

# Structure of Ulvan Isolated from the Edible Green Seaweed, *Ulva pertusa*

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Received 25 August 2015; accepted 27 October 2015; published 30 October 2015

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## Abstract

Ulvan, rhamnan sulfate, was extracted from the edible green seaweed, *Ulva pertusa*, which is grown on the coast of the Okinawa Islands. The yield of ulvan was 8.5% (W/W), and the total carbohydrates, uronic acid and sulfuric acid and ash contents were 67.3%, 23.8%, 19.7% and 22.6%, respectively. L-Rhamnose, D-xylose and D-glucose residues were identified by liquid chromatography, and their molar ratio was 4.0:0.1:0.3. D-Glucuronic and L-idulonic acid residues were also identified in molar ratio of 1.0:0.2. The NMR (<sup>13</sup>C and <sup>1</sup>H) and methylation analysis revealed terminal β-D-glucuronic acid, terminal α-L-idulonic acid, 1,3-linked α-L-rhamnose, 1,4-linked α-L-rhamnose, 1,2,4-linked α-L-rhamnose, 1,3,4-linked α-L-rhamnose, 1,2,3,4-linked α-L-rhamnose and 1,3,4-linked β-D-xylose. The sulfate groups were attached at the C-2 and C-3 positions of the 1,4-linked α-L-rhamnose as well as C-3 of the 1,4-linked β-D-xylose residues. The chemical structure of the ulvan from *Ulva pertusa* was determined.

## Keywords

*Ulva pertusa*, Ulvan, NMR Analysis, Methylation Analysis, Chemical Structure

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## 1. Introduction

In the course of the chemical, physicochemical and biological studies of polysaccharides, we investigated structure-function relationship of κ-carrageenan [1] [2], ι-carrageenan [3], agarose [4], gellan gums [5] [6], rhamnan gums [7] [8], amyloses [9] [10], amylopectins [11]-[15], and starches [16]-[20]. The results and discussions led

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us to realize their gelation, gelatinization, or retrogradation mechanisms in principle [21]-[23].

We isolated many industrially useful polysaccharides, such as agar [24], methylated agar [25],  $\kappa$ -carrageenan [26],  $\iota$ -carrageenan [27], fucoidans [28]-[30], alginates [31] [32], galactomannans [33] [34], pectins [35]-[37], and rhamnan sulfate [38] from the subtropical biomasses grown in Okinawa Islands, Japan.

In the previous studies, we identified fucoidans from commercially cultured Okinawamozuku (*Cladosiphon okamuranus*) [28] and Itomozuku (*Nemacystus decipiens*) [29]. Specifically, a novel fucoidan which was substituted with an acetyl group from the commercially cultured brown seaweed, *Cladosiphon okamuranus* [28] [32] was identified and patented [39]. The acetyl fucoidan exhibits some biological activities, such as antitumor [40] and immune-enhancing abilities [41]. An over-sulfated acetyl fucoidan, the sulfate content of which was 32.8%, showed a significant antitumor activity *in vitro* [40]. The results suggested that the over-sulfated acetyl fucoidan was applicable as an anticancer drug. Recently, the chemical structure of the acetyl fucoidan was determined [41]. The acetyl fucoidan consists of 1,3-linked  $\alpha$ -L-fucopyranosyl residues and a branched  $\beta$ -D-glucuronopyranosyl residue at C-2 on the main-chain. The sulfate and acetyl groups branched at C-4 on the main chain. We also isolated a rhamnan sulfate from commercially cultured Hitoegusa (*Monostroma nitidum*: green seaweed) in Okinawa Prefecture, Japan and determined its chemical structure [38]. The rhamnan sulfate consists of 1,3-linked  $\alpha$ -L-rhamnopyranosyl residues that branch at C-2 with trisaccharide side chains, D-GlcA(1 $\rightarrow$ 2)- $\alpha$ -L-Rha-(1 $\rightarrow$ 2)- $\alpha$ -L-Rha-(1 $\rightarrow$ ). The acetyl fucoidan is now used as a supplement in health food, food and cosmetic industries in the world.

*Ulva pertusa*, an edible green seaweed is widespread in the natural environment of Japan, South Korea and China. The seaweed contains water-soluble rhamnan sulfate, which is referred to as ulvan [42]. The chemical structure of part of the ulvan from *Ulva pertusa* has been reported [43] [44]. This paper describes the fine chemical structure of the ulvan from *Ulva pertusa*.

## 2. Materials and Methods

### 2.1. Materials

*Ulva fertusa* was collected from the coast of Tomishiro City, Okinawa, Japan on April, 2007. The algae were washed with tap water and air dried at 40°C for 48 h before being ground into powder.

The sample (20 g) was suspended in distilled water adjusted to a pH of 3.0 with 0.05 M hydrochloric acid and stirred at 60°C for 3 h to extract the polysaccharide. The extract was neutralized with a 0.01 M NaOH solution, and then centrifuged at 23,000 *g* at 4°C for 20 min. The supernatant was filtered through Celite 545 (Nakarai, Japan). The filtrate was precipitated by adding 2 volumes of ethanol, and the resulting solid was dried *in vacuo*.

The crude polysaccharide was dissolved in distilled water and the solution was passed through Celite 545. The filtrate was deionized by passing through a cation exchange column composed of Amberlite 120A H<sup>+</sup> (Organo, Japan) and neutralized. The solution was subsequently lyophilized.

### 2.2. Chemical Procedures

The total carbohydrate and uronic acid content were determined with the phenol-sulfuric acid [45] and carbazol-sulfuric acid [46] methods using L-rhamnose and D-glucuronic acid as standards, respectively. The purified polysaccharide (70 mg) was dissolved in distilled water (20 mL) and sulfuric acid was added to reach a final concentration of 1.0 M. The mixture was subsequently heated to 100°C for 3 h. The hydrolysate was neutralized with BaCO<sub>3</sub>.

### 2.3. High-Performance Anion Exchange Chromatography Coupled with a Pulse Amperometric Detector (HPAEC-PAD)

The monosaccharides in the hydrolysate of the polysaccharide were identified using a HPAEC (DX-500, Dionex Co., CA, USA), fitted with a CarboPACK PA1 column and a pulsed amperometric detector. The column was eluted at flow rate of 1 mL/min at 35°C with 10 mM NaOH.

### 2.4. Determination of the Sulfate and Uronic Acid Contents

Purified polysaccharide (10 mg) was dissolved in distilled water and hydrochloric acid was added to reach a

final concentration of 1.0 M. The mixture was heated at 100°C for 3 h. The hydrolysate was allied to HPAEC (DX-500, Dionex Co., CA, USA), on a column (A-SC4) equilibrated with 1.7 mM  $\text{NAHCO}_3$  + 1.8 mM  $\text{NaCO}_3$ . The chromatography was carried out at 35°C using a flow rate of 1.0 cm/min.

## 2.5. Desulfation of the Polysaccharide

The aqueous solution of the purified polysaccharide was passed through a cation exchange chromatography column (Dowex 50 W, Dow Chemical Co.) and was neutralized with pyridine. Then it was dialyzed overnight. The dried sample was dissolved in a 90% DMSO+10% methanol solution and heated at 80°C for 4 h under a  $\text{N}_2$  atmosphere [38] [41]. Then the sample was dialyzed, and freeze-dried.

## 2.6. Infrared Spectrum and Specific Rotation of the Polysaccharide

The infrared spectrum of the polysaccharide was recorded in KBr discs using a spectrophotometer (FTS-3000; Bio-Rad Laboratories Inc., CA, U.S.A.) in transmittance mode from 4000 to 400  $\text{cm}^{-1}$ .

The specific rotation was measured at 589 nm using a polarimeter (P-1010; JASCO Inc., Tokyo, Japan) at room temperature. The polysaccharide solution (0.2%) was prepared in distilled water.

## 2.7. $^1\text{H}$ - and $^{13}\text{C}$ -Nuclear Magnetic Resonance (NMR) Spectroscopy

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a  $\alpha$  500 FT-NMR spectrometer (JEOL Ltd, Japan) at 500.00 and 125.65 MHz, respectively. The polysaccharide (2%, W/V) was dissolved in  $\text{D}_2\text{O}$  and recorded at 37°C or 60°C. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR chemical shifts were expressed in parts per million (ppm) relative to sodium 3-(trimethylsilyl) propionic-2,2,3,3-d, acid (TSP, 0.00 ppm), which was used as an internal standard.

## 2.8. Methylation Analysis

The polysaccharide (5 mg) was methylated according to the procedure described by Ciucanu and Kerek [47]. The obtained permethylated polysaccharide was subjected to complete acid hydrolysis using 2 M TFA (2 mL) at 120°C for 2 h. The hydrolysate was dissolved in 1 M  $\text{NH}_4\text{OH}$  (0.2 mL). DMSO (1 mL) containing 20 mg of  $\text{NaBH}_4$  was added and the mixture was incubated at 40°C for 90 min. Subsequently acetic anhydride (0.2 mL) was added to the mixture. Anhydrous 1-methylimidazole (0.2 mL) and acetic anhydride (1 mL) were then added, and the reaction mixture was incubated at ambient temperature for 10 min. After extraction with chloroform and washing with water, partially methylated alditol acetates were obtained.

The partially methylated alditol acetates were analyzed using a gas chromatograph (GC-14A; Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector using a capillary column (DB-1: 40 m  $\times$  0.25 mm, J&W Scientific Inc., CA, U.S.A.). The injector and detector temperatures were 210°C and 270°C, respectively. After injection, the oven temperature was maintained at 150°C for 5 min, and then raised at 5°C/min to 250°C. This temperature was maintained for 5 min. The identities of the peaks were confirmed using GC-MS (GCMS-QP 1000EX; Shimadzu Corp., Kyoto, Japan) under the same conditions.

# 3. Results

## 3.1. Preparation of Polysaccharide

*Ulva pertusa* reached 12 - 20 cm long having two cell layers thick and round blade. The collected seaweed was washed with tap water and then dried in air-dried oven at 40°C for 24 h. A polysaccharide was prepared and purified as described in Materials and methods.

## 3.2. Chemical Components of the Polysaccharide

The yield of purified polysaccharide was estimated to be 21.7% of the algal dry weight after the first ethanol precipitation. The polysaccharide yield was reduced to 8.5% after purification based on the dried weight of algae. The polysaccharide was 67.3% (W/W) carbohydrates. The purified polysaccharide also contained 23.8%, 19.7% and 22.6% of uronic acid, sulfate and ash, respectively. The results are summarized in **Table 1**.

An anion exchange high-performance liquid chromatogram of the hydrolysate of the polysaccharide (**Figure 1**) showed that peaks 1, 2 and 3 were L-rhamnose, D-glucose and D-xylose in the molar ratio of 4.0:0.1:0.3. The result indicates that the polysaccharide isolated from *Ulva pertusa* is an ulvan [43]. D-Dlucuronic acid and L-idulonic acid was identified by anion exchange high-performance liquid chromatography in the molar ratio of 1.0:0.2, as shown in **Figure 2**.

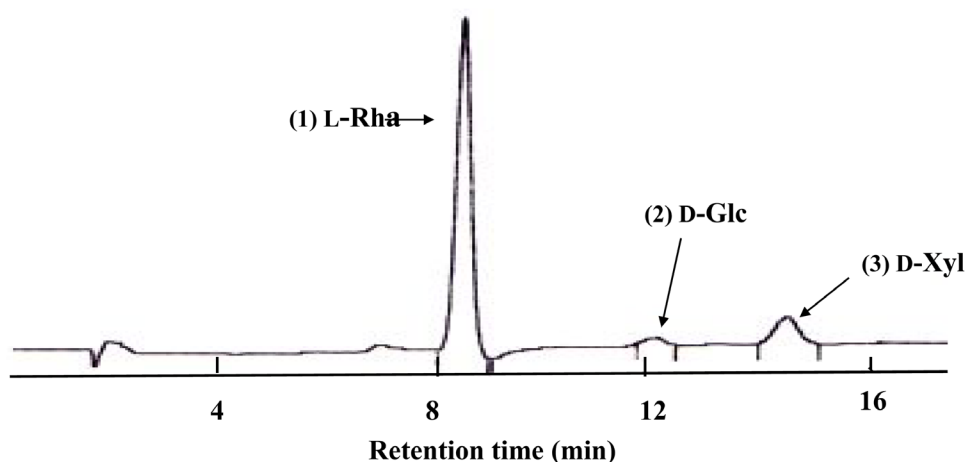
### 3.3. Specific Rotation and Infrared Spectrum (IR) of the Polysaccharide

The specific rotation  $[\alpha]_{589}$  of the ulvan (0.2% in water) at 25°C showed a value of  $-0.006^\circ$ , indicating that  $\alpha$  and  $\beta$  linkages were both involved.

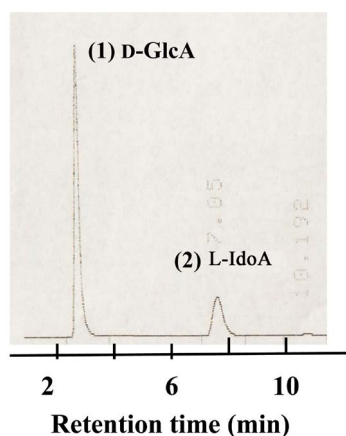
In the IR spectrum of the ulvan presented in **Figure 3**, the major absorption at approximately  $3400\text{ cm}^{-1}$  was attributed to the stretching of hydroxyl groups. Absorption at  $2900\text{ cm}^{-1}$  resulted from C-H stretching of C-H groups. Absorption at  $1638\text{ cm}^{-1}$  resulted from C=O of uronic acid. The absorption at  $1250\text{ cm}^{-1}$  and two shoulder absorptions at  $850$  and  $780\text{ cm}^{-1}$  were indicative of the presence of sulfate ester substitutions. The absorption at the former,  $1250\text{ cm}^{-1}$ , was attributed to C-O-S stretching. The band at  $1053\text{ cm}^{-1}$  corresponds to the stretching of the C=O of uronic acid and the vibration of the C-O-C bridge of the glucosides [43].

**Table 1.** Chemical components of the polysaccharide.

Carbohydrates (% W/W)	Uronic acid (%)	SO <sub>4</sub> (%)	Ash (%)
67.3	23.8	19.7	22.6



**Figure 1.** Liquid chromatogram of hydrolysate of the ulvan.

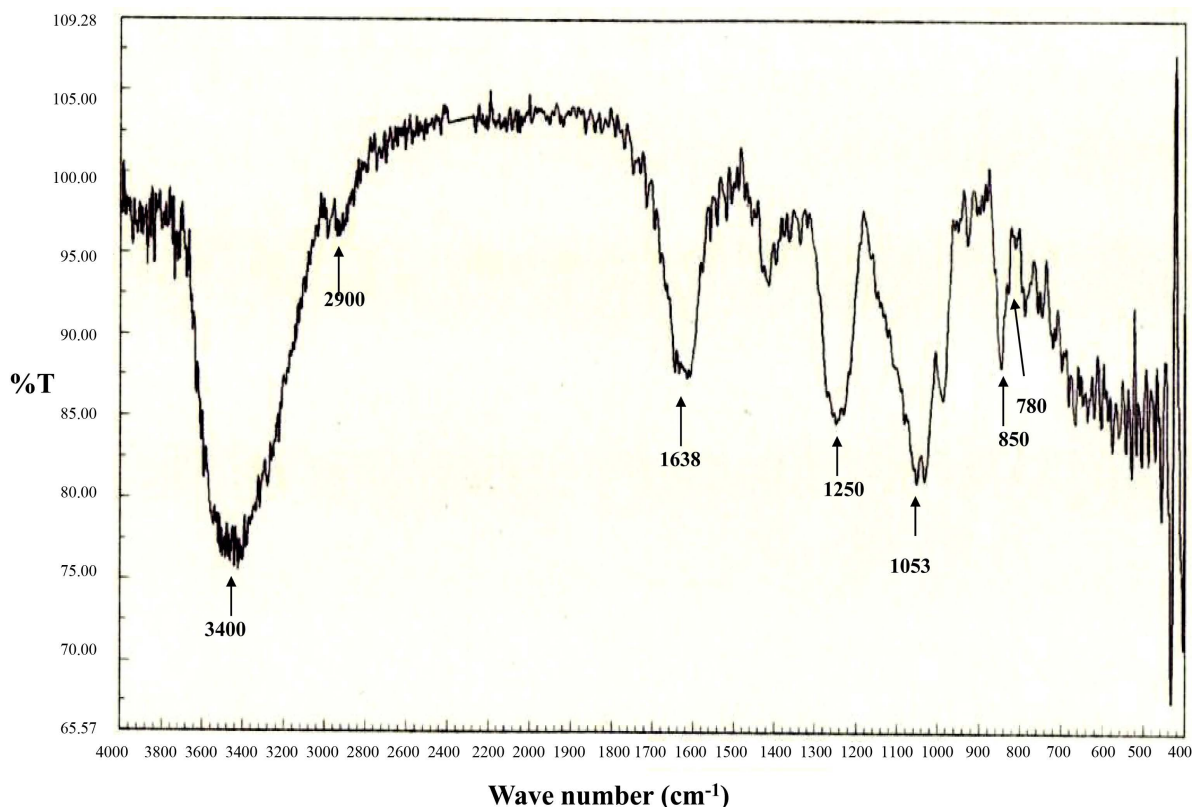


**Figure 2.** Liquid chromatogram of hydrolysate of the ulvan.

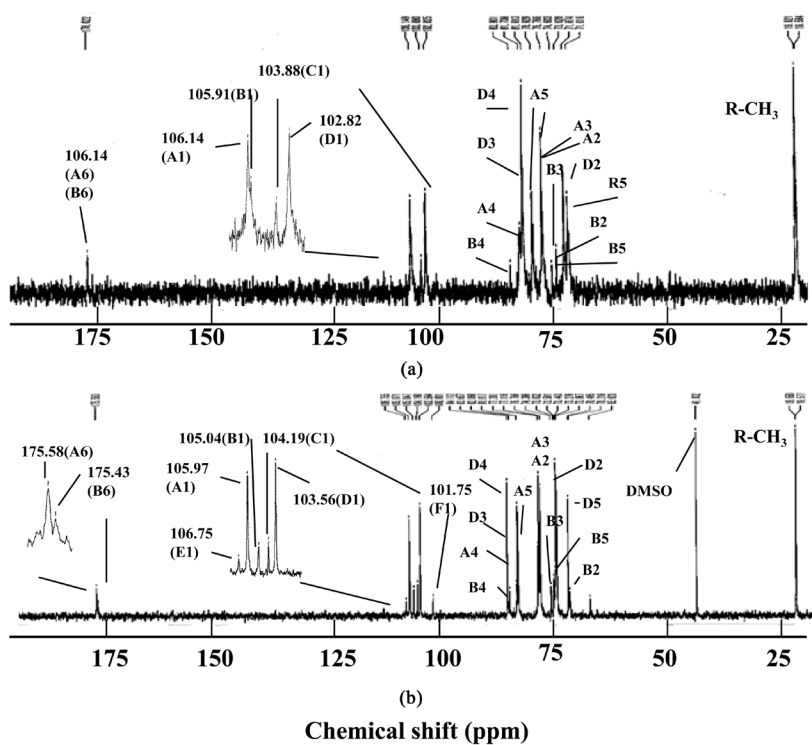
### 3.4. $^{13}\text{C}$ - and $^1\text{H}$ -NMR Spectra of the Ulvan

The  $^{13}\text{C}$ -NMR spectra of native and desulfated ulvan are presented in **Figure 4(a)**, measured at  $37^\circ\text{C}$ , and **4b**, measured at  $60^\circ\text{C}$ . The four sugar moieties were designated as residues A, B, C, and D according to their decreasing anomeric carbon chemical shifts, as shown in **Figure 4(a)**. The  $^{13}\text{C}$ -NMR spectra were well characterized in papers published by Lahaye *et al.* [48]-[52]. The signals A (106.14 ppm), B (105.91), C (103.88) and D (102.82) were assigned to  $\beta$ -D-glucuronic acid,  $\alpha$ -L-idulonic acid, 1,4-linked  $\alpha$ -L-rhamnose substituted with sugar and 1,4-linked  $\alpha$ -L-rhamnose, respectively [50]-[52]. The many ring-carbon signals (70 - 85 ppm) were also assigned as indicated in **Figure 4(a)** and are presented in **Table 2**. Six anomeric signals were observed in desulfated ulvan (**Figure 4(b)**). The new signal E (106.75 ppm) was assigned to  $\beta$ -D-xylose [50], but signal F (101.75 ppm) was unidentified. Methyl signals at 19.82 and 19.58 ppm were observed, indicating that L-rhamnosyl residues were involved in the ulvan [50] [51]. Two carboxyl carbons (A6 and B6) at 175.58 and 175.43 ppm were assigned to D-glucuronic acid and L-idulonic acid residue, respectively [50]-[52]. The many ring-carbon signals (70 - 85 ppm) were also assigned as indicated in **Figure 4(b)** and are presented in **Table 2**.

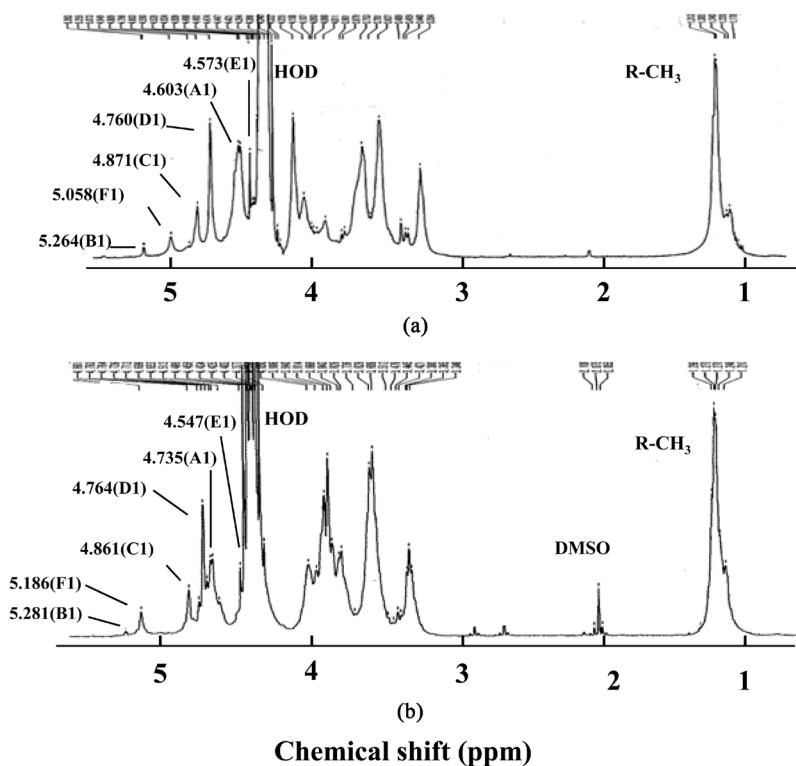
The  $^1\text{H}$  spectra of the native and desulfated ulvan are presented in **Figure 5(a)** ( $37^\circ\text{C}$ ) and **Figure 5(b)** ( $60^\circ\text{C}$ ). For the native sample, six chemical signals were observed in the anomeric region ( $\delta$  5.5 - 4.5) at 5.264, 5.058, 4.871, 4.760, 4.603 and 4.573 ppm. From the published papers [50] [51], signals A (4.603 ppm), B (5.264 ppm), C (4.871 ppm), D (4.760 ppm), E (4.573 ppm) and F (5.058 ppm) were assigned to be  $\beta$ -D-glucuronic acid,  $\alpha$ -L-idulonic acid, 1,4-linked  $\alpha$ -L-rhamnose substituted with side-chain, 1,4-linked  $\alpha$ -L-rhamnose,  $\beta$ -D-xylose, and an unknown sugar, respectively. From a published paper [38], signal F (5.058 ppm) was assigned to be 1,3-linked  $\alpha$ -L-rhamnose. Large signals at 1.340 and 1.329 ppm were observed, indicating that an L-rhamnosyl residue was involved. Six anomeric signals, A (4.735 ppm: D-glucuronic acid), B (5.281 ppm: L-idulonic acid), C (4.861 ppm:  $\alpha$ -1,4-linked L-rhamnose substituted with sugar), D (4.764 ppm:  $\alpha$ -1,4-linked L-rhamnose), E (4.547 ppm: D-xylose) and F (5.186 ppm) were also observed in desulfated ulvan (**Figure 5(b)**). From a published paper [38], signal F (5.18 ppm) was assigned to be  $\alpha$ -1,3-linked  $\alpha$ -L-rhamnose. The results are presented in **Table 3**.



**Figure 3.** Infrared spectrum of the ulvan at 4000 - 400  $\text{cm}^{-1}$ .



**Figure 4.**  $^{13}\text{C}$ -NMR spectra of the ulvan in  $\text{D}_2\text{O}$  at  $37^\circ\text{C}$  for (a) and  $60^\circ\text{C}$  for (b). (a) Native and (b) desulfated ulvan.



**Figure 5.**  $^1\text{H}$ -NMR spectra of the ulvan in  $\text{D}_2\text{O}$  at  $37^\circ\text{C}$  for (a) and  $60^\circ\text{C}$  for (b). (a) Native ulvan and (b) desulfated ulvan.



**Table 2.** Chemical shifts of resonances in the  $^{13}\text{C}$  NMR spectra of native and desulfated ulvan.

Residue	Chemical shifts ( $\delta$ , ppm)					
	$^a\text{C1}/^b\text{C1}$	$^a\text{C2}/^b\text{C2}$	$^a\text{C3}/^b\text{C3}$	$^a\text{C4}/^b\text{C4}$	$^a\text{C5}/^b\text{C5}$	$^a\text{C6}/^b\text{C6}$
A $\beta$ -D-GlcA(1 $\rightarrow$ )	106.14/105.97	76.760/77.11	76.76/77.28	83.90/83.65	78.92/81.81	178.02/175.58
B $\alpha$ -L-IdoA(1 $\rightarrow$ )	105.91/105.04	73.62/73.83	74.60/74.38	83.94/83.65	73.62/73.44	178.02/175.43
C* $\rightarrow$ 4)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	103.88/104.19					19.58/19.57
D $\rightarrow$ 4)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	102.82/103.56	73.87/73.06	81.01/84.11	81.01/84.11	71.01/70.49	19.82/19.58
E $\rightarrow$ 4)- $\beta$ -D-Xyl-(1 $\rightarrow$ )	/106.75					
F $\rightarrow$ 3)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	/101.75					

<sup>a</sup>Native and <sup>b</sup>desulfated ulvan. \* Branching L-rhamnose.

**Table 3.** Chemical shifts of resonances in the anomeric proton NMR spectra of native and desulfated ulvan.

Residue	Chemical shifts ( $\delta$ , ppm)	
	$^a\text{H1}/^b\text{H1}$	
A $\beta$ -D-GlcA(1 $\rightarrow$ )	4.603/4.735	
B $\alpha$ -L-IdoA(1 $\rightarrow$ )	5.264/5.281	
C* $\rightarrow$ 4)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	4.871/4.861	
D $\rightarrow$ 4)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	4.760/4.764	
E $\rightarrow$ 4)- $\beta$ -D-Xyl-(1 $\rightarrow$ )	4.573/4.547	
F $\rightarrow$ 3)- $\alpha$ -L-Rha-(1 $\rightarrow$ )	5.058/5.186	

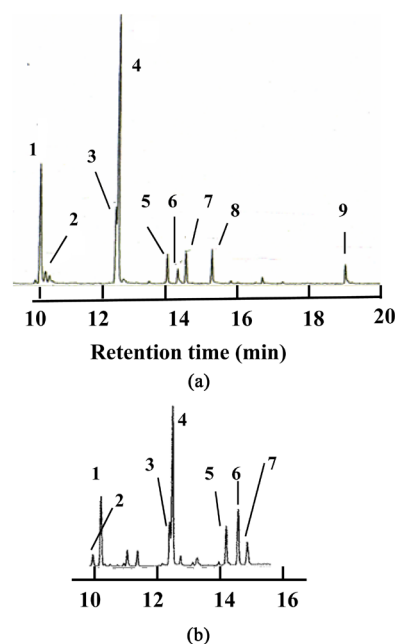
<sup>a</sup>Native and <sup>b</sup>desulfated ulvan. \* Branching L-rhamnose.

### 3.5. Methylation Analysis

The native and desulfated ulvan was methylated according to the procedure described by Ciucanu & Kerek [47]. The obtained permethylated ulvan was subjected to complete acid hydrolysis to furnish mixtures of the methylated sugars which were analyzed as the corresponding alditol acetates using gas-liquid chromatography (GC) and combined gas-liquid chromatography/mass spectroscopy (MS). The chromatograms are shown in **Figure 6(a)** (native ulvan) and **Figure 6(b)** (desulfated ulvan). Partially methylated alditol acetates were identified using published data [53] [54]. For the native ulvan (**Figure 6(a)**), the following 9 peaks were observed: terminal 2,3,4-tri-*O*-methyl-D-glucuronic acid (1), terminal 2,3,4-tri-*O*-methyl-L-idulonic acid (2), 2,4-di-*O*-methyl-L-rhamnose (3), 2,3-di-*O*-methyl-L-rhamnose (4), 2-mono-*O*-methyl-L-rhamnose (5), 2,3-di-*O*-methyl D-xylose (6), 3-mono-*O*-methyl-L-rhamnose (7), 2-mono-*O*-methyl-D-xylose (8) and L-rhamnose (9).

After desulfation (**Figure 6(b)**), peaks 4 and 6 increased, while peaks 8 and 9 disappeared. The results indicated that sulfate groups were attached at C-2 and C-3 of L-rhamnose as well as C-3 of the D-xylose residue. The results are summarized in **Table 4**.

The data show that the native ulvan consists of terminal D-glucuronic acid, terminal L-idulonic acid, 1,4-linked L-rhamnose, 1,3-linked L-rhamnose, 1,3,4-linked L-rhamnose, 1,2,4-linked L-rhamnose, 1,3,4-linked D-xylose and 1,2,3,4-linked L-rhamnose residues.



**Figure 6.** Gas chromatograms of partially methylated alditol acetate of the ulvan. (a) Native ulvan, (b) Desulfated ulvan.

**Table 4.** Methylation analysis of ulvan.

Number	Methylated sugar	Molar ratio		Mode of linkage
		Native	Desulfated	
(1)	2,3,4-tri- <i>O</i> -methyl- $\beta$ -D-glucuronic acid	1.6	1.7	$\beta$ -D-glucuronic acid-(1→
(2)	2,3,4-tri- <i>O</i> -methyl- $\beta$ -L-iduronic acid	0.1	tr	$\alpha$ -L-iduronic acid-(1→
(3)	2,4-di- <i>O</i> -methyl- $\alpha$ -L-rhamnose	1.0	1.0	→3)- $\alpha$ -L-rhamnose-(1→
(4)	2,3-di- <i>O</i> -methyl- $\alpha$ -L-rhamnose	3.0	4.0	→4)- $\alpha$ -L-rhamnose-(1→
(5)	2-mono- <i>O</i> -methyl- $\alpha$ -L-rhamnose	0.3	0.8	→3,4)- $\alpha$ -L-rhamnose-(1→
(6)	2,3-di- <i>O</i> -methyl- $\beta$ -D-xylose	0.1	0.4	→4)- $\beta$ -D-xylose-(1→
(7)	3-mono- <i>O</i> -methyl- $\alpha$ -L-rhamnose	0.7	0.4	→2,4)- $\alpha$ -L-rhamnose-(1→
(8)	2-mono- <i>O</i> -methyl- $\beta$ -D-xylose	0.4	0.0	→3,4)- $\beta$ -D-xylose-(1→
(9)	$\alpha$ -L-rhamnose	0.2	0.0	→2,3,4)- $\alpha$ -L-rhamnose-(1→

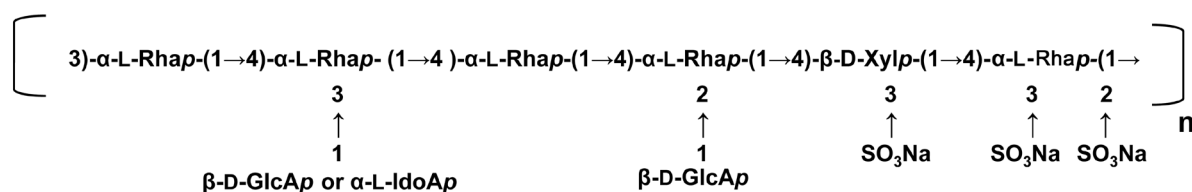
<sup>a</sup>Peak number in **Figure 6**.

## 4. Discussion

Based on the NMR and methylation analysis of the ulvan as well as the chemical shifts reported in the literatures [48]-[52], residue A was assigned as terminal  $\beta$ -D-glucuronic acid, residue B as terminal  $\alpha$ -L-iduronic acid, residue C as 1,4-linked  $\alpha$ -L-rhamnose substituted side-chain, residue D as 1,4-linked  $\alpha$ -L-rhamnose, residue E as 1,4-linked  $\beta$ -D-xylose and residue F as 1,3-linked  $\alpha$ -L-rhamnose. After desulfation, peak 9 in **Figure 5(a)** disappeared in **Figure 6(b)**, suggesting that sulfate groups were attached at C-2 and C-3 of the L-rhamnose residue on the main-chain. Furthermore, a sulfate group was attached at C-3 of the D-xylose residue because 2-mono-*O*-methyl-D-xylose (peak 8 in **Figure 6(a)**) disappeared, but 2,3-di-methyl D-xylose (peak 6) increased after desulfation (**Figure 6(b)**). The residues of peaks 5 (2-mono-L-rhamnose) and 7 (3-mono-L-rhamnose) remained even after desulfation, suggesting that terminal sugars (D-glucuronic acid and L-iduronic acid) are substituted at C-2 and/or C-3 of the L-rhamnose residues.

Consequently, the chemical and structural results showed that the ulvan extracted from *U. pertusa* consisted





**Figure 7.** The structure of ulvan from *Ulva fertusa*.

of 1,4-linked  $\alpha$ -L-rhamnopyranose, 1,3-linked  $\alpha$ -L-rhamnopyranose, 1,4-linked  $\beta$ -D-xylopyranose, terminal  $\beta$ -D-glucuronic acid (>1.5 molar) and terminal L-idulonic acid (<0.5 molar). The terminal sugars were substituted at C-2 and/or C-3 of the 1,4-linked  $\alpha$ -L-rhamnose residue. The sulfate groups were attached at C-2 and C-3 of the L-rhamnopyranose as well as C-3 of the D-xylopyranose residues. In addition, some oligosaccharides, including  $\alpha$ -L-rhamnopyranose 3S-(1→4)- $\beta$ -D-xylopyranose 2S,  $\beta$ -D-glucuronic acid-(1→2)- $\alpha$ -L-rhamnopyranose-(1→4)- $\beta$ -D-xylopyranose,  $\alpha$ -L-rhamnopyranose 3S-(1→4)- $\beta$ -D-xylopyranose 2S-(1→4)- $\alpha$ -L-rhamnopyranose 3S from ulvans were reported [42] [50].

## 5. Conclusion

Thus, on the basis of the results and discussion, we proposed the chemical structure of the ulvan (octa-saccharide repeating units) isolated from *Ulva pertusa*, as shown in **Figure 7**.

## References

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