Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [*Russian original No. 3, May-June, 2011*]

COMPOSITION, STRUCTURAL CHARACTERISTICS, AND ANTITUMOR PROPERTIES OF POLYSACCHARIDES FROM THE BROWN ALGAE *Dictyopteris polypodioides* AND *Sargassum* sp.

R. V. Sokolova,[1*] S. P. Ermakova,[1] S. M. Awada,[2] T. N. Zvyagintseva,[1] and H. M. Kanaan[2]

UDC 582.272.4;582.272.7

The polysaccharide compositions of the brown algae Dictyopteris polypodioides *and* Sargassum *sp. from the Mediterranean Sea were determined. The principal polysaccharide of the studied algae (about 12% of the dry alga weight) was alginic acid. The content of water-soluble polysaccharides was low. The amount of fucoidan was less than 1% of the dry alga weight; of neutral polysaccharides, less than 0.25%. The monosaccharide compositions of fucoidans and neutral polysaccharides were investigated. Experiments on soft agar-agar models showed that fucoidans from* D. polypodioides *and* Sargassum *sp. exhibited antitumor activity against RPMI-7951 human melanoma cells.*

Keywords: brown algae, polysaccharides, *Dictyopteris*, *Sargassum*, laminaran, fucoidan, alginic acid, biological activity.

The search for biologically active compounds from natural sources is currently of great interest. Polysaccharide-type compounds exhibit a broad spectrum of biological activity and low *in vivo* toxicity.

Brown algae contain compounds with structures that differ from those in red and green algae and terrestrial plants. Mannitol and comparatively low-molecular-weight 1,3;1,6-β-D-glucans (laminarans) act as reserve material in them [1]. Salts of alginic acids (alginates) [2] and complex sulfonated polysaccharides (fucoidans) [3, 4] occur in cell walls and intercellular space. The latter exhibit a broad spectrum of biological activity. Structure–activity relationships of the polysaccharides are rather difficult to establish because their structures are heterogeneous, irregular, branched, and highly sulfonated.

The goal of the present work was to study the composition, structural characteristics, and antitumor activity of polysaccharides from the Mediterranean algae *Dictyopteris polypodioides* and *Sargassum* sp.

Alginic acids, neutral polysaccharides, and fucoidans were isolated from these brown algae.

Dried and ground algae were treated with EtOH (70%) to remove low-molecular-weight compounds and then extracted with HCl solution. The extract containing water-soluble polysaccharides was concentrated, dialyzed, and placed on a column with the hydrophobic sorbent Polychrom-1. Charged polysaccharides (Dp-F and S-F) were eluted by H_2O; neutral polysaccharides, by aqueous EtOH (5 and 15%) to afford fractions Dp-5%, Dp-15%, S-5%, and S-15%. Remaining algae were extracted by Na_2CO_3 solution. The extracts were dialyzed. Alginic acid was precipitated by three times the volume of EtOH (96%) to afford Dp-A and S-A. Fractions Dp-F and S-F contained fucoidans after Polychrom-1 and were separated over a column of Macro-Prep DEAE to afford Dp-F1, Dp-F2, Dp-F3, and Dp-F4 and S-F1, S-F2, S-F3, and S-F4, which were lyophilized to produce alginic acids, neutral polysaccharides, and fucoidans. Table 1 presents the yields of polysaccharides.

Table 1 shows that the principal polysaccharide from the studied algae was alginic acid (greater than 12% for both algae), the content of which depended on the species, season, latitude, and ecological habitat [5]. According to the literature, the content of alginic acids in algae of the genus *Sargassum* varies from 3 to 17% [6, 7]. The studied alga could be classified as a *Sargassum* containing significant quantities of alginates.

1) Pacific Institute of Bioorganic Chemistry, Far-East Division, Russian Academy of Sciences, 690022, Vladivostok, Prosp. 100-Letiya Vladivostoka, 159, fax: 8 (4232) 31 40 50, e-mail: Sokolova-R@yandex.ru; 2) Laboratory of Biotechnology of Natural Substances and Health Products, Faculty of Pharmacy, Lebanese University, Beirut, Lebanon. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 297–301, May–June, 2011. Original article submitted October 15, 2010.

0009-3130/11/4703-0329

TABLE 1. Polysaccharide Content of Brown Algae *Dictyopteris polypodioides* and *Sargassum* sp.

Alga	Polysaccharide content, % of dry alga weight			
	alginic acid	neutral polysaccharides		fucoidan
		EtOH elution		
		5%	15%	
Dictyopteris polypodioides	12.04	0.02	0.03	0.15
Sargassum sp.	12.98	0.10	0.12	0.92

TABLE 2. Monosaccharide Composition of Neutral Polysaccharides Obtained by Hydrophobic Chromatography over Polychrom-1

Fraction	Yield, %*	Monosaccharide composition, mol/mol Fuc (Glc**)					
		Rha	Man	Fuc	Gal	Xyl	Glc
Dp-5%	0.02	0.3	0.3	1.0	0.2	0.1	0.6
Dp-15%	0.03	0.1**	0.1**	0.1**	0.1**	0.1**	1.0**
S-5%	0.10	0.4	0.2	1.0	0.4	0.1	0.6
S-15%	0.15	0.4	0.2	1.0	0.4	0.1	0.5

*% of dry alga weight; **monosaccharide composition, mol/mol Glc.

The ratio of mannuronic and glucuronic acid units (M/G) for the Dp-A and S-A fractions isolated by us was calculated from the ratio of intensities of the corresponding resonances in the PMR spectrum. The quantity M/G for *D. polypodioides* and *Sargassum* sp. was 2.0 and 1.5, respectively. According to the literature, the M/G ratio for many algae of the genus *Sargassum* varies from 0.19 to 1.56. Alginates isolated from *S. vulgare* (Brazil) contained a large amount of mannuronic acid [7], in contrast with alginates isolated from *S. dentifolium*, *S. asperifolium*, and *S. latifolium* (Egypt), which had high contents of guluronic acid units [8]. Information on the content of alginic acids in algae of the genus *Dictyopteris* has not been reported.

The algae studied by us, *D. polypodioides* and *Sargassum* sp., contained few water-soluble polysaccharides. The amount of fucoidan in the studied algae was less than 1% of the dry alga weight; of neutral polysaccharides, less than 0.25%. According to the literature, the content of water-soluble polysaccharides isolated from *S. tenerrimum* (India, Okha) was about 3% [6]; of polysaccharides isolated from *S. stenophyllum* (Brazilia, Bombinhas), about 0.4% calculated for dry alga weight [9]. The differences in the polysaccharide compositions of algae of the genus *Sargassum* are probably related to the fact that the specimens were collected at different sites at different times and were different species. The content of water-soluble polysaccharides in algae of the genus *Dictyopteris* has not been reported.

Fractions Dp-5% and S-5%, which were eluted from Polychrom-1 by aqueous EtOH (5%), gave after hydrolysis large amounts of fucose, mannose, rhamnose, and galactose and minor amounts of xylose (Table 2). The structures of these compounds were not analyzed by ^{13}C NMR and mass spectrometry because their monosaccharide compositions were highly heterogeneous.

Fraction DP-15% gave a large amount of glucose and minor amounts of other monosaccharides upon hydrolysis. The ^{13}C NMR spectrum of isolated fraction Dp-15% (not shown) showed resonances with chemical shifts 103.21 (C-1), 73.98 (C-2), 84.86 (C-3), 68.81 (C-4), 76.31 (C-5), and 61.41 (C-6) ppm that were characteristic of 1,3-bound β-D-glucopyranose [10]. The ^{13}C NMR spectrum also showed a resonance with chemical shift 64.5 ppm that was characteristic of mannitol. In addition to these strong resonances, there were additional weaker resonances with chemical shifts 96.58 and 92.77 ppm that corresponded to C-1 of α- and β-Glc*p* and indicated that the compound was an oligosaccharide. This hypothesis was confirmed by data obtained from MALDI-TOF mass spectrometry.

Fraction Dp-15% was a mixture of 1,3-β-D-glucooligo- and polysaccharides, the degree of polymerization of which was in the range 7–30.

In contrast with Dp-15%, fraction S-15%, which was eluted from Polychrom-1 by aqueous EtOH (15%), was a heterogeneous polysaccharide. Table 2 presents the monosaccharide composition, which was also confirmed by ^{13}C NMR spectroscopy (spectrum not shown). It is known that many algae of the genus *Sargassum* contain practically no laminaran [6]. This agreed with our results.

TABLE 3. Characteristics of Fucoidans Obtained by Anion-Exchange Chromatography over Macro-Prep DEAE*

Fucoidan	Eluent [NaCl], M	Yield, %**	Content, %***		Monosaccharide composition, mol/mol Fuc					
			$NaSO_3^-$	polyphenols	Rha	Man	Fuc	Gal	Xyl	Glc
Dp-F1	0–0.3	2.30	5.78	–	0.2	0.3	1.0	0.3	0.2	0.3
Dp-F2	0.4–0.7	22.50	12.67	–	0.2	0.3	1.0	0.1	0.4	0.1
Dp-F3	0.7–0.8	6.52	13.06	–	0.2	0.3	1.0	0.2	0.2	0.1
Dp-F4	0.9–1.0	15.73	13.41	–	0.3	0.1	1.0	0.8	0.2	0.2
S-F1	0.1–0.2	2.07	6.20	1.8	0.2	0.3	1.0	0.4	0.2	0.1
S-F2	0.5–0.8	13.99	19.64	4.9	0.1	0.2	1.0	0.3	0.2	0.1
S-F3	0.9–1.0	10.40	23.33	7.6	0.1	0.1	1.0	0.4	0.1	0.1
S-F4	1.2–1.4	12.78	28.79	6.0	0.1	0.1	1.0	0.3	0.1	0.1

*Protein-type compounds determined by the Bradford method [26] were absent in all fractions; **% of water-soluble fraction content; ***% of weight.

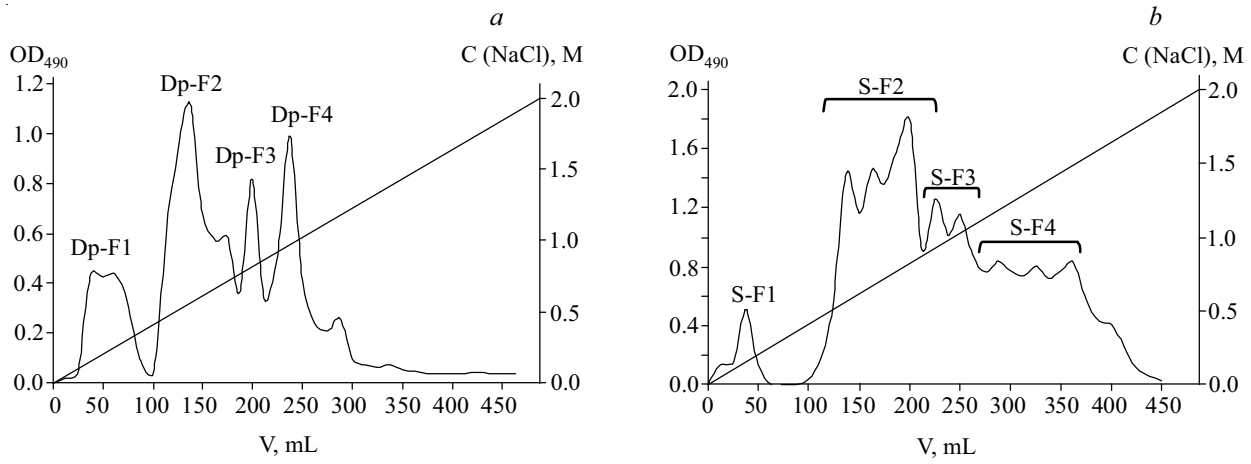

Fig. 1. Ion-exchange chromatography over Macro-Prep DEAE (Cl^--form, 8 × 2.5 cm) of fucoidans from *Dictyopteris polypodioides* (*a*) and *Sargassum* sp. (*b*).

Fractions Dp-F and S-F contained after Polychrom-1 fucoidans that were separated over Macro-Prep DEAE by elution with a linear gradient H_2O–NaCl (2 M). Figure 1 shows the elution profile for *D. polypodioides* and *Sargassum* sp.

Each alga afforded four fractions: Dp-F1, Dp-F2, Dp-F3, and Dp-F4 and S-F1, S-F2, S-F3, and S-F4. Table 3 presents their characteristics.

Table 3 presents the monosaccharide compositions of the isolated polysaccharides. Fractions obtained from anion-exchange chromatography of polysaccharides Dp-F and S-F gave upon hydrolysis large quantities of fucose, galactose, rhamnose, mannose, and xylose in addition to minor amounts of glucose. The contents of mannose and rhamnose decreased in both algae on going from the first to the fourth fraction.

Fucoidans (S-F1, S-F2, S-F3, and S-F4) isolated from *Sargassum* sp. were sulfonated heterofucans, like analogous polysaccharides isolated from algae of this genus (*S. stenophyllum* [9], *S. tenerrimum* [6], and *S. hemiphyllum* [10]).

Structural characteristics of fucoidans isolated from algae of the genus *Dictyopteris* (family Dictyotaceae) have not been reported. However, it is known that other algae belonging to this family have afforded both practically pure sulfonated fucans (*Stoechospermum marginatum* [11]) and heterogeneous polysaccharides (*Spatoglossum schroederi* [12], *Lobophroa variedata* [13], *Padina gymnospora* [14], and *Dictyota menstrualis* [15]).

Fucoidans isolated from *D. polypodioides* did not contain polyphenols whereas the polyphenol content in fucoidans isolated from *Sargassum* sp. varied from 1.8 to 7.6%. All isolated fucoidans contained no proteins. The degree of sulfonation in the fucoidans increased with increasing eluent concentration. Thus, we isolated fucoidans with both low and high degrees of sulfonation. Table 3 gives the values.

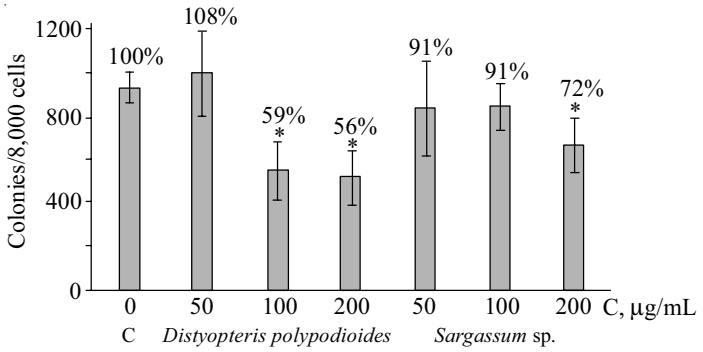

Fig. 2. Growth inhibition of RPMI-7951 human melanoma cell colonies by fucoidans (Dp-F4, S-F4).

Fucoidans isolated from *D. polypodioides* and *Sargassum* sp., like many other natural algal fucoidans, have complicated ^{13}C NMR spectra (not shown) that are difficult to interpret fully. They all contained strong resonances in the anomeric region (96–102 ppm) and at high frequencies (15–17 ppm) that were typical of α-fucopyranosides. Resonances at 19–21 ppm (CH_3) and 173–176 (C=O) corresponded to acetyls in spectra of fractions Dp-F3, Dp-F4, S-F1, and S-F4.

Polysaccharides isolated from algae are known to exhibit various biological activities such as antitumor, antiviral, anti-inflammatory, antiangiogenic, and immunostimulating. Thus, water-soluble polysaccharides and alginates isolated from algae of the genus *Sargassum* exhibited antitumor properties [16–20]; sulfonated polysaccharides, antiviral properties [6, 21–23].

According to the literature, sulfonated polysaccharides isolated from *D. delicatula* exhibited antioxidant and anticoagulant activity [24]. Fucoidans isolated from algae of the family Dictyotaceae exhibited antiviral [11], antithrombotic [12], and anticoagulant [13–15] activity. Therefore, it seemed interesting to determine the antitumor activity of the polysaccharides isolated by us. Treatment of RPMI-751 cells with fucoidans from *D. polypodioides* and *Sargassum* sp. (Dp-F4 and S-F4) at concentrations up to 200 μg/mL did not inhibit their growth and were not associated with extensive cell death. The growth inhibition of RPMI-7951 cell colonies treated with fucoidan from *D. polypodioides* at a dose of 200 μg/mL was 44%. Fucoidan from *Sargassum* sp. was a weaker inhibitor (28%) (Fig. 2).

The studied fucoidans had different structural characteristics with respect to both the monosaccharide composition and degree of sulfonation. Apparently the differences in the antitumor activities among fucoidans from various sources are due to these structural features.

EXPERIMENTAL

Reagents and Materials. EtOH, acetone, NaOH, and inorganic acids and salts were commercial products (Diaem, Russia). Standards (mannose, rhamnose, maltose, glucose, galactose, xylose, bovine serum albumin) were obtained from Sigma (USA). Sorbents for chromatography were Polychrom-1 (Reakhim, Russia) and Macro-Prep DEAE (Bio-Rad, USA).

Brown algae *Dictyopteris polypodioides* and *Sargassum* sp. were collected on the Mediterranean coast (Lebanon) in July 2009 (Batroun: N 34° 15.069′, E 35° 39.3852 ; Barbara: N 34° 12.034′, E 35° 38.180′). Dried and ground algae were treated with EtOH (70%, 1:1) for subsequent isolation of polysaccharides.

Carbohydrate contents were determined colorimetrically using the reaction with phenol and H_2SO_4 [25]; **protein contents**, by the Bradford method [26] using bovine serum albumin as a standard; **polyphenol contents**, colorimetrically by the Folin–Ciocalteau method [27].

Extraction of Polysaccharides. Dried defatted algae *Dictyopteris polypodioides* and *Sargassum* sp. (50 g) were extracted with HCl solution (0.1 M, 1:20 ratio, 2 ×) for 2 h at 60°C. The extracts were neutralized and centrifuged. The supernatant was concentrated in a rotary evaporator, dialyzed against distilled water, and evaporated to dryness to afford fractions containing water-soluble polysaccharides. The algae remaining after the extraction were extracted with Na_2CO_3 solution (2%, 1:30 ratio) for 2 h at 55°C. The extracts were centrifuged. The supernatant was dialyzed against distilled water. Alginic acid was precipitated from the extracts by three times the volume of EtOH (96%). The precipitates were washed successively with EtOH (96%) and acetone and dried in air to afford Dp-A (6.9 g) and S-A (8.4 g).

Hydrophobic Chromatography over Polychrom-1 (Polytetrafluoroethylene). The extract containing water-soluble polysaccharides was placed on a column of Polychrom-1 (15 × 6.5 cm). Charged polysaccharides (fucoidans Dp-F, 0.16 g; S-F, 1.52 g) were eluted with water (until the reaction of the eluent for total carbohydrates was negative; phenol–H_2SO_4 method). Neutral polysaccharides were eluted by aqueous EtOH (5 and 15%, until the reaction of the eluent for total hydrocarbons was negative). The resulting fractions were evaporated in a rotary evaporator, frozen, and lyophilized to afford Dp-5%, Dp-15%, S-5%, and S-15% (0.01, 0.02, 0.05, and 0.06 g, respectively).

Anion-Exchange Chromatography. Polysaccharide fractions (Dp-F and S-F) were dissolved in HCl (0.04 M), placed on a column of Macro-Prep DEAE (8 × 2.5 cm) and eluted by a linear gradient of NaCl (2 M). The resulting fractions Dp-F1, Dp-F2, Dp-F3, and Dp-F4 and S-F1, S-F2, S-F3, and S-F4 (0.01, 0.04, 0.01, 0.03, 0.03, 0.21, 0.16, 0.20 g, respectively) were dialyzed against distilled water, evaporated in a rotary evaporator, and dried (lyophilized).

Acid Hydrolysis of Polysaccharides. A sample (5 mg) was dissolved in TFA (0.5 mL, 2 N). The hydrolysis was performed at 100°C for 6 h. The sample was neutralized by aqueous ammonia and evaporated to dryness.

Monosaccharide compositions of the polysaccharides after acid hydrolysis were determined on a Biotronik IC-5000 analyzer (Germany) using a Shim-pack ISA-07/S2504 column (0.4 × 25 cm) with elution by potassium borate buffer at 0.6 mL/min. Detection used the bicinchonate method and integration by the Shimadzu C-R2 AX system. The standards were maltose, rhamnose, mannose, fucose, galactose, xylose, and glucose.

Sulfonate contents in the polysaccharides were determined by turbidimetry [28].

PMR and ^{13}C NMR spectra of D_2O solutions of the compounds were taken on a Bruker DPX-500 NMR spectrometer at 303 K.

Cell Cultivation. RPMI-7951 human melanoma cells were cultivated in MEM growth medium with FBS (10%), penicillin (100 U/L), and streptomycin (100 μg/L) in an MCO-18AIC incubator (Sanyo, Japan) at 37°C with 5% CO_2.

Determination of Fucoidan Cytotoxicity. RPMI-7951 human melanoma cells (2×10^4/mL) were seeded in a 96-well plate and cultivated in RPMI-1640 (10%, 200 μL) in a CO_2-incubator at 37°C for 24 h. Then, cells were treated with fucoidans at various concentrations (10, 50, 100, and 200 μg/mL), incubated for 24 h, treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT-reagent), and incubated for 4 h (37°C, 5% CO_2). The optical density (OD) was measured on a Bio-Tek Instruments spectrophotometer (USA) at 490/630 nm ($A_{490/630}$).

Neoplastic Transformation of Cells (soft agar-agar model). The activity of the fucoidans from brown algae on the formation and growth of colonies of RPMI-7951 human melanoma cells was determined by the soft agar-agar model. Melanoma cells (8×10^3 cells) were treated with fucoidans from brown algae *D. polypodioides* and *Sargassum* sp. (50, 100, and 200 μg/mL) in BME agar-agar containing FBS (10%). Cells were cultivated at 37°C and 5% CO_2 for 20 d. Colonies of RPMI-7951 human melanoma cells were estimated using a Motic AE 20 inverted microscope (China) and the Motic Image Plus program.

Statistical processing of results was carried out using Student's *t*-criterion with 95% confidence probability (SigmaPlot 2000 program, Version 6, SPSS Inc., USA)

ACKNOWLEDGMENT

The work was supported by RFBR Grant No. 09-04-00761-a and the Molecular Cellular Biology Program. We thank Prof. V. V. Isakov and Cand. Chem. Sci. S. D. Anastyuk (PIBOC, FEB, RAS) for recording spectra and assisting with their interpretation.

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