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Stabilization of Drugs. II.[1] Neighboring Amide Group Participation in Acidic Hydrolysis of Amide[2]

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Intramolecular catalyzed hydrolysis of amide bond by a second amide group was investigated in the acidic hydrolysis of 2-acylamidobenzamide as model compound. In the 2-arylamido series, the hydrolysis rate decreases as the substituent becomes more electron withdrawing. A Hammett reaction constant was found to be −1.05 and is consistent with the intramolecular catalyzed mechanism. The connection between the present results and penicillin stability in an acidic medium was discussed.

Bender, *et al.*[4] have discussed the possibility of the participation of a neighboring amide group as a nucleophile in the acidic hydrolysis of carboxylic acid derivatives. More recently, Cohen and Lipowitz[5] have realized this situation for the hydrolysis of 2-benzamido-N,N-dicyclohexylbenzamide in acetic acid and sulfuric acid mixtures, of which hydrolysis rate is about 10^4 times faster than N,N-dicyclohexylbenzamide under same conditions. Their observations, however, have been discussed in semi-quantitative terms. It is somewhat surprising that the mechanism and structure-reactivity relationship of the intramolecular catalyzed hydrolysis of amide by a second amide group are still only poorly understood.

We now report the kinetics and mechanism of the neighboring amide group participation and discuss the linear free-energy relationship, in the acidic hydrolyses of 2-acylamidobenzamides as model compound. The results are consistent with the view[6] on the penicillin-reactivity that the introduction of electron-attracting substituents into the side-chain inhibits the hydrolysis in acidic solution.

Experimental

Materials——2-Acylamidobenzamides (a—i) were prepared from anthranilamide (l) and the corresponding acyl chlorides according to the method of Cohen and Lipowitz.[5b] 2-(Benzenesulfonamido)benzamide (j) and 2-(benzylamino)benzamide (k) were prepared by the methods of Franke[7] and Armarego,[8] respectively. Analytical and physical data for these compounds are listed in Table I.

All other chemicals were of reagent grade and used without further purification.

Kinetics——The rates of hydrolysis were determined by following the liberated ammonia in the same manner as reported previously.[9] Reaction were initiated by adding 5 ml of 5.00×10^{-3}M solution of amides in ethanol into 100 ml of preheated hydrochloric acid solution. The ionic strength of all solutions was maintained at 1M with KCl.

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1) Part I: T. Yamana, A. Tsuji and Y. Mizukami, *Chem. Pharm. Bull.* (Tokyo), **21**, 721 (1973).
2) This work was presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April, 1973.
3) Location: *Takara-machi, Kanazawa.*
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5) *a*) T. Cohen and J. Lipowitz, *J. Am. Chem. Soc.*, **83**, 4866 (1961); *b*) *Idem, ibid.*, **86**, 5611 (1964).
6) *a*) F.P. Doyle, J.H.C. Nayler, H. Smith and E.R. Stove, *Nature*, **191**, 1091 (1961); *b*) F.P. Doyle, J.H.C. Nayler, H.R.J. Waddington, J.C. Hansen and G.R. Thomas, *J. Chem. Soc.*, **1963**, 497; *c*) E.P. Abraham, "Topics in Pharmaceutical Sciences," Vol. 1, ed. by D. Perlman, Wiley, New York, 1968, p. 1; *d*) M.A. Schwartz and F.H. Buckwalter, *J. Pharm. Sci.*, **51**, 1119 (1962).
7) E. Franke, *J. Praktishe Chemie*, **44**, 417 (1891).
8) W.L.F. Armarego, *J. Chem. Soc.*, **1961**, 2697.
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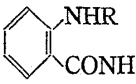
Table I. Analytical and Physical Properties of Compounds



	Amide R	mp[a] (°C) (lit.)	Formula	Analysis (%)					
				Calcd.			Found		
				C	H	N	C	H	N
a	$(CH_3)_3CCO-$	206	$C_{12}H_{16}O_2N_2$	65.42	7.34	12.72	65.78	7.44	12.97
b	CH_3CO-	178(177)[b]	$C_9H_{10}O_2N_2$	60.65	5.67	15.72	60.76	5.80	15.92
c	$C_6H_5CH_2CO-$	127(127)[c]	$C_{15}H_{14}O_2N_2$	70.84	5.56	11.02	70.97	5.61	11.03
d	$C_6H_5OCH_2CO-$	199	$C_{15}H_{14}O_3N_2$	66.65	5.23	10.37	66.87	5.23	10.57
e	$ClCH_2CO-$	185(183—185)[d]	$C_9H_9O_2N_2Cl$	50.83	4.27	13.18	50.99	4.32	13.14
f	$4-MeC_6H_4CO-$	205(204—205)[e]	$C_{15}H_{14}O_2N_2$	70.84	5.56	11.02	70.62	5.52	11.23
g	C_6H_5CO-	215(216)[f]	$C_{14}H_{12}O_2N_2$	69.97	5.04	11.66	70.02	5.21	11.77
h	$4-ClC_6H_4CO-$	204(205)[e]	$C_{14}H_{11}O_2N_2Cl$	61.20	4.04	10.20	61.47	3.77	10.32
i	$4-NO_2C_6H_4CO-$	234(235—236)[e]	$C_{14}H_{11}O_4N_3$	58.94	3.89	14.73	59.02	3.82	14.96
j	$C_6H_5SO_2-$	170[g](167)[h]	$C_{13}H_{12}O_3N_2S$	56.50	4.39	10.14	56.81	4.42	10.29
k	$C_6H_5CH_2-$	173(171—172)[i]	$C_{14}H_{14}ON_2$	74.30	6.25	12.38	74.74	6.19	12.48

a) recrystallized from EtOH
b) R. Anschütz, O. Schmidt and A. Greiffenberg, *Chem. Ber.*, **35**, 3480 (1902)
c) S.C. Pakrashi, J. Bhattacharyya, L.F Johnson and H. Budzikiewicz, *Tetrahedron* **19**, 1011 (1963)
d) W.A. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, **39**, 1439 (1917)
e) D.T. Zentmyer and E.C. Wagner, *J. Org. Chem.*, **14**, 967 (1949)
f) J. Pinnow and C. Sämann, *Chem. Ber.*, **29**, 623 (1896)
g) recrystallized from benzene
h) lit. 7
i) lit. 8

Result

Figure 1 illustrates a typical semilogarithmic plot of the residual ratio of amide against time and shows that the hydrolysis is first-order respect to amides.

The hydrolysis was monitored by absorbance change of the ultraviolet (UV) chromophore as a function of time. The typical spectral curves are illustrated in Fig. 2. One or more isosbestic points were observed.

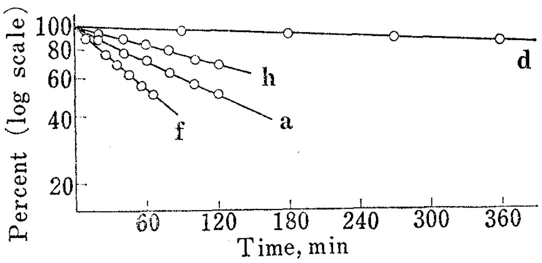


Fig. 1. Typical First-order Plots for the Hydrolyses of 2-Acylamidobenzamides (**a**, **d**, **f** and **h**) in 1 N HCl at 80°

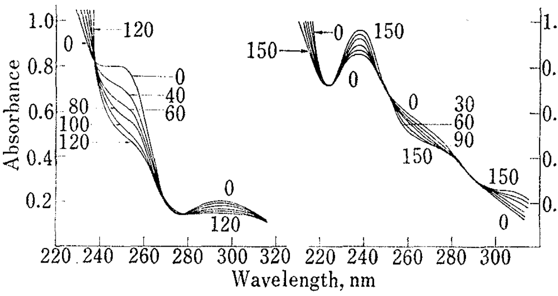


Fig. 2. Typical Spectral Changes for the Hydrolyses of 2-Acylamidobenzamides (**a**, 9.80×10^{-5} M and **h**, 4.95×10^{-5} M) in 1N HCl at 80°

numbers in curves refer to the time in minutes after the start of the reaction

Catalysis of amide hydrolysis by hydronium ion was examined in various concentration range up to *ca.* 1M hydrochloric acid. In this region, all the compounds studied obey the rate law for simple hydronium ion catalysis of Eq. 1

$$k_{obs} = k_{H^+}[H_3O^+] \qquad (1)$$

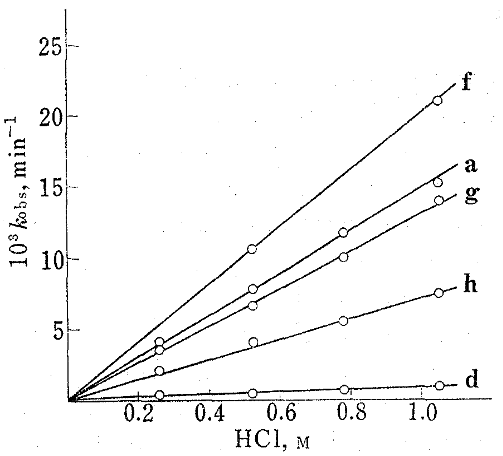


Fig. 3. Observed First-order Rate Constants of the Hydrolyses of 2-Acylamidobenzamides at 90° as a Function of Hydrochloric Acid Concentration

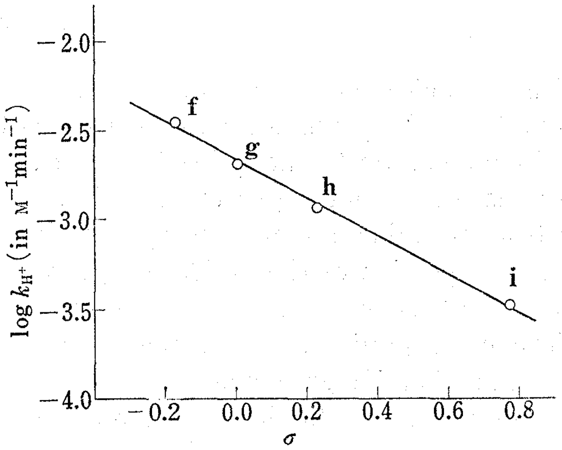


Fig. 4. Hammett Plot of the Catalytic Rate Constants for the Acidic Hydrolyses of 2-Arylamidobenzamides (f—i) at 70° *vs.* σ for the *para* Substituent

where k_{obs} is the observed first-order rate constant and k_{H^+} is the second-order rate constant for hydronium ion catalysis. Example of plots of k_{obs} *vs.* [HCl] is shown in Fig. 3 for five 2-acylamidobenzamides. The k_{H^+} values are listed in Table II.

The rate of acid-catalyzed hydrolysis of 2-arylamidobenzamide decreases as the substituent becomes more electron withdrawing. A Hammett plot of log k_{H^+} *vs.* σ provides a linear correlation with a slope, ρ of $-1.05$10) and with correlation coefficient of 0.997 as shown in Fig. 4.

The effects of temperature on the second-order rate constants, k_{H^+}, for the acidic hydrolyses of various 2-substituted benzamides are shown in Table II. The energies of activation were determined by least squares treatment from Arrhenius plots. The thermodynamic activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, at 70° were calculated according to transition-state theory. These activation parameters are listed in Table II.

TABLE II. Rate Constants and Activation Parameters for the Acidic Hydrolyses of Amides

Amide	Rate constant, $10^3 k_{H^+}$ M^{-1} min^{-1}					Activation parameter at 70°	
	60°	70°	80°	90°	98°	$\Delta H^{\ddagger}$ kcal/mole	$\Delta S^{\ddagger}$ e.u.
a	0.837	2.63	5.68	15.0	30.9	22.0	−14.6
b		3.80	10.4	20.0	41.7	20.5	−18.3
c		0.872	2.12	5.12	10.1	21.4	−18.6
d		0.165	0.402	0.960	2.48	23.4	−16.0
e		0.177	0.462	1.09	2.40	23.0	−17.1
f	1.32	3.55	10.3	20.3		21.7	−14.9
g	0.686	2.02	5.01	13.1		23.0	−12.2
h		1.13	3.30	7.27	15.2	22.6	−14.6
i		0.339	1.04	2.61	4.36	22.7	−16.7
j		0.0565	0.139	0.339	0.728	22.4	−21.1
k		0.0662	0.197	0.412	0.844	22.0	−21.9
l		0.174	0.396	1.06	1.97	21.7	−20.9

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10) The effect of temperature on the ρ values was negligible, suggesting that the isokinetic temperature for the reaction series might be significantly different from the temperature utilized in this study.

Discussion

The second-order rate constants of the acidic hydrolyses of 2-acylamidobezamides (**a**—**i**) are found to be larger than the rate constants for the 2-substituted benzamides not containing second amide group (**k** and **l**) (see Table II). For example, the rate constants of **a** and **b** are approximately 20 times greater than that of **l** under the same conditions. Since the acidic hydrolysis of amide is almost completely insensitive to the electronic effects of substituents on the acyl portion of the amide,[11] this rate enhancement is probably caused by the intramolecular participation of 2-acylamido group to the amide bond cleavage.

The nucleophilic center of neutral amide is known to be the carbonyl oxygen.[12] If the oxygen of amide groups as nucleophile can catalyze the acidic hydrolysis of neighboring amide bond, the introduction of electron-donating substituents to the attacking amide moiety would cause rate acceleration, whereas electron-attracting substituents would retard the reaction. The magnitude and sign of Hammett's reaction constant found for 2-arylamidobenzamides ($\rho = -1.05$) in Fig. 4 are in accordance with this expected direction and with intramolecular mechanism.

In the intramolecularly facilitated hydrolysis of 2-benzamido-N,N-dicyclohexylbenzamide, Cohen and Lipowitz[5] isolated the intermediate, 2-phenyl-4H-3,1-benzoxazin-4-one, from the reaction mixture in dry dioxane with hydrogen chloride. From the result these authors have claimed that the rate facilitation in this diamide hydrolysis is due to intramolecular nucleophilic catalysis by the neighboring amide group rather than to other kinetically equivalent intramolecular catalyses. A similar mechanism, as shown in Chart 1, probably operates with 2-acylamidobenzamides (I) in the acidic hydrolyses, although no direct evidence for the existance of intermediate (II) is available.

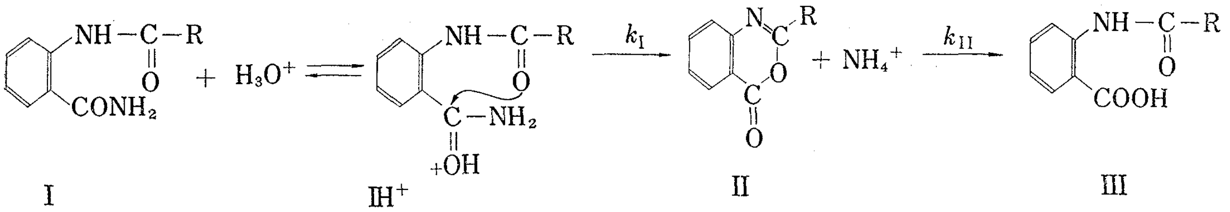

Chart 1

In the present reaction series, no detectable hydrolysis of anilide bond was observed by following liberation of the 2-amino group with diazotization assay. Under the experimental conditions, only the benzamide moiety of I was found to be hydrolyzed by a first-order process. Most amides are extensively protonated on the oxygen atom and these conjugated acids of amides are the reactive species.[12a,13] This common feature to the acidic hydrolysis of amide is reflected in the fact that the reaction rates for the intramolecular catalyzed hydrolyses of 2-acylamidobenzamides have a first-order dependence on the hydronium ion concentration (see Eq. 1 and Fig. 3).

Most recently Williams and Salvadori[14] reported the kinetics for the hydrolysis of benzoxazinones (II). A comparison of their data and present hydrolysis rate constants indicates that benzoxazinones hydrolyze approximately 10^5 times faster than the corresponding diamides

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11) *a*) J.A. Leisten, *J. Chem. Soc.*, **1959**, 765; *b*) J.T. Edwards, H.S. Chang, K. Yates and S. Stewart, *Am. J. Chem.*, **38**, 2271 (1960).
12) *a*) M.L. Bender, "Mechanism of Homogeneous Catalysis from Protons to Proteins," Wiley Interscience, New York, 1971, p. 306; *b*) B.C. Challis and J.A. Challis, "The Chemistry of Amides," ed. by J. Zabicky, Wiley Interscience, 1971, Chap. 13.
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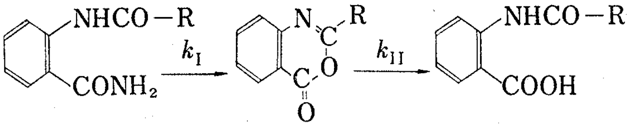
14) A. Williams and G. Salvadori, *J. Chem. Soc.* (*B*), **1971**, 1105.

Table III. Comparison of Rate Constants for the Proposed Consecutive Reaction in the Hydrolyses of 2-Acylamidobenzamides



I II III

R	k_I (25°)[a] M^{-1} min^{-1}	k_{II} (25°)[b] M^{-1} min^{-1}
	$\times 10^{-6}$	$\times 10^{-1}$
CH_3	29.3	19.0[c]
C_6H_5	10.9	12.0[d]
4-MeC_6H_4	23.1	10.5[d]
4-$NO_2C_6H_4$	2.19	4.50[d]
4-BrC_6H_4	—	6.30[d]
4-ClC_6H_4	6.30	—

a) calculated from activation parameters in Table II
b) data of Williams and Salvadori (ref. 14)
c) $\mu=0.1$, in water
d) $\mu=0.6$, in 40% dioxane–HCl aqueous solution

(I) under the same condition as shown in Table III. The ratio of the rate constants (k_I/k_{II}) for the consecutive reaction, $I\xrightarrow{k_I}II\xrightarrow{k_{II}}III$, predicts apparently no detection of this intermediate (II) in the significant concentration in the reaction mixture. This is consistent with the observation of simple time-spectral change for the hydrolysis of diamides as illustrated in Fig. 2.

Instability of penicillin in acidic solution is known to be attributed to the intramolecular catalyzed hydrolysis of β-lactam by side chain amideg roup.[6] This penicillin–penicillenic acid transformation can be inhibited by the introduction of electron-attracting substituent into attacking group side-chain.[6] Doyle, *et al.*[6a,b] found a qualitative correlation between pK_a of the side chain acid and acid stability of the semi-synthetic penicillins. The present series of the intramolecular catalyzed amide hydrolysis by second amide group considerably correlate with pK_a of RCOOH (for attacking group) and the logarithm of their second-order rate constants and verify qualitative penicillin reactivity in acidic medium as discussed by Doyle, *et al.*[6a,b]

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