Polysaccharides of Algae

71*. Polysaccharides of the Pacific brown alga *Alaria marginata*

*M. I. Bilan,[a] N. G. Klochkova,[b] A. S. Shashkov,[a] and A. I. Usov[a]**

[a]N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences,
47 Leninsky prosp., 119991 Moscow, Russian Federation.
Fax: +7 (499) 135 5328. E-mail: usov@ioc.ac.ru
[b]Kamchatka State Technical University,
35 ul. Klyuchevskaya, 683003 Petropavlovsk-Kamchatsky, Russian Federation.
E-mail: ninakl@mail.ru

The polysaccharide composition in sporophylls of the brown alga *Alaria marginata* enriched with laminaran and sulfated polysaccharides was studied. It was shown that laminaran molecules had an average degree of polymerization about 30 and consisted mainly of 3-linked β-D-glucopyranose residues, having no more than 10% of 1→6 linkages. The majority of chains (about 60%) were terminated at "reducing" end by mannitol residue. Alginic acid of sporophylls contained mannuronic (M) and guluronic (G) acids residues distributed along the linear polymer molecules as MM, MG, and GG blocks at a ratio of 4 : 1 : 1. Fucoidan was found to be composed of fucose, galactose, and sulfate as the major constituents, while xylose, mannose, glucuronic acid, and acetate were the minor components. It was shown that fucoidan contained two major components: fucan sulfate, molecules of which are built up of 3-linked fucopyranose residues with branches and sulfate groups at different positions, and fucogalactan, also containing chains of 3-linked fucopyranose residues with branches at positions 4 together with highly branched galactan chains terminated by fucose residues. The fucoidan contained also sulfated glucuronomannan and sulfated glucuronan as minor components.

Key words: *Alaria marginata*, brown algae, polysaccharides, laminaran, fucoidan, alginate.

Brown algae, representatives of the class Phaeophyceae, have a unique carbohydrate composition and may be used as a rich source of mannitol, laminaran, alginic acids, and alginates, and also of sulfated polysaccharides (fucoidans), which are intensively studied as potential components for new medicines.[2—4] In particular, the structures and biological activity of laminarans and sulfated galactofucans from the two Pacific brown algae, *Alaria angusta*[5] and *Alaria marginata* (*A. marginata*),[6] were reported recently. Those works are aimed at polysaccharide preparations isolated from whole algal thalli. It is worth noting that *Alaria* species, as well as other representatives of the family Alariaceae, have the thallus differentiated into a blade with a midrib, a quite long stipe, and anchoring organs (rhizoids) (Fig. 1). Special spore-bearing organs (sporophylls) grow on the stipe close to the blade base.[7] In previous studies of the two *Alaria* species: *A. fistulosa*[8] and *A. marginata*,[9] we demonstrated that distinct parts of the thallus have different polysaccharide compositions, wherein sporophylls significantly exceed other parts of algae in the content of sulfated polysaccharides. These data for *A. marginata* were acquired by analytical techniques which allowed one to quantify individual polysaccharides without their isolation from the algal biomass. The present work is aimed at the more detailed study of the polysaccharide composition in *A. marginata* sporophylls *via* the isolation and structural analysis of individual biopolymers.

Results and Discussion

A. marginata P. *et* R. is widespread in the Russian Far East from the Chukchi Peninsula to the Middle Primorye.[7] Two samples of this alga were used in this study: the first one (A) was harvested at the coast of Is. Shikotan in August, 1987 and the second (B) was collected at the coast of Kamchatka, in Avacha Bay, in July, 1990. Each thallus was separated into a blade, a midrib, sporophylls, and rhizoids (see Fig. 1). These parts of the thalli were dried in air (under a fan) and ground. The information about a carbohydrate composition in samples from the Kamchatka was obtained earlier by the hydrolysis of bio-

* For Part 70 see Ref. 1.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 0137—0143, January, 2018.

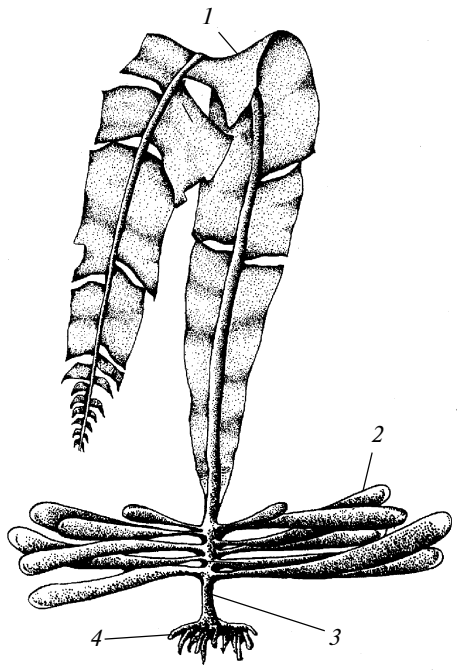

Fig. 1. The structure of the brown alga *A. marginata* thallus: blade with the midrib (*1*), sporophylls (*2*), stipe (*3*), and rhizoids (*4*).

mass and the determination of neutral monosaccharides by a GLC method, and also by the spectrophotometric determination of fucoidan and alginate in acidic and alkaline extracts, respectively (these results were reported in our previous work[9]). The content of neutral monosaccharides in the hydrolysates of the Shikotan biomass samples was also analyzed by the GLC method, and there was no fundamental difference in comparison with results of the Kamchatka algae analysis. To summarize those observations, one can conclude that mannitol was accumulated mainly in the blade, while laminaran and fucoidan were located in the sporophylls, and the galactose content in hydrolysates was comparable with the content of fucose.

Sporophylls from both algal samples (A and B) were taken for the preparative isolation of polysaccharides. The detailed procedure for the extraction of water-soluble polysaccharides was described in our previous reports.[8,10] Sporophylls from the sample A were treated with 2% aqueous solution of calcium chloride at 85 °C, and anionic polysaccharides were precipitated from the extract by an addition of hexadecyltrimethylammonium bromide (Cetavlon). Laminaran was obtained from the mother liquor after dialysis and freeze-drying in 8.1% yield. The precipitate of Cetavlon salts was converted into water-soluble sodium salts and a resulting preparation fucoidan-A was obtained in 12.7% yield.

In the laminaran hydrolyzate, mannitol was found in addition to glucose. The ratio of glucose : mannitol was 52 : 1. When laminaran was preliminarily reduced by sodium borohydride, its hydrolyzate contained mannitol and sorbitol at the ratio of ~1.5 : 1. This evidence indicated that approximately 3/5 of the laminaran molecules contained a residue of mannitol at the "reducing" ends (M-chains), while the other molecules were terminated by reducing glucose residues (G-chains). The average degree of polymerization (DP) for laminaran molecules was calculated on these data and was about 30 monosaccharide residues in the chain. The ^{13}C NMR spectrum of laminaran (Fig. 2) contained six major signals related to the carbon atoms from 3-substituted glucose residues and characteristic for linear polymer molecules that consisting of (1→3)-linked β-glucopyranose residues.[11]. Minor signals could be assigned to a small number of (1→6)-linked β-glucopyranose residues. In general, the spectrum was almost identical to the spectrum of a similar laminaran preparation from Sigma company,[12] wherein the content of (1→6)-bonds was 10%. The optical activity of the polysaccharide, $[\alpha]_D$ −9.7, was also typical for laminarans[2] and confirmed the D-configuration of glucose residues.

The total fucoidans were fractionated by anion-exchange chromatography on DEAE-Sephacel. Fucoidan-A was separated by this method into four fractions (A-1—A-4) using the elution with 0.75, 1.0, 1.25, and 1.5 *M* NaCl solutions. The yields and analytical characteristics of the obtained fractions are given in Table 1. As can be seen from this table, the major monosaccharide in the fractions A-1 and A-2 was fucose, in addition to which small amounts of other monosaccharides were also present. There was no significant difference between A-1 and A-2 in the degree of sulfation. As might be expected based on the results of anion-exchange chromatography, the fractions A-3 and A-4 were more sulfated. In comparison with the previous fractions, their monosaccharide composition was much simpler: they contained almost only fucose and galactose, and the latter was the major con-

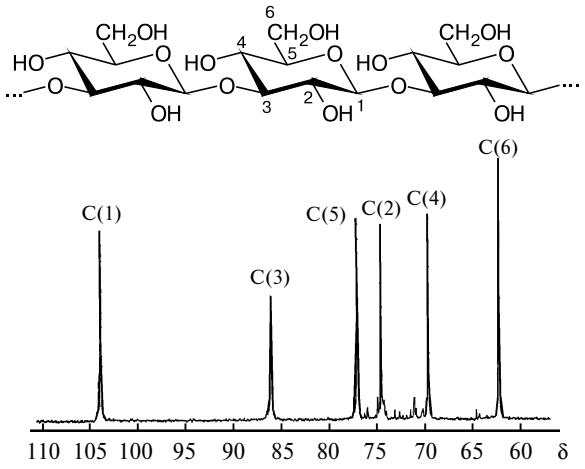

Fig. 2. ^{13}C NMR spectrum of the laminaran in D_2O.

Table 1. Yields and compositions (% of the weighed sample) of fucoidan-A and fractions obtained in its separation by the anion-exchange chromatography and subsequent desulfation

Sample	Eluent (NaCl) /mol L^{-1}	Yield (%)	Composition						
			Fuc	Xyl	Man	Glc	Gal	Uronic acids	SO_3Na
Fucoidan-A	—	—	30.9	1.0	1.4	2.3	18.9	6.3	29.8
A-1	0.75	8.0[a]	34.8	5.6	4.2	1.5	4.9	—[b]	17.4
A-2	1.00	13.4[a]	46.3	2.9	2.1	—	5.7	—[b]	20.1
A-3	1.25	27.7[a]	27.1	1.4	—	—	21.6	—[b]	28.4
A-4	1.50	7.1[a]	12.8	1.2	—	—	28.5	—[b]	30.3
A-4deS	—	30.9[c]	18.3	4.1	1.0	1.1	70.0	—[b]	1.9

[a] The yield with respect to fucoidan-A. [b] Not determined. [c] The yield with respect to the fraction A-4.

stituent in the preparation A-4. These data indicated the presence in the total fucoidan-A of two main components: sulfated fucan (A-1 + A-2) and sulfated fucogalactan (A-3 + A-4). Fraction A-4 was used in further studies to obtain information about the structure of fucogalactan.

NMR spectra of preparation A-4 (Fig. 3) were too much intricate for the interpretation, which was similar to most other native fucoidans of brown algae. A chemical modification was carried out to simplify the structure of A-4. The sulfate groups were removed by solvolytic desulfation to give preparation A-4deS (see Table 1, Fig. 4). An additional procedure for deacetylation of A-4deS was performed since A-4 and A-4deS contained acetyl groups according to their NMR spectra. However, the NMR spectra interpretation for obtained preparation A-4deSdeAc encountered difficulties, which are common in the assignment of signals for this class of polysaccharides. Therefore, the methylation analysis was used for the structural study of A-4.

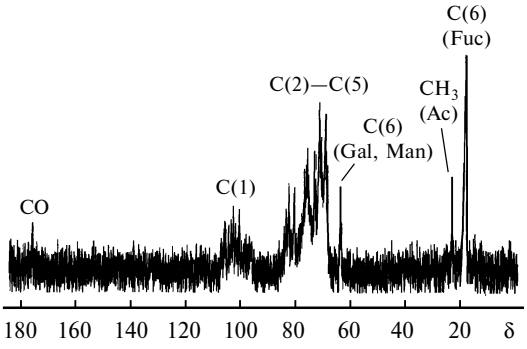

Fig. 3. ^{13}C NMR spectrum of the fraction A-4 in D_2O.

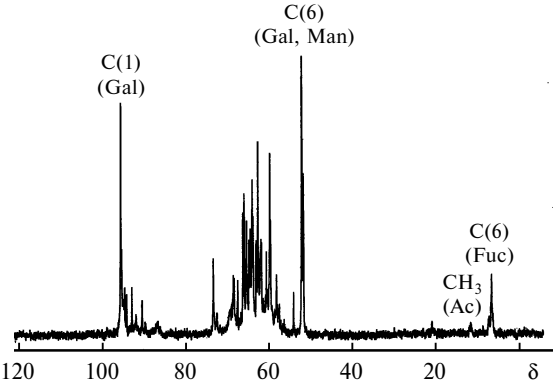

Fig. 4. ^{13}C NMR spectrum of the fraction A-4deS in D_2O.

Desulfated preparation A-4deS was treated with methyl iodide and sodium hydroxide in DMSO under appropriate conditions to remove the acetyl groups with subsequent total methylation of all hydroxy groups in the polysaccharide. The methylated polysaccharide was hydrolyzed, the resulting mixture of partially methylated monosaccharides was analyzed, and it was found that the substance contained carbohydrate chains that consisted of (1→3)-linked fucose residues with branches at position 4 of every fifth residue (Table 2). The galactan sections of the molecules were more branched and contained 1→3, 1→4, and 1→6 links in linear chains, while in the branches there were only 6-linked galactose residues having addi-

Table 2. Methylation analysis of the desulfated preparation A4-deS from the *A. marginata* sporophylls

Positions of O—Me groups	Substitution positions	A4-deS (mol. %)
Xyl:		
2,3,4	Xyl*p*→	<1
2,3 (3,4)	→4(2)Xyl→	4
Fuc:		
2,3,4	Fuc*p*→	7
2,4	→3Fuc*p*→	9
2	→3,4Fuc*p*→	2
Gal:		
2,3,4,6	Gal*p*→	11
2,3,6	→4Gal*p*→	20
2,4,6	→3Gal*p*→	21
2,3,4	→6Gal*p*→	10
3,4	→2,6Gal*p*→	5
2,3	→4,6Gal*p*→	9
2,4	→3,6Gal*p*→	2

tional substituents at positions 4, 2, or 3. The ratio between the number of branch points and terminal nonreducing monosaccharide residues indicated that at the branches of the galactan chains there must be fucopyranose residues or chains of such residues.

The procedure for the extraction of water-soluble polysaccharides from the sporophylls of sample B was different in one aspect: laminaran and fucoidan were not separated by Cetavlon precipitation. The resulting preparation contained both of these polysaccharides (fucoidan-B) and was obtained in the yield of 19.5%. The remaining biomass in this experiment was treated with a dilute acid and then extracted under heating with 3% sodium carbonate solution. Alginic acid was precipitated from this extract by acidification. The crude preparation of alginic acid contained 89.1% of uronic acids and was obtained in the yield of 39.0%, based on the residue after the extraction of water-soluble polysaccharides.

Fucoidan-B was dissolved in water (some precipitate was formed) and fractionated by the chromatography on DEAE-Sephacel column as described hereinbefore. The yields and composition of the obtained fractions are shown in Table 3 and one can see that the precipitate was enriched with laminaran due to the limited solubility of this polysaccharide in water. The aqueous eluate from the ion exchange column, containing neutral substances, was pure laminaran. The saline eluates are similar to the fractions obtained by the separation of fucoidan-A. Fractions B-3 and B-4 were similar in the content of monosaccharides and different in the degree of sulfation, resembling fractions A-1 and A-2, and obviously consisted mostly of sulfated fucan; while fractions B-5 and B-6 were similar to fractions A-3 and A-4, and contained sulfated fucogalactan. Fraction B-4 was selected for a more detailed characterisation of the sulfated fucan.

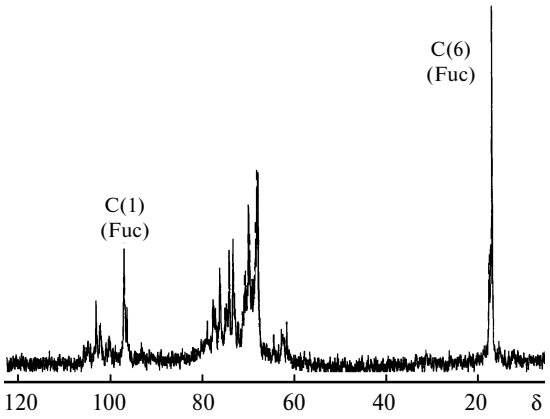

Fig. 5. ^{13}C NMR spectrum of the fraction B-4deSdeAc in D_2O.

NMR spectra of this substance were difficult for interpretation even after desulfation and deacetylation (the spectrum of desulfated and deacetylated preparation B-4deSdeAc is shown in Fig. 5). The diversity of observed signals could be partially explained by a presence of several minor polysaccharides in the fraction as impurities to the major component, such as common for the laminaria algae sulfated glucuronomannan and glucuronan.[13,14] The Smith degradation of B-4deSdeAc was performed to further simplify its structure. A part of the substance lost its solubility in water after the periodate oxidation, subsequent reduction, and partial acid hydrolysis. Compositions of the insoluble (Sm-1) and soluble (Sm-2) fractions obtained by Smith degradation of B-4deSdeAc are shown in Table 3.

It is well known that a low solubility in water is common for linear polymer molecules constructed from 3-linked residues of α-L-fucopyranose. Indeed, the ^{13}C NMR spectrum of fraction Sm-1 (Fig. 6) was almost

Table 3. Yields and compositions (% of the weighed sample) of fucoidan-B and fractions obtained in its separation by the anion-exchange chromatography, and also the product from desulfation and deacetylation of fraction B-4 (B-4deSdeAc); and two preparations (Sm-1 and Sm-2) obtained by Smith degradation of B-4deSdeAc

Sample	Eluent (NaCl) /mol L^{-1}	Yield (%)	Composition						
			Fuc	Xyl	Man	Glc	Gal	Uronic acids	SO_3Na
Fucoidan-B	—	—	21.9	1.1	2.1	34.1	11.0	—[a]	17.3
Осадок	—	6.6[b]	2.0	—	2.3	58.7	6.0	—[a]	—[a]
B-1	0.00	22.4[b]	—	—	4.2	98.9	—	—[a]	—[a]
B-2	0.50	3.9[b]	25.1	2.9	3.9	19.8	3.5	17.0	3.6
B-3	0.75	5.9[b]	41.8	6.6	4.2	2.4	5.8	8.3	11.0
B-4	1.00	9.9[b]	41.3	—	2.1	1.9	6.7	4.1	20.1
B-5	1.25	20.4[b]	34.3	—	—	—	21.6	2.1	33.7
B-6	1.50	5.3[b]	16.0	1.2	—	—	32.3	2.7	27.7
B-4deSdeAc	—	37.1[c]	49.7	2.1	4.2	1.4	13.1	15.7	—[a]
Sm-1	—	18.0[d]	75.4	—	—	—	—	—[a]	—[a]
Sm-2	—	28.0[d]	17.7	3.0	8.6	—	1.4	23.0	—[a]

[a] Not determined. [b] The yield with respect to fucoidan-B. [c] The yield with respect to the fraction B-4. [d] The yield with respect to the fraction B-4deSdeAc.

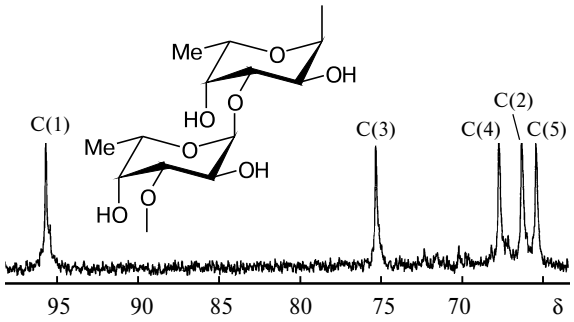

Fig. 6. ^{13}C NMR spectrum [C(1)—C(5) region] of the preparation Sm-1 (solution in DMSO-d_6 containing 10%LiCl).

identical to an analogous spectrum of linear fucan obtained earlier by the Smith degradation of fucoidan from the brown alga *Analipus japonicus*.[15] The spectrum contained six major signals belonging to carbon atoms of the 3-substituted fucopyranose residues. Several minor signals could be assigned to terminal non-reducing monosaccharide residues. At the same time, methylation of Sm-1 showed that in addition to linear sections of the carbohydrate chains the substance also contained branches (approximately one branch per every five fucose residues), wherein the main branch points were at 2,3-disubstituted residues and minors were at 3,4-disubstituted fucose residues (Table 4). Branched carbohydrate chains were previously observed in several fucoidans, wherein the side substituents in most cases were single residues of fucose or glucuronic acid.[16] It is obvious that in our case, in order to preserve branches in Sm-1 during the periodate oxidation, the side chains in B-4deSdeAc should have a more complex structure, for example, to be residues of 3-linked disaccharides. The methylation analysis data for fraction Sm-2 confirmed the branched structure of fucan molecules. At the same time, short chains of 4-linked xylose and long chains containing 2-linked mannose residues were found in this fraction, which could most likely be fragments of glucuronomannan (glucuronic acid residues were lost during polymer analysis by methylation), although there was no reasonable explaination for the preservation of such molecular fragments under the periodate oxidation conditions. A comparison between the methylation results for preparations B-4 and B-4deS (see Table 4) showed that fucose residues in B-4 were sulfated at different positions, although a significant number of terminal non-reducing fucose residues was not sulfated.

^{13}C NMR spectrum of sodium alginate isolated from the sporophylls of sample A was typical for polymers of this class[17,18] and indicated that the substance contained residues of mannuronic (M) and guluronic (G) acids at the ratio of 1.76 : 1. To characterize the block distribution for these acids residues along the polymer chains, the substance was partially hydrolyzed[19] with subsequent preparative isolation of the fractions corresponding to MM, MG, and GG blocks, which were obtained in the yields of 41, 10, and 10%, respectively.

Table 4. Methylation analysis (mol.%) of the native preparation B-4, the product of its desulfation B-4deS, and two fractions (Sm-1 and Sm-2) obtained by Smith degradation of B-4deSdeAc

Positions of O—Me groups	Substitution positions	B-4	B-4deS	Sm-1	Sm-2
Xyl:					
2,3,4	Xyl→	—	1	—	5
2,3 (3,4)	→4(2)Xyl→	—	2	—	7
Fuc:					
2,3,4	Fuc→	14	24	9	10
2,3	→4Fuc→	6	1	—	—
2,4	→3Fuc→	22	38	77	34
2	→3,4Fuc→	13	11	3	4
4	→2,3Fuc→	17	5	11	3
Fuc	→2,3,4Fuc→	21	—	—	—
Hex:					
2,3,4,6-Gal	Gal→	—	2	—	—
2,3,4,6-Hex	Man→	—	—	—	9
2,3,6-Gal	→4Gal→	—	6	—	—
2,4,6-Gal	→3Gal→	—	2	—	—
3,4,6-Hex	→2Man→	—	—	—	28
2,3,4	→6Hex→	—	3	—	—
2,6	→3,4Hex→	2	—	—	—
4,6	→2,3Hex→	—	3	—	—
2,3	→4,6Hex→	—	2	—	—
2,4	→3,6Hex→	1	—	—	—
2	→3,4,6Hex→	1	—	—	—
3 (4)	—	3	—	—	—

Therefore, this study confirmed previous data that various parts of *A. marginata* thallus were significantly different in carbohydrate composition, and showed that these differences were preserved for samples collected in particular geographical areas. It was established that laminaran accumulated in sporophylls was a typical representative from this class of brown algae reserve polysaccharides and contained slightly branched molecules with an average DP about 30. Laminaran molecules were consisted of 3-linked β-D-glucopyranose residues, while 3/5 of these molecules were terminated with mannitol residues at the reducing end. The alginate from sporophylls was also a typical representative from this class of structural polyuronides, and contained fairly elongated blocks constructed from mannuronic (poly-M) and guluronic (poly-G) acid residues, separated by relatively short poly-MG fragments. The data about the presence in the biomass of two major sulfated polysaccharides, fucan and fucogalactan, were obtained from separation of the total fucoidan preparations of sporophylls by anion-exchange chromatography. Fucan molecules contained the main chain built from 3-linked fucose residues with numerous

branches and sulfate groups at different positions. Fucogalactan contained chains of 3-linked fucopyranose residues with branch points at C(4) atom in every fifth residue, and also a chain of 3-, 4-, and 6-linked galactose residues, while some of the 6-linked galactose residues had branches at positions 2, 3, or 4; and part of the branched galactan chains was terminated by non-reducing fucose residues. The presence of sulfated glucuronomannan and glucuronan as minor polysaccharide components in sporophylls was suggested. The presence of all said types of sulfated polysaccharides was observed earlier in the compositions from representatives of other brown algae species. The accumulation of the sulfated polysaccharides in sporophylls could be related to their important role in a sporulation process.[20] Improved isolation methods from the total fucoidan preparations will be required for individual polysaccharides in order to acquire more detailed data about their structures.

Experimental

General analytical methods. Gas-liquid chromatography (GLC) was performed on an Agilent Technologies 7820A chromatograph equipped with a flame-ionization detector in a nitrogen flow in a temperature gradient from 160 to 290 °C at a rate of 7 deg min^{-1}. To perform acid hydrolysis, 2 *M* trifluoroacetic acid (1 mL) containing *myo*-inositol (1.0 mg mL^{-1}, internal standard) was added to weighed samples of polysaccharide preparations (10—12 mg), the mixture was heated at 100 °C for 8 h, and the acid was distilled off with ethanol *in vacuo*. The released neutral monosaccharides were transformed into alditol acetates[21] and determined by GLC. The quantitative processing of the chromatograms was performed using the EZ Chrom Elite software.

The sulfate content in polysaccharides was evaluated by turbidimetry[22] after the hydrolysis in 2 *M* trifluoroacetic acid (8 h, 100 °C). The uronic acid content was determined by spectrophotometry using the reaction with 3,5-dimethylphenol and sulfuric acid.[23]. The quantitative measurements were carried out on an Ultrospec 4050 spectrophotometer (LKB Biochrom). The NMR spectra were recorded on a Bruker Avance 600 spectrometer at 303 K after one—two-fold freeze-drying from D_2O followed by dissolution in 99.96% D_2O (2—3% solutions). To obtain the ^{13}C NMR spectrum of the water-insoluble preparation Sm-1, the substance was dissolved in DMSO-d_6 containing 10% of anhydrous LiCl. Sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (internal standard, $\delta_H = 0.0$, $\delta_C = -1.6$) was used for the spectral calibration. The reliability of the signal assignment was monitored using 2D $^1H—^1H$ and $^1H—^{13}C$ correlation NMR spectra. The spectra were processed with the standard Bruker software. Gas chromatography-mass spectrometry analysis of partially methylated alditol acetates was performed on an Agilent 7890B—5977B GC-MSD instrument equipped with an HP-5 capillary column; the temperature was programmed as follows: at 150 °C for 1 min and then an increase to 280 °C at a rate of 10 deg min^{-1}.

Extraction of polysaccharides. Dried and crushed thallus parts of the alga *A. marginata* (the polysaccharide composition of the Kamchatka algal samples was given in the study,[9] samples No. 19—22) were used for extraction. A 2% $CaCl_2$ solution (300 mL) was added to sporophylls from the sample A (35 g). The mixture was stirred and kept overnight for swelling. Then the mixture was stirred at 85 °C for 4 h and centrifuged. A 10% aqueous hexadecyltrimethylammonium bromide (Cetavlon) solution (100 mL) was added to the extract. The residue of the alga was three more times extracted with 2% $CaCl_2$ (300 mL) under the same conditions. The extracts were added to the mixture containing Cetavlon. The precipitate of Cetavlon salts was separated by centrifugation, washed with water, stirred for four days with a saturated NaI solution in ethanol (4×50 mL), dissolved in water (50 mL), dialyzed, filtered, concentrated *in vacuo*, and freeze-dried to obtain the total preparation fucoidan-A in the form of sodium salt in the yield of 4.43 g. After the separation of Cetavlon salts of anionic polysaccharides, the mother liquor was dialyzed, concentrated, diluted fourfold with ethanol; and the formed precipitate was dissolved in water; the solution was freeze-dried to obtain the laminaran preparation in the yield of 2.84 g.

Sporophylls from the sample B (35 g) were extracted with 2% $CaCl_2$ solution (4×250 mL) as described for sample A. The combined extracts were dialyzed, concentrated, and freeze-dried to obtain the total preparation fucoidan-B in the yield of 4.9 g.

Anion-exchange chromatography. Fucoidan-A (400 mg) was dissolved in 0.1 *M* NaCl (10 mL) and loaded on a 30×2 cm column packed with DEAE Sephacel in 0.1 *M* NaCl. The column was washed with 0.1 *M* NaCl until the eluate was free of carbohydrates, which was assayed by the phenol reaction,[24] followed by successive washings with 0.75, 1.0, 1.25, and 1.5 *M* NaCl solutions, each procedure being performed until carbohydrates were not detected in the eluates by the phenol reaction. The fractions were dialyzed and freeze-dried to obtain the preparations A-1, A-2, A-3, and A-4, respectively. The yields and compositions of these preparations are given in Table 1.

Fucoidan-B (1.52 g) was dissolved in water (60 mL). The remaining precipitate was separated, washed with ethanol, acetone, and dried. The solution was loaded on a 90×3 cm column packed with DEAE Sephacel in water. The column was washed with water, followed by successive washings with 0.5, 0.75, 1.0, 1.25, and 1.5 *M* NaCl solutions, each washing being performed until carbohydrates were not detected in the eluates by the phenol reaction.[24] The fractions were dialyzed and freeze-dried, the yields and compositions of obtained preparations B-1—B-6 are given in Table 3.

Solvolytic desulfation[10] and methylation of fucoidans. The preparations A-4 or B-4 (100 mg) were dissolved in water, transformed into the pyridinium salts by passing through a column packed with the Dowex-50w×4 cation-exchange resin (PyH^+ form), and freeze-dried. The resulting preparations were dissolved in a mixture of DMSO (4.5 mL) and methanol (0.5 mL), heated at 80 °C for 5 h, dialyzed, and freeze-dried to obtain A-4deS or B-4deS. For a deacetylation, these preparations were dissolved in 1 *M* NH_3(aq), kept at 37 °C for 16 h, and freeze-dried. The yields and compositions of the obtained preparations are given in Tables 1 and 3. For the methylation analysis, these preparations or pyridinium salts of the fractions A-4 and B-4 (5—7 mg) were suspended in DMSO (0.5 mL), finely powdered NaOH (30—40 mg) and CH_3I (0.2 mL) were added, and the suspension was stirred for 1 h. After 30 min, CH_3I (0.2 mL) was added followed by the addition of water (3 mL) and chloro-

form (3 mL). The resulting mixture was dialyzed, concentrated to remove chloroform, and freeze-dried. A 2 *M* CF_3COOH solution (1 mL) was added to the methylated polysaccharide, the mixture was heated at 100 °C for 8 h, and the acid was distilled off with ethanol. The resulting monosaccharide derivatives were reduced with $NaBH_4$ and acetylated with a mixture of Ac_2O and pyridine (0.2 mL each). Partially methylated alditol acetates were analyzed by GLC and GLC/MS according to known procedures.[21]

Smith degradation. A sample of preparation B-4deSdeAc (70 mg) in water (24 mL) was oxidized with aq. 0.021 *M* $NaIO_4$ (12 mL) in the dark (48 h, until the stopped consumption of the oxidant which was controlled by decreasing in the optical density of the solution at 305 nm). Ethylene glycol (0.5 mL) was added to the solution, the mixture was dialyzed, concentrated, and reduced with $NaBH_4$ (300 mg) for 12 h. The resulting solution was neutralized with acetic acid, dialyzed, and freeze-dried to obtain the modified polysaccharide (53 mg) containing fucose (30.6%), uronic acids (18.8%), mannose (3.5%), galactose (3.5%), and xylose (1.7%). This preparation was dissolved in 1% AcOH (10 mL) and heated at 100 °C for 2 h. The precipitate formed during the partial hydrolysis was separated by centrifugation, washed with water, acetone and dried to obtain fraction Sm-1 in the yield of 9 mg. The supernatant was concentrated (until 2 mL volume) and loaded on a 2.7×76 cm column packed with Toyopearl TSK HW-40 (S) gel. Water was used for the elution at the 1 mL min^{-1} rate, while a differential refractometer was used as the detector. The polymer fraction was concentrated and freeze-dried to obtain the preparation Sm-2 in the yield of 14 mg.

This work was financially supported by the Russian Science Foundation (Project No. 14-50-00126).

References

1. M. I. Bilan, N. E. Ustyuzhanina, A. S. Shashkov, T. T. T. Thanh, M. L. Bui, T. T. V. Tran, V. N. Bui, A. I. Usov, *Carbohydr. Res.*, 2017, **449**, 23.
2. T. J. Painter, in *The Polysaccharides,* Vol. 2, Ed. G. O. Aspinall, New York, Academic Press, 1983, 195.
3. M. V. Sukhoveeva, A. V. Podkorytova, *Promyslovye vodorosli i travy morei Dal´nego Vostoka: Biologiya, rasprostranenie, zapasy, tekhnologiya pererabotki* [*Commercial Algae and Herbs of Far East Seas: Biology*, *Distribution*, *Stocks*, *Processing Technology*], TINRO-tsentr, Vladivostok, 2006, p. 244 (in Russian).
4. A. I. Usov, *Voprosy sovremennoi al´gologii (Spets. vypusk)* [*Issues of Modern Algology (Special Issue)*], 2017, 20 (in Russian).
5. R. V. Menshova, S. D. Anastyuk, S. P. Ermakova, N. M. Shevchenko, V. I. Isakov, T. N. Zvyagintseva, *Carbohydr. Polym.*, 2015, **132**, 118.
6. R. V. Usoltseva (Menshova), S. D. Anastyuk, N. M. Shevchenko, T. N. Zvyagintseva, S. P. Ermakova, *Carbohydr. Polym.*, 2016, **153**, 258.
7. N. G. Klochkova, V. A. Berezovskaya, *Vodorosli kamchatskogo shel´fa. Rasprostranenie, biologiya, khimicheskii sostav* [*Algae of Kamchatka Shelf: Distribution, Biology, Chemical Composition*], Dalnauka, Vladivostok, 1997, p. 155 (in Russian).
8. A. I. Usov, G. P. Smirnova, N. G. Klochkova, *Russ. Chem. Bull.*, 2005, **54**, 1282.
9. A. I. Usov, G. P. Smirnova, N. G. Klochkova, *Russ. J. Bioorg. Chem.*, 2001, **27**, 450.
10. A. I. Usov, G. P. Smirnova, A. S. Shashkov, M. I. Bilan, *Russ. J. Bioorg. Chem.*, 1998, **24**, 382.
11. H. E. Ensley, B. Tobias, H. A. Pretus, R. B. McNamee, E. L. Jones, I. W. Browder, D. L. Williams, *Carbohydr. Res.*, 1994, **258**, 307.
12. A. Mueller, J. Raptis, P. J. Rice, J. H. Kalbfleisch, R. D. Stout, H. E. Ensley, W. Browder, D. L. Williams, *Glycobiology*, 2000, **10**, 339.
13. M. I. Bilan, A. A. Grachev, A. S. Shashkov, M. Kelly, C. J. Sanderson, N. E. Nifantiev, A. I. Usov, *Carbohydr. Res.*, 2010, **345**, 2038.
14. M. I. Bilan, N. G. Klochkova, N. E. Ustyuzhanina, A. O. Chizhov, A. S. Shashkov, N. E. Nifantiev, A. I. Usov, *Russ. Chem. Bull.*, 2016, **65**, 2729.
15. M. I. Bilan, A. N. Zakharova, A. A. Grachev, A. S. Shashkov, N. E. Nifantiev, A. I. Usov, *Russ. J. Bioorg. Chem.*, 2007, **33**, 38.
16. A. I. Usov, M. I. Bilan, *Russ. Chem. Rev.*, 2009, **78**, 785.
17. H. Grasdalen, B. Larsen, O. Smidsrød, *Carbohydr. Res.*, 1981, **89**, 179.
18. A. Heyraud, C. Gey, C. Leonard, C. Rochas, S. Girond, B. Kloareg, *Carbohydr. Res.*, 1996, **289**, 11.
19. A. Haug, B. Larsen, O. Smidsrød, *Acta Chem. Scand.*, 1966, **20**, 183.
20. A. V. Skriptsova, N. M. Shevchenko, D. V. Tarbeeva, T. N. Zvyagintseva, *Mar. Biotechnol.*, 2012, **14**, 304.
21. F. A. Pettolino, C. Walsh, G. B. Fincher, A. Bacic, *Nature Protocols*, 2012, **7**, 1590.
22. K. S. Dodgson, R. G. Price, *Biochem. J.*, 1962, **84**, 106.
23. A. I. Usov, M. I. Bilan, N. G. Klochkova, *Bot. Mar.*, 1995, **38**, 43.
24. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.*, 1956, **28**, 350.

Received August 28, 2017;
in revised form October 27, 2017;
accepted November 15, 2017