POLYSACCHARIDES OF BROWN ALGAE

VIII. A STUDY OF PELVECYAN AND SARGASSAN BY METHYLATION

A. F. Pavlenko, A. V. Kurika,
V. A. Khomenko, and Yu. S. Ovodov

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It has been shown previously [1] that pelvecyan and sargassan are highly branched sulfated polysaccharides based on a linear glucuronomannan chain. In the present communication we give the results of an investigation of the structures of these polysaccharides by the methylation method. The presence of sulfate groups in the polysaccharides complicates the use of this method [2]. Consequently, the pelvecyan and sargassan were previously desulfurated with a solution of hydrogen chloride in absolute methanol [3]. In the desulfuration process, the partial splitting off of the fucose and, to a certain extent, of the xylose and a reduction in the sulfur content from 5.8 to 0.8% were observed. It is impossible to achieve complete desulfuration by this method.

We methylated the resulting desulfurated polysaccharides by Hakomori's method [4], with methyl iodide in dimethyl sulfoxide in the presence of the methylsulfinyl carbanion. On methylation, the sulfate groups are completely eliminated, and by a single treatment a partially methylated polysaccharide is obtained. The repetition of this operation, although it led to completely methylated compounds gave them in low yield because of accompanying degradation. For this reason, the further methylation was performed by Purdie's method [5] with methyl iodide in methanol in the presence of silver oxide. After two treatments by this method, permethylated pelvecyan and permethylated sargassan were obtained, and these were hydrolyzed with sulfuric acid or with a solution of hydrochloric acid in methanol. The results of an investigation of the hydrolyzates by paper, thin-layer, and gas–liquid chromatographies showed that they consisted of complex mixtures of methylated sugars or methyl glycosides.

The hydrolyzates were fractionated on columns of silica gel. For each polysaccharide we obtained five groups of methylated sugars according to the degree of methylation of the individual components. Each of them was demethylated with boron tribromide [6]. On subsequent chromatography, the initial monosaccharides were identified for each of the groups of methylated sugars obtained (Table 1).

The highest degree of methylation was characteristic for the fucose, xylose, and galactose residues which corresponded to their terminal position in the molecules of the two polysaccharides. The low degree of methylation (groups IV and V) of a number of galactose and mannose residues showed that these monosaccharides are points of branching of the carbohydrate chains of the polysaccharides.

The further separation of the groups of methylated sugars into individual components was performed by column and preparative thin-layer chromatographies. The individual compounds were compared with authentic samples chromatographically, and in a number of cases by the mass spectrometry of the corresponding aldononitrile acetates [7]. If the mixtures could not be separated preparatively, the methylated sugars were identified by the chromato-mass spectrometry of the aldononitrile acetates [7] and of the acetates of the methylated methyl glycosides [8].

By gas–liquid chromatography (of the substances in the form of acetates of methyl glycosides) in the hydrolyzates of the methylated pelvecyan and methylated sargassan we identified the following methylated sugars: 2,3,4-tri- and 3,4-di-O-methyl-D-xylose; 2,3,4-tri- and 2,4-di-O-methyl-L-fucose; 2,3,4,6-tetra-, 2,3,6-tri-, 2,3-di-, and 6-mono-O-methyl-D-galactose; 3,4,6- and 2,3,4-tri-, 3,6-di-, and 4,6-di-O-methyl-D-mannose; and 2,4-di-O-methyl-D-glucuronic acid. In addition, the pelvecyan hydrolyzate contained 3-O-

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TABLE 1. Original Monosaccharides for Various Groups of Methylated Sugars from Pelvecyan and Sargassan

Group	Degree of methylation of the components of the group	Original polysaccharides	
		pelvecyan	sargassan
I	High	Fucose, xylose, galactose	
II	Medium	Fucose, xylose, galactose, mannose	Fucose, mannose
III		Galactose, glucuronic acid, xylose (traces)	Galactose, glucuronic acid
IV	Low	Galactose, mannose	
V			

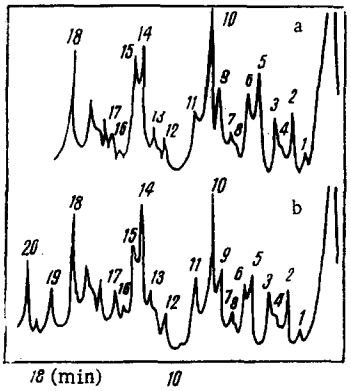

Fig. 1. Gas–liquid chromatogram of methanolyzates of permethylated pelvecyan (a) and permethylated sargassan (b) (conditions of separation given in the experimental part); derivatives: acetates of methyl glycosides; peaks: 1, 2) 2,3,4-tri-O-methyl-D-xylose; 3, 4) 2,3,4-tri-O-methyl-L-fucose; 5, 6) 3,4-di-O-methyl-D-xylose; 7, 8) 2,3,4,6-tetra-O-methyl-D-galacose; 9, 11) 2,4-di-O-methyl-L-fucose; 10) 3,4,6-tri-O-methyl-D-mannose; 12) 2,3,6-tri-O-methyl-D-galactose; 13) 2,3,4-tri-O-methyl-D-mannose; 14) 3,6-di-O-methyl-D-mannose; 15) 4,6-di-O-methyl-D-mannose; 16, 17) 2,3-di-O-methyl-D-galactose; 18) 6-mono-O-methyl-D-galactose; 19, 20) 3-mono-O-methyl-D-galactose.

methyl-D-galactose, which was absent from the sargassan hydrolyzate. The results obtained confirm the high degree of branching of the molecules of pelvecyan and sargassan (Fig. 1).

Thus, the xylose molecules are terminal and are partially linked to the carbohydrate chain by 1–2 bonds. The mannose residues are linked mainly by 1–2 and partly by 1–6 bonds, and some of the mannose residues act as points of branching of the carbohydrate chain and contain substituents in positions 3 and 4. The majority of the galactose molecules are highly substituted and are centers of branching of the carbohydrate chains. A few of these monosaccharides are located at nonreducing ends of the molecules or have substituents in position 4. The fucose residues are terminal or are connected by a 1–3 bond. In addition to the direct identification of methylated glucuronic acid by gas–liquid chromatography, the fractions containing its fragments were reduced with potassium tetrahydroborate. A hydrolyzate of the reduced fragment was found to contain 2,4,6-tri-O-methyl-D-glucose, which confirmed the presence of a 1–3 bond with the glucuronic acid residue.

Although part of the information is lost in the desulfuration of the polysaccharides, nevertheless methylation gives us a knowledge of the nature of the links between the monosaccharide residues in pelvecyan and sargassan. The great similarity of the molecules of the two polysaccharides must be noted.

EXPERIMENTAL

Preparative chromatography was performed in columns of KSK silica gel (100-150 mesh) that had been washed with hydrochloric acid and heated at 100-110°C for 24 h. The substances were eluted from the column with mixtures of chloroform and methanol (with a gradient change in the methanol content).

For thin-layer chromatography we used KSK silica gel (> 250 mesh) impregnated with a 0.25 M solution of sodium dihydrogen phosphate. The chromatograms were run in the following solvent systems (proportions by volume): 1) benzene–ethanol–water–aqueous ammonia (200:47:15:1, upper layer); 2) chloroform–methanol (10:1); 3) ethyl acetate; 4) methyl ethyl ketone saturated with 1% aqueous ammonia; 5) butan-1-ol–acetone–water (4:5:1); and 6) butan-1-ol–CH_3COOH–water (4:1:5, upper layer).

The monosaccharides were chromatographed on FN-4 paper in system 6, and the methylated sugars on Whatman No. 1 paper in system 4. The substances were detected on the chromatograms with the following reagents: aniline phthalate (10 min/105-110°C), and alkaline solution of silver nitrate, and concentrated sulfuric acid (5 min/115-120°C).

Gas–liquid chromatography was performed on a "Pye Unicam 104" chromatograph with a flame-ionization detector. The best separation of the acetates of the methylated methyl glycosides was found on

a helical glass column (6× 150 mm) using 3% of neopentyl glycol succinate on Chromosorb W (60-80 mesh) that had been washed with acid and treated with dichlorodimethylsilane. Argon was used as the carrier gas at a rate of 30 ml/min. The temperature was programmed from 125 to 223°C at the rate of 5°C/min.

The chromato-mass spectrometry was performed on a LKB-9000 chromato-mass spectrometer, as described previously [7]. The aldononitrile acetates of the monosaccharides were obtained in the usual way [9]. All the solutions were evaporated in vacuum at 40-45°C.

Desulfuration of the Polysaccharides. A mixture of 5 g of a polysaccharide and 350 ml of a 0.15 M solution of dry HCl in absolute methanol was stirred at 20°C for 48 h. The precipitate was filtered off and was treated further with the same mixture at 5°C for 48 h. The polysaccharides were separated off, dissolved in a small amount of water, dialyzed, and freeze-dried. This gave partially desulfurated pelvecyan with a yield of 3.7% (0.8% S) and partially desulfurated sargassan with a yield of 3.5 g (0.7% S).

Preparation of the Permethylated Polysaccharides. A. A solution in 100 ml of absolute dimethyl sulfoxide of 2.5 g of a partially desulfurated polysaccharide that had been dried in vacuum over phosphorus pentoxide was methylated by Hakomori's method [4]. This gave partially methylated pelvecyan (2.1 g) containing 30% of OCH_3 groups and 0.0% of sulfur, and partially methylated sargassan (2.1 g) with 33% of OCH_3 groups and 0.0% of sulfur. The IR spectrum of each compound had the absorption band of a hydroxy group.

B. A partially methylated polysaccharide (1 g) was dissolved in a mixture of 25 ml of absolute methyl iodide and 8 ml of methanol. The solution was heated to 45°C and was treated by Purdie's method [5]. After two methylations, permethylated pelvecyan was obtained with a yield of 70% and an OCH_3 content of 40.1% and permethylated sargassan was obtained with a yield of 65% and an OCH_3 content of 39.5%.

On further methylation, the amount of methoxy groups did not change. The IR spectra of the compounds obtained lacked the absorption band of a hydroxy group.

Hydrolysis of the Methylated Polysaccharides. A. A fully-methylated polysaccharide (0.05 g) was dissolved at 0°C in 0.5 ml of 72% sulfuric acid and the solution was kept in a tube at room temperature for 1 h, and then 4 ml of water was added and the tube was sealed and was heated at 100°C for 5 h. The reaction mixture was diluted with water, neutralized with $BaCO_3$, and filtered. The filtrate was treated with Amberlite IR-120 (H^+) and was examined by paper chromatography in system 4 and by thin-layer chromatography in silica gel in systems 1, 2, and 4. This syrup was separated into five groups of substances by preparative column chromatography on silica gel. The separation was monitored by thin-layer chromatography in system 1.

B. The methylated polysaccharide (0.05 g) was heated with 0.5 ml of a 1:10 mixture of 72% $HClO_4$ and methanol in a sealed tube at 100°C for 3 h. Then the reaction mixture was diluted with methanol, treated with Dowex 1 (HCO_3^-), filtered, and evaporated. The residue was chromatographed in a thin layer of silica gel in system 3. For gas–liquid chromatography, the mixture was acetylated with acetic anhydride in pyridine in the usual way (for the results of GLC, see Fig. 1).

Demethylation of the Methylated Monosaccharides. A methylated sugar (or a mixture of sugars of groups I-V) (1-3 mg) was demethylated with 0.5 ml of boron tribromide as described previously [6]. The monosaccharide (or mixture of monosaccharides) obtained was chromatographed on paper in system 6. The results of a study of groups I-V are given in Table 1.

CONCLUSIONS

It has been shown by the methylation method that pelvecyan and sargassan are polysaccharides of similar structures with a high degree of branching of the carbohydrate chains. Fucose, xylose, and galactose are located at the nonreducing ends of their molecules, and mannose and galactose form points of branching of the carbohydrate chains of these polysaccharides.

The positions of the bonds between the monosaccharide residues in pelvecyan and sargassan have been determined.

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