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The Novel Pyruvated Glucogalactan Sulfate Isolated from the Red Seaweed, *Hypnea pannosa*

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Abstract

The polysaccharide was isolated from *Hypnea pannosa* which was grown in Okinawa, Japan. The yield of the polysaccharide was 17.2%, and the total carbohydrates, pyruvic acid, sulfuric acid and ash contents were 55.2%, 3.8%, 35.2% and 24.3%, respectively. 3,6-Anhydro-*α*-D-galactose, *β*-D-galactose, *α*-D-galactose and D-glucose were identified by liquid and thin-layer chromatography. Fourier transform infrared (FTIR) spectra of the polysaccharide resembled that of *ι*-carrageenan. From the ^{1}H- and ^{13}C-NMR spectra, 1,3-linked *β*-D-galactose, 1,4-linked anhydro-*α*-D-galactose, 1,4-linked *α*-D-galactose, 1,4-linked *β*-D-glucose and pyruvic acid (carboxyl acetal, methyl proton and methyl carbon) were assigned. Methylation analysis revealed terminal D-galactose 0.1 mol), 1,4-linked D-glucose (1.0 mol) and 1,2,3,4,6-linked D-galactose (3.7 mol) for native polysaccharide, and terminal D-galactose, 1,4-linked D-galactose (1.9 mol), 1,4-linked D-glucose (1.0 mol), 1,3-linked D-galactose (1.7 mol), and 1,3,4,6-linked D-galactose (0.3 mol) which substituted with pyruvate group at 4 and 6 positions for desulfated polysaccharide. The polysaccharide was the novel pyruvated glucogalactan sulfate, the structure of which was proposed.

Keywords

***Hypnea pannosa*, Pyruvated Glucogalactan Sulfate, ^{1}H- and ^{13}C-NMR Analysis, Methylation Analysis, Chemical Structure**

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1. Introduction

One of the authors, Tako, has isolated agar [1], methylated agar [2], fucoidan [3]-[6], alginate [5] [7], κ-carrageenan [8], ι-carrageenan [9], galactomannan [10] [11], pectin [12]-[14], rhamnan sulfate [15] and ulvan [16] from the subtropical biomasses grown in Okinawa Islands, Japan. Recently, we discovered the novel deoxy-D-altrose [17] [18] and α-glucan [19] from the edible mushrooms. Especially, we isolated the novel acetylfucoidan from commercially cultured *Cladosiphon okamuranus* [5] and patented [20]. The acetyl fucoidan exhibited some biological activities, such as antitumor [21] and immune-enhancing abilities [22]. An over-sulfated acetylfucoidan, the sulfate content of which was 32.8%, showed a significant antitumor activity *in vitro* [21]. The results suggest that the over-sulfated acetyl fucoidan is applicable as an anticancer drug. The acetylfucoidan is now used as a supplement in health food, food and cosmetic industries in the world.

On the other hand, Tako discussed the structure-function relationship and proposed gelation mechanism of κ-carrageenan [23]-[25], ι-carrageenan [26] [27], agarose [28], gellan gum [29] [30], amylose [31] [32], alginate [33] [34] and deacetylated rhamsan gum [35]. We also proposed gelatinization and retrogradation mechanism of amylopectin [36]-[38] and starch [39]-[44]. Tako realizes that there are some basic rules in gel-formation process including water molecules in principle [45]-[47].

Carrageenans are water-extractable sulfated galactans from red algae Rhodophyta. They are essentially linear polymers composed of repeating disaccharide units of β-(1→3)-linked D-galactose and α-(1→4)-linked 3,6-anhydro-D-galactose residues. Sulfate groups substituted at C-4 position of D-galactose residue for κ-carrageenan and, C-4 and C-2 position of D-galactose and 3,6-anhydro-D-galactose residue for ι-carrageenan. We report herein the isolation and structural characterization of the pyruvated glucogalactan sulfate from *Hypnea pannosa* which belong to red seaweed and grow in the coast of Okinawa Island, Japan.

2. Materials and Methods

2.1. Materials

Hypnea pannosa was collected on April 2007 at Uruma, Okinawa Prefecture, Japan. The collected seaweed was washed with tap water and then air-dried at 40˚C for 24 h. The dried seaweed was powdered using a mixer. The powder was stored in refrigerator (4˚C) until extraction.

2.2. Polysaccharide Extraction

The powdered seaweed sample (3 g) was suspended in 500 mL of distilled water and stirred at 100˚C for 1 h. The suspension was filtered, and the filtrate was concentrated at 40˚C using a rotary evaporator. Ethanol (2 - 3 vol.) was added to the concentrated solution to precipitate polysaccharide. The precipitate was washed with ethanol twice and then dried in a vacuum chamber at 40˚C.

The dried precipitate was dissolved in distilled water and then filtered through a suction filter (Celite 545). The filtrate was passed through a column of Amberlite IR-120B (ø 5 × 30 cm, H^+ form). The eluate was adjusted to pH 7 with 0.05 m NaOH and concentrated using a rotary evaporator at 40˚C. The concentrated solution was dialyzed against distilled water, and the dialyzed solution was freeze-dried.

2.3. Chemical Component Analysis

Total carbohydrate was determined by the phenol-sulfuric acid method using D-galactose as a standard [48]. 3,6-Anhydro-D-galactose was determined by method of Yaphe and Arsenault [49] using anhydro-β-D-galactose as a standard. Ash content was determined by incinerating the polysaccharide for 24 h in a muffle furnace at 550˚C and then weighed the residue. Sulfate content was measured by turbidimetric method [50] as follows: the polysaccharide (20 mg) was dissolved in 1.5 mL of distilled water, and 11.5 m HCl solution was added to a final concentration of 3.0 M. The solution was heated at 100˚C for 2 h. The hydrolyzate was dried, then dissolved in 1 mL of distilled water and centrifuged at 2150 × *g* for 20 min. To the supernatant, 3.8 mL of 4% trichloroacetic acid and 1 mL of 1% gelatin + 2% $BaCl_2$ were added, then mixed sufficiently, and left for 20 min. Absorbance was measured at 360 nm. Pyruvic acid content was measured by using 2,4-dinitrophenylhydrazine [51].

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

For the quantitative determination of constituent sugar, the polysaccharide (10 mg) was hydrolyzed with 3.0 M trifluoroacetic acid (TFA) at 121˚C for 1 h. The hydrolyzate was dried using a compressor. Isopropanol (500 µL) was added and dried again. The dried hydrolyzate was dissolved in purified water and centrifuged. The supernatant was analyzed by high performance anion exchange chromatography (HPLC) on a DX 500 liquid chromatograph (Dionex Co., Ltd., Sunnyvale, CA), fitted with a column of CarboPac PA1 (ø 4 × 250 mm) and a pulsed amperometric detector. The column was eluted at a flow rate of 1 mL/min at 35˚C with 15 mm NaOH.

2.5. Methanolysis

The polysaccharide (10 mg) was treated with 0.5 m hydrogen chloride in methanol at 105˚C for 12 h in a sealed tube. The reaction mixture was neutralized with silver carbonate at 60˚C, and then filtered and evaporated [2] [7] [8].

2.6. Thin-Layer Chromatography

Thin-layer chromatography was carried out on glass plate (20 cm in length) treated with silica gel containing calcium sulfate as the binder and using a solvent of butanol-ethanol-water (4:1:5). Chromatograms were sprayed with 10% sulfuric acid in water and heated at 100˚C for 15 min [2] [7] [8].

2.7. Molecular Mass Determination

The molecular mass of the polysaccharide was determined by high-performance liquid chromatography (HPLC) (Shimadzu SCL-6B; Shimadzu Seisakusho, Co., Ltd, Japan) on a Superdex 200 column (TSK gel G 4000 PWXL) with a sample loop of 200 µL. The HPLC operation was performed at room temperature. The column was developed with 50 mM phosphate buffer, and the same buffer supplemented with 150 mM sodium chloride and fractions (3 mL each) were collected at a flow rate of 0.2 mL/min. Standard pullulan (P-82), P-400 (molecular mass, 4.0×10^5), P-100 (1.0×10^5), P-20 (2.0×10^4), and P-5 (0.5×10^4), (Pharmacia Chemicals Co., Ltd., Sweden), having definite molecular mass were used for calibration.

2.8. Fourier Transform Infrared (FTIR) Spectroscopy and Specific Rotation

FTIR spectrum was measured using an FTS-3000 spectrophotometer (Bio-Rad Laboratories, Hercules, CA) in transmittance mode from 4000 to 400 cm^{-1} in KBr disc. The KBr disc was prepared by dispersing solid sample in the KBr salt.

The specific rotation of the polysaccharide was measured at 589 nm on a polaimeter (P-1010, Japan Spectroscopic Co., Ltd., Japan) for a 0.2% (W/V) solution in distilled water at 25˚C.

2.9. ^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H- and ^{13}C-NMR spectra were measured on an FT-NMR spectrometer at 500 and 125 MHz (JNM α500, JEOL Ltd., Ltd., Tokyo, Japan) at 80˚C. The ^{1}H and ^{13}C spectra were recorded using 45˚ pulse width, 32,768 data points, and 1 s pulse delay for ^{1}H; and 60˚ pulse width, 16,384 data points, and 0.5 s pulse delay for ^{13}C, respectively [13]. Sodium 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP, 0.00 ppm) was used as an internal standard. As the polysaccharide solution in D_2O showed too large viscosity to measure, it was partially hydrolyzed with 0.1 m HCl at 55˚C for 1 h, neutralized with 0.3 m NaOH, and then freeze-dried. The partially hydrolyzed polysaccharide (2.0%) was dissolved in D_2O at room temperature [13] [16].

2.10. Methylation Analysis

Methylation of the polysaccharide was carried out by Ciucanu and Kerek method [52]. The polysaccharide (5 mg) was dissolved in 2 mL of DMSO and powder of NaOH (5 mg) was added to the solution and stirred at room temperature for 90 min. CH_3I (1 mL) was added to the solution and stirred for 60 min. Distilled water (4 mL) was added to the solution and dialyzed against tap water followed by distilled water. The dialyzed solution was evaporated to dryness. The methylated polysaccharide was extracted with $CHCl_3$ (2 mL) and washed with dis-

tilled water (3 mL) five times. The extracted methylated polysaccharide was hydrolyzed with 2 M TFA (2 mL) at 120˚C for 2 h. The hydrolyzate was dissolved in 1 M NH_4OH (100 µL), and then DMSO (500 µL) containing 10 mg of $NaBH_4$ was added. The mixture was incubated at 40˚C for 90 min. Glacial acetic acid (100 µL) was added to the mixture. Anhydrous 1-methylimidazole (100 µL) and acetic anhydride (0.5 µL) were added and then incubated at ambient temperature for 10 min. Partially methylated alditol acetates (PMAAs) were obtained after extracting with $CHCl_3$ and washing with distilled water. The PMAAs in $CHCl_3$ were dried with Na_2SO_4 and filtered. The PMAAs were analyzed using a GC-MS (GCMS-QP5000, Shimadzu Co., Ltd.) equipped with a capillary column (DB-1, ø 0.25 mm × 30 m, J & W Scientific). Helium was used as carrier gas (125 kPa). The injector and interface temperatures were 210˚C and 270˚C, respectively. Oven temperature was maintained at 150˚C for 5 min after injection, then raised at 5˚C/min to 250˚C, and this temperature was kept for 5 min [13] [16].

3. Results

3.1. Preparation of Polysaccharide from *Hypnea pannosa*

One of red seaweed, *H. pannosa*, which was collected in April from Uruma City, Okinawa Prefecture, Japan, reached 5 - 10 cm long, having many thin branches, which was about *φ*2.0 - 3.0 mm. The collected seaweeds were washed with tap water and then dried by an air-dried oven at 40˚C for 24 h. The polysaccharide was prepared and purified as described in Materials and Methods. The purified polysaccharide was a colorless, fibrous powder, with yield of 17.2% (w/w) based on dried seaweed. Precipitation or gel-formation did not occur when KCl (1.0%) or $CaCl_2$ (1.0%) was added into the polysaccharide aqueous solution (0.5%) (**Table 1**).

3.2. Identification of Sugar Components of the Polysaccharide

The total carbohydrate, 3,6-anhydro-*α*-D-galactose, pyruvic acid, sulfuric acid and ash of the purified polysaccharide were estimated to be 55.2%, 12.5%, 3.8%, 35.2% and 24.3%, respectively.

As shown in **Figure 1**, the HPLC of the acid hydrolyzate showed the presence of D-galactose and D-glucose the molar ratio of which was estimated to be 2.9:1.0.

The examination of methanolysis product of the polysaccharide by thin-layer chromatography indicated the presence of 3,6-anhydro-methy-*α*-D-galactoside (spot 2), methyl-*β*-D-galactoside (4), and methyl-*α*-D-galactoside (spot 5) [8] [9]. A spot 3, which was located at higher than that of spot 4, might be methyl-*α*-and/or -*β*-D-glucosides (**Figure 2**).

Table 1. Chemical components of the polysaccharide isolated from *Hypnea panossa*.

(%, w/w)

	Carbohydrates	Pyruvic acid	sulfate	Ash
Polysaccharide	55.2	3.8	35.2	24.3

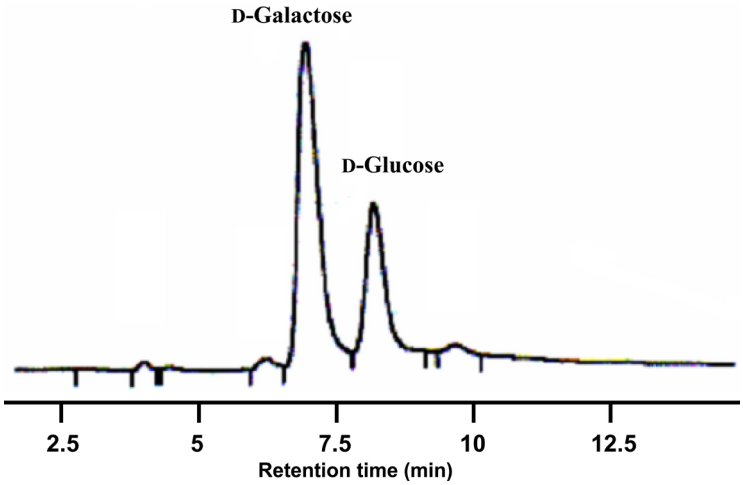

Figure 1. Liquid chromatogram of hydrolysate of the polysaccharide isolated from *H. pannosa*.

3.3. Molecular Mass

The molecular mass of purified polysaccharide was measured by the gel chromatography on a Superdex 200 column (**Figure 3**). According to the standard calibration curve obtained from the definite molecular mass pullulan, the molecular mass of the polysaccharide was calculated to be approximately 7.9×10^5.

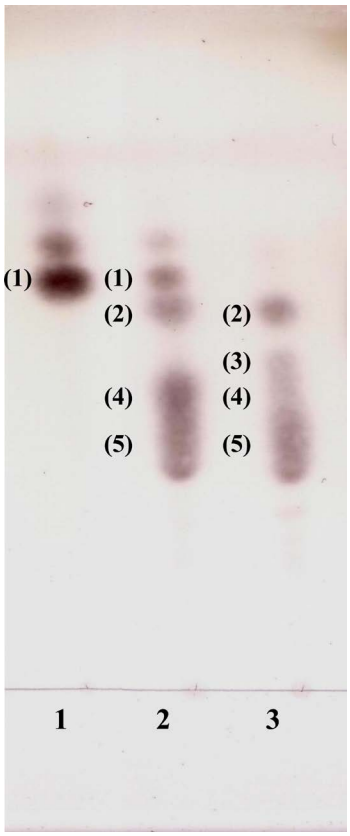

Figure 2. Thin-layer chromatogram of methanolyzate of the polysaccharide isolated from *H. pannosa*. 1. Methyl-3,6-anhydro-*β*-D-galactoside; 2. Methanolyzate of standard *ι*-carrageenan; 3. Methanolyzate of the polysaccharide.

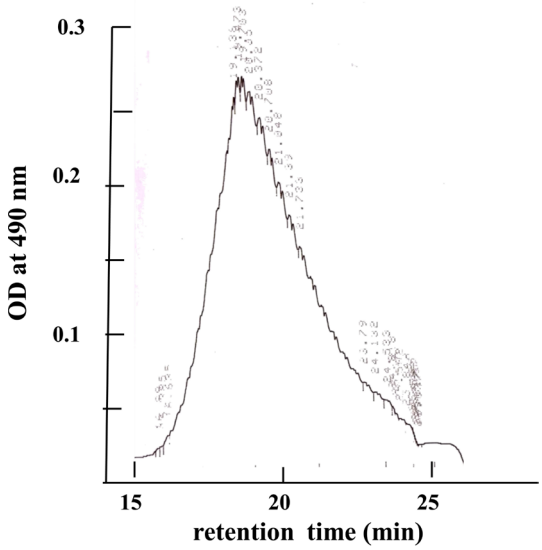

Figure 3. Gel permeation chromatogram of the polysaccharide from *H. pannosa*.

3.4. FTIR Spectrum and Specific Rotation

Figure 4 shows spectra of the polysaccharide and standard *ι*-carrageenan. A broad absorption at 1245 cm^{-1} was common to all the sulfated polysaccharides due to sulfate absorption. An absorption at 850 cm^{-1} (strong) was assigned to be ester sulfate on C-4 of 3-linked D-galactose residue. An absorption at 939 cm^{-1} (weak) was assigned to C-O ether bond of 3,6-anhydro-galactose residue. The peak at 805 cm^{-1} (medium) was assigned to be an ester sulfate on C-2 of the 4-linked 3,6-anhydro-D-galactose residue. The band at 900 cm^{-1} (very weak) indicated 3-linked D-galactose residues bearing pyruvate acetal substitution [53] [54]. These data were consistent in part with those of standard *ι*-carrageenan prepared from *Euchemua spinosum*.

The specific rotation $[\alpha]_{589}$ of the polysaccharide at 25˚C was estimated to be a value of +16.8˚ (c 0.2%, H_2O). The value was lower than that of standard *ι*-carrageenan (+26.5˚). The result suggests that D-glucosyl residue consisted of *β*-conformation on the polysaccharide.

3.5. ^{13}C- and ^{1}H-NMR Spectra Analysis

As the polysaccharide solution in D_2O showed too large viscosity to measure, it was partially hydrolyzed with 0.1 M HCl at 55˚C for 1 h. The partially hydrolyzed polysaccharide (2.0%) was dissolved in D_2O. The seven sugar moieties were designated as residues A, B, C, D, E, F and G according to their decreasing anomeric carbon chemical shifts. **Figure 5** shows ^{13}C-NMR spectrum. From published papers [53]-[59], the signal at B (105.753 ppm)), C (103.526), D (103.102), E (97.266), F (97.009) and G (96.700) was assigned to be anomeric carbon of 3-linked *β*-D-galactopyranose adjacent to 4-linked *α*-D-galactopyranose (B), *β*-D-galactopyranose (C), pyruvated *β*-D-galactopyranose (D), anhydro-*α*-D-galactopyranose (E), sulfated anhydro-*α*-D-galactopyranoe (F) and *α*-D-galactopyranose (G), respectively. The chemical shift at 106.49 ppm (A) is assigned to *β*-1,4-linked D-glucopyranosyl residue [60] [61]. The carboxyl, acetal and methyl carbon was assigned at 186.673, 101.361 and 27.919 ppm, respectively [53] [54] [57].

^{1}H-NMR spectrum is shown in **Figure 6**. The chemical shift of the envelope of anomeric signals are consis-

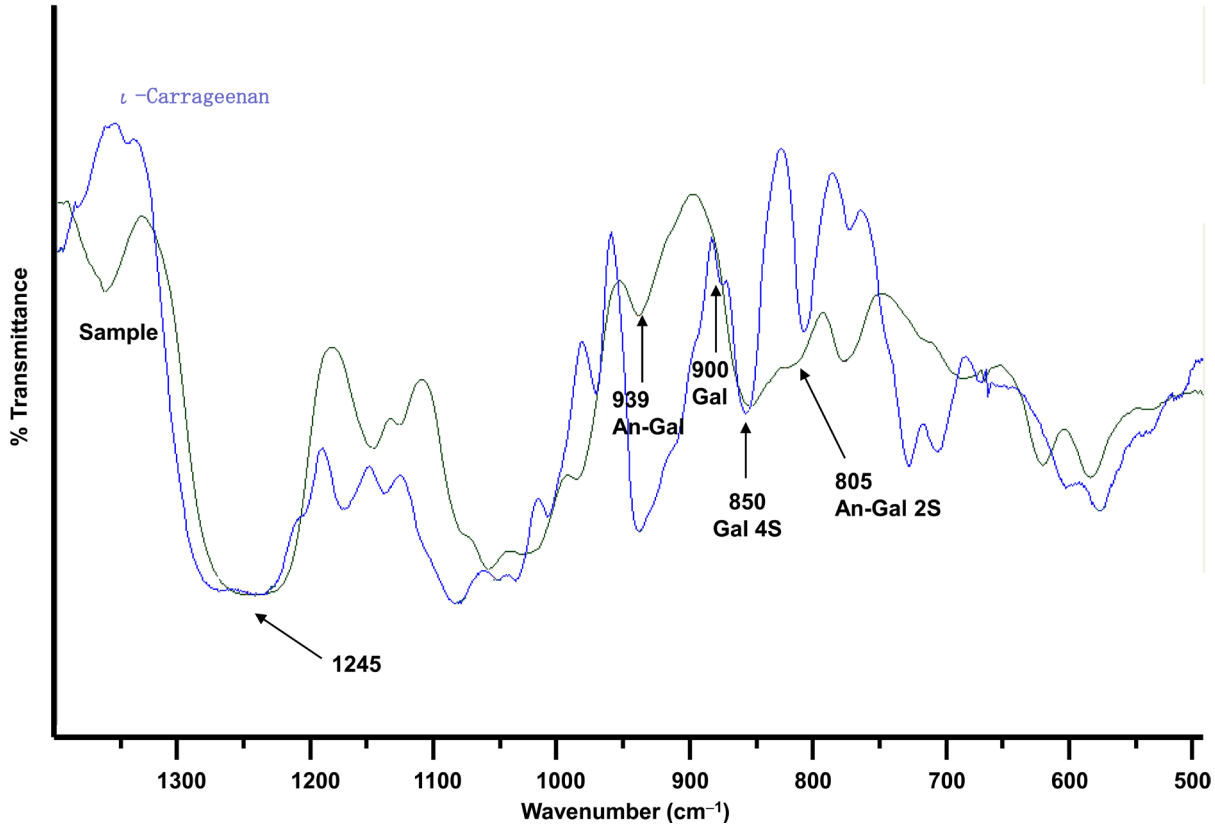

Figure 4. Infrared spectra of the polysaccharide from *H. pannosa* and standard *ι*-carrageenan.

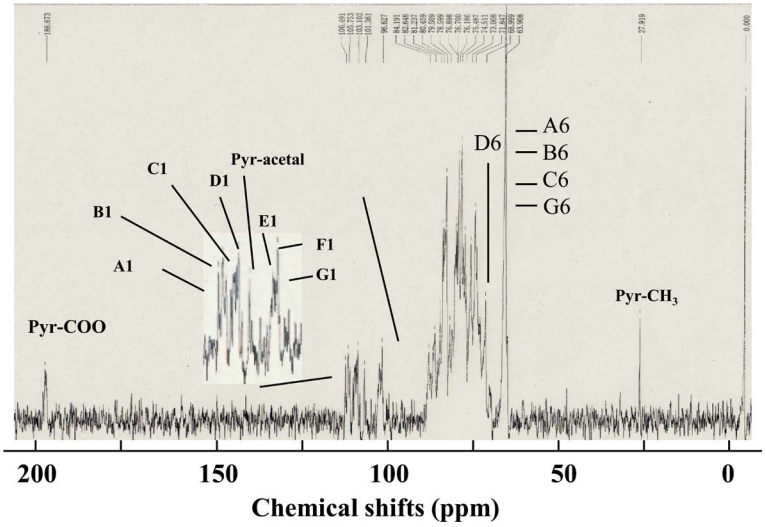

Figure 5. ^{13}C NMR Spectrum of the polysaccharide isolated from *H. pannosa* at 60˚C.

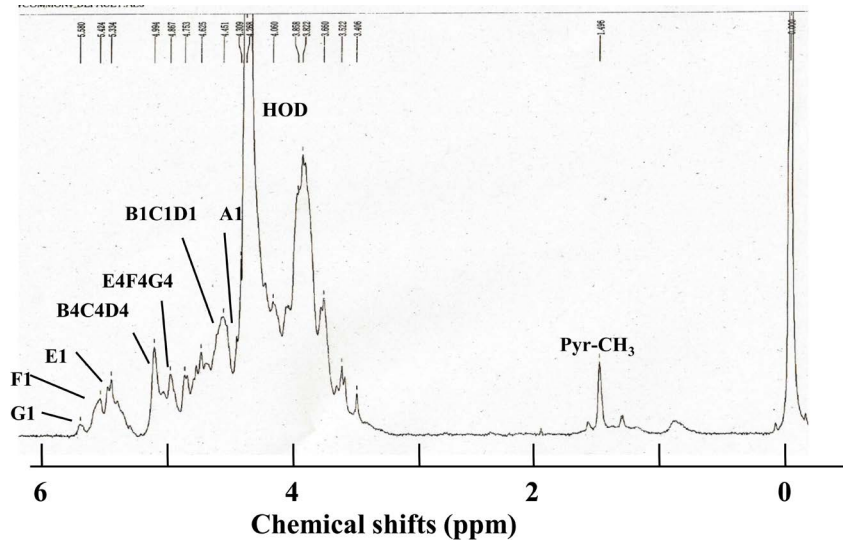

Figure 6. ^{1}H NMR Spectrum of the polysaccharide isolated from *H. pannosa* at 60˚C.

tence with presence of *α*-D-galactopyranose (G), C-2 sulfated 3,6-anhydro-*α*-D-galactopyranose (F), 3,6-anhydro-*α*-D-galactopyranose (E), and *β*-D-galactopyranose (B, C and D) at 5.690, 5.425, 5.337, and 4.625 ppm, respectively [53] [57] [59] [62]. The anomeric proton of *β*-D-glucopyranose was assigned at 4.454 ppm [60] [61]. The signal at 1.493 ppm can be assigned to methyl proton of pyruvic acid [53] [54] [57]. The ring proton signals of the spectrum were overlapped due to high viscosity even after partially hydrolysis. The results are summarized in **Table 2**.

3.6. Methylation Analysis

The native and desulfated polysaccharide was methylated according to the procedure described by Ciucanu & Kerek [52]. The obtained permethylated polysaccharide 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 chromatogram is shown in **Figure 7** (desulfated polysaccharide). Partially methylated alditol acetates were identified using published data [63] [64]. For the native polysaccharide (not shown in Figure), the 3 peaks were observed: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactose (peak 1), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucose (3), and 1,2,

3,4,5,6-hexa-*O*-acetyl-D-galactose (8) which originated from terminal D-galactose (0.1 mol), 1,4-linked D-glucose (1.0 mol) and 1,2,3,4,5,6-linked D-galactose (3.8 mol) residues, respectively.

After desulfation (**Figure 7**), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucose (peak 3; 1.0 mol) 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactose (peak 2: 1.9 mol)=1,5-di-*O*-acetyl-2.3.4.6-tetra-*O*-methyl-D-galactose (peak 1; 0.6 mol), 1,3,5-tri-*O*-acetyl-2,4,6-*O*-methyl-D-galactose (peak 4:1.7 mol), 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-galactose (peak 5; 0.3 mol), 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-galactose (peak 6; 0.3 mol), and 1,3,4,5,6-penta-*O*-acetyl-2-mono-*O*-methyl-D-galactose (peak 7; 0.3 mol) were observed, but peak 8 was disappeared. The results indicated that the 1,4-linked D-glucose residue (peak 3) was free from sulfuric acid and pyruvic acid. The peak 7 suggested that the pyruvate group substituted with acetal linkage on the position of C-4 and C-6 of 1,3-linked D-galactose. The small amount of peak 7 (0.3 mol) was due to association of pyruvate group even after desulfation. The results are summarized in **Table 3**.

4. Discussion

The polysaccharide isolated from *Hypnea pannosa* had high content of sulfate (35.2%) combination with pyruvic acid (3.8%). The polysaccharide consisted of 3,6-anhydro-*α*-D-galactose (12.5%), *α*-D-galactose, *β*-D-galactose, and D-glucose residues. IR spectrum indicated the presence of *β*-D-galactose 4 sulfate and 3,6-anhydro-*α*-D-galactose 2-sulfate residues. The result suggested that the polysaccharide involved *ι*-carrabiose units. ^{13}C and ^{1}H NMR spectra showed that it contained 1,3-linked *β*-D-galactose, 1,4-linked *α*-D-galactose, 1,4-linked 3,6-anhydro-D-galactose, 1,4-linked *β*-D-glucose, and pyruvic acid residues, respectively. The pyruvate group was associated in acetal linkage on the main chain. The methylation analysis of native polysaccharide showed all hydroxyl groups of D-galactose residues substituted with sulfate or pyruvate groups, because 2,3,4,6-tetra-*O*-methyl-D-galactose (peak 1: 0.1 mol), 2,3,6-tri-*O*-methyl-D-glucose (peak 3: 1.0 mol) and D-galactose

Table 2. Chemical shifts of resonances in the ^{13}C and ^{1}HNMR spectra of the polysaccharide isolated from *H. pannosa*.

Residue	Chemical shifts (*δ*, ppm)			
	C1/H1	Pyruvic acid		
		CH3	Acetal	Carboxyl
A →4)-*β*-D-Glc(1→	106.491/4.454			
B →4)*β*-D-Gal(1→	105.753/4.625			
C* →3)-*β*-D-Gal-(1 →	103.526/4.625			
D** →3)-*β*-D-Gal(1→	103.1024.625	27.919/1.493	101.361	186.673
E →4)-*α*-D-An-Gal-(1→	97.880/5.337			
F*** →4)-*α*-D—An-Gal –(1→	97.009/5.425			
G**** →4)-*α*-D--Gal –(1→	96.740/5.690			

*Sulfated *β*-D-galactose, **Pyruvated *β*-D-galactose, ***Sulfated Anhydro-*α*-D-galactose, ****Sulfated *α*-D-galactose.

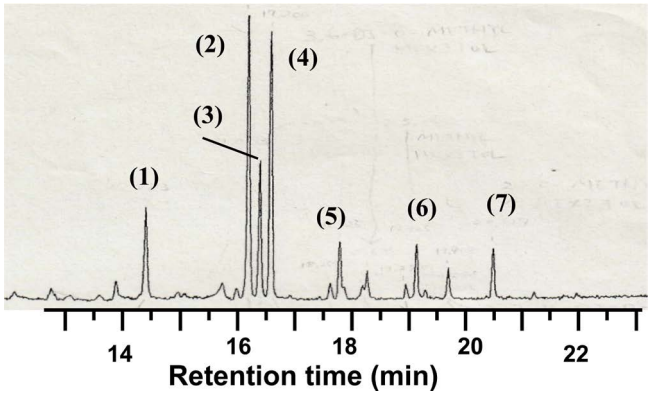

Figure 7. Gas chromatogram of partially methylated alditol acetates of the desulfated polysaccharide isolated from *Hypnea pannosa*.

Table 3. Methylation analysis of the polysaccharide isolated from *Hypnea pannosa*.

[a]Number	Methylated sugar	Molar ratio		Mode of linkage
		Desulfated	Native	
(1)	2,3,4,6-tetra-*O*-methyl-*β*-D-galactose	0.6	0.1	*β*-D-galactose-(1→
(2)	2,3,6-tri-*O*-methyl-*α*-D-galactose	1.9	0	→4)-*β*-D-galactose-(1→
(3)	2,3,6-tri-*O*-methyl-*β*-D-glucose	1.0	1.0	→4)-*α*-D-glucose-(1→
(4)	2,4,6-tri-*O*-methyl-*β*-D-galactose	1.7	0	→3)-*β*-D-galactose-(1→
(5)	2,6-di-*O*-methyl-*α*-D-galactose	0.3	0	→3,4)-*α*-D-galactose-(1→
(6)	2,3-di-*O*-methyl-D-galactose	0.3	0	→4,6)-D-galactose-(1→
(7)	2-mono-*O*-methyl-D-galactose	0.3	0	→3,4,6)-*β*-D-galactose-(1→
(8)	D-galactose	0	3.8	→2,3,4,6)-*β*-D-galactose-(1→

[a]Peak number in **Figure 7**.

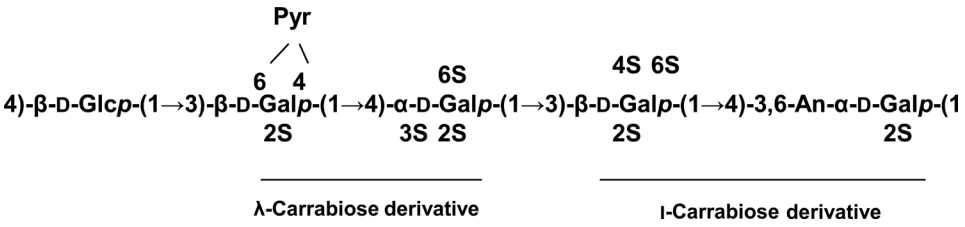

Figure 8. The chemical structure of the pyruvated glucogalactan sulfate isolated from *Hypnea pannosa*.

(peak 8: 3.8 mol) were identified for native polysaccharide. After desulfation, peak 8 was disappeared, and 2,3,6-tri-*O*-methyl-D-galactose (peak 2: 1.9 mol), 2,4,6-tri-*O*-methyl-D-galactose (peak 4: 1.7 mol) and 2-mono-*O*-methyl-D-galactose (peak 7: 0.3 mol) were identified. The pyruvate group might substitute at 4 and 6 positions of the 3-linked D-galactose (peak 7) residue. About 50% of 2,3,6-tri-*O*-methyl-D-galactose (peak 2) might be derived from 3,6-anhydro-*α*-D-galactose (12.5%) and the rest from *α*-D-galactose residue. The results suggested that both *ι*-carrabiose derivative, *λ*-carrabiose derivative and 1,4-linked *β*-D-glucose units are involved in the polysaccharide (five sugar repeating units).

5. Conclusion

From the results and discussion, we concluded that the polysaccharide isolated from *Hypnea pannosa* was the pyruvated glucogalactan sulfate. The chemical structure of the polysaccharide was illustrated in **Figure 8**. The polysaccharide consisted of *ι*-carrabiose derivative, *λ*-carrabiose derivative and 1,4-linked *β*-D-glucose units. However, it was not neglected that the polysaccharide from *Hypnea pannosa* was the mixture of *ι*-carrageenan derivative, *λ*-carrageenan derivative and 1,4-linked *β*-D-glucan, because such pyruvated glucogalactan sulfate had not been reported.

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