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Note

An improved method for the preparation of crystalline sodium salts of 2-deoxy-2-sulfoamino-D-glucose and methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside

YUKO INOUE AND KINZO NAGASAWA*

School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane 5-chome, Minato-ku, Tokyo 108 (Japan)

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The presence of a 2-deoxy-2-sulfoamino-D-glucose component in the polysaccharide structure of heparin and heparan sulfate differentiates these polysaccharides from other sulfated or nonsulfated glycosaminoglycuronans[1]. In order to study the biological significance of the sulfoamino group in heparin, 2-deoxy-2-sulfoamino-D-glucose and methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside were synthesized through different routes[2–7], and their chemical and physical properties were reported[3–5,7–9]. The present paper describes a simplified and improved method for the preparation of the crystalline sodium salts of these two sulfoamino derivatives in an excellent yield.

For the preparation of 2-deoxy-2-sulfoamino-D-glucose and its methyl glycosides, two different methods have been used. One, reported by Wolfrom *et al.*[2], Foster *et al.*[3], and Onodera and Komano[4], is based on sulfation of suitably blocked *O*-acetyl derivatives, followed by removal of the protecting groups. Such method obviously favors the formation of *N*-sulfate derivatives. It necessitates, however, a number of chemical manipulations for preparative procedures. Furthermore, *N*-sulfation and final de-*O*-acetylation are inevitably accompanied with $O \rightarrow N$ acetyl migration[3]. On the other hand, in the second method, sulfation of the free amino groups of unprotected 2-amino-2-deoxy-D-glucose under the conditions of Warner and Coleman[10] resulted in the formation of crystalline potassium[5,6] and sodium[7] salts of 2-deoxy-2-sulfoamino-D-glucose.

We have reinvestigated the conditions of the second method[7], and found that the formation of alkali-isomerized by-products derived from 2-amino-2-deoxy-D-glucose was the cause of difficulty in crystallizing the sodium salt. The formation of by-products could be avoided, while maintaining the efficiency of *N*-sulfation, by adjusting the pH of the reaction medium to 8.5–9.0. The reaction at 50° was faster

*To whom correspondence should be addressed.

TABLE I

EFFECT OF THE PROPORTION OF PYRIDINE–SULFUR TRIOXIDE ON THE YIELD OF SODIUM SALT OF 2-DEOXY-2-SULFOAMINO-D-GLUCOSE

Molar ratio of pyridine–SO_3 to 2-amino-2-deoxy-D-glucose	*Yield of Na salt of 2-deoxy-2-sulfoamino-D-glucose*	
	Ethanol-precipitated product (%)	*Crystalline product (%)*
1.5	84	68
2.0	89	59
3.0	89	67

than that at room temperature, but a tendency for the formation of by-products was observed. The reactivity of the pyridine–sulfur trioxide reagent was higher than that of the trimethylamine–sulfur trioxide reagent[6,11] at pH 8.5–9.0, but some increase of coloration during the reaction took place. The effect of the molar ratio of the reactants was examined (see Table I). The smaller the amount of pyridine–sulfur trioxide used, the larger was the amount of unreacted starting material. When 3 molar equivalents of pyridine–sulfur trioxide was used, the reaction mixture became significantly colored, and a small amount of over-sulfated by-products was formed. From these results, a proportion of 1.5 molar equivalent of the reagent was selected.

For crystallization of the sodium salt of 2-deoxy-2-sulfoamino-D-glucose, it was essential to minimize the presence of contaminants. After removal of inorganic sulfate and chloride ions by successive treatment with barium acetate and silver carbonate, all the basic substances and cations were removed by passing through a column of Dowex 50-W (H^+) cation-exchange resin. 2-Deoxy-2-sulfoamino-D-glucose was stable enough to be handled at room temperature under such an acidic condition resulting from contact with Dowex 50-W (H^+) cation-exchange resin. Repeated precipitation with ethanol freed the final product of coexistent sodium acetate, which seriously interferes with crystallization of the product. Under these revised conditions, the crystalline sodium salt of 2-deoxy-2-sulfoamino-D-glucose was prepared in an excellent yield.

These conditions were successfully applied to the preparation of the crystalline sodium salt of methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside (see the Experimental section), excluding the precipitation with ethanol.

EXPERIMENTAL

Materials. — All the reagents used were of special reagent grade. Methyl 2-amino-2-deoxy-α-D-glucopyranoside was prepared by the procedure previously reported[12] in 32% yield, based on 2-acetamido-2-deoxy-D-glucose.

Analytical methods. — The bound sulfate and *N*-sulfate were determined by the methods previously reported[13,14].

Preparation of 2-deoxy-2-sulfoamino-D-glucose, sodium salt. — 2-Amino-2-deoxy-D-glucose hydrochloride (2 g) was suspended in water (5 ml), and the pH of the solution was adjusted to 9.0 by the addition of 5% sodium hydroxide (7.4 ml). Pyridine–sulfur trioxide (2.2 g, 1.5 mol. equiv.) was added to the well-stirred solution over a period of 1 h at room temperature (27°). During this addition, the pH was maintained at 8.5–9.0 by the addition of 5% sodium hydroxide (14.6 ml). After being stirred for an additional 1 h, the solution was adjusted to pH 6.6 by the addition of 5% hydrochloric acid (0.8 ml), and 10% barium acetate (11.5 ml) was added. Precipitated barium sulfate was centrifuged off, the supernatant solution was concentrated to ~10 ml, and passed through a column of Dowex 50-WX8 (H^+) cation-exchange resin. Powdery silver carbonate was added to the eluate, the precipitated silver chloride and excess silver carbonate were centrifuged off, and the supernatant solution was again passed through a column of Dowex 50-WX8 (H^+) cation-exchange resin. The eluate was immediately neutralized to pH 6.8 by the addition of 5% sodium hydroxide (12.5 ml) and concentrated to ~5 ml. The solution was treated with activated charcoal for 5 min at 50°, and ethanol (150 ml) was added to the solution. The precipitate was filtered off, redissolved, and once more precipitated. The precipitate was washed successively with ethanol (2 × 50 ml) and ether (50 ml), and dried (phosphorus pentaoxide) at room temperature under reduced pressure. The product, which was a chromatographically homogeneous, white powder (2.18 g, 83.6%), was crystallized from 3:1 (v/v) ethanol–water to give colorless crystals (1.77 g, 67.9%), m.p. 141–143° (darkening at ~170°)*, $[\alpha]_D^{25}$ +58.3° (*c* 2.013, water, equilibrium after 20 h). The analytical sample was dried for 10 h at 80° under reduced pressure in the presence of phosphorus pentaoxide.

Anal. Calc. for $C_6H_{12}NNaO_8S$: C, 25.62; H, 4.30; N, 4.98; S, 11.40. Found: C, 25.58; H, 4.49; N, 4.91; S (bound sulfate), 11.38; S (*N*-sulfate), 11.27.

Methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside, sodium salt monohydrate. — Methyl 2-amino-2-deoxy-α-D-glucopyranoside (100 mg) was dissolved in water (2.5 ml), and the pH of the solution was adjusted to 9.0 by the addition of 5% sodium hydroxide. Pyridine–sulfur trioxide (110 mg, 1.34 mol. equiv.) was added to the solution. The reaction conditions and the purification procedure were virtually identical with those described for the sodium salt of 2-deoxy-2-sulfoamino-D-glucose, except the procedure for its precipitation with ethanol. As the solubility of the sodium salt of methyl 2-deoxy-2-sulfoamino-α-D-glucopyranoside in ethanol or aqueous ethanol is far greater than that of the sodium salt of 2-deoxy-2-sulfoamino-D-glucose, the concentrated solution was precipitated once with ethanol and washed with a limited volume of ethanol to give the product, which was a chromatographically homogeneous, white powder (90 mg, 58.9%). Crystallization of the amorphous powder from 3:1 (v/v) ethanol–water gave colorless crystals (78 mg, 51.1%), m.p. 148–150°, $[\alpha]_D^{25}$ +91.7° (*c* 1.01, water); lit.[2] m.p. 159–161°, $[\alpha]_D^{27}$ +103.1° (*c* 2.1,

*The m.p. for the sodium salt of 2-deoxy-2-sulfoamino-D-glucose previously prepared by the present authors[7] had been erroneously reported as 235° (dec.).

water); lit.[4] m.p. 175–178° (dec.), $[\alpha]_D^{25}$ +105.9° (*c* 1, water). The analytical sample was dried at room temperature overnight under reduced pressure in the presence of phosphorus pentaoxide.

Anal. Calc. for $C_7H_{14}NNaO_8S \cdot H_2O$: C, 26.48; H, 5.15; N, 4.47; S, 10.24. Found: C, 26.48; H, 5.44; N, 4.33; S (bound sulfate), 10.22; S (*N*-sulfate), 10.55.

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