.		n.d.	35,500
4′	1.5–2.0	59.9	81.6	16.4	2.0	26.0 (~ 32)	20:72:100	4.8	21,900
5	2.0–3.0	18.2	n.d.	n.d.	n.d.	n.d.		n.d.	14,300
5′	2.0–3.0	32.2	97.5	2.5	–	15.3 (~ 16)	2:31:100	5.9	6,400
6	3.0–4.0	14.9	n.d.	n.d.	n.d.	31.3		n.d.	n.d.
6′	3.0–4.0	39.8	97.9	2.1	–	31.7 (~ 33)	2:82:100	6.6	30,000

[a] All data correspond to the new fractionation.
[b] Xylose:sulfate:mannose.
[c] Yields are given as percentages of the recovered (52.7% of the water-soluble polysaccharides).
[d] n.d. = not determined.
[e] Yields for 2′–6′ are given as percentages of the recovered after treatment with NaCl solutions of the corresponding concentration.
[f] In parentheses percentage calculated from methylation analysis.

amount of the single stubs of D-xylose and that only fractions with similar quantities of side chains are separated according to their percentage of sulfate (i.e., fractions 2′ and 3; fractions 3 and 4′; fractions 5′ and 6′).

Treatment of fractions 2′ and 4′ with 0.1 M methanolic hydrogen chloride led to the elimination of 75.0% and 77.7% of the sulfate content, respectively, and produced the corresponding desulfated derivatives (desulfated fraction 2′: Xyl 29.3 mol%, Man 57.3%, Gal 13.4%; desulfated fraction 4′: Xyl 22.2%, Man 76.1%, Gal 1.7%).

When fraction 2′ was converted into the triethylammonium salt [16] and subjected to methylation by the Hakomori procedure using sodium methylsulfinylmethanide–iodomethane in dimethyl sulfoxide [14], a high degree of undermethylation was achieved. Methylation of fraction 2′ using MMNO monohydrate–dimethyl sulfoxide [17] and lithium chloride–dimethyl sulfoxide [18] as solvents, with the same base and methylating agent as above, again gave in both cases a high degree of undermethylation. Methylation of fraction 2′ and of its desulfated derivative was accomplished when the samples were first treated by the Haworth method [13] and then fully methylated according to Hakomori [14]. Attempts at interpretation of the data (Table 2) on the basis of a xylogalactomannan sulfate were unsuccessful, but analysis was straightforward when the samples were considered as complexes of a xylan (7–8 mol% and 9–10%, for fraction 2′ and its desulfated derivative, respectively), a galactan (10–11% and 15–16%, respectively) and a xylomannan (81–83% and 74–76%, respectively) (Table 2) [6]. According to that model, the dimethylated xyloses are derived from the 'mixed linkage' xylan, the methylated galactoses from the agaroid galactan with very little, if any, xylose and sulfate substitution, and the mannose derivatives together with the trimethylated xylose from the xylomannan.

When fraction 4′ was converted into the triethylammonium salt and methylated according to the Hakomori procedure, undermethylation was observed again; but methylation was achieved when the aforementioned two-step method was used (Table 3); for desulfated fraction 4′, permethylation was accomplished in one step with the Hakomori procedure. No xylan and only a small amount of galactan (1–3%) was observed in the methylated derivatives of fraction 4′ and desulfated fraction 4′ (Table 3). In the four cases a small undermethylation (Tables 2 and 3) precluded a rigorous balance of substituents.

FTIR spectra of fractions 2′ and 4′ showed broad bands with a maximum at 843 cm^{-1} and a shoulder

Table 2
Composition (mol%) of permethylated fraction 2′, permethylated xylomannan 2′, permethylated desulfated fraction 2′, and permethylated desulfated xylomannan 2′ [a,b]

Monosaccharide	Fraction 2′	Xylomannan 2′	Desulfated fraction 2′	Desulfated xylomannan 2′
2,3,4-Me_3 Xyl	24.8	29.1	17.6	23.4
2,3,4,6-Me_4 Gal	–	–	1.4	–
2,4-Me_2 Xyl	1.2	–	2.0	–
2,3-Me_2 Xyl	6.7	–	7.3	–
2,4,6-Me_3 Man	7.8	9.8	32.2	42.9
2,4,6-Me_3 Gal	6.6	–	6.9	–
2,3,6-Me_3 Man	1.3	1.6	–	–
2,3,6-Me_3 Gal	1.6	–	5.3	–
4,6-Me_2 Man	29.3	36.8	18.2	24.2
2,6-Me_2 Gal	1.0	–	2.0	–
2,4-Me_2 Man	4.1	5.1	2.3	3.1
6-Me Man	1.2	1.5	1.4	1.9
2-Me Gal	1.6	–	–	–
4-Me Man	10.9	13.7	2.1	2.8
Man	1.9	2.4	1.3	1.7

[a] When fraction 2′ and its desulfated derivative were analyzed on the basis of the xylan–galactan–xylomannan complex model, 2,4- and 2,3-di-*O*-methylxylose were assigned to the xylan, and 2,3,4,6-tetra-*O*-methylgalactose, 2,4,6- and 2,3,6-tri-*O*-methylgalactose, 2,6-di-*O*-methylgalactose and 2-*O*-methylgalactose to the galactan. The rest of the partially methylated monosaccharides were derived from the xylomannan.
[b] Percentages lower than 1% are not considered.

Table 3
Composition (mol%) of permethylated fraction 4′, permethylated xylomannan 4′, permethylated desulfated fraction 4′, and permethylated desulfated xylomannan 4′ [a,b]

Monosaccharide	Fraction 4′	Xylomannan 4′	Desulfated fraction 4′ [c]	Desulfated xylomannan 4′
2,3,4-Me_3 Xyl	11.6	12.1	19.2	19.7
2,4,6-Me_3 Man	5.0	5.2	47.1	48.5
2,4,6-Me_3 Gal	2.6	–	1.3	–
2,3,6-Me_3 Gal	1.6	–	–	–
4,6-Me_2 Man	42.7	44.7	22.9	23.5
2,4-Me_2 Man	9.7	10.1	–	–
6-Me Man	2.5	2.6	1.4	1.4
2-Me Man	1.1	1.1	–	–
4-Me Man	20.7	21.6	–	–
Xyl	–	–	1.4	–
Man	2.5	2.6	6.7	6.9

[a] When fraction 4′ was analyzed on the basis of the xylan–galactan–xylomannan complex model, no 2,4- and 2,3-di-*O*-methylxylose corresponding to the xylan were detected; 2,4,6- and 2,3,6-tri-*O*-methylgalactose were derived from the galactan and the rest of the partially methylated monosaccharides from the xylomannan. For desulfated fraction 4′, only 2,4,6-tri-*O*-methylgalactose (1.3%) was detected.
[b] Percentages lower than 1% are not considered.
[c] Glc (1.1%) was also detected in permethylated desulfated fraction 4′.

at 820–824 cm^{-1} due to axial and primary sulfate groups, respectively [8]. These results are in agreement with the pattern of sulfation observed by the other methods.

The anomeric signals at 5.52, 5.21, and 5.12 ppm, observed in the ^1H NMR spectra of fractions 2′ and 4′, were assigned to mannose 2-sulfate, mannose with single stubs of β-(1 → 2)-linked D-xylose, and mannose and/or mannose 6-sulfate, respectively. This assignment was made using *xylo*-mannans 3, 5′, and 6′ as model compounds [7,8,19].

The ^{13}C NMR spectrum of fraction 2′ (Table 4) showed, in the anomeric region, five strong signals at 104.6, 104.1, 102.6, 101.9, and 100.5 ppm. The resonance at 104.6 ppm was assigned to the single stubs of β-(1 → 2)-linked D-xylose [20] while the peak at 101.9 ppm (broad) was mainly due to 3-linked α-D-mannose or α-D-mannose 6-sulfate units,

Table 4
^{13}C NMR spectral assignment of xylomannans 2′ and 4′

Unit	C-1	C-2	C-3	C-4	C-5	C-6
Xylomannan 2′ [a]						
β-D-Xyl	104.6	73.7	76.3	70.1	65.9	
α-D-Man	103.2	70.6	79.3	67.2	74.2	61.6
α-D-Man 6-sulfate	103.2	70.6	79.3	67.2	72.2	68.2
α-D-Man 6-sulfate [b]	101.9	79.3	79.3	68.2	72.2	68.2
α-D-Man [b]	101.9	79.3	79.3	68.2	74.2	61.6
α-D-Man 2-sulfate	100.5	77.3	77.3	67.2	74.2	61.6
Xylomannan 4′						
β-D-Xyl	104.7	73.6	76.3	70.1	65.9	
α-D-Man	103.2	70.6	79.1	67.2	74.2	61.8
α-D-Man 6-sulfate	103.2	70.6	79.1	67.2	72.2	68.2
α-D-Man 6-sulfate [b]	102.0	79.1	79.1	68.2	72.2	68.2
α-D-Man [b]	102.0	79.1	79.1	68.2	74.2	61.6
α-D-Man 2-sulfate	100.4	77.4	77.4	67.2	74.2	61.8

[a] Resonances at 102.6 (C-1), 73.7 (C-2), 74.7 (C-3), 77.7 (C-4), and 63.9 (C-5) were assigned to the 4-linked β-D-xylopyranosyl units. The anomeric signal of the galactan at δ 104.1 (3-linked β-D-galactose) was also observed; the anomeric signal corresponding to the 4-linked α-L-galactose unit is included in the peak at δ 101.9 [6].
[b] With single stubs of β-(1 → 2)-linked D-xylose.

Table 5
Antiviral activity of *xylo*-mannans isolated from *Nothogenia fastigiata*

Compound	IC_{50} ($\mu g\ mL^{-1}$)				CC_{50} Vero cells ($\mu g\ mL^{-1}$)
	HSV-1 F	HSV-2G	HSV-1 TK$^-$ B2006	HSV-1 TK$^-$ Field	
2′	7.1	7.1	2.6	2.0	> 1000
3	14.4	6.1	19.6	9.5	300
4′	28.2	27.6	12.9	7.3	> 1000
5′	> 100	96.3	> 100	73.0	250
6′	0.7	0.7	1.3	0.6	120
ds 8000	2.1	1.0	1.7	2.7	> 1000
Heparin	1.3	2.1	3.6	5.0	> 1000

both with single stubs of β-(1 → 2)-linked D-xylose [21–23]. It is noteworthy that even though 3-linked α-D-mannose and α-D-mannose 6-sulfate total ~ 12% in this fraction, only a small broad signal was observed at 103.2 ppm [21,22]. The absorption at 100.5 ppm corresponds to 3-linked α-D-mannose 2-sulfate units. The signals at 104.1 and 101.9 ppm are consistent with the presence of the galactan sulfate previously studied [6]. The peak at 102.6 ppm could be due to the C-1 of a xylose linked to the 4-position of another residue, in the 'mixed linkage' xylan also associated to the xylomannan [4].

The ^{13}C NMR spectrum of fraction 4′ (Table 4) gave, in the anomeric region, four absorptions at 104.7, 103.2. 102.0, and 100.4 ppm that were assigned as described above. It is important to note that all the peaks of the α-D-mannose 2,6-disulfate residues are overlapped with the signals of the α-D-mannose 2-sulfate and 6-sulfate units. No peaks associated with the galactan were observed in agreement with the small amount of this product detected in the fraction.

Optical rotations of fractions 2′ and 4′ were measured in water and in 0.5 M NaCl. Fraction 4′ showed similar values in both solvents (+21.7° and +23.3°, respectively); for fraction 2′ a considerably lower negative optical rotation was obtained in 0.5 M NaCl (−13.0° against −1.3° in water).

When the *xylo*-mannans were evaluated for antiviral activity against herpesviruses, mannan 6′ was found to be the most active compound with IC_{50} values in the range 0.6–1.3 $\mu g\ mL^{-1}$, whereas the activities of xylomannans 2′ and 3 were slightly lower. In contrast, mannan 5′ showed a very weak or no inhibitory effect against herpesvirus replication. Dextran sulfate with molecular weight of 8000 (ds 8000) and heparin were tested as reference compounds in order to compare with the *xylo*-mannans. As shown in Table 5, the antiherpetic activities of the most active sample, mannan 6′, were comparable to

Table 6
The compositions (mol%) in structural units of the α-(1 → 3)-linked *xylo*-mannans [a]

Xylo-mannan	3-linked mannose sulfated at the position indicated						3-linked mannose with single stubs of xylose at the 2-position		Xylose
	None	2	6	2,4	2,6	4,6	6-sulfate 2-Xyl	2-Xyl	Single stubs
2′ [b]	10	22	5	1	–	–	14	16	30
3 [c]	5	32	12	3	–	–	22	2	24
4′	5	46	10	3	10	1	12	–	12
5′	61	–	29	–	–	2	–	3	5
6′	9	52	31	1	1	–	3	–	3

[a] The complexity of the samples only permits a semiquantitative approximation. Xylose was supposed to be mainly linked to mannose 6-sulfate units. The remaining xylose was assigned to the 2-position of nonsulfated mannose units.
[b] Fraction 2′ has 2% of 4-linked D-mannose units. The presence of 3-linked mannose 2,6-disulfate units cannot be ruled out from data in Table 2.
[c] The composition was calculated from data reported previously [7].

those of ds 8000 and heparin, with a similar wide spectrum of action against both serotypes of HSV and TK^- variants resistant to acyclovir. Furthermore, the compounds were nontoxic for Vero cells at concentrations up to 120–300 μg mL^{-1} or higher (Table 5).

The TT was measured to assay the anticoagulant activity of the *xylo*-mannans. The TT value of the untreated plasma was 15.6 s. Treatment of the plasma with any of the five sulfated xylomannans (5–200 μg mL^{-1}) did not significantly change the control TT value (data not shown).

4. Discussion

The red seaweed *Nothogenia fastigiata* synthesizes a system of a α-(1 → 3)-linked D-mannans 2- and 6-sulfated and having single stubs of β-(1 → 2)-linked D-xylose [7,8]. The *xylo-mannans* 2′ and 4′ follow this general pattern but differ in the quantitative distribution of the different structural units. Xylomannan 4′ has a lesser amount of D-xylose side chains but a higher sulfate content than xylomannan 2′ and also shows an unusual disulfation on C-2 and C-6 (Table 6). Both xylomannans were obtained as complexes with a 'mixed linkage' xylan and an agaroid galactan co-existing in the seaweed [2–6]. Similar complexes in which a xylogalactan sulfate predominates were previously isolated from the same crude extract [5,6].

The fact that the fractionation of the Cetrimide salts does not depend on the sulfate percentage (Table 1), suggests that the major factor of insolubilization is the formation of packed aggregates of molecules, being this process facilitated by the formation of the insoluble Cetrimide salts [24]. Previous work [5,6] showed that the sulfated *xylo*-galactans, *xylo*-mannans and the 'mixed linkage' xylans produced by this seaweed form insoluble aggregates, and possibly soluble ones. The aggregation properties of these compounds could be explained by the 'sugar zipper' model of carbohydrate–carbohydrate interactions [25]. The β-D-(1 → 3)-, β-D-(1 → 4)-'mixed linkage' xylan could interact with a regular array of xylose branches or with an elongated sulfated galactan and/or mannan backbone.

The present evaluation of the antiherpetic properties of these sulfated α-D-(1 → 3)-linked *xylo*-mannans revealed a selective inhibitory activity against several strains of HSV-1 and HSV-2 replication in vitro. Mannan 6′ was the most active with IC_{50} values of 0.6–1.3 μg mL^{-1}. The xylomannan 2′, although less efficient, could be considered an effective antiherpetic compound since its IC_{50} values are in the range 2.0–7.1 μg mL^{-1}.

The conformation of the α-D-(1 → 3)-linked mannan backbone as well as the molecular size and amount of sulfate groups of the samples fulfill the requirements for antiviral activity [8,26].

The most adequate distribution of the sulfate groups for the mannan antiviral activity could be visualized considering the strong interactions between the cell surface heparan sulfate receptors and the viral glycoproteins [28]. Moreover, the *N*-sulfation and the 6-*O*-sulfation of the α-D-glucosamine residues of heparan sulfate play a key role for viral binding [27]. Those polysaccharides with a sulfate distribution structurally resembling the negative charge distribution of the binding sites of heparan sulfate will competitively inhibit viral binding. The presence of 2- and 6-sulfate groups seems to be important, but their appropriate distribution into domains, which would mimic the negative binding site of heparan sulfate, might be necessary in order to achieve antiviral activity.

Although sulfated polysaccharides are generally endowed with anticoagulant properties, the evaluation of the antithrombin activity of the *xylo*-mannans showed that they were inactive. As determined for chemically modified heparin [28,29] and carrageenans [30], data obtained for the *xylo*-mannans confirm that there is no correlation between the antiviral and anticoagulant properties.

Acknowledgements

The authors are indebted to UMYMFOR (FCEyN-CONICET) for technical assistance. This work was supported by grants from CONICET (PID-BID 458/92), OAS and the University of Buenos Aires.

References

[1] P. Parkinson, *Taxon*, 32 (1983) 601–610.
[2] A.S. Cerezo, A. Lezerovich, R. Labriola, and D.A. Rees, *Carbohydr. Res.*, 19 (1971) 289–296.
[3] A.S. Cerezo, *Carbohydr. Res.*, 22 (1972) 209–211.
[4] M.C. Matulewicz, A.S. Cerezo, R.J. Jarret, and N. Syn, *Int. J. Biol. Macromol.*, 14 (1992) 29–32.
[5] H.H. Haines, M.C. Matulewicz, and A.S. Cerezo, *Hydrobiologia*, 204–205 (1990) 637–643.

[6] M.C. Matulewicz, H.H. Haines, and A.S. Cerezo, *Phytochemistry*, 36 (1994) 97–103.
[7] M.C. Matulewicz and A.S. Cerezo, *Carbohydr. Polym.*, 7 (1987) 121–132.
[8] A.A. Kolender, M.C. Matulewicz, and A.S. Cerezo, *Carbohydr. Res.*, 273 (1995) 179–185.
[9] K.S. Dodgson and R.G. Price, *Biochem. J.*, 84 (1962) 106–110.
[10] J.T. Park and M.J. Johnson, *J. Biol. Chem.*, 181 (1949) 149–151.
[11] G. Ingram, in *Methods of Organic Elemental Microanalysis*, Chapman and Hall, London, 1962, pp 105–127.
[12] D.P. Sweet, R.H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1971) 217–225.
[13] A.S. Cerezo, *Carbohydr. Res.*, 26 (1973) 335–340.
[14] S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
[15] E.B. Damonte, M.C. Matulewicz, A.S. Cerezo, and C.E. Coto, *Chemotherapy*, 42 (1996) 57–64.
[16] T.T. Stevenson and R.H. Furneaux, *Carbohydr. Res.*, 210 (1991) 277–298.
[17] J.-P. Joseleau, G. Chambat, and B. Chumpitazi-Hermoza, *Carbohydr. Res.*, 90 (1981) 339–344.
[18] L. Petruš, D.G. Gray, and J.N. BeMiller, *Carbohydr. Res.*, 268 (1995) 319–323.
[19] J.-R. Brisson, F.M. Winnik, J.J. Krepinsky, and J.P. Carver, *J. Carbohydr. Chem.*, 2 (1983) 41–55.
[20] K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.
[21] A. Allerhand and E. Berman, *J. Am. Chem. Soc.*, 106 (1984) 2400–2412.
[22] A.I. Usov, S.V. Yarotskii, and L.K. Vasayina, *Bioorg. Khim.*, 1 (1975) 1583–1588.
[23] A.I. Usov and I.M. Dobkina, *Bioorg. Khim.*, 17 (1991) 1051–1058.
[24] F. Kong and C. Schuerch, *Macromolecules*, 17 (1984) 983–989.
[25] D. Spillmann, *Glycoconjugate J.*, 11 (1994) 169–171.
[26] M. Witvrouw, J. Desmyter, and E. De Clercq, *Antiviral Chem. Chemother.*, 5 (1994) 345–359.
[27] B.C. Herold, S.I. Gerber, T. Polonsky, B.J. Belval, P.N. Shaklee, and K. Holme, *Virology*, 206 (1995) 1108–1116.
[28] M. Baba, E. De Clercq, D. Schols, R. Pauwels, R. Snoeck, C. Van Boeckel, G. Ven Dedem, N. Kraaijeveld, P. Hobbelen, H. Ottenheijm, and F. Den Hollander, *J. Infect. Dis.*, 161 (1990) 208–213.
[29] T. Barzu, M. Level, M. Petitou, J.C. Lormeau, J. Choay, D. Schols, M. Baba, R. Pauwels, M. Witvrouw, and E. De Clercq, *J. Med. Chem.*, 36 (1993) 3546–3555.
[30] M.J. Carlucci, C.A. Pujol, M. Ciancia, M.D. Noseda, M.C. Matulewicz, E.B. Damonte, and A.S. Cerezo, *Int. J. Biol. Macromol.*, 20 (1997) 97–105.