Cell-wall polysaccharides from the marine green alga Ulva "rigida" (Ulvales, Chlorophyta) - NMR analysis of ulvan oligosaccharides

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Cell-wall polysaccharides from the marine green alga *Ulva "rigida"* (Ulvales, Chlorophyta) — NMR analysis of ulvan oligosaccharides [1]

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Abstract

In order to obtain information on sugar sequences in the water soluble polysaccharides from *Ulva "rigida"*, oligosaccharides were purified by anion exchange and gel permeation chromatography from the partial acid hydrolysate of the native or desulfated ulvan. The chemical structure and sugar sequence of these oligomers were determined by ^{1}H and ^{13}C NMR spectroscopy to be α-L-rhamnosyl $(1 \rightarrow 4)$ D-xylose, β-D-glucuronosyluronic acid $(1 \rightarrow 2)$-α-L-rhamnosyl $(1 \rightarrow 4)$ D-xylose, β-D-glucuronosyluronic acid $(1 \rightarrow 4)$ L-rhamnose 3 sulfate, β-D-glucuronosyluronic acid $(1 \rightarrow 4)$ [β-D-glucuronosyluronic acid $(1 \rightarrow 2)$] L-rhamnose and β-D-glucuronosyluronic acid $(1 \rightarrow 4)$ [β-D-glucuronosyluronic acid $(1 \rightarrow 2)$] α-L-rhamnosyl $(1 \rightarrow 4)$ D-xylose. The sugar linkages and particularly the branching and the sulfate position were in total agreement with previous chemical results.

Keywords: *Ulva*; Chlorophyceae; Algae; Ulvan; NMR spectroscopy; Oligosaccharides

1. Introduction

The cell wall of the marine green algae *Ulva "rigida"* is composed of a water-soluble sulfated glucuronorhamnoxyloglycan (referred to as ulvan for simplicity), alkali-soluble linear β-1,4 glucoxylan, and β-1,4-glucuronan [1,2]. The alkali-insoluble

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polysaccharides of a closely related species, *U. lactuca*, are composed of cellulose and linear β-1,4 xyloglucans [3]. Methylation analysis of native and chemically modified ulvan indicated that this polysaccharide is composed of 1,4 and 1,2,4-linked rhamnose 3-sulfate, 1,4- and terminally-linked glucuronic acid and 1,4-linked xylose partially sulfated on O-2 [4]. Previous chemical studies on the sequence of these sugars in *U. lactuca* ulvan showed the presence of a majority of 4-*O*-(β-D-glucuronosyluronic acid)-L-rhamnose and small quantities of two other aldobiouronic acids tentatively identified as 3-*O*- and 4-*O*-(D-glucuronosyluronic acid)-D-xylose [5]. An acidic tetrasaccharide identified as D-glucuronosyluronic acid-(1 → 4)-L-rhamnosyl-(1 → 3/4)-D-glucuronosyluronic acid-(1 → 3)-D-xylose and particularly the trisaccharide L-rhamnosyl-(1 → 4)-D-xylosyl-(1 → 3)-D-glucose, obtained after partial acid hydrolysis of desulfated and carboxy-reduced ulvan, demonstrated the association of the three different sugars in the polysaccharide [5]. Ulvan is part of the polysaccharide that resists degradation by human digestive enzymes (dietary fibers) in the edible seaweed "sea-lettuce" [6,7] and can form weak gels in the presence of borate and calcium ions in a slightly alkaline environment [2,8,9]. In order to determine sugar sequences of this polysaccharide, oligosaccharides were generated by mild acid hydrolysis of native and chemically desulfated ulvan from *U. "rigida"* [4] and their chemical structure established by NMR analysis. The ^{13}C and ^{1}H NMR signal assignments of the major disaccharide obtained, the aldobiuronic acid 4-*O*-(β-D-glucuronosyluronic acid)-L-rhamnose, have already been reported [2] and we now report on the chemical structure of sulfated or branched oligosaccharides.

2. Materials and methods

Oligosaccharides.—Native and desulfated ulvan from *U. "rigida"* [1,4] were hydrolyzed with 0.1 M TFA at 100 °C for 75 min. Oligosaccharides were recovered as described [2] after DEAE-Sepharose CL6B (17.5 × 3.4 cm, Pharmacia) and Bio-Gel P2 (GPC, 96 × 2.6 cm, Bio-Rad) chromatographies with orcinol colorimetry or differential refractive index detection. Elution of the oligosaccharides from GPC was achieved with deionized water for neutral fractions and with $NaNO_3$ (0.1 M) for charged oligosaccharides and is expressed as K_{av} [$K_{av} = (V_e - V_0)/(V_t - V_0)$ with V_t, V_0 being the total and void volume of the column and V_e the elution volume of the sample]. Fractions corresponding to oligosaccharide peaks were pooled, concentrated, and desalted by permeation through Sephadex G10 (100 × 1.6 cm, Pharmacia) eluted with deionized water and freeze-dried.

NMR analysis.—^{13}C and ^{1}H NMR spectra of oligosaccharide solutions in D_2O were recorded on a Bruker ARX 400 spectrometer. Carbon and proton chemical shifts of polymers were referenced to acetone assigned to 31.4 and 2.225 ppm, respectively. Oligosaccharides were deuterium-exchanged twice in 99.9% D_2O before solubilization in 0.5 mL 100% D_2O with a trace of acetone as internal reference. Non-exchangeable proton assignments of oligosaccharides and sugar sequences were determined from 2D, COSY90, COSY-Relay, COSY-DQF, TOCSY (HOHAHA), and NOESY spectra with water suppression using the conventional pulse sequences provided by Bruker. Carbon

chemical shifts were determined from ^{1}H-^{13}C HMQC and HMQC-TOCSY experiments. The pD values were measured with a pH electrode calibrated with aqueous buffer solutions and without correction for the deuterium.

3. Results and discussion

Oligosaccharides.—Oligosaccharides from native ulvan were fractionated into neutral and charged fractions by chromatography through DEAE-Sepharose CL6B. The charged fractions were eluted into two major populations by 0.15 and 0.40 M NaCl in a 0 to 1 M gradient. Only the neutral and the charged oligosaccharides eluting first were further studied. The neutral population was fractionated on Bio-Gel P2 into several oligosaccharides. The major one, eluting at K_{av} 0.47, was identified by NMR spectroscopy as the aldobiuronic acid β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose [2]. The chemical shift for H-5/C-5 and H-4/C-4 reported in ref. [2] have been reversed in agreement with published data [10] and results obtained from oligosaccharides in this study (see below). Other oligosaccharides eluted at K_{av} 0.67, 0.57, 0.38, and 0.16, and only the oligomer eluting at K_{av} 0.38, referred to as **N1**, was studied further. The charged population was fractionated on Bio-Gel P2 into one major spreading peak probably due to excess of salts, and only the portion of the peak eluting at $K_{av} \sim 0.4$ (referred to as **N2**) was studied further. After partial acid hydrolysis of desulfated ulvan, neutral and charged oligomers were obtained as above. The neutral population yielded two major peaks on chromatography through Bio-Gel P2 with K_{av} of 0.81 and 0.71. Only the latter oligosaccharide was studied and is referred to as **D1**. The charged oligosaccharides eluted from the anion exchanger into a major wide fraction and a smaller population eluting with 0.1–0.3 M and 0.5 M NaCl, respectively. Only the first population was studied and yielded several peaks on chromatography through Bio-Gel P2. The major one (K_{av} 0.61) was identified by NMR spectroscopy as the aldobiuronic acid, β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose [2]. Two minor peaks eluted at K_{av} 0.37 and 0.27 were studied further and are referred to as **D2** and **D3**, respectively.

From the chemical composition of the parent polysaccharides [4], the oligosaccharides were expected to be composed of only xylose, rhamnose, and glucuronic acid.

Determination of the chemical structure of the neutral oligosaccharides **D1** *and* **N1**. —The ^{1}H NMR spectrum of oligosaccharide **D1** shows 4 major signals in the region of the anomeric protons (5.195, 4.926, 4.921, 4.586 ppm, see Fig. 1) with intensities indicating that this oligomer is a disaccharide. From their chemical shifts and $J_{1,2}$ coupling constants (Table 1) two of the anomeric signals (5.195 and 4.586 ppm) were attributed to a reducing-end xylose in the α and β configuration [11]. The two overlapping anomeric signals (4.926 and 4.921 ppm) with a small coupling constant (1.9 Hz) and the methyl proton (1.317 ppm) were attributed to H-1 of an α-rhamnose residue [11] affected by the configuration of the reducing end sugar to which it is linked and its H-6, respectively. The effect of the reducing-end on the chemical shifts of the rhamnose protons is observed up to H-3. 2D COSY90 and Relay-COSY allowed the full chemical shift assignment of all the protons in this disaccharide (Table 1) and the coupling constants measured are in agreement with those reported in the literature [11]. The

Table 1
^{1}H and ^{13}C NMR chemical shifts of ulvan oligosaccharides

Oligosaccharide	Sugar [a]	^{1}H							^{13}C					
		1	2	3	4	5a [b]	5e [b]	6	1	2	3	4	5	6
Aldobiuronic acid	Rha α	5.10	3.92	4.01	3.67	3.94		1.33	94.9	72.0	71.1	82.3	68.0	18.1
(ref. [2]) [c]	Rha β	4.86	3.93	3.82	3.61	3.50		1.35	94.5	72.5	73.9	81.8	71.7	18.1
	GlcA β	4.73	3.35		~3.53	3.73			104.0	74.8	76.7	72.9	77.4	176.5
D1	Xyl α	5.195 (3.6)	3.554 (9.4)	3.794 (9.6)	3.662	3.73 (5.5)	3.840 (10.2, 11.0)		93.3	72.7	72.3	75.2	59.8	
	Xyl β	4.586 (7.8)	3.264 (9.2)	3.584 (9.2)	3.687	3.357 (5.2)	4.081 (10.3, 11.7)		97.9	75.3	75.3	75.2	63.9	
	Rha α													
	α [d]	4.926	4.259	4.483										
					3.615	4.040		1.317	98.9	70.1	79.8	71.1	70.1	17.9
	β [d]	4.921 (1.9)	4.246 (3.2)	4.475 (9.6)	(9.6)	(6.3)								
N1	Xyl α	5.195 (3.4)	3.545	3.725	~3.70	~3.66	~3.80		93.3	72.7	72.3	75.3 [e]	59.7	
	Xyl β	4.568 (7.9)	3.248	3.522	3.676	3.313	4.054		97.9	75.3 [e]	75.2 [e]	75.1	63.9	
	Rha α	5.173	4.015	3.864	3.496	3.939		1.285	98.3	83.3	71.2	73.5	69.9	17.8
	GlcA' β	4.609 (7.8)	3.403	~3.50		3.700			105.4	74.6	76.7	73.0	77.7	nd [f]
N2	Rha α	5.119 (2.3)	4.236	4.657	3.832	4.007		1.363	94.6	70.5	79.3	79.4	68.8	18.4
	Rha β	4.907 (1.1)	4.265	4.458	3.744	3.539		1.380	94.2	70.9	81.2	79.0	72.0	18.4
	GlcA β	4.648 (7.9)	3.333	~3.54	3.691				104.3	74.5	76.5	72.7	76.6	174.9

D2	Rha α	5.349 (2.1)	4.026	4.091	3.758	3.921		1.330						
	Rha β	4.823	4.145	3.931	3.715	3.524		1.349						
	GlcA′ β													
	α [d]	4.639	3.414	~ 3.52		3.700								
		4.655	3.476	~ 3.55		3.761								
	β [d]	(7.8)												
	GlcA β	4.739 (7.9)	3.355	~ 3.53		3.683								
D3	Xyl α	5.185 (3.6)	3.551 (9.2)	3.769	~ 3.69	3.705	3.827		93.3	72.7	72.3	75.5	59.8	
	Xyl β	4.568 (7.8)	3.261 (9.2)	3.538 (9.2)	3.687 (11.0, 5.5)	3.325 (11.6)	4.070		97.9	75.4	75.3	75.4	63.9	
	Rha α													
	α [d]	5.157												
			4.025	4.085	3.773	4.002		1.343	98.2	80.9	71.3	81.8	68.5	17.9
		5.152	multiplet (9.5)		(9.5)	(6.3)								
	β [d]	(~ 2.1)												
	GlcA′ β	4.618 (7.8)	3.410	~ 3.52		3.688			105.2	74.6	76.7	72.9	77.3	176.7
	GlcA β	4.737 (7.9)	3.346	~ 3.53	3.681				103.9	74.9	76.9	73.0	77.5	176.7

[a] Rha, Xyl, GlcA, and GlcA′ refer to rhamnose, xylose, glucuronic acid linked to O-4 and O-2 of rhamnose, respectively.
[b] a and e refers to axial and equatorial protons, respectively.
[c] Chemical shifts measured at 40 °C from acetone (δC: 31.4, δH: 2.23), assignments for H/C-4 and H/C-5 of glucuronic have been reversed (see text).
[d] α and β refer to the effect of the α and β configurations of the reducing sugar.
[e] May be interchanged.
[f] Not determined.

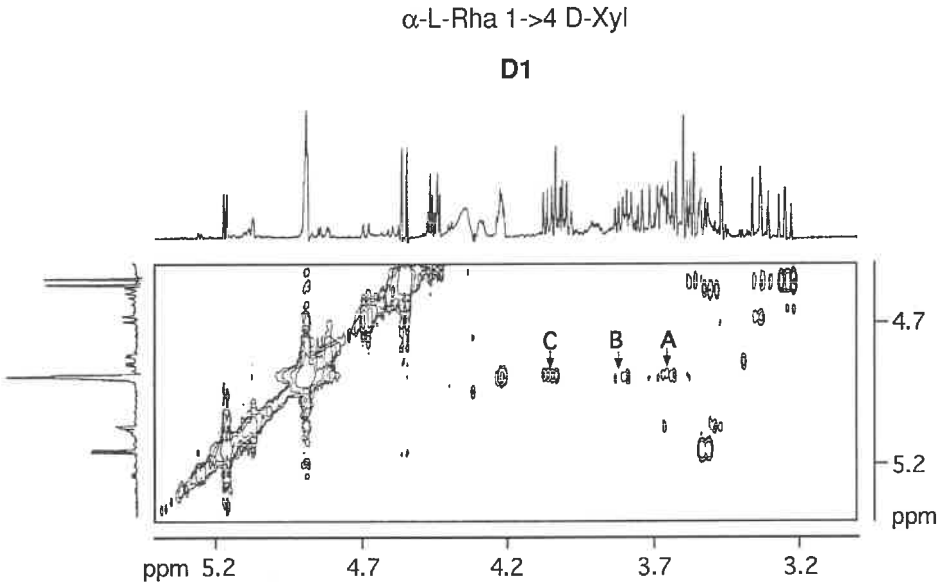

Fig. 1. Part of 2D NOESY spectrum of oligosaccharide **D1** at 55 °C with a mixing time of 700 ms. Along the F1 and F2 axes are high resolution 1D spectra of the oligomer. The following inter-residue cross-peak are indicated: **A**, Rha H-1/Xyl H-4α and H-4β; **B**, Rha H-1/Xyl H-5α axial; **C**, Rha H-1/Xyl H-5β axial.

linkage of rhamnose to the reducing-end xylose was established by a NOESY experiment (Fig. 1). Cross-peaks were observed between rhamnose H-1 and xylose H-4α/β and H-5′α/β clearly indicating the rhamnose linkage to O-4 of xylose. No NOE was observed between rhamnose H-1 and rhamnose H-3 and H-5 and, together with the $J_{1,2}$ coupling constant, confirmed the α configuration of the sugar [12]. Oligosaccharide **D1** was thus deduced to be α-L-rhamnosyl-(1 → 4)-D-xylose. The ^{13}C NMR chemical shifts were deduced from an HMQC experiment (Table 1).

Oligosaccharide **N1** gave a ^{1}H NMR spectrum with 4 major anomeric protons at 5.195 ($J_{1,2}$ 3.4 Hz), 4.568 ($J_{1,2}$ 7.9 Hz), 5.173 and 4.609 ppm ($J_{1,2}$ 7.8 Hz) (see Fig. 2) which, based on their intensities, indicated that **N1** is a trisaccharide. Three of these anomeric protons corresponded to those of rhamnose and reducing end xylose of oligosaccharide **D1**. The identity of the last sugar residue was determined to be β-GlcA from its coupling network and by comparison with chemical shifts obtained for the aldobiuronic acid, β-D-GlcA-(1 → 4)-L-rhamnose (Table 1). All the proton chemical shifts were determined by COSY-DQF and TOCSY experiments (Table 1). The linkage of these sugars was determined from the NOESY cross-peaks observed between H-1 of the glucuronic acid residue with rhamnose H-2 and H-1 of rhamnose with xylose H-4α and H4-β (Fig. 3). Thus, this trisaccharide is identified as β-D-GlcA-(1 → 2)-α-L-Rha-(1 → 4)-D-Xyl. The ^{13}C NMR chemical shifts were attributed from HMQC and HMQC-TOCSY experiments and by comparison with chemical shifts observed for these carbons in oligosaccharides **N2** and **D3** (Table 1).

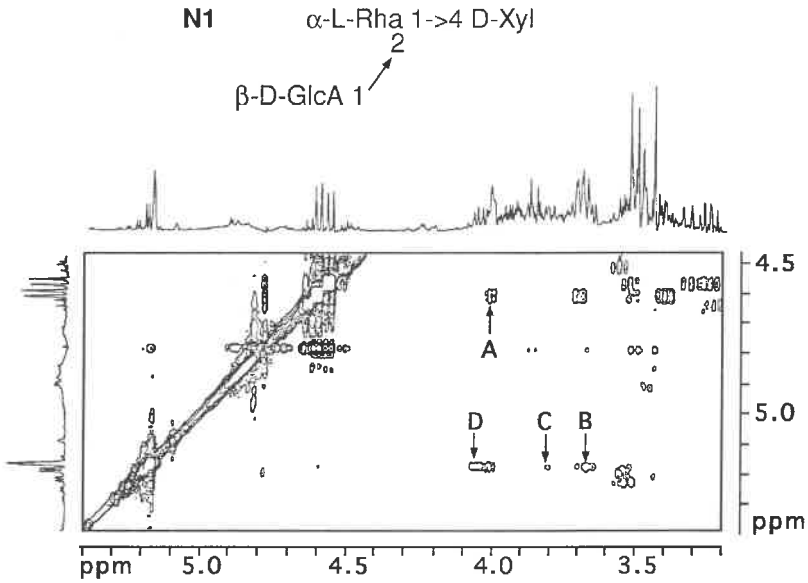

Fig. 2. Part of 2D NOESY spectrum of oligosaccharide **N1** at 55 °C (pD 6.8) with a mixing time of 500 ms. Along the F1 and F2 axes are high resolution 1D spectra of the oligomer. The following inter-residue cross-peak are indicated: **A**, GlcA H-1/Rha H-2; **B**, Rha H-1/Xyl H-4α and H-4β; **C**, Rha H-1/Xyl H-5α axial; **D**, Rha H-1/Xyl H-5β axial.

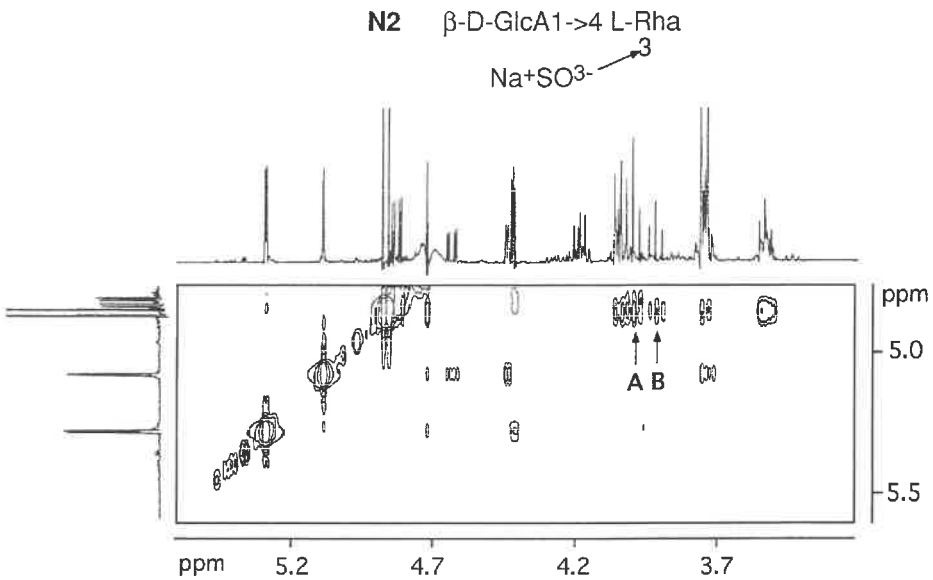

Fig. 3. Part of 2D NOESY spectrum of oligosaccharide **N2** at 55 °C (pD 3.5) with a mixing time of 700 ms. Along the F1 and F2 axes are high resolution 1D spectra of the oligomer. The following inter-residue cross-peak are indicated: **A**, GlcA H-1/Rha H-4α; **B**, GlcA H-1/Rha H-4β.

Determination of the chemical structure of the oligosaccharides **N2**, **D2**, *and* **D3** *eluted with a salt gradient from the anion exchanger.*—The higher charge density of these oligosaccharides is due to a greater amount of uronic acids and/or the presence of sulfate groups. Oligosaccharide **N2** gave a ^{1}H NMR spectrum (see Fig. 3) showing 3 anomeric protons of chemical shifts close to those of the aldobiuronic acid (β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose) (Table 1). Taking into account their intensity, **N2** is a disaccharide with rhamnose at the reducing-end. The chemical shift of the other protons was determined by COSY-DQF and TOCSY experiments (Table 1). Non-first-order couplings between the glucuronic acid protons were observed (particularly H-2 and H-5, data not shown) and were probably related to the virtual long-range spin–spin couplings described for this sugar [13]. Such couplings were also observed for the aldobiuronic acid (data not shown). As for the latter acidic disaccharide, the glucuronic acid in **N2** is linked to rhamnose O-4 as demonstrated by the cross-peaks observed on the NOESY contour plot (Fig. 3) between glucuronic H-1/rhamnose H-4α and β. A minor temperature effect was seen for the chemical shift of H-5 of the glucuronic acid residue (+0.01 ppm from 56 to 25 °C) but, as reported [10], strong effects were seen for glucuronic acid protons at different pD (+0.05, +0.02, +0.06 and +0.29 ppm for H-1, 2, 3/4 and 5, respectively between pD 7.6 and 2.3). Similar effects of pD were observed for the aldobiuronic acid H-5 and H-3/4 of glucuronic acid (data not shown) and indicates the strong influence of the ionic status of the carboxyl group on the conformation of the glucuronic acid residue. No effect was observed for the rhamnose protons. The ^{13}C NMR chemical shifts were determined for this oligosaccharide by HMQC and HMQC-TOCSY experiments as for oligosaccharide **N1** (Table

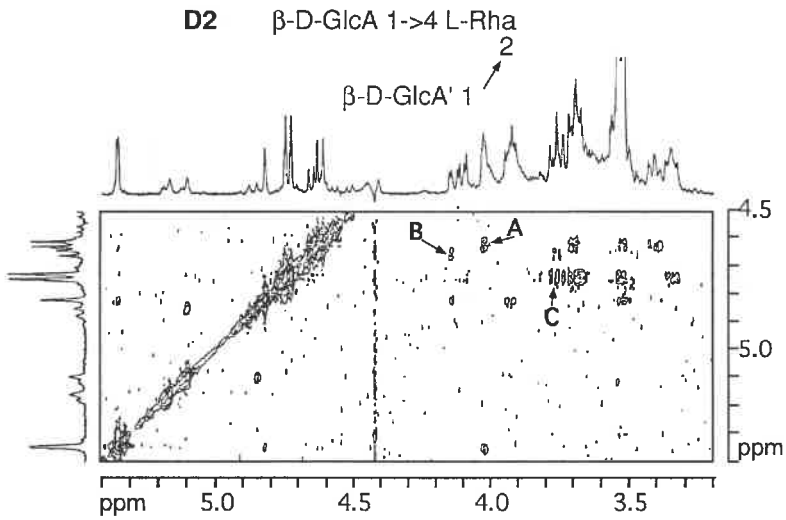

Fig. 4. Part of 2D NOESY spectrum of oligosaccharide **D2** at 55 °C (pD 7.8) with a mixing time of 700 ms. Along the F1 and F2 axes are high resolution 1D spectra of the oligomer. The following inter-residue cross-peak are indicated: **A**, GlcA′ H-1/Rha H-α2; **B**, GlcA′ H-1/Rha H-β2; **C**, GlcA H-1/Rha H-4; the prime denotes the GlcA linked to O-2 of Rha.

1). Considering the marked downfield shifts of rhamnose H-3α/β, H-2α/β, and H-4α/β resonances (about 0.65, 0.33, and 0.15 ppm, respectively), the major downfield shift for C-3α/β (8.2 and 7.3 ppm for the α and β configuration, respectively) and the upfield shifts for the C-2α/β and C-4α/β resonances (1.6 and 2.9 ppm, respectively) compared to the respective nuclei in the aldobiuronic acid (Table 1), O-3 of rhamnose is deduced to be the site of sulfate substitution. Oligosaccharide **N2** is thus identified as β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose 3-sulfate.

Oligosaccharide **D2** showed a ^{1}H NMR spectrum with 5 anomeric signals at 5.349 ($J_{1,2}$, 2.1 Hz), 4.823, 4.639 ($J_{1,2}$, 7.8 Hz), 4.655 ($J_{1,2}$, 7.8 Hz) and 4.739 ppm ($J_{1,2}$, 7.9 Hz) (see Fig. 4) which, taking their intensity into account, corresponded to a trisaccharide with one residue markedly affected by the configuration of the reducing end sugar. The different saccharides were identified as two β-D-GlcAs and one reducing end rhamnose from the proton chemical shifts and coupling constants determined by COSY-DQF and TOCSY experiments (Table 1). Some of the chemical shifts presented similarities with those of the GlcAs of oligosaccharide **N1** and of the aldobiuronic acid (Table 1). Proton resonances of the β-rhamnose residue were close to those reported for a β-(1,2,4)-linked rhamnose residue in a polysaccharide extracted from the stipules of the tree *Musanga cercropoides* [14]. Linkages of the sugars were deduced from the NOESY experiment (Fig. 4): GlcAs are linked to O-4 and O-2 of the rhamnose located at the reducing-end. The protons of the GlcA linked to O-2 of rhamnose are markedly affected by the anomeric configuration of the reducing end sugar. The NOESY cross-peaks between the GlcA doublet at 4.655 ppm with H-2 of β-rhamnose and that at 4.639 ppm with H-2 of α-rhamnose clearly demonstrated it (Fig. 4). Such reducing end configurations have only a minor effect on the anomeric proton of the GlcA linked to

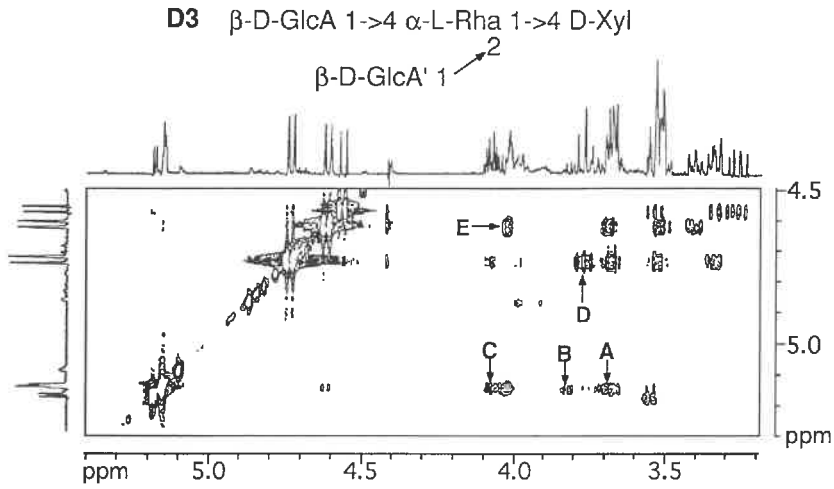

Fig. 5. Part of 2D NOESY spectrum of oligosaccharide **D3** at 55 °C (pD 7.9) with a mixing time of 700 ms. Along the F1 and F2 axes are high resolution 1D spectra of the oligomer. The following inter-residue cross-peak are indicated: **A**, Rha H-1/Xyl H-4α-β; **B**, Rha H-1/Xyl H-5α axial; **C**, Rha H-1/Xyl H-5β axial; **D**, GlcA H-1/Rha H-4; **E**, GlcA′ H-1/Rha H-2; the prime denotes the GlcA linked to O-2 of Rha.

O-4 of rhamnose: only a small enlargement at the base of the doublet signal at 4.739 ppm can be observed. This oligosaccharide is thus identified as β-D-glucuronosyluronic acid-(1 → 4)-[β-D-glucuronosyluronic acid-(1 → 2)-]-L-rhamnose. The low oligosaccharide concentration precluded 1D ^{13}C and 2D $^{1}H/^{13}C$ correlation spectra to be recorded and thus, carbon chemical shifts were not determined.

Oligosaccharide **D3** ^{1}H NMR spectrum shows 5 anomeric signals and considering their intensity, corresponds to a tetrasaccharide (see Fig. 5). Two glucuronic acids, one rhamnose and one reducing end xylose residues were identified by COSY90 and RELAY-COSY experiments (Table 1). ^{1}H NMR chemical shifts of **D3** were close to those for the glucuronic acids and some of the rhamnose protons of oligosaccharide **D2** and for the xylose and some of the rhamnose protons of oligosaccharide **N1** (Table 1).

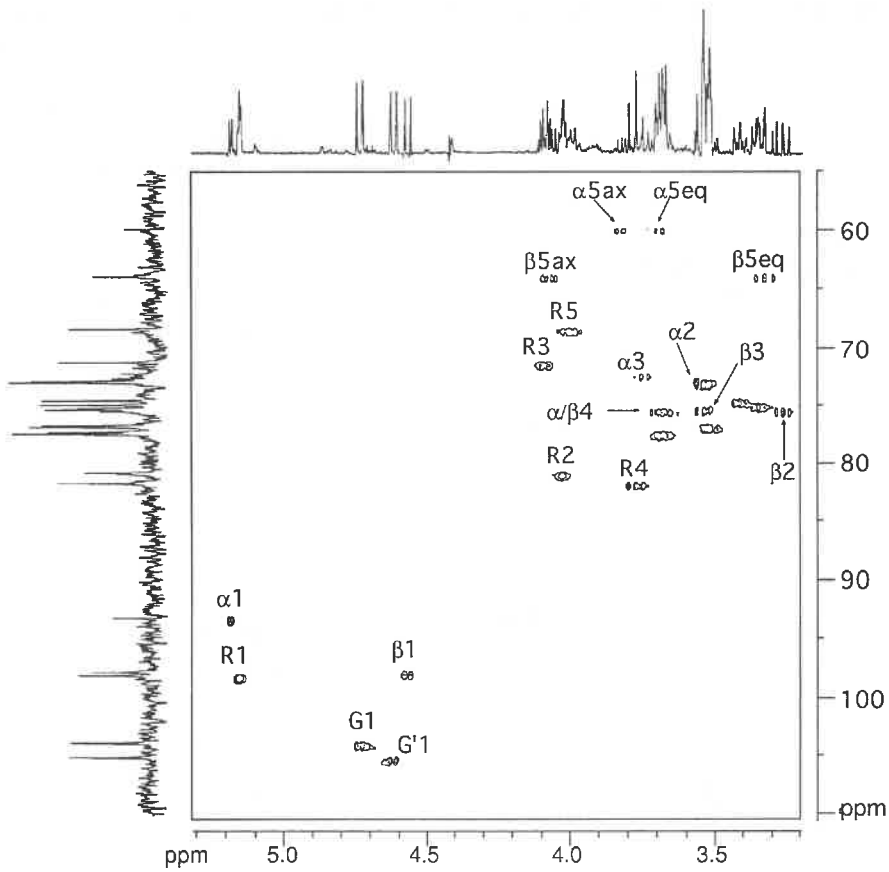

Fig. 6. 2D ^{1}H-^{13}C chemical shift correlation spectroscopy (HMQC) contour plot obtained for oligosaccharide **D3** at 55 °C. Letters R, G, G′, α and β refer to Rha, GlcA linked to O-4 of Rha, GlcA linked to O-2 of Rha, Xyl α and Xyl β, respectively; numbers correspond to the proton/carbon in the residues; ax and eq refer to axial and equatorial, respectively. High resolution 1D ^{1}H and ^{13}C spectra are represented along the F2 and F1 axes, respectively.

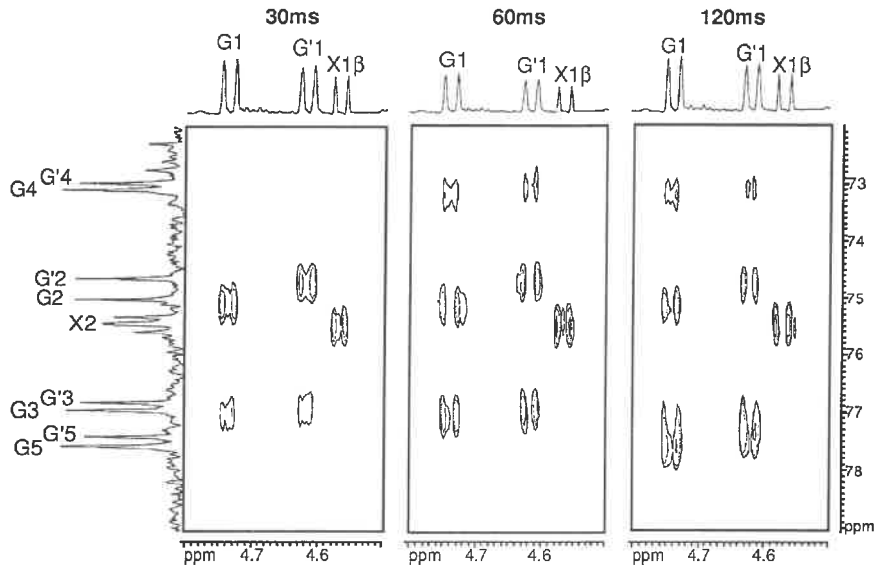

Fig. 7. Part of 2D HMQC-TOCSY of oligosaccharide **D3** at 55 °C in D_2O. Symbols are as in Fig. 6. High resolution ^{1}H and ^{13}C NMR spectra are shown along the F2 and F1 axes, respectively.

The NOESY experiment allowed the determination of the linkages between the sugars (Fig. 5). Thus, glucuronic acid residues are linked to O-4 and O-2 of rhamnose itself linked to O-4 of xylose at the reducing end. This oligosaccharide is identified as β-D-glucuronosyluronic acid-(1 → 4)-[β-D-glucuronosyluronic acid-(1 → 2)-]-α-L-rhamnosyl-(1 → 4)-D-xylose. Most of the ^{13}C NMR chemical shifts were attributed by a HMQC experiment (Table 1, Fig. 6). HMQC-TOCSY experiments with different mixing times (30–120 ms) allowed for the unambiguous attribution of the carbons C2–C5 of the two glucuronic acid residues (Fig. 7).

4. Conclusion

The oligosaccharides obtained after partial acid hydrolysis of native and desulfated ulvan allowed identification of sugar sequences in ulvan and to confirm linkages and substitutions determined by methylation analysis of the native and chemically modified polysaccharide [4]. In agreement with literature data obtained from *U. lactuca* [5], the major oligosaccharide obtained from *U. "rigida"* ulvan was identified in a previous study to be the aldobiuronic acid, β-D-glucuronosyluronic acid-(1 → 4)-L-rhamnose [2] that we propose to refer to as ulvanobiuronic acid for simplicity. Other basic repeating sequences isolated from the acid hydrolysate of native and desulfated ulvan provided the definitive proof that all three sugars are linked together in the same polymer and that branching by glucuronic acid occurs on O-2 of rhamnose. Considering that glucuronic acid is found by methylation analysis to be located for a good part at non-reducing ends

of ulvan [4], the present data suggest that the branch is composed of single glucuronic acid residue. However, future work using milder and more specific degradation methods are required to determine the composition and the length of the side-chain in the native polymer that may have been partially degraded by the hydrolysis condition used in this study. Rhamnose is often found in bacterial exopolysaccharides [15] or plant pectic polysaccharides [16] but branching on O-2 of a (1 → 4)-linked α-L-rhamnose residue by a β-D-glucuronic acid residue has, to our knowledge, only been described for the exopolysaccharide produced by the bacterium *Arthrobacter* sp. [17]. Apart from the ulvanobiuronic acid, the oligosaccharides obtained in this study differ from those of Haq and Percival [5]. The only common features between these works are the linkages of rhamnose to O-4 of D-xylose and that of glucuronic acid to O-4 of rhamnose. In contrast to the branching on O-3 of rhamnose deduced by these authors from methylation analysis, no such branching was observed in the oligosaccharides isolated in the present work. Concerning the site of sulfate substitution on ulvan, the comparison between ^{1}H and ^{13}C NMR chemical shifts of ulvanobiuronic acid and that of oligosaccharide **N2** clearly confirmed the location of sulfate group on O-3 of rhamnose determined by chemical analysis [4]. Unfortunately, oligosaccharides containing sulfated xylose residues were not isolated at this time and the site of sulfation on this residue only rely on data obtained from chemical analyses. Further works is under way to determine if this sulfation and sugar linkages are common to the ulvan from different species of *Ulva*.

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