

# DETERMINATION OF THE POSITIONS OF THE SULFATE GROUPS IN SULFATED POLYSACCHARIDES

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Sulfated polysaccharides form an important class of biopolymers possessing various biological activities [1, 2]. In establishing the structures of sulfated polysaccharides, considerable difficulty is presented by the determination of the positions of the sulfate groups. In a preceding communication [3], we suggested a method for determining the positions of the sulfate groups in the sulfated monosaccharides which consists of the desulfation of the acetyl derivatives of the monosaccharide sulfates followed by methylation and identification of the methyl ethers formed.

In the present paper we give information on the determination of the positions of the sulfate groups by this method in the following sulfated polysaccharides: a fucan from *Pelvetia wrightii* [4], in which the positions of the sulfate groups were already reliably known, and also other sulfated polysaccharides being studied in our laboratory - sargassan from *Sargassum pallidum* [5] and pelvecyan from *Pelvetia wrightii* [6].

In the determination of the positions of the sulfate groups in sulfated polysaccharides we first performed partial acid hydrolysis [7] and acetylation of the hydrolyzate [8]. The working up of the acetylated hydrolyzate yielded a water-soluble fraction of acetylated monosaccharide sulfates. Subsequent desulfation of these compounds with thionyl chloride [3] and methylation with diazomethane [9] gave a set of acetates of monosaccharide methyl ethers which were identified by chromato-mass spectrometry. The gas-liquid chromatography of acetates of the methyl ethers from fucan, sargassan, and pelvecyan are shown in Fig. 1, a-c, respectively.

The mass spectrum of the acetate of 3-O-methylhexose (Fig. 2a) is similar to that published by Bieman [7].

Since the degree of difference between the mass spectra of fully acetylated 2- and 4-O-methyl hexoses cannot be predicted on the basis of Bieman's paper [10], the mass spectrum of the peracetylated 4-O-methylhexose (Fig. 2b) was identified by comparing it with the mass spectra of synthetic samples of the tetraacetates of 2- and 4-O-methylglucoses. The mass spectrum of the triacetate of 4-O-methylfucose was identified similarly (Fig. 3a); here series C (m/e 129, 87), B (m/e 156, 114), and A (m/e 245, 185, 143) show the presence of a methyl group in position 2 or 4. The mass spectrum of the acetate of 3-O-methylfucose (Fig. 3b) contains all the expected fragments: C (m/e 157, 115), B (m/e 184, 142, 100), and A (m/e 245, 153).

Since the spectrometric method does not make it possible to distinguish between the monosaccharides - in this case, between Gal and Man -, we additionally subjected the acetates of the methyl ethers of the hexoses to preparative gas-liquid chromatography. Their subsequent deacetylation [12], demethylation [11], and paper chromatography enabled the original monosaccharides to be identified.

Thus, the position of the sulfate group in fucan [4] at C-4 found previously was confirmed by the results obtained. By the methods described above we detected only 4-O-methylfucose in the fucan.

In sargassan we identified the following monosaccharide sulfates: Fuc 4-sulfate, Fuc 3-sulfate, Man 3-sulfate, Gal 3-sulfate, and Gal 4-sulfate; and in pelvecyan Xyl 3-sulfate, Fuc 4-sulfate, and Fuc 3-sulfate. These results are confirmed by those of periodate oxidation and IR spectroscopy performed previously [13, 14].

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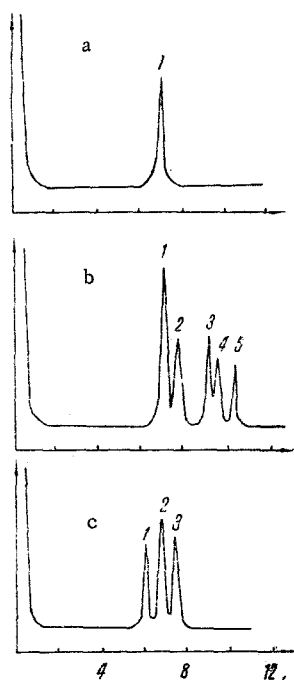


Fig. 1. Gas-liquid chromatography of the acetates of the methyl ethers of fucan, sargassan, and pelvecyan: a: 1) 4-O-methyl-6-deoxyhexose (Fuc); b: 1) 4-O-methyl-6-deoxyhexose (Fuc); 2) 3-O-methyl-6-deoxyhexose (Fuc); 3) 3-O-methylhexose (Man); 4) 3-O-methylhexose (Gal); 5) 4-O-methylhexose (Gal); c: 1) 3-O-methylpentose (Xyl); 2) 4-O-methyl-6-deoxyhexose (Fuc); 3) 3-O-methyl-6-deoxyhexose (Fuc).

The results obtained agree well with what was known previously, which shows the applicability of the proposed approach to the determination of the positions of the sulfate groups in sulfated polysaccharides. A defect of the method is that during acid hydrolysis partial desulfation may take place, leading to some loss of information.

#### EXPERIMENTAL

Thin-layer chromatography was performed in a fixed layer of silica gel. Partition chromatography was performed on FN-4 paper. For chromatography we used the following solvent systems (ratios by volume): benzene-ethyl acetate (95:5); chloroform-methanol-acetic acid (6:1:1); chloroform-acetone (98:2); and butanol-acetone-water (4:5:1). The spots were detected with concentrated  $\text{H}_2\text{SO}_4$  at  $115^\circ\text{C}$  for 10 min and with a solution of aniline hydrogen phthalate at  $105-110^\circ\text{C}$ . The GLC of the monomethyl ethers of the monosaccharide tetraacetates was performed on a Pye-Unicam-104 chromatograph in spiral glass columns  $150 \times 0.6$  cm using the following stationary phases and conditions of separation: A) 3% of QF-1 on Gas-Chrom, 100-120 mesh,  $170 \rightarrow 215^\circ\text{C}$ ,  $\Delta = 3$  deg/min; and B) 3% of neopentyl glycol succinate on Chromosorb W, 60-80 mesh,  $170 \rightarrow 220^\circ\text{C}$ ,  $\Delta = 3$  deg/min. Preparative gas-liquid chromatography was performed on a Pye-Unicam-104 chromatograph with a spiral glass column  $300 \times 0.8$  cm using the following conditions of separation: 3% QF-1 on Gas-Chrom, 100-120 mesh,  $170 \rightarrow 225^\circ\text{C}$ ,  $\Delta = 2$  deg/min.

**Acid Hydrolysis.** The polysaccharide under investigation (2.5 g) was treated with 37 ml of water and then 37 ml of 2 N  $\text{H}_2\text{SO}_4$  was added. The resulting solution of the polysaccharide was heated at  $95^\circ\text{C}$  for 30 min. The precipitate formed was separated off by filtration, and the filtrate was neutralized with a saturated solution of  $\text{Ba}(\text{OH})_2$ . The reaction mixture obtained was centrifuged, the supernatant was poured into a fourfold excess by volume of ethanol, and the mixture was left overnight and the supernatant was evaporated at  $40^\circ\text{C}$ . The resi-

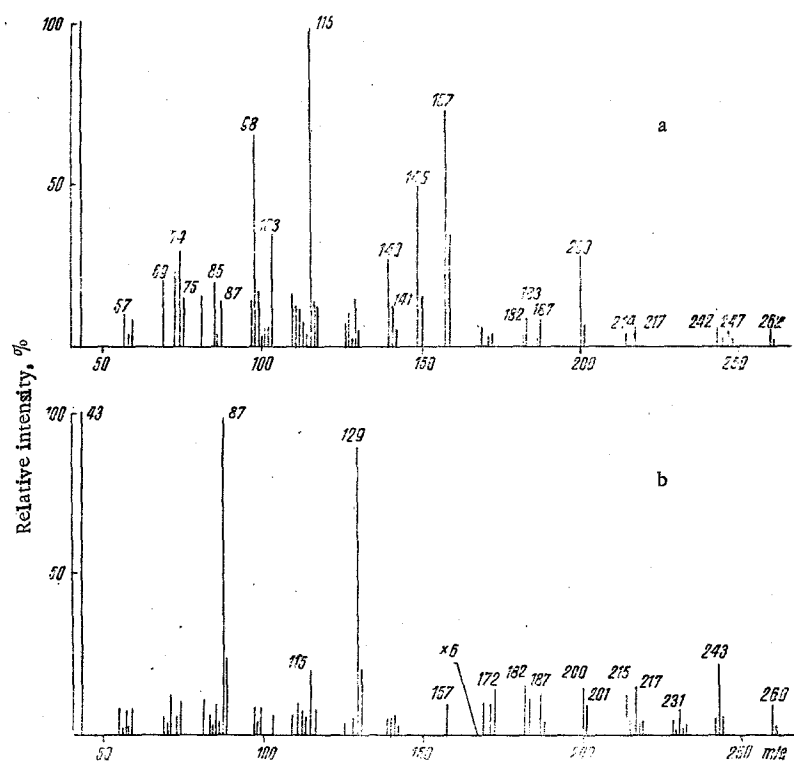


Fig. 2

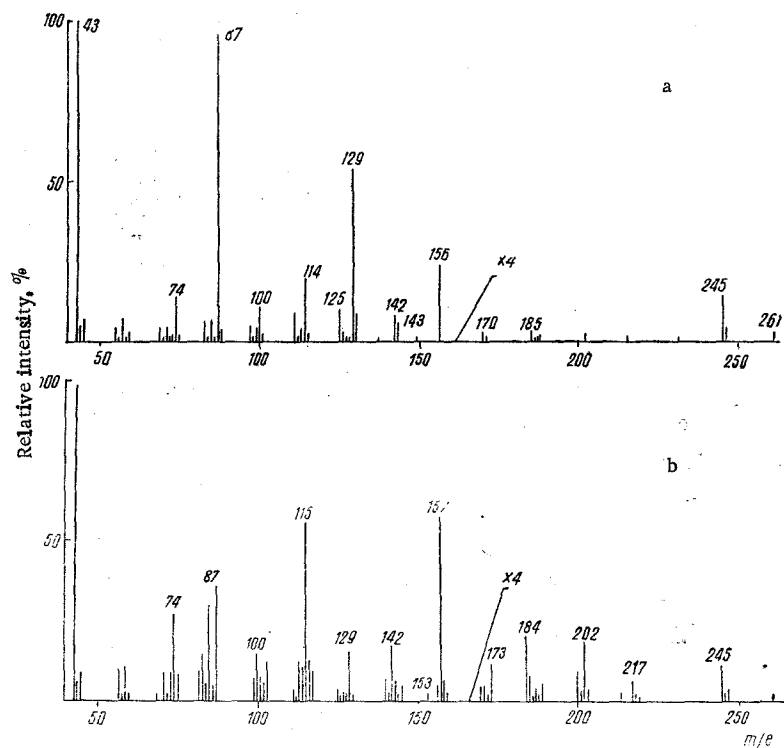


Fig. 3

due was dried over  $P_2O_5$ . A mixture of monosaccharides and their sulfates was obtained in a yield of 1.8 g.

Acetylation of the Hydrolyzate. A mixture of 6 ml of acetic anhydride and 7.5 ml of absolute pyridine was cooled to  $0^\circ C$ , and 1.8 g of the polysaccharide of hydrolyzate was added. The solution was kept at room temperature for 18 h and was then poured into a 50-ml funnel 1/3-filled with ice. The aqueous solution was extracted with chloroform ( $4 \times 6$  ml), and the chloroform solution was evaporated to dryness. This gave a mixture of acetylated monosaccharide sulfates in a yield of 0.75 g.

Desulfation with Thionyl Chloride. A solution of 0.7 g of the mixture of acetylated monosaccharide sulfates in 6 ml of freshly distilled  $SOCl_2$  was kept at room temperature for 6 h and was then evaporated in vacuum. The residue was dissolved in  $CHCl_3$ , and the solution was washed with 5%  $NaHCO_3$  solution and with water and was dried over calcined  $Na_2SO_4$ . The chloroform was evaporated and the residue was dried over  $P_2O_5$ . Yield 0.35 g.

Methylation. A solution of 0.2 g of the mixture of partially methylated monosaccharides in 10 ml of absolute methylene chloride was treated with 0.01 ml of  $BF_3$ . The solution was cooled to  $-5^\circ C$  and a solution of diazomethane in methylene chloride was added until a permanent yellow coloration has been formed. After 30 min, the precipitate of polymethylene was filtered off, the filtrate was washed with 10%  $NaHCO_3$  solution and then with water, and was dried over  $MgSO_4$ , and the methylene chloride was separated off in vacuum. Yield 0.15 g.

The acetates of the methyl ethers obtained were identified by chromato-mass spectrometry.

#### SUMMARY

1. The possibility has been shown of using a method proposed by us previously to determine the positions of the sulfate groups in sulfated polysaccharides.
2. The positions of the sulfate groups in the following sulfated polysaccharides have been determined: fucan and pelvecyan from Pelvetia wrightii and sargassan from Sargassum pallidum.

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