Polysaccharide sulfates
I. Conversion of sodium sulfates of starch, carboxymethylstarch, and (4-*O*-methyl-D-glucurono)-D-xylan into hydrogen sulfates

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Received 2 August 1989

Sodium sulfates of starch, carboxymethylstarch, and (4-*O*-methyl-D-glucurono)-D-xylan were prepared using the sulfur trioxide—pyridine complex or chlorosulfonic acid. Conversion of derivatives prepared into the corresponding hydrogen sulfates was carried out with hydrochloric acid in isopropyl alcohol in the presence of a little amount of water. Approximately 20 and 3 % decrease in the sulfur content was observed with derivatives of starch and (4-*O*-methyl-D-glucurono)-D-xylan, respectively, on conversion under the given conditions; the latter revealed a substantially lower elimination of the sulfate group. Determination of acid groups by differential potentiometric method (titration with sodium methoxide in dimethyl sulfoxide) afforded a little lower values than those of methods employed so far.

Polysaccharide sulfates are a noticeable group of derivatives utilizable in various branches; derivatives of starch and (4-*O*-methyl-D-glucurono)-D-xylan are valuable especially in pharmacology. Investigated were physiological effects of starch sulfates and their components aiming to prepare a substitute for the natural anticoagulant heparin [1—3]. (4-*O*-Methyl-D-glucurono)-D-xylan sulfates were prepared to examine the relationship between their structure and antithrombotic or antilipemic activities [4].

Sulfatation of the above-mentioned polysaccharides with sulfuric acid is unfavourable because of considerable degradation of the substrate especially of starch [5]. More advantageous is the treatment with chlorosulfonic acid in a suitable solvent, as *e.g.* in pyridine [6], or in the presence of *N*,*N*-dimethylformamide [7]. Polysaccharides can also be sulfatized with complexes of sulfur trioxide in a suited solvent, as *e.g.* pyridine, triethylamine, *N*,*N*-dimethylformamide or dioxane [8].

Polysaccharide sulfates are known to be stable in the form of their sodium salts. Their conversion into hydrogen sulfates is associated with some problems. Thus, *Whistler* and *Spencer* [5] reported that some hydrogen sulfates of polysaccharides undergo an easy autohydrolysis due to a high dissociation of the functional group. According to Japanese authors [9] the dissociation degree of the hydrogen sulfate group of cellulose hydrogen sulfate derivative equals 1

irrespectively of the pH of the medium in the actual region; on the other hand, the dissociation degree of the carboxyl group in the afore-mentioned type of derivatives is pH dependent.

This paper concerns the examination of sodium polysaccharide sulfates conversion (of starch, carboxymethylstarch, and (4-*O*-methyl-D-glucurono)-D--xylan) to hydrogen sulfates. The possibility to determine the acid groups by differential potentiometric titration in a nonaqueous medium was verified.

Experimental

Potato starch and carboxymethylstarch ($w(COCH_3) = 8.65\,\%$) are technical products (České škrobárny, Havlíčkův Brod), (4-*O*-methyl-D-glucurono)-D-xylan ($\bar{M}_n = 18\,000$, w(4-*O*-methyl-D-glucuronic acid) = 17.30 %) was isolated from beech wood according to [10]. The low-molecular xylan ($\bar{M}_n = 8000$, w(4-*O*-methyl-D-glucuronic acid) = 17.20 %) was prepared from the above-mentioned product according to [11]. Solution of 0.1 M sodium methoxide was obtained according to [12] in the mixture methanol—dimethyl sulfoxide ($\varphi_r = 1:20$). 4 M-HCl was prepared by mixing concentrated hydrochloric acid (37 %) and isopropyl alcohol in the appropriate ratio. The sulfur trioxide—pyridine complex is a commercial product (Merck, FRG).

Determination of sulfur after mineralization of samples was done gravimetrically through barium sulfate according to [13]. The content of uronic acids was determined by the carbazole method according to [14]. All samples were dried under reduced pressure prior to determination at 60 °C for 5 h. Digital pH-meter OP-208/1 (Radelkis, Budapest) equipped with antimony electrode of a usual type and a calomel electrode in which the saturated aqueous KCl solution was replaced by a saturated methanolic KCl solution was employed for potentiometric titrations.

Starch and carboxymethylstarch sulfates

Suspension of the respective polysaccharide (4 g) in *N*,*N*-dimethylformamide (50 cm^3) was added to the sulfur trioxide—pyridine complex (8 g) in *N*,*N*-dimethylformamide (100 cm^3) at room temperature at which the mixture was shaken for 16 h. Aqueous solution of sodium hydrogencarbonate (50 cm^3; 10 %) was added to the product; methanol (100 cm^3) was introduced into the stirred suspension, the product was filtered off and dried. Yield = 4.10 g and 4.05 g of starch and carboxymethylstarch sulfates, respectively.

(4-O-Methyl-D-glucurono)-D-xylan sulfates

To (4-*O*-methyl-D-glucurono)-D-xylan (6 g) dissolved in *N*,*N*-dimethylformamide (200 cm^3) a mixture of pyridine (32.5 cm^3) and chlorosulfonic acid (8.1 cm^3) was dropwise

added with stirring at 0 °C. The mixture was then heated to 75 °C and stirred for 4.5 h, cooled and poured into ethanol (2.2 dm^3). The precipitate was filtered off, washed three times with ethanol (100 cm^3 each) and dissolved in distilled water (500 cm^3). The pH of the solution was adjusted with 1 M-NaOH to 8.8, the solution was concentrated to *ca.* 120 cm^3 and the product was precipitated with acetone—ethanol ($\varphi_r = 3:1$; 2.5 dm^3). The lightbrown precipitate was filtered off, dissolved in distilled water (100 cm^3) and the solution was left to dialyze against distilled water for 72 h. Yield = 6.5 g, $\bar{M}_n = 11\,500$.

For sulfatation of the low-molecular xylan the same procedure was applied. The yield from 0.60 g of the original material was 0.62 g, $\bar{M}_n = 4900$.

Conversion of sodium sulfates of polysaccharides to the corresponding hydrogen sulfates

A solution of 4 M hydrochloric acid in isopropyl alcohol (10 cm^3) was added to a stirred suspension of sodium polysaccharide sulfate (1 g) in isopropyl alcohol (50 cm^3). After 5 min stirring in a stoppered flask the suspension was filtered off and washed with methanol till the absence of chloride ions. The product was dried and the content of hydrogen sulfate and carboxyl groups was titrimetrically determined immediately.

Determination of acid groups in hydrogen sulfates by differential titration

The sample (50 mg) was dissolved in dimethyl sulfoxide (20 cm^3) in a stoppered flask. The air was removed by washing with gaseous nitrogen for 10 min and the content was determined by titration with 0.1 M sodium methoxide added in 0.1 cm^3 portions by means of potentiometric curve reading.

Results and discussion

Starch and carboxymethylstarch were sulfatized by treatment with the sulfur trioxide—pyridine complex. The required substitution degree corresponding to 4.0—6.5 mass % of sulfur was achieved in *N*,*N*-dimethylformamide during 16 h at room temperature. The substitution degree was lower when applying pyridine as a solvent.

(4-*O*-Methyl-D-glucurono)-D-xylan prepared according to [10] was depolymerized according to [11], because relative molecular mass of the product required for sulfatation should not exceed 10 000. The so-called low-molecular xylan thus obtained was reacted under the same conditions.

Conversion of sodium polysaccharide sulfates to the corresponding hydrogen sulfates is accompanied with autohydrolysis [5]. Also the hydrogen sulfate

Table 1

Determination of sulfur and uronic acid in sulfated polysaccharides

Parent polysaccharide	w(S)/%			w(uronic acid)/%		
	Sulfate	Hydrogen sulfate form		Sulfate	Hydrogen sulfate form	
	via $BaSO_4$	*via* $BaSO_4$	MeONa	Carbazole	Carbazole	MeONa
Potato starch	6.50	4.82	4.75	—	—	—
Carboxymethylstarch	4.30	3.22	3.03	—	—	—
(4-*O*-Methyl-D-glucurono)-D-xylan	14.20	13.80	13.28	15.60	15.10	14.50
Low-molecular xylan	16.10	15.60	15.10	10.50	10.05	9.90

group is very reactive and therefore we investigated several conversion procedures. The most favourable agent was found to be hydrochloric acid in isopropyl alcohol.

The products were characterized by routine methods employed for sulfur and uronic acid determinations. At the same time we tried to differentiate and determine the acid groups by potentiometric titration with sodium methoxide in *N*,*N*-dimethylformamide. The results are presented in Table 1.

It has been found that conversion of sodium polysaccharide sulfates (values for the starting sulfates in Table 1 are reduced to H^+ form to be comparable) into hydrogen sulfates was accompanied by a decrease in the sulfur content in starch and carboxymethylstarch by *ca.* 20 %, which means a considerable elimination of the functional group; on the other hand, with (4-*O*-methyl-D-glucurono)-D-xylan derivatives only a 3 % decrease of the sulfur content occurred. Some analogy might be seen with the alkaline desulfatation of some polysaccharide derivatives, where elimination of sulfate groups proceeds more easily when the saccharide unit of the polysaccharide bore a free hydroxyl group at C-6 [5]; this is, however, not the case with xylan derivatives.

Routine method was employed for titrimetric determination of hydrogen sulfate samples with sodium methoxide in dimethyl sulfoxide. (Evaluation of potentiometric curves will be presented separately.) With starch derivatives the hydrogen sulfate group was estimated for 10 parallel titrations. The calculated standard deviation *s* and variation coefficient were 0.326 and 7.43 %, respectively. Comparison of results obtained by gravimetric and titration methods of sulfur determination showed a little lower values with the second one.

Content of uronic acid was examined in both xylan types of compounds; as found no considerable decarboxylation occurred under the given conditions. Values obtained by titration are again but unsignificantly lower than those of carbazole method.

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Translated by Z. Votický