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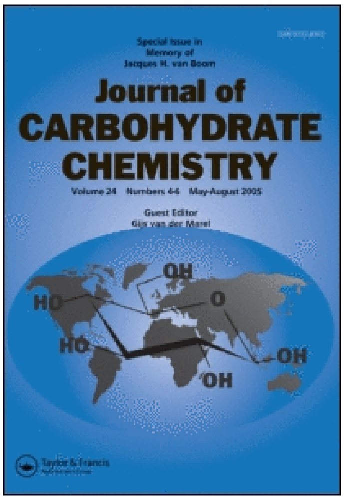

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# SULFATION OF POLYSACCHARIDES USING MONOMETHYL SULFATE

Ryo Takano, Tomomi Nagai, Xiaofen Wu, Xin-Yan Xu,
Nguyen Tien Huy, Kaeko Kamei and Saburo Hara*

Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of
Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, JAPAN

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## ABSTRACT

The polysaccharides, curdlan, starch and dextran were sulfated when heated in DMSO with sodium methyl sulfate and a catalytic amount of $H_2SO_4$ or with pyridinium methyl sulfate. Use of diminished pressure and anhydrous $CaSO_4$ as a desiccant improved the degree of sulfation and recovery. Under conditions using sodium methyl sulfate, $H_2SO_4$ and $CaSO_4$ *in vacuo,* sulfation at O-6 was predominant in the cases of curdlan and starch, while sulfation at O-2 and O-3 was preferential in the case of dextran.

## INTRODUCTION

In relation to the biological activities and the physical properties of a variety of sulfated polysaccharides,[1,2] several methods have been developed for their preparation.[3,4] Many of these methods, however, are inconvenient due to the high reactivity or toxicity of sulfating reagents such as chlorosulfuric acid, $SO_3$, $SO_3$-amine complexes and piperidine-*N*-sulfate. Esters of non-volatile alcohols are synthesized from corresponding esters of volatile alcohols, such as MeOH, using transesterification procedures. On the other hand, polysaccharides containing sulfate ester groups yield monomethyl sulfate when treated with methanolic HCl.[5] We here describe a method for sulfation of polysaccharides based on transesterification of the sulfate group from the

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monomethyl ester of sulfuric acid, a relatively mild sulfating reagent when compared with the other reagents.

## RESULTS AND DISCUSSION

As expected, no reaction occurred when starch, curdlan or dextran was simply heated with a large excess of sodium methyl sulfate in DMSO (data not shown). We then attempted sulfation under various conditions using sodium methyl sulfate as the reagent, curdlan as the polysaccharide and a small amount of $H_2SO_4$ as the catalyst (Table 1). Although an elevated reaction temperature (100 °C) and prolonged reaction time (6 h) improved the degree of sulfation in comparison with the reaction at 80 °C for 3 h, a significant reduction in the yield occurred (Nos. 1—3). The polysaccharide was sulfated only in low degree of sulfation and with low recovery, based on the weight of the starting polysaccharide, even under more favorable conditions (No. 1). The low recovery is possibly due to hydrolysis of the polysaccharide chain caused by residual moisture. In addition, MeOH, which is formed as a co-product in the following equilibrium or as a by-product due to hydrolysis of sodium methyl sulfate, may interfere with the sulfation reaction, by solvolyzing the polysaccharide sulftate.

$$\text{R-OH} + \text{Me-OSO}_3^- \text{X}^+ \rightleftharpoons \text{R-OSO}_3^- \text{X}^+ + \text{MeOH}$$

Removal of the moisture and MeOH from the system is thus expected to reduce the depolymerization and to improve the degree of sulfation. Methanol can be removed *in vacuo*. For removal of the moisture, anhydrous $CaSO_4$ may be suitable, since it is employed as a desiccant for methanolysis of polysaccharides in MeOH containing water and $H_2SO_4$.[6] We thus tried sulfation reactions *in vacuo* with and without anhydrous $CaSO_4$. As expected, a much higher degree of sulfation was achieved in the case of the reaction *in vacuo* (No. 6), and use of the desiccant improved the recovery (No. 7). Under the conditions identical to No.7, starch and dextran were also successfully sulfated (1.10 and 1.06 mole $\text{-SO}_4^-$ per Glc residue in 152% and 146% yield, respectively, based on weight of starting material).

Pyridinium salts of sulfated polysaccharides are solvolytically desulfated when heated in aprotic solvents such as DMSO, dioxane[7] and DMSO containing MeOH.[8] As MeOH acts as an acceptor of $SO_3$ liberated by the solvolytic desulfation,[9] polysaccharides containing free -OH groups may also act as acceptors when mixed with the pyridinium salt of sulfated alcohol. As expected, when curdlan was heated in DMSO with an

**Table 1. Sulfation of curdlan with sodium methyl sulfate**

| No. | Temperature (°C) | Time (h) | Reagent mole/-OH | Desic-cant | Evacua-tion | Degree of sulfation (mole/Glc residue) | Yield (%)* |
|---|---|---|---|---|---|---|---|
| 1 | 80 | 3 | 8 | - | - | 0.11 | 26 |
| 2 | 100 | 3 | 8 | - | - | 0.51 | 6 |
| 3 | 80 | 6 | 8 | - | - | 0.18 | 6 |
| 4 | 80 | 3 | 4 | - | - | 0.06 | 26 |
| 5 | 80 | 3 | 6 | - | - | 0.07 | 30 |
| 6 | 80 | 3 | 8 | - | + | 1.05 | 28 |
| 7 | 80 | 3 | 8 | + | + | 1.17 | 128 |

*Based on weight of starting material

**Table 2. Sulfation of curdlan with pyridinium methyl sulfate**

| No. | Temperature (°C) | Time (h) | Reagent mole/-OH | Desic-cant | Evacua-tion | Degree of sulfation (mole/Glc residue) | Yield (%)* |
|---|---|---|---|---|---|---|---|
| 1 | 60 | 3 | 8 | - | - | 0.15 | 104 |
| 2 | 80 | 3 | 8 | - | - | 0.50 | 128 |
| 3 | 100 | 3 | 8 | - | - | 0.56 | 90 |
| 4 | 80 | 1 | 8 | - | - | 0.38 | 128 |
| 5 | 80 | 6 | 8 | - | - | 0.51 | 130 |
| 6 | 80 | 3 | 4 | - | - | 0.42 | 124 |
| 7 | 80 | 3 | 12 | - | - | 0.47 | 130 |
| 8 | 80 | 3 | 8 | - | + | 1.04 | 120 |
| 9 | 80 | 3 | 8 | + | + | 1.13 | 148 |

*Based on weight of starting material

excess of pyridinium methyl sulfate even without desiccant and evacuation, the polysaccharide was significantly sulfated with fairly good recovery (Table 2) in comparison with the reaction using sodium methyl sulfate. The reaction conditions of Nos. 2 and 5 appeared to be suitable for sulfation of curdlan among the reactions tested (Nos. 1—7). As in the case of the reaction with sodium methyl sulfate, diminished pressure and use of anhydrous $CaSO_4$ were effective for improving degree of sulfation and recovery (Nos. 8 and 9). When starch and dextran were sulfated under the conditions identical to experiment No. 9, 1.50 and 1.67 mole of -$SO_4^-$ per Glc residue were respectively introduced in yields of 162% and 164% (yields are based on weight of starting material).

Location of the sulfate groups on the above polysaccharides after reaction with sodium methyl sulfate and the desiccant *in vacuo* was further investigated with the aid of methylation (Table 3). Since 2,4-di-*O*-methylglucose was the predominant product from acid hydrolyzed, methylated and sulfated curdlan, sulfation is most likely to have oc-

**Table 3. Methylation analyses of polysaccharides sulfated with sodium methyl sulfate and desiccant under diminished pressure**

| Product | Linkage | Mole % | | |
|---|---|---|---|---|
| | | Curdlan | Starch | Dextran |
| 2,3,4-$Me_3$-Glc[a] | 6 | | | 12.3 |
| 2,3,6-$Me_3$-Glc | 4 | | 8.3 | |
| 2,4,6-$Me_3$-Glc | 3 | 4.5 | | |
| 2,3-$Me_2$-Glc | 4,6 | | 44.7 | |
| 2,4-$Me_2$-Glc | 3,6 | 86.0 | | 25.6 |
| 2,6-$Me_2$-Glc | 3,4 | | 4.3 | |
| 3,4-$Me_2$-Glc | 2,6 | | | 36.9 |
| 3,6-$Me_2$-Glc | 2,4 | | 5.3 | |
| 2-Me-Glc | 3,4,6 | 9.5 | 15.3 | |
| 3-Me-Glc | 2,4,6 | | 22.1 | 9.6 |
| 4-Me-Glc | 2,3,6 | | | 15.6 |

[a] Analyzed as *O*-methylated-*O*-acetylated glucose products.

curred predominantly at O-6 possibly due to steric allowance in comparison with the other -OH groups. Regardless of a small amount of 1,6-branching in the amylopectin moiety, starch, like curdlan, is also likely to have been sulfated predominantly at O-6, because 2,3-di-*O*-methylglucose is observed as the main methylated product. In the case of dextran, in which free O-6 is not available, sulfation at O-2 and O-3 appears to be dominant, yielding 3,4-di-*O*-methylglucose and 2,4-di-*O*-methylglucose as the final methylation products.

In summary, the polysaccharide sulfation method described here, especially using sodium methyl sulfate may be superior in safety and convenience to more conventional methods employed, and may be suitable for sulfation in both laboratory scale and industrial scale. More effective means for removing moisture and MeOH would overcome the defects of this method.

## EXPERIMENTAL

**Materials and general methods.** Soluble starch and dextran T-500 were purchased from Nacalai Tesque Co. and Pharmacia Fine Chemicals, respectively. Anhydrous $CaSO_4$ (Drierite) was a product of W. A. Hammond Drierite Co. Curdlan and other reagents were obtained from Wako Pure Chemical Co. Colorimetric determination of Glc was carried out by the anthrone test.[10] Sulfate content was determined by ion chromatography using IC-Anion-PWXL (Tosoh Co.) after hydrolysis of the polysaccharides with 1 N HCl at 100 °C for 5 h. Gas-liquid chromatography was carried out using a gas chromatograph, GC-7A (Shimadzu Corp.) equipped with an FID. The capillary columns employed were CP-Sil-88 (0.25 mm × 30 m, Chrompack Co.) operated at 205 °C and a PEG-20M bonded column (0.25 mm × 30 m, GL Science Co.) operated at 200 °C. Nitrogen was used as the carrier gas; flow rate, 2 mL/min; split ratio, 20:1. Combined GLC-MS was performed using a GC/MS QP-1000 (Shimadzu Corp.), under the conditions identical to GLC above except for use of He as the carrier gas.

**Sodium methyl sulfate.** Methanol (120 mL) and $H_2SO_4$ (60 mL) were slowly mixed in an ice bath and allowed to stand overnight at room temperature. The mixture was dissolved in water (1.5 L), immediately neutralized with $BaCO_3$, and the resulting $BaSO_4$ and the excess $BaCO_3$ were removed by centrifugation and filtration. To the solution of barium methyl sulfate was added 1 M $Na_2SO_4$ until no more $BaSO_4$ precipitated. After removal of $BaSO_4$ by centrifugation and filtration, the solution was concentrated to dryness and the solid was further dried *in vacuo* at 60 °C with $P_4O_{10}$ to give sodium methyl sulfate as a white powder (112 g).

**Pyridinium methyl sulfate.** Methanol (175 mL) and $H_2SO_4$ (95 mL) were slowly mixed in an ice bath and allowed to stand overnight at room temperature. The mixture was neutralized with pyridine and kept overnight at 4 °C. The resulting crystals were collected by filtration, washed with pyridine and dried *in vacuo* at 60 °C with $P_4O_{10}$ to give pyridinium methyl sulfate (193 g).

**Sulfation of polysaccharides with sodium methyl sulfate.** Polysaccharide (0.5 g) was dissolved in DMSO (15 mL). To the solution were added $H_2SO_4$ (10 mg) and sodium methyl sulfate, and the mixture was heated under the conditions described in Table 1 (Nos. 1—5). After crushed ice (15 g) was added, the reaction mixture was immediately neutralized with NaOH. Then the solution was dialyzed and lyophilized to afford sulfated polysaccharide.

**Sulfation of polysaccharides with pyridinium methyl sulfate.** Polysaccharide (0.5 g) was dissolved in DMSO (15 mL). To the solution was added pyridinium methyl sulfate, and the mixture was heated under the conditions described in Table 2 (Nos. 1—7). After crushed ice (15 g) was added, the reaction mixture was neutralized with NaOH. Then the solution was dialyzed and lyophilized to afford sulfated polysaccharide.

**Sulfation in the presence of anhydrous calcium sulfate under diminished pressure.** A solution of polysaccharide (0.5 g) in DMSO (15 mL) was mixed with anhydrous $CaSO_4$ (1 g) and stirred overnight at room temperature. To the mixture were added sodium methyl sulfate (9.92 g) and $H_2SO_4$ (10 mg), or pyridinium methyl sulfate (14.16 g). The mixture was stirred at 80 °C for 3 h *in vacuo* (14 torr). In another experiment, a solution of the polysaccharide and the reagent was simply heated *in vacuo*. The reaction mixture was then treated similarly as described in the previous sections.

**Methylation analyses.** Before methylation, the sulfated polysaccharide was converted into its triethylammonium salt according to the recommendation by Stevenson and Furneaux[11] in order to improve solubility. Methylation was carried out by the methods of Isogai *et al.*[12] and Ciucanu and Kerek[13] using powdered NaOH and $CH_3I$ in DMSO. The methylated polysaccharide was hydrolyzed with 1 N $H_2SO_4$ for 16 h at 100 °C and the hydrolyzate was analyzed as a mixture of partially methylated alditol acetates[14] using GLC and GLC-MS.

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