+39.2° (340). CD (c=0.215, MeOH) [θ] (mμ): 0 (400), −108 (345) (negative maximum), −32.4 (330). ORD (c=0.063, 4% $NaHCO_3$) [α] (mμ): +25.4° (590), +38.2° (550), +57.2° (500), +102° (450), +286° (400), +429° (370) (peak), −349° (318) (trough), −254° (310). *Anal.* Calcd. for $C_{18}H_{15}O_5N_3$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.13; H, 4.21; N, 12.03.

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General Base Catalyzed Hydrolysis of Furylmethylketone Isonicotinoylhydrazone[1]

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Aminolysis of esters[3,4] and a number of acyl transfer reactions of acetylimidazole, including hydrolysis[5] have been reported to be subject to classical general base catalysis. Schowen and Zuorick[6] have reported the superimposed general-base catalysis by glycine-sodium glycinate buffer solution in the hydrolysis of anilides. Schwartz has recently reported the participation by general base catalysis in the aminolysis of benzylpenicillin by aliphatic diamines.[7]

However, very little work has been reported concerning the general catalysis of hydrolysis of hydrazones.

In our previous investigation on the hydrolysis of antitubercular agents like furylmethylketone isonicotinoylhydrazone (FKI)[8] and other isoniazid hydrazones in biological media,[9] amino acids such as glycine, asparagine, and glutamic acid exhibited a dominant effect to accelerate the degradative reaction and the data seemed to support the view that nonprotonated amino group acts as catalytic species.

To test this hypothesis, studies were conducted of the reaction of FKI with a series of amino acids and a few aliphatic amines, and kinetic deuterium solvent isotope effect was tested for the certification of general catalysis. An investigation of the mechanism of the catalytic reaction should aid in a better understanding of biopharmaceutical mechanisms involving such compounds.

Experimental

Materials——FKI (Daiichi Seiyaku Co., Ltd.) was used as received. Amino acids, aliphatic amines, and all other chemicals were of reagent grade. Deuterium oxide (Merck Sharp & Dohme of Canada Ltd.) was more than 99.7% in its isotopic purity.

1) This paper forms Part III of "Studies on the Stability of Drugs in Biological Media," Preceding paper, Part II: K. Kakemi, H. Sezaki, K. Iwamoto, and Y. Sano, *Chem. Pharm. Bull.* (Tokyo), **17**, 1623 (1969).
2) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) J.F. Bunnett and D.T. Davis, *J. Am. Chem. Soc.*, **82**, 665 (1960).
4) W.P. Jenks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 675 (1960).
5) W.P. Jenks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).
6) R.L. Schowen and G.W. Zuorick, *J. Am. Chem. Soc.*, **88**, 1223 (1966).
7) M.A. Schwartz, *J. Pharm. Sci.*, **57**, 1209(1968).
8) K. Kakemi, H. Sezaki, N. Takasugi, and K. Iwamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1481 (1968).
9) K. Kakemi, H. Sezaki, K. Iwamoto, and Y. Sano, *Chem. Pharm. Bull.* (Tokyo), **17**, 1623 (1969).

Kinetic Studies——The kinetic procedures employed were essentially the same as those reported previously.[8] A series of amino acids and aliphatic amines were dissolved in pH 7.0 phosphate buffer solution (0.05M, $\mu=0.2$) to maintain effective concentrations as catalyst (0.01 to 0.05M). The hydrolysis of FKI was carried out in duplicate in a volumetric flask which was immersed in a constant temperature bath kept at 37°. Five milliliter aliquots were withdrawn periodically and made to 50 ml with the same buffer solution. FKI concentrations were determined by reading the absorbance of the quenched solution at 310 mμ using a Shimadzu Model QV-50 spectrophotometer. Catalytic rate constants were calculated as the mean value of the duplicates. In the case of methyl amine, effect of pH was tested over the pH range 5.0 to 8.0. Deuterium oxide effect was tested at a single pH of 7.0 using glycine and methyl amine as model catalysts. In each kinetic run the pH value of reaction mixture was maintained within ±0.02.

Results and Discussion

Catalytic Rate Constant

As was shown in the previous report,[8] plots of apparent first-order rate constant (k_{app}) of hydrolysis at constant pH against amino acid or aliphatic amine concentration showed a linear relationships. The rate expression for the reaction can be given:

$$\text{Rate}=k_{app}(\text{F})=\{k_0+C(\text{A})\}(\text{F}) \qquad \text{(Eq. 1)}$$

where k_0 is the apparent first-order rate constant in the absence of amino acid or amine (A), C is the over-all catalytic rate constant, and (F) is the concentration of FKI.

Since there was no contribution of carboxyl group of amino acids,[8] this rate law can be rewritten in terms of non-protonated and protonated amino groups to give Eq. 2:

$$\text{Rate}=\{k_0+k_1(\text{B})+k_2(\text{BH}^+)\}(\text{F}) \qquad \text{(Eq. 2)}$$

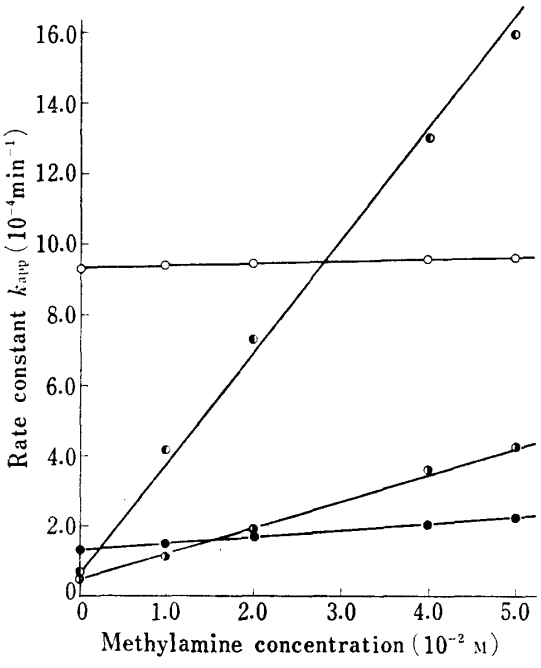

Fig. 1. Effect of Methylamine on the Stability of FKI in 0.05M Phosphate Buffer Solution at 37°

—○—: pH 5.0 —●—: pH 6.0
—◑—: pH 7.0 —◐—: pH 8.0

where k_1 and k_2 denote second-order catalytic rate constants. Previous results[8] revealed that the increase of the non-protonated to protonated ratio of amino group with pH was directly proportional to the increase of the over-all catalytic rate constant suggesting that the non-protonated amino group (B) acts as the primary catalytic species. To confirm this finding, catalytic effect of methylamine was investigated over the pH range 5.0 to 8.0. At a single pH, the observed first-order rate constant was found to increase linearly with the increase of the amine concentration, and more rapidly at higher pH as shown in Fig. 1.

The value of k_2 calculated from the data at two different pH was nearly equal to zero, indicating no participation of protonated amino group (BH^+) in the reaction. Equation 2, therefore, simplified to:

$$\text{Rate}=\{k_0+k_1(\text{B})\}(\text{F}) \qquad \text{(Eq. 3)}$$

The values of k_1 were obtained from the slopes of the plots of observed apparent first-order rate constant against the concentration of catalyst at pH 7.0 and are summarized in Table I.

Table I. Catalytic Rate Constants for the Hydrolysis of FKI in 0.05M Phosphate Buffer Solution (pH 7.0 and 37°) and Basicities of the Catalysts

No.	Catalyst[a]	k_1 ($min^{-1}M^{-1}$)	log k_1/q[b] ($min^{-1}M^{-1}$)	pK_a' ($-\log p/qK_a$)[c]
1	glycine	12.3 (5.01)[d]	1.088	9.78
2	β-alanine	7.49	0.899	10.19
3	L-alanine	3.30	0.518	9.97
4	taurine	1.08	0.033	8.75
5	serine	1.31	0.116	9.15
6	aspartic acid	4.51	0.353	9.52
7	asparagine	0.97	−0.019	8.86
8	glutamic acid	6.24	0.494	9.66
9	glutamine	1.19	0.075	8.96
10	arginine	2600	3.114	12.78
11	lysine	48.2	1.382	10.83
12	histidine	3.29	0.517	9.18
13	tryptophan	3.22	0.508	9.39
14	glycylglycine	2.35	0.371	8.25[e]
15	methylamine	28.8 (10.9)[d]	1.459	10.62
16	ethanolamine	2.69	0.430	9.50

a) Compounds from 5 to 13 are L-isomers. *b*) corrected according to Eq. 4
c) referred to lit.[10] and corrected *d*) data in 0.05M phosphate buffer solution of deuterium oxide
e) referred to lit.[11]

General Base Catalysis

The Brönsted plot of the catalytic rate constant (k_1) is given in Fig. 2, having a slope of approximately 0.67. The experimental values were plotted with correction for multi-valent bases according to the following equation:[12,13]

$$\frac{k_b}{q} = G_b\left(\frac{p}{qK_a}\right)^{\beta} \qquad \text{(Eq. 4)}$$

where k_b is base catalytic constant (k_1), G_b and β (slope) are constants, K_a is the dissociation constant of catalyst, q is the number of positions in the catalyzing base to which a proton may be attached, and p is the number of equivalent dissociable protons in the conjugate acid. The plot serves to emphasize the rather adequate correlation between catalytic rate constant and pK_a.

In FKI hydrolysis, this linearity in the Brönsted plot with a slope smaller than unity suggests significantly the absolute participation of general base catalysis by non-protonated amino group of a series of bases used. The pK_a' values of these bases do not extend over a relatively large range as previously discussed by Bruice and Benkovic.[14] Slightly upward deviations were observed with glycine, arginine, and glycylglycine. This discrepancies might be explained by the weaker but probably effective role of the following species: 1) another base like imino or carboxyl group, 2) multi-amino groups which act synergistically, and 3) some groups which act as acid catalyst such as onium group.

10) *a*) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases, A Laboratory Manual," 1-st Edition, Methuen & Co., Ltd., London, 1962, pp. 134—143; *b*) P.G. Stecher, "The Merck Index," 8-th Edition, Merck & Co., Inc., Rahway, 1968.
11) J.T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. II, Academic Press, Inc., New York, 1958, p. 453.
12) K.J. Laidler, "Reaction Kinetics," Pergamon Press (London), 1963, Vol. II, p. 64.
13) A.A. Frost and P.G. Pearson, "Kinetics and Mechanisms," John Wiley & Sons, Inc., New York, 1966, p. 218.
14) T.C. Bruice and S.J. Benkovic, "Bioorganic Mechanisms," Vol. I, W.A. Benzamin, Inc., New York, 1966, pp. 32—37.

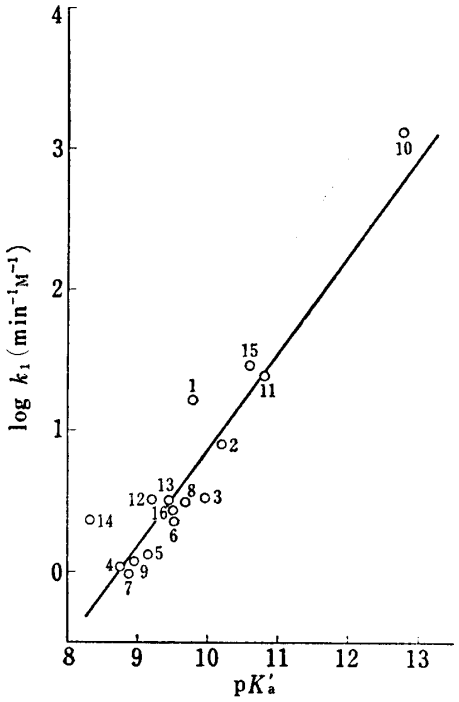

Fig. 2. Brönsted Plot of the Catalytic Rate Constants for the Hydrolysis of FKI (at pH 7.0 and 37°)

The Amine compounds are summarized as in Table I.

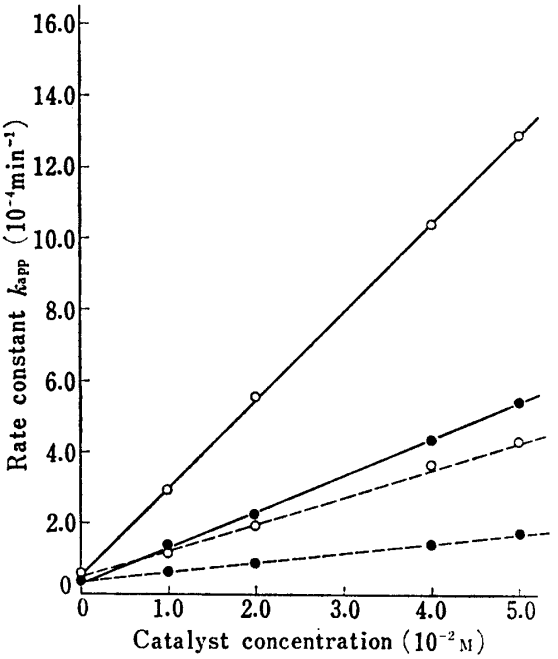

Fig. 3. Deuterium Solvent Isotope Effect for the General Base Catalyzed Hydrolysis of FKI at pH 7.0 and 37°

In a general base catalyzed reaction, the position of the proton in the transition state is dependent on the basicity of the catalyst as well as the basicity of the conjugate base of the substrate. The magnitude of the deuterium solvent isotope effect (k^{H_2O}/k^{D_2O}) is related to the position of the proton in the transition state. When $\beta=0$ no isotope effect should be observed, since the H–C or D–C bonds have not been stretched in the critical transition state. In the area of general-base catalysis a small isotope effect should be obtained at $\beta=0.1$, which increases, more than two in the ratio of k^{H_2O}/k^{D_2O}, to a maximum at $\beta=0.5$, and again decreases to a small value at $\beta=0.9$. This effect was investigated to prove the participation of general-base catalysis. In Fig. 3, the isotope effect is shown, indicating the value of 2.45 for glycine and 2.63 for methylamine. While the absence of such a deuterium isotope effect does not rule out general-base catalysis,[4,15] its presence strongly suggests that a bond to hydrogen is

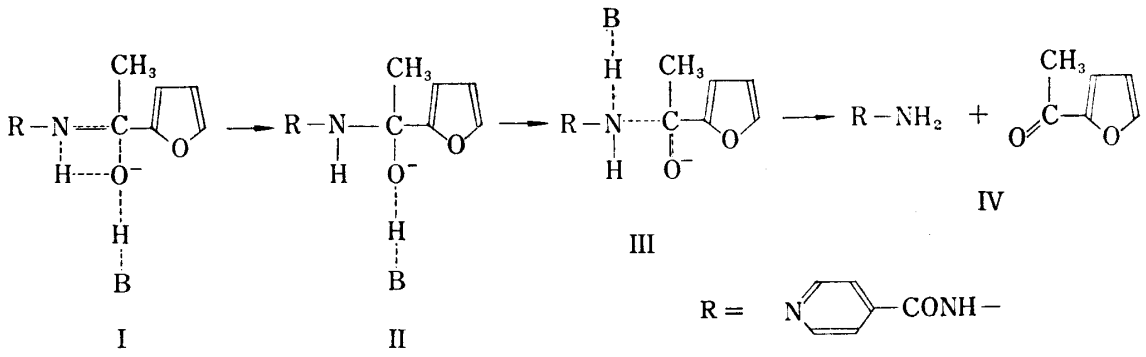

Chart 1

15) S.D. Ross, M. Finkelstein, and R.C. Peterson, *J. Am. Chem. Soc.*, **81**, 5336 (1959).

stretched in the transition state of the catalyzed reaction and that the catalysis involves proton transfer.

From the results described above, the catalysis of FKI hydrolysis by amino group may be considered as general-base catalysis and proceed through the following kinetically indistinguishable transition states which may be preceded by one or more pre-equilibrium steps (Chart 1).

Namely, amino group (B) may react as general base in the intramolecular catalysis of FKI hydrolysis. Nitrogen atom with higher electron density may attract a proton to make a hydrogen bonding, while the base may stretch the other hydrogen bonding between oxygen and itself (I). In the step from (II) to (III), proton transfer may be involved by the base. And the bond cleavage between nitrogen (hydrazide) and carbon (ketone) may occur as the final stage of the hydrolysis.

The above considerations suggest that in the case of hydrazone with a poor leaving group relative to the attacking amino reagent the transition state reflects the expulsion of this group from a species which resembles a tetrahedral addition compound, while with a relatively good leaving group the rate-limiting step reflects principally nucleophilic attack on the hydrazone group and is sensitive to the nucleophilic reactivity of the attacking reagent. In the former case proton transfer becomes an important, or even a necessary, part of the reaction to aid expulsion of the attacking group. It is furthermore suggested that the step from (II) to (III) may be the rate-limiting one, since the decrease of catalytic rate constant in deuterium oxide solvent was found.[14]

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UDC 615.356.011.5 : 577.164.14

Investigations on Pantothenic Acid and Its Related Compounds. XXIV. Chemical Studies. (11).[1] Chemical Synthesis of Coenzyme A Analogs of a Modified Pantetheine Moiety

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In parallel with the synthetic works on coenzyme A (CoA) analogs,[1,3] the approach to the mechanism of enzymatic transacetylation with phosphotransacetylase is in progress by the biochemical research group in this laboratory, during which the importance of pantothenic acid moiety of CoA has been noticed concerning the interaction between CoA and the enzyme. The requirement for the suitable analogs led to initiate the present work. The chemical and trivial names of the synthesized analogs are shown in Table I together with their structures in the simplified forms. The trivial names were given by putting the eliminated moiety with added prefix de- in accordance with the precedent of 3′-de-phospho-CoA.

In the previous work,[4] the thiazoline method developed by us was successfully applied for the total synthesis of CoA from P^1-adenosine 3′-phosphate 5′-P^2-D-pantothenonitrile 4′-

1) Part XXIII: M. Shimizu, O. Nagase, S. Okada, and Y. Hosokawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 313 (1970).
2) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo, 132, Japan.*
3) M. Shimizu, O. Nagase, Y. Hosokawa, and H. Tagawa, *Tetrahedron*, **24**, 5241(1968).
4) M. Shimizu, O. Nagase, S. Okada, Y. Hosokawa, H. Tagawa, Y. Abiko. and T. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **15**, 655 (1967).