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Studies on Decomposition and Stabilization of Drugs in Solution. XVIII.[1,2] The Hydrolysis of Phthalylsulfathiazole

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The hydrolysis of phthalylsulfathiazole at 60° was studied. The pH–rate profile was similar to that of phthalamic acid in acidic region, and rate constant of intramolecular reaction was 1.12×10^{-3} sec^{-1}. In strong alkaline region the rate was proportional to hydroxide ion concentration and the rate constant was 3.80×10^{-6} liter·mole^{-1} sec^{-1}. Reaction species were discussed in relation to observed rate constants and the rate expression was presented. $\Delta H^{\neq}$ was 18.1 kcal/mole and $\Delta S^{\neq}$ was −17.9 e.u.

Intramolecular catalysis in the hydrolyses of esters and amides of polycarboxylic acid have been widely studied since Bender reported the hydrolysis of phthalamic acid.[4] Intramolecular catalysis means that a group at suitable position to the reactive site of a molecule play a role of catalyst. Usually, intramolecular catalysis increases reaction rates remarkably more than intermolecular catalysis. Although intramolecular catalysis was originally proposed as a model of enzyme action,[4] it became one of the important concepts in the studies on the stability and the reactivity of drugs.[5] The hydrolyses of monoesters of polycarboxylic acids have been studied by several workers,[6–11] but those of monoamides especially in drugs have not been studied so extensively.[12–16]

This paper deals with the hydrolysis of phthalylsulfathiazole (PST) as a model of an amide which might play an intramolecular catalysis in the mechanism of its hydrolysis.

The products of the hydrolysis of PST in wide pH range were found as sulfathiazole (ST) and phthalic acid. These were identified by thin-layer chromatography. It was proposed that phthalamic acid was hydrolyzed through phthalic acid anhydride to phthalic acid.[4] Thus in PST, phthalic acid anhydride would be produced in the first step and then hydrolyzed to phthalic acid.

The concentration of ST was determined by diazo coupling method.

A typical plot of the residual PST concentration *v.s.* reaction time was shown in Fig. 1.

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2) A part of this work was reported at Kanto Branch Meeting of Pharmaceutical Society of Japan, June, 1965.
3) Location: *Hongo, Tokyo.*
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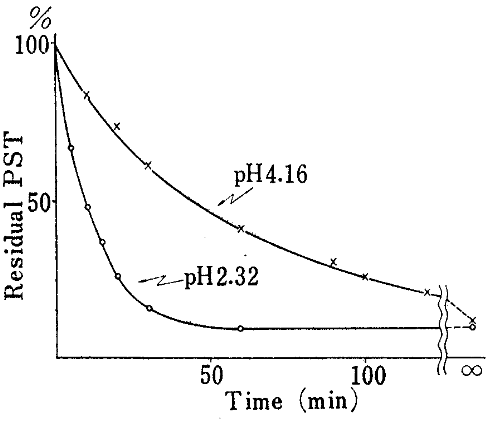

Fig. 1. Degradation Curve of Phthalylsulfathiazole at 60°

PST: phthalylsulfathiazole

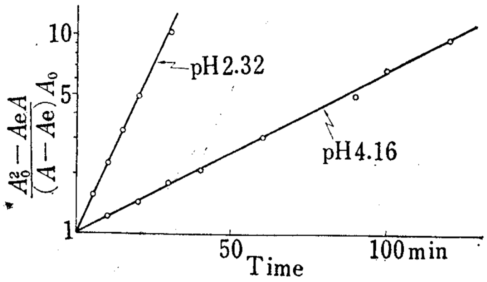

Fig. 2. Plots of the Hydrolysis of Phthalylsulfathiazole according to the Equilibrium Equation at 60°

A_0: initial concentration
A : concentration at timet
A_e: final concentration

It was found that PST was not hydrolyzed completely between pH 2 and pH 7, and seemed to reach to the equilibrium at about 90% hydrolyzed or more. The reverse reaction is probable since some dicarboxylic acids and polycarboxylic acids have been found to react with aromatic amines in aqueous solutions to form the corresponding amides.[12] To ascertain the equilibrium reaction in PST, the reverse reaction from equimolar mixtures of ST and phthalic acid to form PST was tried in the same concentration as in the hydrolysis of PST. But the change of the concentration of ST was so small (it would be expected at about 10% at most), that the change of the absorbance of azo-compound could not be followed in accuracy. When large amounts of ST were used to make the change of the absorbance large, ST could not be soluble. Thus the reverse reaction was not investigated further.

When the concentration of PST was plotted according to the integrated equation of the equilibrium reaction,[17] a straight line was obtained as shown in Fig. 2.

The rate constants calculated from the above plots were nearly equal to the constants calculated from the pseudo first order equation or from the initial velocities within an accuracy of 15%. This would be caused by the fact that A_e was very small. Furthermore the final concentration could not be obtained in the whole pH range examined. Therefore usual semilog plots of pseudo first order and above pH 8, Guggenheim method were used to calculate the rate constant K_{obs} for further discussion.

The pH-rate profile was shown in Fig. 3.

The k_{obs} were constant from pH 1 to pH 3, decreased from pH 4 to pH 10 and began to increase from pH 11. The pH-rate profile was very similar in acidic region to those of phthalamic acid,[4] phthalanilic acid[7] and succinanilic acid,[16] where were found intramolecular catalysis.

Bender obtained a linear relation between $1/k_{obs}$ and $1/[H^+]$ in his intramolecular catalysis study[18] and confirmed the kinetic dependency of the undissociated species of phthalamic

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17) $\mathrm{A} \underset{k'}{\overset{k}{\rightleftarrows}} \mathrm{B}+\mathrm{C}$ $\quad \ln\frac{A_0^2-A_eA}{(A-A_e)A_0}=k\frac{(A_0+A_e)}{(A_0-A_e)}t$

A.A. Frost and R.G. Pearson, "Kinetics and Mechanism," 2nd Ed., John Wiley & Sons, Inc., N.Y., 1962, p. 186.

18) Assuming the following equation; $\mathrm{RCOOH} \overset{K}{\rightleftarrows} \mathrm{RCOO^-} + \mathrm{H^+}$ and $\mathrm{RCOOH} \xrightarrow{k}$ products, then $k_{obs} = k\Big/\left(1+\frac{K}{\mathrm{H^+}}\right)$, thus $\frac{1}{k_{obs}}=\frac{1}{k}+\frac{K}{k}\times\frac{1}{[\mathrm{H^+}]}$ is obtained.

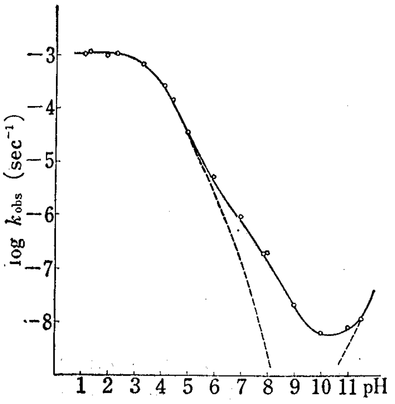

Fig. 3. The pH–rate Profile of the Hydrolysis of Phthalylsulfathiazole at 60°

——— calculated from eq. 1,2,3 and 4
– – – – calculated from eq. 1 and 4

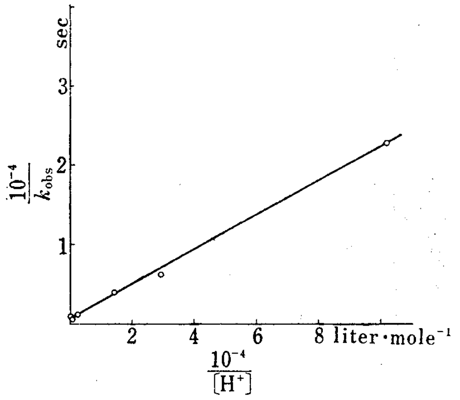

Fig. 4. Relationship between Rate Constants and Reciprocal of Hydrogen Ion Concentration in the Hydrolysis of Phthalylsulfathiazole at 60°

acid and independency of the external hydrogen ion.[4] In the hydrolysis of PST, a similar straight line was obtained as in Fig. 4.

The rate constant k and the dissociation constant K were calculated from the reciprocal of the intercept and the value of slope/intercept, respectively. The value of K and pK were 1.12×10^{-3} sec^{-1} and 3.35 at 60°, respectively. The value of pK_{a_1} of PST could not be obtained in aqueous solution because of its low solubility. It was found, however, that the value, of 3.35 as pK_{a_1} obtained from the kinetic data was favourably agreed with that of the analogous compound, phthalamic acid, 3.70 at 47.3°.[4]

Thus the mechanism of the hydrolysis of PST between pH 1 to pH 5 would be similar to that of phthalamic acid, as shown in Chart 1. The kinetic constants of PST were incidentally close to those of phthalamic acid as in Table 1.

Table I. Intramolecular Rate Constants and Thermodynamic Constants in the Hydrolyses of Amides

Compound	k_1 (sec^{-1}) at 47.3°($\times 10^{-4}$)	k_1 (sec^{-1}) at 60°($\times 10^{-3}$)	$\Delta H^{\ddagger}$ (kcal·mole^{-1})	$\Delta S^{\ddagger}$ (e.u.)
Phthalsulfathiazole	4.07	1.12	18.1	−17.9
Phthalamic acid	2.24	—	20.7	−12.4

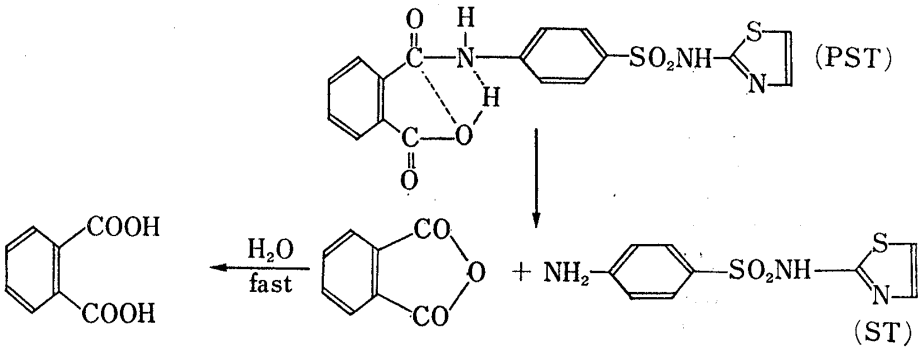

Chart 1

In sodium hydroxide solution, the pseudo first order rate constants were proportional to hydroxide concentrations. Thus hydroxide catalyzed hydrolysis should prevail in this region. The second order rate constant was calculated as 3.80×10^{-6} liter•mole^{-1} sec^{-1} from the slop of the line in Fig. 5.

Between pH 7 and pH 10.5 the calculated velocities, assuming the reaction species given in Chart 2 and using the above two rate constants, did not give a good agreement with the observed value, as was shown in broken line in Fig. 3.

In this pH range, another form of PST (II) might exist in considerable amount and II would also produce ST and phthalic acid. Furthermore, the second dissociation exponent, pK_{a_2}, of PST was found about 7.00 at 60°, spectrophotometrically. So it is possible that form III would be hydrolyzed spontaneously in aqueous solution.

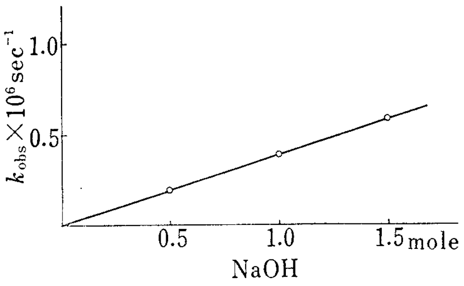

Fig. 5. Relationship between Rate Constants and Alkaline Concentration in the Hydrolysis of Phthalylsulfathiazole at 60°

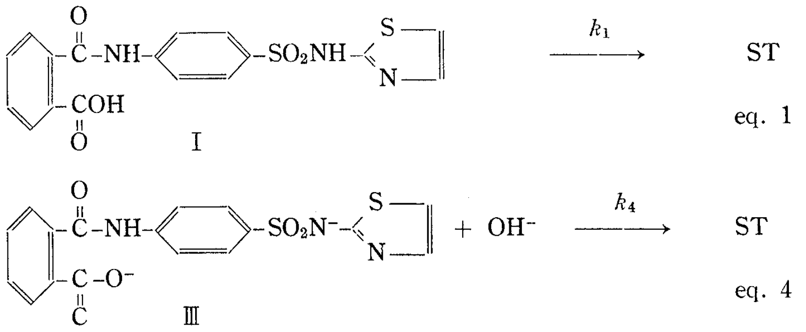

Chart 2

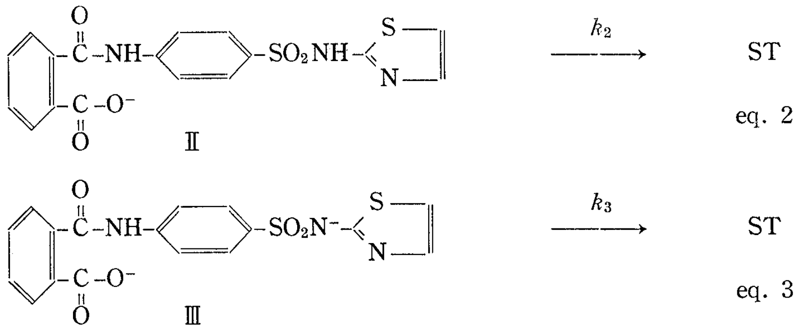

Chart 3

Thus other species as shown in Chart 3 may be necessary to explain the curve shown in Fig. 3 and then the following equation could be obtained.

$$\frac{d(\mathrm{ST})}{dt} = -\frac{d(\mathrm{PST})}{dt} = k_{\mathrm{obs}}(\mathrm{PST})$$

$$= k_1(\mathrm{I}) + k_2(\mathrm{II}) + [k_3 + k_4(\mathrm{OH}^-)](\mathrm{III})$$

thus

$$k_{\mathrm{obs}} = \frac{k_1}{1 + \frac{K_{a_1}}{[\mathrm{H}^+]} + \frac{K_{a_1} \cdot K_{a_2}}{[\mathrm{H}^+]^2}} + \frac{k_2}{\frac{[\mathrm{H}^+]}{K_{a_1}} + 1 + \frac{K_{a_2}}{[\mathrm{H}^+]}} + \frac{k_3 + k_4[\mathrm{OH}^-]}{\frac{[\mathrm{H}^+]^2}{K_{a_1} \cdot K_{a_2}} + \frac{[\mathrm{H}^+]}{K_{a_2}} + 1}$$

The rate constants k_2 and k_3 were determined by trial and error method using k_{obs} and the following values: $pK_{a_1} = 3.35$, $pK_{a_2} = 7.00$, $k_1 = 1.12 \times 10^{-3}$ sec^{-1}, $k_4 = 3.80 \times 10^{-6}$ liter·mole^{-1} sec^{-1}. The rate constants k_2 and k_3 were 1.47×10^{-6} sec^{-1} and 4.18×10^{-9} sec^{-1}, respectively.

It was reviewed that intramolecular catalysis have some roles in the ester hydrolyses of aspirin, dialkyl aminoalcohol esters, hydrocortisone hemisuccinate, *etc.*[5] An example of intramolecular catalysis of amide hydrolysis of drugs was given in this report.

Experimental

Materials——The commercial phthalylsulfathiazole and sulfathiazole were used after recrystallization from EtOH. The melting points were found to agree with the literature values.[19] Tsuda reagent was purchased and used without further purification. Other reagents were of the purest grade.

Identification of Products——Thin-layer chromatography was used. Layer: Wakogel B-5, solvent: $CHCl_3$–MeOH–water (15:15:1), thickness: 0.25 mm. Average *Rf* values: 0.52 for phthalylsulfathiazole, 0.32 for sulfathiazole, 0.0 for phthalic acid.

Analytical Procedure——The concentration of sulfathiazole which was a hydrolyzed product of phthalylsulfathiazole was measured with Tsuda reagent. General procedure was as follows; one ml of reaction mixture was taken into a volumetric glass tube and cooled in an ice bath. Two ml of chilled 1 N HCl and 4 drops of 0.2% $NaNO_2$ were added to the sample, and cooled for 10 min in the ice bath. Then 2 drops of 10% NH_4SO_3-NH_2 and about 4 ml of water were added and left for 10 min at room temperature. Then 5 drops of 0.2 % Tsuda reagent were added to the sample solution and diluted to final volume of 10 ml with water. The final solution had a color of reddish violet. After 15 min, the absorbance of the solution was measured at 550 mμ with Hitachi Perkin–Elmer spectrophotometer 139. Since the effects of residual phthalylsulfathiazole and phthalic acid on the analytical procedure could be ignored, the amount of hydrolyzed phthalylsulfathiazole was obtained directly from the absorbance.

Kinetic Procedure——Most of kinetic runs were as follows; The solution of phthalylsulfathiazole in absolute MeOH (5 mg/ml) was used a stock solution. A volumetric flask (200 ml) containig 100 ml of buffer solution was placed in a thermostated water bath. After the buffer solution reached to the desired temperature, one ml of the stock solution was added, mixed well and left in the bath. At intervals, one ml of the sample solution was withdrawn from the reaction mixture. Between pH 7 and pH 11, the hydrolyses were so slow that the reaction mixtures were sealed in ampules. It was found that a change in the ionic strength from 0.5 to 2.0M did not produce any change in the rate constant both at pH 2 and pH 3. But in almost every kinetic run the ionic strength was adjusted to 0.1M with NaCl.

Determination of p*K*a of Phthalylsulfathiazole——pKa_1: Calculated from the kinetic data (See in the text.).

pKa_2: Determined by spectrophotometry. One hundred mg of phthalylsulfathiazole was dissolved in 100 ml of absolute MeOH and 1 ml of the solution was poured to 200 ml of a buffer solution. Within 5 min after mixing, the absorbance at 285 mμ was measured by Hitachi Perkin–Elmer Spectrophotometer 139. The pKa_2 was calculated from the following equation,

$$pKa_2 = pH + \log \frac{d-d_1}{d_2-d}$$

where d was the absorbance at the pH, d_1 was the absorbance in alkaline media, d_2 was the absorbance in acidic media, respectively. The value of pKa_2 was about 7.00 at 60°.

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[19] Merck Index (7th edition).