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# Alcoholysis Reaction of Sulfamic Acids and Its Application to the Preparation of biochemically Related Sulfate Esters

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The alcoholysis reaction of sulfamic acids catalyzed by pyridine was investigated with piperidine-N-sulfonic acid–cyclohexanol system and a possible formation and participation of the intermediate "pyridine-$SO_3$ adduct" in the reaction was suggested.

To apply this reaction to the preparation of sulfate esters, the experimental conditions such as the kind of sulfamic acids, molar ratio of reactants, and temperature and time of reaction were examined. Biochemically related sulfate esters, adenosine 5′-sulfate, riboflavin 5′-sulfate, and D-galactose 6-sulfate, were synthesized by this reaction.

It has been known that sulfamic acid undergoes alcoholysis with alcoholic or phenolic compounds to form its O-sulfated derivatives[2,3] and that the reaction is accelerated by pyridine.[4–6] It has later been reported that its N-substituted derivatives, such as N-cyclohexylsulfamic acid, piperidine-N-sulfonic acid, *etc.*, are more reactive than sulfamic acid itself.[7,8]

Recently, we have reported that sulfamic acid and its N-substituted derivatives are decomposed catalytically by polar solvents such as pyridine, dimethylformamide, dimethyl sulfoxide, *etc.*, to an equimolar amount of inorganic sulfate and the constitutional amine.[9] In the previous paper, the mechanism of the decomposition of sulfamic acids catalyzed by pyridine in which "pyridine-$SO_3$ adduct" participates as an intermediate was proposed as indicated in Chart 1 (I→II→III).

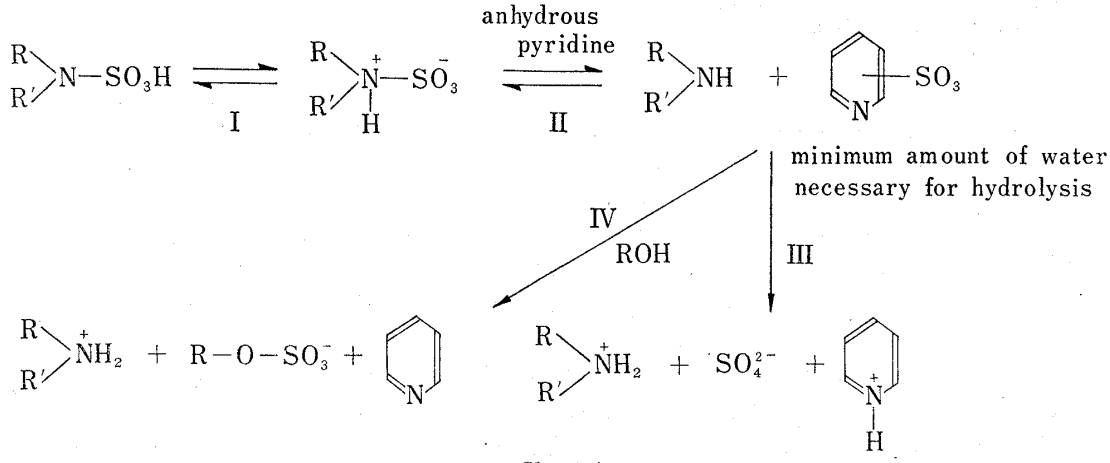


Chart 1

1) Location: *Shirokane 5–9–1, Minato-ku, Tokyo.*
2) F. Kraft and W. Bourgeios, *Ber.*, **25**, 472 (1892); K. Hofmann and E. Biesalski, *Ber.*, **45**, 1394 (1912).
3) M.E. Cupery, *Ind. Eng. Chem.*, **30**, 627 (1938).
4) W.F. Short and P. Oxley, Brit. Pat. 577666 (1946) [*C.A.*, **41**, 2084 (1947)].
5) R.L. Burwell, Jr., *J. Am. Chem. Soc.*, **71**, 1769 (1949).
6) K. Yamaguchi, *Nippon Kagaku Zasshi*, **80**, 171 (1959).
7) K. Nagasawa, A. Yūki and K. Yamazaki, The 18th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1963; K. Nagasawa, Japan Pat. 464379 (1966).
8) K. Nakano and H. Yamaguchi, *Kogyo Kagaku Zasshi*, **67**, 2055 (1964).
9) K. Nagasawa and H. Yoshidome, *Chem. Pharm. Bull.* (Tokyo), **17**, 1316 (1969).

Result of the previous work suggested that a similar reaction mechanism could be involved in the alcoholysis of sulfamic acids catalyzed by pyridine as indicated in Chart 1 (I→II→IV). Therefore, an experiment analogous to the previous work was carried out to clarify the mechanism of this alcoholysis reaction. To the equilibrium mixture[10] composed of piperidine-N-sulfonic acid (84.2%) and pyridine-$SO_3$ adduct (15.8%), cyclohexanol was added, and the course of reaction was examined periodically.

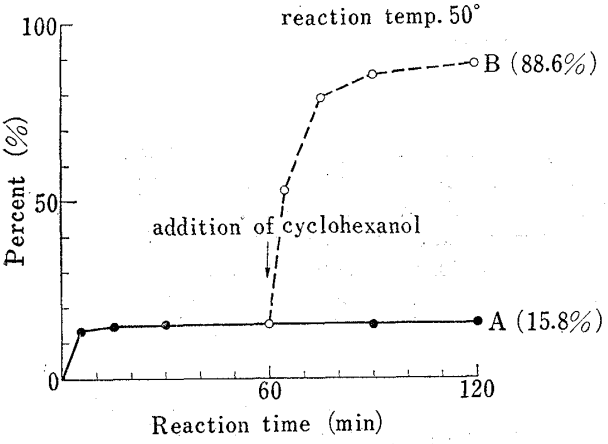


Fig. 1. Influence of Cyclohexanol on the Degradation Process of Piperidine-N-sulfonic Acid in Anhydrous Pyridine

—●—●—: anhydrous pyridine
------○------○------: pyridine containing 2.5% (w/v) of cyclohexanol
A(%)=Ratio of pyridine-$SO_3$ adduct formed in the equilibrium mixture in the absence of cyclohexanol.
100-A(%)=Ratio of piperidine-N-sulfonic acid present in the equilibrium mixture in the absence of cyclohexanol.
B(%)=Ratio of cyclohexyl sulfate formed in the equilibrium mixture added with cyclohexanol.
100-B(%)=Ratio of piperidine-N-sulfonic acid and pyridine-$SO_3$ adduct present in the equilibrium mixture added with cyclohexanol.

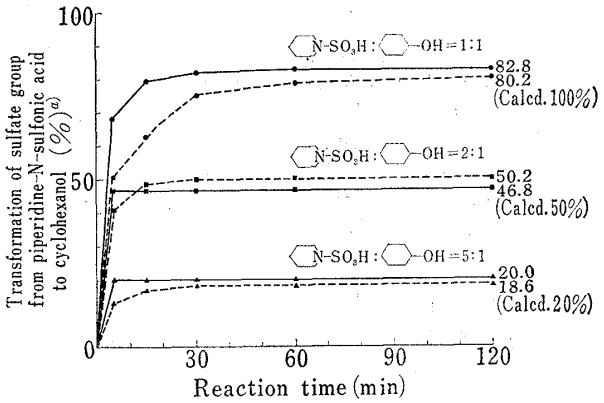


Fig. 2. Influence of the Molar Ratio of Reactants on the Sulfation of Cyclohexanol with Piperidine-N-sulfonic Acid

——: reacted at 80°; ------: reacted at 50°
*a*) The ratio of N→O transformation of sulfate is represented by a ratio of the amount of cyclohexyl sulfate formed to the original amount of piperidin-N-sulfonic acid used for the reaction.

As shown in Fig. 1, addition of cyclohexanol broke the equilibrium (solid line in Fig. 1) and resulted in the formation of cyclohexyl sulfate, the amount of which corresponded to 88.6% of total S in the reaction mixture. The course of this alcoholysis reaction (---○--- in Fig. 1) instituted by cyclohexanol added was almost identical to those in the previous work where water was added in place of cyclohexanol. The above results suggest that the alcoholysis of piperidine-N-sulfonic acid with cyclohexanol in pyridine proceeds according to the reaction scheme I→II→IV in Chart 1.

To examine the influence of molar ratio of the reactants, reaction temperature, and reaction time in this reaction, piperidine-N-sulfonic acid was reacted with cyclohexanol in pyridine at different molar ratio. It was found from Fig. 2 that the N→O transformation of sulfate group will be almost quantitative at a molar ratio of 1:1 within 30 min if moisture is excluded completely.

For the application of this alcoholysis to the preparation of biochemically related sulfate esters, two fundamental experiments were carried out with 2′,3′-O-isopropylideneadenosine and riboflavin. 2′,3′-O-Isopropylideneadenosine was reacted in pyridine–dimethylformamide (1:4) with piperidine-N-sulfonic acid at molar ratios of 1:1—5. As shown in Table I, excess of piperidine-N-sulfonic acid resulted in an almost quantitative transformation of 2′,3′-O-iso-

10) The equilibrium mixture is prepared by dissolving piperidine-N-sulfonic acid in anhydrous pyridine at 50°. See reference 9.

Table I. Influence of the Molar Ratio of Reactants on the Sulfation of 2′,3′-O-Isopropylideneadenosine with Piperidine-N-Sulfonic Acid

| Molar ratio of Ip-adenosine[a] to piperidine-N-sulfonic acid | Yield of Ip-adenosine 5′-sulfate[a] (%) Reaction time (hr) | |
|---|---|---|
| | 2 | 4 |
| 1 : 1 | 44.3 | 45.9 |
| 1 : 2 | 81.9 | 85.3 |
| 1 : 5 | 95.3 | 99.3 |

*a*) Ip-adenosine=2′,3′-O-isopropylideneadenosine
Ip-adenosine 5′-sulfate=2′,3′-O-isopropylideneadenosine 5′-sulfate

Table II. Yield and Composition of Riboflavin O-Sulfates formed by the Reaction between Riboflavin and a Variety of Sulfamic Acid Compounds

| Sulfamic acid compound | Yield and composition of riboflavin O-sulfate (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Unreacted riboflavin | Riboflavin O-sulfate | | | Unreacted riboflavin | Riboflavin O-sulfate | | |
| | | Mono- | Di- | Tri-and tetra- | | Mono- | Di- | Tri-and tetra- |
| | (Reacted at 36—37° for 72 hr) | | | | (Reacted at 95° for15 min) | | | |
| Sulfamic acid | 96.3 | 3.7 | 0 | 0 | 61.5 | 35.5 | 3.1 | 0 |
| N-Cyclohexylsulfamic acid | 36.4 | 55.6 | 7.4 | 0.6 | 32.7 | 53.3 | 13.4 | 0.7 |
| Morpholine-N-sulfonic acid | 19.9 | 62.4 | 16.4 | 1.3 | 44.7 | 47.4 | 7.6 | 0.3 |
| Piperidine-N-sulfonic acid | 29.2 | 66.1 | 4.6 | 0.1 | 31.0 | 53.8 | 14.5 | 0.5 |
| N-Benzylsulfamic acid | — | — | — | — | 44.1 | 48.1 | 7.2 | 0.2 |

A mixture of sulfamic acid compound and riboflavin is reacted at a 4:1 molar ratio in dimethyl sulfoxide and pyridine (4:1).

propylideneadenosine to its 5′-O-sulfate, but the reaction at an equimolar amount yielded the sulfate only in 45.9%.

The sulfating activity of various sulfamic acids was estimated by their alcoholyses of riboflavin. The results shown in Table II indicate that the reactivity of unsubstituted sulfamic acid was less than that of N-substituted derivatives which were slightly different from each other.

Based on the results obtained as above, biochemically related sulfate esters, adenosine 5′-sulfate, riboflavin 5′-sulfate, and D-galactose 6-sulfate, were synthesized by this reaction.

## Experimental

**Reagent and Solvent**——Pyridine, dimethyl sulfoxide, and dimethylformamide were dehydrated and purified by conventional methods. Other solvents and reagents, which were all special reagent grade, were used without further purification.

**Sulfamic Acids**——N-Cyclohexylsulfamic acid was prepared from commercially available sodium cyclamate.[9,11] Piperidine-N-sulfonic acid, morpholine-N-sulfonic acid and N-benzylsulfamic acid were synthesized from chlorosulfonic acid and the corresponding amine.[9,11]

**Paper Electrophoresis**——Paper electrophoreses were carried out on Toyo Roshi No. 50 filter paper (25×9 cm) at 22 V/cm for 30—60 min, using one of the following buffer solutions.

(a) 0.05M ammonium formate, pH 4.0.

(b) pyridinium acetate solution, pH 5.8 (pyridine:acetic acid:butanol:water=5:1:5:250 v/v).

Samples were applied on line positioned at 4.5 cm from the center of the filter paper.

11) L.F. Audrieth and M. Sveda, *J. Org. Chem.*, **9**, 89 (1944).

**Determination of Inorganic Sulfate**——Inorganic sulfate in test solutions was determined by Dodgson's turbidmetric method.[9,12]

**Influence of Cyclohexanol on the Degradation Process of Piperidine-N-sulfonic Acid in Anhydrous Pyridine**——A solution of 20 ml of pyridine containing 330 mg of piperidine-N-sulfonic acid was kept at 50°, an aliquot (1 ml) was removed at 5, 15, 30, 60, and 120 min after the start, and was immediately diluted with water to 20 ml. The reaction II in Chart 1 was stopped by the addition of excess water as described above. The amount of inorganic sulfate analyzed on the test solutions corresponds to those of pyridine-$SO_3$ adduct present in the reaction mixture.

Another aliquot (10 ml) at 60 min was removed separately, and it was added with 10 ml of pyridine containing cyclohexanol (5%, w/v) to start the reaction IV in Chart 1. The solution was kept at 50°, and an aliquot (1 ml) was removed from the solution at 5, 15, 30, and 60 min after the start. To the aliquot was immediately added 9 ml of pyridine containing 1% water and the mixture was heated at 100° for 10 min to decompose unreacted piperidine-N-sulfonic acid. The cyclohexyl sulfate formed in this reaction was not affected by this treatment. The reaction mixture was diluted with water to an appropriate concentration to analyze the inorganic sulfate. The amount of inorganic sulfate obtained corresponds to the sum of unreacted piperidine-N-sulfonic acid and pyridine-$SO_3$ adduct present in the reaction mixture.

**Determination of Cyclohexyl Sulfate formed by the Reaction of Cyclyhexanol with Piperidine-N-sulfonic Acid**——The solutions composed of piperidine-N-sulfonic acid and cyclohexanol at 1:1, 2:1, or 5:1 molar ratios in anhydrous pyridine were prepared according to the following procedures:

1:1 solution (10 ml): Prepared from 165 mg of piperidine-N-sulfonic acid and 100 mg of cyclohexanol in pyridine.
2:1 solution (10 ml): Prepared from 330 mg of piperidine-N-sulfonic acid and 100 mg of cyclohexanol in pyridine.
5:1 solution (10 ml): Prepared from 825 mg of piperidine-N-sulfonic acid and 100 mg of cyclohexanol in pyridine.

Each solution prepared in a tightly stoppered flask was reacted at the indicated temperature (50° and 80°) and 1 ml of aliquot was withdrawn from the flask at 5, 15, 30, 60, and 120 min after the start. To each aliquot was immediately added 1 ml of pyridine containing 1% water and the solution was heated at 100° for 10 min to decompose unreacted piperidine-N-sulfonic acid. The reaction mixture was diluted with water to an appropriate concentration and analyzed for inorganic sulfate. The amount of cyclohexyl sulfate formed is calculated by substracting the amount of inorganic sulfate obtained as above from the original amount of piperidine-N-sulfonic acid used for the reaction.

**Determination of Ip-Adenosine 5′-Sulfate formed by the Reaction of Ip-Adenosine with Piperidine-N-sulfonic Acid**——Three reaction mixtures (2 ml each) containing piperidine-N-sulfonic acid (0.1, 0.2, or 0.5 mmole) and Ip-adenosine (0.1 mmole each) in dimethylformamide–pyridine (4:1) were reacted at 50° in glass-stoppered flasks and 0.5 ml of aliquot was withdrawn at 2 and 4 hr after the start. To each aliquot was added 0.5 ml of pyridine containing 1% water and the solution was heated at 100° for 10 min to decompose unreacted piperidine-N-sulfonic acid. An appropriate amount of the test solution obtained as above was applied to the paper electrophoresis using buffer solution (a). Each paper zone corresponding to Ip-adenosine and its 5′-sulfate was cut off and extracted with water. The absorbance of each extract was measured at 260 m$\mu$ and the relative amount of Ip-adenosine and its 5′-sulfate was determined.

**Determination of Riboflavin Sulfates formed by the Reaction of Riboflavin with a Variety of Sulfamic Acids**——A mixture of riboflavin (0.05 mmole) and sulfamic acid compound (0.2 mmole) in dimethyl sulfoxide–pyridine (4:1, 2 ml) was reacted under the indicated conditions. After the reaction, an equal volume of water was added to the reaction mixture. An appropriate amount of this solution was applied to the paper electrophoresis using buffer solution (b). Each paper zone corresponding to unreacted riboflavin and riboflavin sulfates resolved was cut off and extracted with water. Absorbance of each extract was measured at 450 m$\mu$ and the relative amount of the components in the reaction mixture was determined.

The mobilities of riboflavin and its sulfate esters on the paper electrophoresis are shown in Table III.

**Preparation of Adenosine 5′-Sulfate**——A mixture of 2′,3′-O-isopropylideneadenosine (154 mg, 0.5 mmole) and N-cyclohexylsulfamic acid (300 mg, 1.65 mmoles) in anhydrous pyridine (10 ml) was reacted at 60° for 30 min. The reaction mixture was diluted with 0.5 ml of water and heated at 100° for 10 min to decompose excess N-cyclohexylsulfamic acid. After evaporation of pyridine, the residue was dissolved in 5 ml of water and its pH was adjusted to 2.5 with Dowex 50 $H^+$ resin. The acid solution was heated at 95° for 60 min to remove the isopropylidene group, cooled, and added with satd. $Ca(OH)_2$ to remove inorganic sulfate. After filtration, the clear solution (pH 9.0) was concentrated *in vacuo* to *ca.* 5 ml and its pH was adjusted to 6.5 with Dowex 50 $H^+$ resin. The solution (pH 6.5) was concentrated to *ca.* 2 ml *in vacuo*, and filtered again to remove a small amount of impurities. The clear concentrate was added gradually with 20 ml of EtOH to crystallize calcium salt of adenosine 5′-sulfate. Yield, 135 mg (65%). UV $\lambda_{max}^{H_2O\,(pH6)}$ 260 m$\mu$.

12) K.S. Dodgson, *Biochem. J.*, **78**, 312 (1961).

Table III. Paper Electrophoretic Mobilities of Riboflavin and Its Sulfate Esters

| Compound[a)] | Distance from starting line (cm) | Relative migration to riboflavin |
|---|---|---|
| Riboflavin | 2.0 | 1 |
| Riboflavin monosulfate | 3.9 | 1.95 |
| Riboflavin disulfate | 5.8 | 2.90 |
| Riboflavin trisulfate | 7.1 | 3.55 |
| Riboflavin tetrasulfate | 7.8 | 3.90 |

a) Idenification of each of mono-, di-, tri-, and tetrasulfates of riboflavin was carried out in a separate experiment which will be published later.
conditions: 22 V/cm for 30 min, buffer solution (b)

*Anal.* Calcd. for $C_{10}H_{12}O_7N_5SCa_{1/2}\cdot 3H_2O$ (Adenosine 5′-sulfate, calcium): C, 28.55; H, 4.32; N, 16.75; S, 7.63. Found: C, 28.73; H, 4.00; N, 17.02; S, 7.91.

**Preparation of Riboflavin Monosulfate**——A mixture of riboflavin (376 mg, 1 mmole) and morpholine-N-sulfonic acid (670 mg, 4 mmoles) in dimethyl sulfoxide–pyridine (4:1, 15 ml) was reacted at 85° for 60 min with stirring. The paper electrophoresis of the reaction mixture revealed that the mixture was composed of unreacted riboflavin (2.1%), riboflavin monosulfate (49.7%), reboflavin disulfate (38.3%), and riboflavin tri- and tetrasulfates (9.9%). The reaction mixture was mixed with 4 mmoles of 10% $Na_2CO_3$, and shaken well, and then added successively with EtOH (25 ml) and ether (50 ml). The precipitated sodium salts of riboflavin sulfates and inorganic sulfate were separated and redissolved in water. This solution was added with an adequate amount of 10% $Ba(OAc)_2$ to remove inorganic sulfate alone. After centrifugation, the supernatant was adjusted to pH 6.5, and concentrated to *ca.* 4 ml *in vacuo*. The concentrate freed from some impurities was mixed with EtOH (20 ml) and ether (10 ml), and allowed to stand overnight to precipitate sodium salts of riboflavin sulfates. The precipitate was collected and washed with EtOH and ether, and dried in the dark. Yield, 380 mg.

To prepare sodium riboflavin monosulfate, the mixed sulfate esters were chromatographed on a cellulose column (6.5×45 cm, Toyo Roshi cellulose powder, 200—300 mesh) using the solvent system of benzyl alcohol: EtOH:$H_2O$=3:1:1.[13] The fractions containing riboflavin monosulfate were collected, and were extracted with ether to remove benzyl alcohol. The aqueous layer was separated and concentrated to *ca.* 5 ml *in vacuo*, filtered to remove a small amount of insoluble materials. To the filtrate, EtOH (20 ml) and ether (10 ml) were added and allowed to stand overnight in the drak. The yellow precipitate formed was collected, washed with EtOH and ether, and dried over $P_2O_5$ *in vacuo* at room temperature. Yield, 110 mg (23.0%). *Anal.* Calcd. for $C_{17}H_{19}O_9N_4SNa$ (riboflavin monosulfate, sodium): N, 11.71; S, 6.69. Found: N, 11.90; S, 6.77. UV $\lambda_{max}$: 225, 266, 372, 445 mμ (in 0.1M phosphate buffer, pH 7.0).

Periodate oxidation of riboflavin monosulfate revealed that it was mainly composed of riboflavin 5′-monosulfate because a consumption of 1.8 moles of periodate per mole of the sample was measured.

**Preparation of D-Galactose 6-Sulfate**——A mixture of 1,2:3,4-di-O-isopropylidene-D-galactose (5.2 g, 20 mmoles) and N-cyclohexylsulfamic acid (11.8 g, 66 mmoles) in anhydrous pyridine (80 ml) was reacted at 26—28° for 24 hr. The reaction mixture was diluted with 4 ml of water and heated at 100° for 10 min to decompose excess N-cyclohexylsulfamic acid. Pyridine was evaporated, the residue was dissolved in 100 ml of water, and the solution was passed through a column of Dowex 50 $H^+$ resin. The acid effluent (about 160 ml) was heated at 80° for 2.5 hr, neutralized with satd. $Ba(OH)_2$, and the precipitate formed was removed by centrifugation. The supernatant was concentrated to *ca.* 50 ml *in vacuo*. A large excess of EtOH was added to this concentrate to precipitate a product composed mainly of barium salt of D-galactose 6-sulfate. The precipitate was dissolved in 80 ml of water and freed from barium ion by passing through a column of Dowex 50 $H^+$ resin. The effluent and washings were combined and mixed with stirring with 100 ml of ethanol containing brucine base (9.33 g, 20 mmoles). The mixture was evaporated *in vacuo* to *ca.* 40 ml, and the excess free brucine that separated was filtered off. To the filtrate, MeOH (325 ml) was added with stirring and the mixture was allowed to stand overnight to crystallize brucinium salt of D-galactose 6-sulfate as needles. The crystals were collected, successively washed with MeOH and ether, and dried over $P_2O_5$ *in vacuo* at room temperature. Yield, 7.2 g (55.1%), mp 195—197° (decomp.). *Anal.* Calcd. for $C_6H_{12}O_9S\cdot C_{23}H_{26}O_4N_2$ (D-Galactose 6-sulfate, brucinium): N, 4.28; S, 4.90. Found: N, 4.31; S, 4.85.

Additional 1.9 g of the crystals were recovered from the mother liquor by addition of ether (300 ml).

**Acknowledgement** The authors are indebted to Mmes. A. Yūki and Yamazaki for their technical assistance.

13) R. Takahashi, K. Yagi and F. Egami, *Nippon Kagaku Zasshi*, **78**, 1287 (1957).