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HYDROLYTIC PROPERTIES OF *N*-SULFATED COMPOUNDS CONTAINING NEIGHBORING HYDROXYL OR SULFATE GROUPS, WITH REFERENCE TO CHEMICAL PROPERTIES OF NATURAL HEPARIN

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ABSTRACT

Hydrolytic properties of synthetic acyclic and cyclic sulfoamino compounds having a neighboring hydroxyl or *O*-sulfate group were examined. In all the compounds examined, the *O*-sulfate group in vicinal position accelerated the hydrolysis of the sulfoamino group more than did the hydroxyl group in vicinal position, and the hydrolysis of the sulfoamino group linked to a cyclic compound was faster than that of a group linked to an acyclic compound, with the substituent in *trans* position having a greater effect than that in *cis* position. The effect of a substituent removed by one carbon atom from the sulfoamino group was much smaller than that of the vicinal substituent. The marked lability of the sulfoamino group in 2-deoxy-2-sulfoamino-D-glucose seems to be due to the overlapping of the multiple effects of hydroxyl groups, pyranose ring structure, and cyclic oxygen atom. Based on the liberation of amino and inorganic sulfate groups from amino alcohol sulfoamino groups, the possibility of trans-sulfation from N to O under the experimental conditions used is discussed.

INTRODUCTION

In general, alkylsulfamic acids are relatively stable to acid hydrolysis, but the sulfoamino group of heparin, a natural *N*-sulfated compound, is labile under acid conditions[1–4]. Hydrolysis of *ca.* 7–8% of the *N*-sulfate groups in heparin with acetic acid results in almost complete loss of its anticoagulant activity[5], and the relationship between the content of *N*-sulfate groups of heparin and its biological activity is of interest[6–8]. An explanation for the lability of the *N*-sulfate group in heparin may be either the neighboring-group effect of the hydroxyl and *O*-sulfate groups or the macromolecular size. In order to examine the effect of neighboring groups on the stability of *N*-sulfate groups in heparin, the hydrolysis rate of the *N*-sulfate group of synthetic sulfoamino compounds having neighboring hydroxyl or *O*-sulfate groups was examined.

RESULTS AND DISCUSSION

The amino groups liberated by the acid hydrolysis of *N*-sulfated amino alcohols can be determined accurately by the use of the corresponding nonsulfated, 2,4,6-trinitrophenyl derivative of the amino alcohol as a standard. The four different amino alcohol *N,O*-disulfates, *i.e.*, 2-sulfoaminoethanol 1-sulfate, 3-sulfoaminopropanol 1-sulfate, and *cis*- and *trans*-2-sulfoaminocyclohexanol 1-sulfates, gave by acid hydrolysis the amino alcohol *O*-sulfates besides the corresponding nonsulfated amino alcohols. As shown in Table I, the absorption coefficient of any of the *N*-(2,4,6-trinitrophenyl) derivatives of *O*-sulfated amino alcohols, is greater than that of the corresponding amino alcohol 2,4,6-trinitrophenyl derivatives. Consequently, the accurate

TABLE I

EXTINCTION COEFFICIENT OF 2,4,6-TRINITROPHENYLAMINO ALCOHOL *O*-SULFATES RELATIVE TO THAT OF THE CORRESPONDING 2,4,6-TRINITROPHENYLAMINO ALCOHOL

N-2,4,6-Trinitrophenyl derivative of	*Relative extinction coefficient*
2-Aminoethanol 1-sulfate	1.13
3-Aminopropanol 1-sulfate	1.28
cis-2-Aminocyclohexanol 1-sulfate	1.22
trans-2-Aminocyclohexanol 1-sulfate	1.58

determination of the total amount of amino groups liberated from these amino alcohol *N,O*-disulfates, requires the separation of the amino alcohols formed from their *O*-sulfates, followed by the quantitative determination based on the absorption coefficient of their *N*-(2,4,6-trinitrophenyl) derivatives, and addition of the results. Since, in the present work, the determination of the liberation of the amino groups from all the sulfated derivatives of amino alcohols was based only on the correspond-

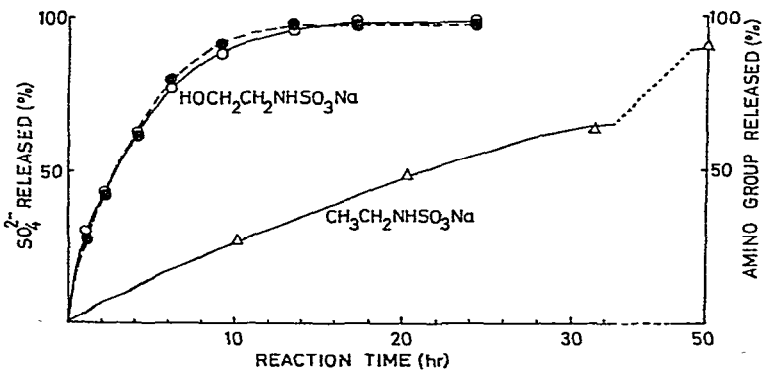

Fig. 1. Rate of sulfate groups and amino groups released from 2-sulfoaminoethanol and *N*-ethylsulfamic acid in 0.1M hydrochloric acid at 100°; O———O and △———△, sulfate release; ●- - - - -●, amino group release.

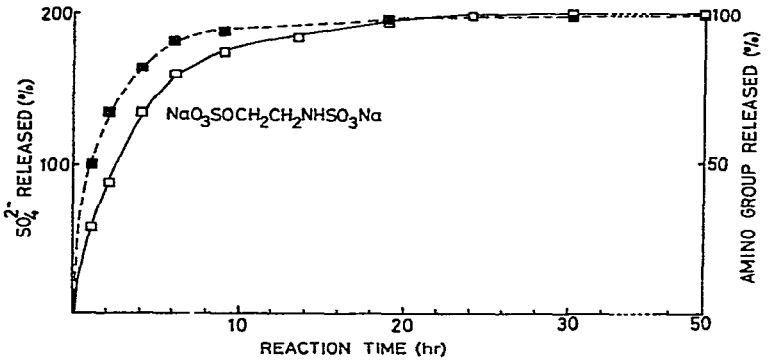

Fig. 2. Rate of sulfate groups and amino groups released from 2-sulfoaminoethanol 1-sulfate in 0.1M hydrochloric acid at 100°; □———□, sulfate release; ■- - - - -■, amino group release.

ing nonsulfated amino alcohol 2,4,6-trinitrophenyl derivative as a standard, it is not possible to discuss accurately the course of this liberation.

The hydrolysis of *N*-sulfated and *N,O*-disulfated 2-aminoethanol (2-sulfoaminoethanol and 2-sulfoaminoethanol 1-sulfate) is shown in Figs. 1 and 2, respectively. The *N*-sulfate group of *N*-sulfated compounds having hydroxyl or *O*-sulfate substituent groups in vicinal position is hydrolyzed much faster than that of *N*-ethylsulfamic acid. Comparison of the effect of both substituents on the hydrolysis of the *N*-sulfate group suggests that the correct proportions of amino groups released are smaller than those shown in Fig. 2, since the 2,4,6-trinitrophenyl derivative of 2-aminoethanol 1-sulfate has an absorption coefficient higher than that of 2-aminoethanol. Nevertheless, the observed value of amino groups released from 2-sulfoaminoethanol 1-sulfate is higher than that of groups released from 2-sulfoaminoethanol ,which indicates that the effect of the *O*-sulfate group is greater than that of

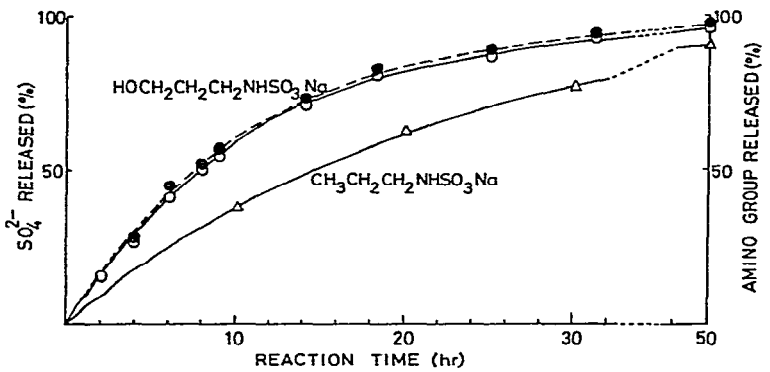

Fig. 3. Rate of sulfate groups and amino groups released from 3-sulfoaminopropanol and *N*-propylsulfamic acid in 0.1M hydrochloric acid at 100°; O———O and △———△ sulfate release; ●- - - - -●, amino group release.

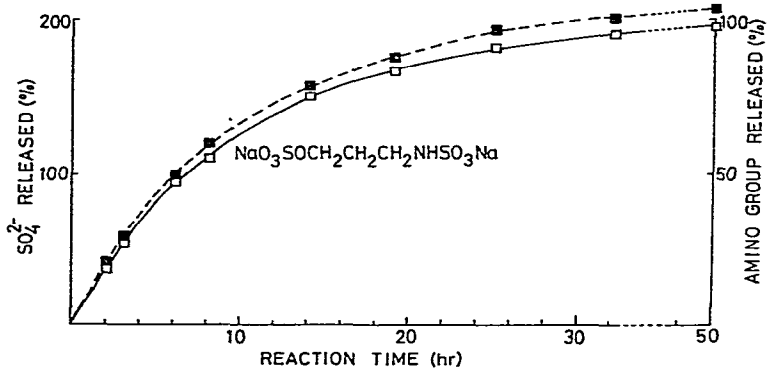

Fig. 4. Rate of sulfate groups and amino groups released from 3-sulfoaminopropanol 1-sulfate in 0.1M hydrochloric acid at 100°; □———□, sulfate release; ■------■, amino group release.

the hydroxyl group. The rate of hydrolysis of the *N*-sulfate group is faster than that of the *O*-sulfate group in 2-sulfoaminoethanol 1-sulfate.

The hydrolysis rate of the *N*-sulfate group in the *N*-sulfate derivative of 3-aminopropanol (see Fig. 3) and in the *N,O*-disulfate derivative (see Fig. 4), which have functional groups separated by one carbon atom, is not as fast as that of the *N*-sulfate group in 2-sulfoaminoethanol, as expected, although it is faster than that of *N*-propylsulfamic acid (Fig. 3). The effect, in 3-aminopropanol, of the hydroxyl and *O*-sulfate groups on the hydrolysis of the *N*-sulfate group is hardly perceptible, which indicates the strong participation of the functional groups located in vicinal positions. There is also virtually no difference between the hydrolysis rates of the *N*-sulfate and *O*-sulfate groups in 3-sulfoaminopropanol 1-sulfate.

The isomeric 2-aminocyclohexanol and its *O*-sulfate, formed by acid hydrolysis of *cis*- or *trans*-2-sulfoaminocyclohexanol 1-sulfates, were not separated and the total free amino groups were determined with the corresponding isomeric 2-aminocyclohexanol as a standard. As shown in Table I, the absorption coefficient of 2-(2,4,6-trinitrophenyl)aminocyclohexanol 1-sulfate is greater than that of 2-(2,4,6-trinitrophenyl)aminocyclohexanol and this difference is especially marked in the *trans* isomer. Consequently, the observed value of amino groups liberated during the acid hydrolysis of these *N,O*-disulfates is larger than the theoretical one. Thus, results above 100% are observed as shown in Figs. 5 and 6, which indicate that the *N*-sulfate group in 2-sulfoaminocyclohexanol 1-sulfate is more rapidly hydrolyzed than the *N*-sulfate group of the corresponding 2-sulfoaminocyclohexanol, even when the overestimation of the amino group released from *N,O*-disulfate is taken into consideration. This suggests that the *O*-sulfate group in vicinal position on the cyclohexane ring has a greater effect than the hydroxyl group in the same position. The release of the *N*-sulfate group is followed by a considerably slower hydrolysis of the *O*-sulfate group in the *N,O*-disulfate derivative, which agrees well with the observed hydrolysis rate of the corresponding 2-aminocyclohexanol 1-sulfate.

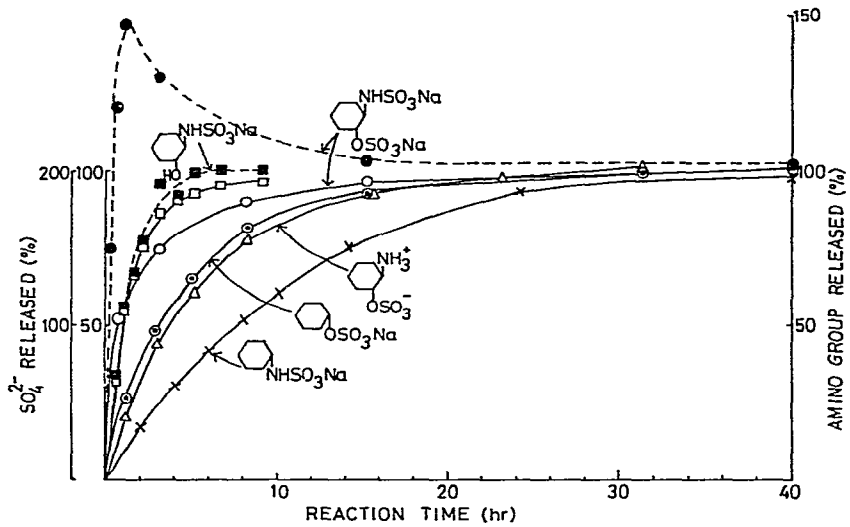

Fig. 5. Rate of sulfate groups and amino groups released from *trans*-2-aminocyclohexanol, *N*- and *O*-sulfate and *N,O*-disulfate, *N*-cyclohexylsulfamic acid, and cyclohexylsulfate in 0.1M hydrochloric acid at 100°; ○———○, □———□, △———△, ⊙———⊙, and ×———×, sulfate release; ●-----● and ■-----■, amino group release.

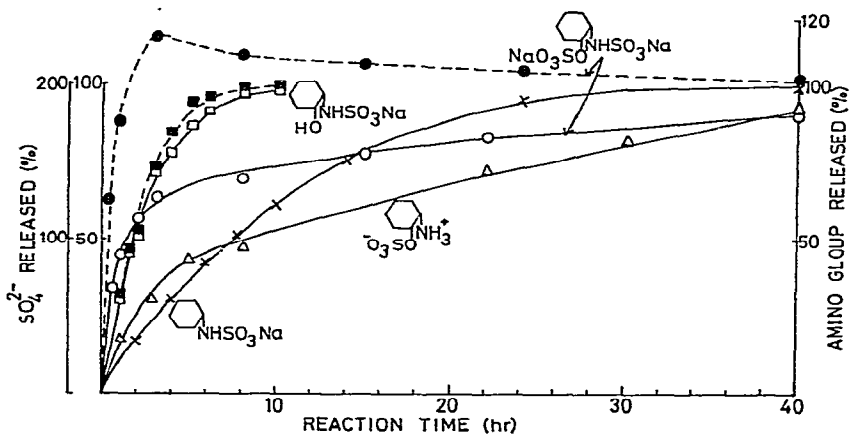

Fig. 6. Rate of sulfate groups and amino groups released from *cis*-2-aminocyclohexanol *N*- and *O*-sulfate and *N,O*-disulfate, and *N*-cyclohexylsulfamic acid in 0.1M hydrochloric acid at 100°; ○———○, □———□, △———△, and ×———×, sulfate release; ●-----● and ■-----■, amino group release.

The results just mentioned suggest that the *O*-sulfate group linked to C-3 of 2-deoxy-2-sulfoamino-D-glucose would greatly accelerate the hydrolysis rate of the *N*-sulfate group, in agreement with the observations that 7–8% of the *N*-sulfate groups in heparin are markedly acid-labile[5] and that heparin contains a small proportion of

2-deoxy-2-sulfoamino-D-glucose 3,6-disulfate residues[9]. The vicinal, free amino group inhibits the hydrolysis of the *O*-sulfate group more in the *cis*-2-aminocyclohexanol 1-sulfate than in the *trans* isomer (see Figs. 5 and 6), owing probably to the interference, by the NH_3^+ group, of the attack of H^+ ions on the *O*-sulfate bond.

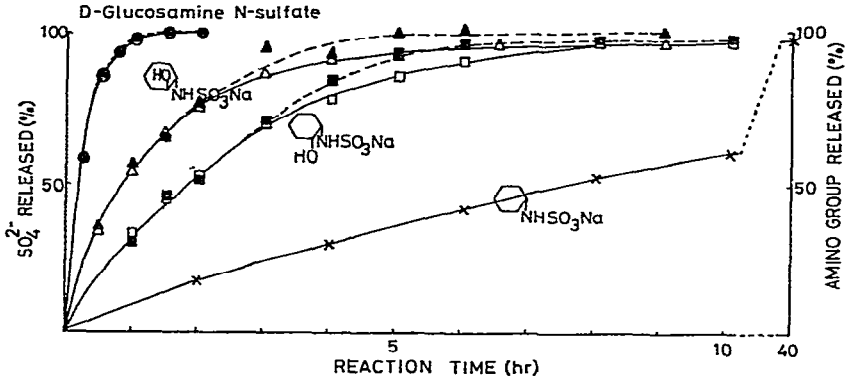

Fig. 7. Rate of sulfate groups and amino groups released from *N*-sulfated derivatives of 2-deoxy-2-amino-D-glucose, *cis*- and *trans*-2-aminocyclohexanols and *N*-cyclohexylsulfamic acid, in 0.1M hydrochloric acid at 100°; O———O, □———□, △———△, and ×———×, sulfate release; ●------●, ■------■, and ▲------▲, amino group release.

The liberation of inorganic sulfate is markedly faster from 2-deoxy-2-sulfoamino-D-glucose than from 2-sulfoaminocyclohexanol (see Fig. 7), and this is probably due to the concerted effect of the four hydroxyl groups, pyranose ring structure, and ring oxygen atom in the former compound. Yoshizawa *et al.*[5] reported that treatment of α- or ω-heparin with 40% acetic acid for 24 h at 37° resulted in the liberation of 7–8% of the *N*-sulfate groups and loss of the anticoagulant activity; almost no change of the total sulfur content was observed, and the lipemia-clearing activity was retained. These observations suggest a trans-sulfation from the amino to a hydroxyl group in heparin. In 2-deoxy-2-sulfoamino-D-glucose the rates of liberation of inorganic sulfate and of appearance of free amino groups are almost equal, but in *cis*- and *trans*-2-sulfoaminocyclohexanol these rates begin to diverge about 2 h after the start of the reaction, and the difference is the greatest after 5 h (see Fig. 7). This difference between rates suggests a trans-sulfation from the amino to the hydroxyl groups in 2-sulfoaminocyclohexanols. However, consideration of the absorption coefficients of the 2,4,6-trinitrophenyl derivatives of aminocyclohexanol *O*-sulfates (Table I) indicates that such a trans-sulfation, if produced, is very limited. Examination of the hydrolyzates, by electrophoresis, for 5 h after the start of the reaction failed to show any product of the trans-sulfation from N to O.

The rate constants for the hydrolysis of sulfoamino compounds and of the corresponding hydroxylated derivatives agree with the first-order equation of chemical kinetics (Table II).

TABLE II

RATE CONSTANTS FOR THE HYDROLYSIS OF SULFOAMINO COMPOUNDS AND CORRESPONDING HYDROXYLATED DERIVATIVES[a]

Sodium salt of	$k\ 10^{-3}\ min^{-1}$[b]
N-Ethylsulfamic acid	0.546
2-Sulfoaminoethanol	4.03
N-Propylsulfamic acid	0.803
3-Sulfoaminopropanol	1.43
N-Cyclohexylsulfamic acid	1.64
trans-2-Sulfoaminocyclohexanol	10.1
cis-2-Sulfoaminocyclohexanol	6.33
2-Deoxy-2-sulfoamino-D-glucose	74.6 (56.5[c])
Heparin	(62.0[d])

[a]In 0.1M hydrochloric acid at 100°. [b]Calculated from the rate of liberation of inorganic sulfate. [c]Potassium salt, 0.1M hydrochloric acid at 99.5° (Ref. 1). [d]Sodium salt, 0.1M hydrochloric acid at 99.5° (Ref. 1).

EXPERIMENTAL

Materials. — The preparation of the alkylamine and amino alcohol sulfates used in this study has previously[10] been described.

Sulfate and amino group analysis. — The inorganic sulfate and free amino groups liberated were determined by turbidimetry[11] and by the 2,4,6-trinitrobenzenesulfonic acid method[5], respectively.

Hydrolysis procedures. — The sample (21–42 mg) was dissolved in 0.1M hydrochloric acid (10 ml), and the solution was distributed into nine tubes, which were sealed. The tubes were heated at 100°, cooled in ice, and opened. The hydrolyzates were neutralized with an equal volume of 0.1M sodium hydroxide and analyzed for inorganic sulfate and amino groups.

Paper electrophoresis. — The paper electrophoresis of hydrolyzates was performed on Toyo Roshi No. 51 paper with a buffer solution (pH 5.8) consisting of pyridine (5 ml), acetic acid (1 ml), butanol (5 ml), and water (250 ml), at a potential of 23 V/cm, for 25 min. The spots were detected under u.v. light as dark spots on a light background, and then sprayed with the ninhydrin reagent[12].

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