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Solvent Effect of Dimethyl Sulfoxide on Alkaline Hydrolysis of Amide[1]

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Dimethyl sulfoxide (DMSO) effect on the alkaline hydrolysis rate of amide was studied. Among seven amides studied, *i.e.*, benzamide, *p*-nitrobenzamide, N-methyl-*p*-nitrobenzamide, acetanilide, N-methylacetanilide, N,N-dimethylbenzamide and N,N-dimethyl-*p*-nitrobenzamide, DMSO increased the rates of the last two N,N-dimethylamides, and decreased the rates of the other five amides. Since DMSO increases the ester alkaline hydrolysis rates with desolvation of hydroxide ion, the rate determining step in N,N-dimethylbenzamides hydrolysis was assumed to be the OH^- addition to carbonyl carbon as in ester. And in the other five amides, the rate determining step was assumed to be the leaving of amino group from the tetrahedral intermediate. This assumption was supported by pH dependence of DMSO effect on 2,2,2-trifluoro-N-methylacetanilide hydrolysis of which rate determining step depends on hydroxide ion concentration. From this standpoint, as DMSO decreased the hydrolysis rates of hexobarbital and thiamylal, the rate determining step of barbitals was considered to be C–N bond fission.

To predict chemical reactivity of pharmaceuticals in water-organic mixed solvent is not a new problem. Marcus[3] reported that the increase of acidic degradation rate of chloramphenicol in water-propylene glycol system was explainable with the term of reciprocal dielectric constant of the mixed solvent system. Ikeda[4] analyzed in detail the decrease of hydrolysis rate of hexobarbital in water-ethanol or water-methanol system similarly with the term of reciprocal dielectric constant. The difference between the increase of rate in the former and the decrease in the latter was ascribed to the difference of charges of the reaction species. But the relation between charges of reactants and dielectric constant of system has not always been a successful tool.[5] Garrett[5b] reported that both ethanol and dioxane decreased the dielectric constant of an aqueous solution, however, the former increased the hydrolytic rate of acetylsalicylic acid in a neutral pH range, while the latter did not.

As for dipolar aprotic solvent, such as dimethyl sulfoxide (DMSO), the solvent effect on reaction rate has been studied extensively from the standpoint of synthetic chemistry and reaction mechanism. According to the Parker's review,[6] anionic reactant is desolvated by dipolar aprotic solvent and the rates of various reactions, such as bimolecular substitution, elimination reaction and alkaline hydrolysis of ester, are accelerated, since the activity of reactant anion is increased with desolvation. And he pointed out the usefulness of the solvent effect for the transition state analysis. But it seems unlikely that desolvation of reactant increases always the reaction rate. Watanabe[7] reported that N,N-dimethyl acetamide, one of dipolar aprotic solvents, decreased the hydrolysis rate of acetylsalicylic acid in a pH region where OH^- was operative. Using DMSO, the dipolar aprotic solvent effect on alkaline

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[3] A.D. Marcus and A.J. Taraszka, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 77 (1959).

[4] K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **8**, 504 (1960).

[5] *a*) R. Alexander, E.C.F. Ko, A.J. Parker, and T.J. Broxton, *J. Am. Chem. Soc.*, **90**, 5049 (1968); *b*) E.R. Garrett, *ibid.*, **79**, 3401 (1957).

[6] A.J. Parker, *Chem. Rev.*, **69**, 1 (1969).

[7] K. Watanabe, *Yakugaku Zasshi*, **90**, 1572 (1970).

hydrolysis rate has been studied mainly on ester, while there are many pharmaceuticals which have amide linkage. Accordingly, the authors intended to study dipolar aprotic solvent effect on alkaline hydrolysis of amide. In the present study, anomalous DMSO effect on amide hydrolysis is reported and it is elucidated with the hydrolytic mechanism of amide.

Experimental

Materials——Commercial products were purified as follows. Hexobarbital, thiamylal and ethyl *p*-nitrobenzoate were recrystallized from EtOH–H_2O. Acetanilide, N-methylacetanilide, *p*-nitrobenzamide, benzamide and *p*-nitrobenzoic acid were recrystallized from H_2O. Phenyl acetate was distilled. N,N-dimethylbenzamide was vacuum distilled and then recrystallized from petroleum ether according to Bunton.[8] DMSO was dried in the presence of molecular sieve and then vacuum distilled under the flow of N_2 gas, bp 81° (14 mmHg). N-methylaniline was distilled, bp 65—68° (5 mmHg), and aniline was distilled in the presence of Zn-powder. N-methyl-*p*-nitrobenzamide was prepared according to Wenker,[9] mp 216° (lit. 217°). *Anal.* Calcd. for $C_8H_8O_3N_2$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53.21; H, 4.53; N, 15.60. N,N-dimethyl-*p*-nitrobenzamide was prepared also according to Wenker,[9] mp 97° (lit. 97°). *Anal.* Calcd. for $C_9H_{10}O_3N_2$: C, 55.66; H, 5.19; N, 14.42. Found: C, 55.65; H, 5.07; N, 14.31. 2,2,2-Trifluoro-N-methylacetanilide was prepared according to E.J. Bourne, *et al.*[10] mp 26° (lit. 26—27°). All other reagents were of the purest grade and used without further purification.

Kinetic Procedure——1. For rapid reaction of phenyl acetate, ethyl *p*-nitrobenzoate and 2,2,2-trifluoro-N-methylacetanilide, change of the optical density was recorded directly from the spectrophotometer with temperature controlled cell holder at 25±0.1°. Only exception was that 2,2,2-trifluoro-N-methylacetanilide was hydrolyzed in 60% (v/v) DMSO-glycinate buffer solution. The reaction was too slow to be measured directly as above. In this case, a reaction solution was kept in a thermostatted bath at 25±0.05°, and 3 ml aliquots were pipetted into ultraviolet (UV) cell at approriate times and the optical density was determined immediately.

2. For slow reaction of compounds not mentioned in (1), after a reaction solution was adjusted to 100 ml in a volumetric flask at 25°, the flask was kept in a thermostatted bath at 60±0.1°, and after 15 min for equilibration 2 ml aliquots were pipetted off at approriate times and adjusted to 50 ml with buffer solutions listed in Table I. And then the optical density was determined.

Table I. Details for Kinetic Runs

Compound	Wave length (nm)		Reaction temp. (°C)	Initial concn. $\times 10^4$(M)	Buffer soln. for dten.
	H_2O	DMSO–H_2O			
A. Phenyl acetate	291[a)]	295	25.0	1.93	—[b)]
Ethyl *p*-nitrobenzoate	292[a)]	293	25.0	1.22	—[b)]
p-Nitrobenzamide	295	295	60.0	10.0	Sörensen buffer (pH=10)
Benzamide	280	280	60.0	10.0	0.1N HCl
N,N-Dimethylbenzamide	280	280	60.0	10.0	0.1N HCl
Acetanilide	290	290	60.0	37.5	Sörensen Buffer (pH=10)
N-Methylacetanilide	285	285	60.0	80.0	Sörensen Buffer (pH=10)
2,2,2-Trifluoro-N-methylacetanilide	286[c)]	297	25.0	3.25	—[b)]
B. N-Methyl-*p*-nitrobenzamide	285	285	60.0	15.0	0.1N HCl
N,N-Dimethyl-*p*-nitrobenzamide	291	291	60.0	20.0	0.1N HCl
Hexobarbital	243	243	60.0	20.0	Sörensen buffer (pH=10)
C. Thiamylal	306	306	60.0	3.0	Menzel buffer (pH=10.1)

a) 2% (v/v) DMSO–H_2O soln. b) determined directly in a reaction medium c) 0.5% (v/v) DMSO–H_2O soln.
A: Product was determined.
B: Wave length where the difference of optical density between hydrolysate and product was maximum was used.
C: Optical density of product was negligible.

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8) C.A. Bunton, B. Nayak, and C. O'Connor, *J. Org. Chem.*, 33, 572 (1968).
9) H. Wenker, *J. Am. Chem. Soc.*, 60, 1081 (1938).
10) E.J. Bourne, S.H. Henry, C.E.M. Tatlow, and J.C. Tatlow, *J. Chem. Soc.*, 1952, 4014.

First order rate constants were calculated with the usual method. The infinite optical density necessary for calculation was determined at more than 10 times half life of each condition. Wave lengths for determination and other conditions for kinetic procedure were listed in Table I.

Instruments——Toa Dempa pH meter model HM-5A was used for pH measurement, and Hitachi spectrophotometer model 124 was used for spectroscopy.

Result and Discussion

DMSO Effect on Hydrolysis Rate of Hexobarbital and Thiamylal

Since barbituric acid derivatives are very important pharmaceuticals which have -CO-NH- or -CS-NH- linkage, DMSO effect on alkaline hydrolysis of hexobarbital and thiamylal was studied. As shown in Fig. 1, DMSO decreases the hydrolysis rates of them for a wide pH region where a specific hydroxide ion catalysis is dominant.[11] Although there have been many reactions of which rates are increased by DMSO,[6] such a DMSO effect to decrease the reaction rate in a wide pH region is not reported. As far as the hydrolysis rate, ester has been a usual test compound for DMSO effect in the scope of the authors' literature survey.[12] Therefore, the possibility was considered that the decreasing effect of DMSO was specific for amide hydrolysis.

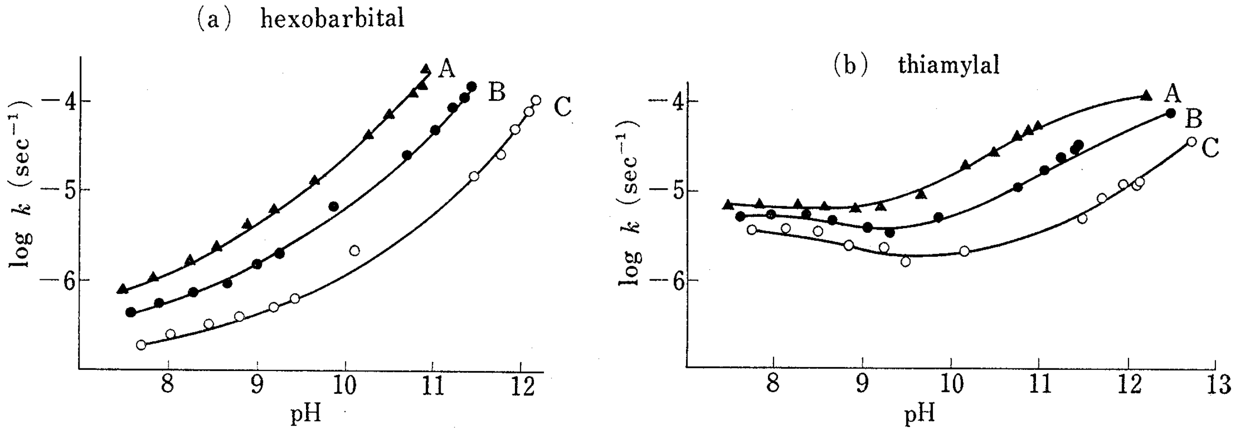

Fig. 1. pH Profile of Hydrolysis Rate Constants of Hexobarbital and Thiamylal in Water and Dimethyl Sulfoxide (DMSO)–Water Mixtures at 60°

A: water, B: 20% (v/v) DMSO–water, C: 40% (v/v) DMSO–water
Values of pH read from glass electrode were used in abscissa both for water and DMSO–water mixtures.

DMSO Effect on Hydrolysis Rates of Various Esters and Amides

In order to check the above possibility, DMSO effect was studied for various esters and amides. The compounds were chosen for convenience sake of determination. The results were written in Table II. Hydrolysis rates of all esters examined are increased with DMSO. This coincides well with past works for substituted ethyl benzoates,[12a,b] ethyl acetate[12c] and dicarboxylic esters, such as diethyl succinate.[12d] On the other hand, DMSO decreases or increases hydrolysis rates of amide depending on its structure. Accordingly the decreasing effect of DMSO is not necessarily observed in amide.

Mechanism of DMSO Effect

The mechanism of ester hydrolysis has been generally known as Chart 1.[13] Since the experimentally observed rate constant (k_{obs}) is described as $k_{obs}=k_1[OH^-]/(k_2/k_3+1)$, therefore,

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[11] E.R. Garrett, J.T. Bojarski, and G.J. Yakatan, *J. Pharm. Sci.*, **60**, 1145 (1971).

[12] *a*) M. Hojo, M. Utaka, and Z. Yoshida, *Yuki Gosei Kagaku Kyokaishi*, **23**, 1040 (1965); *b*) E. Tommila and I. Palenius, *Chem. Acta Scand.*, **17**, 1980 (1963); *c*) E. Tommila and M. Murto, *ibid.*, **17**, 1947 (1963); *d*) N. Venkatasubramanian and G.V. Rao, *Tetrahedron Letters*, **52**, 5275 (1967).

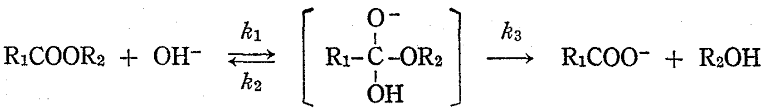
[13] M.L. Bender, *Chem. Rev.*, **60**, 53 (1960).

Table II. Dimethyl Sulfoxide (DMSO) Effect on Hydrolysis Rates of Amides and Esters

Compound	NaOH(M)	Temp. (°C)	k_0(sec^{-1})	k_{20}/k_0	k_{40}/k_0	k_{60}/k_0
Benzamide	0.1	60	9.76×10^{-6}	0.52	0.22	0.11
p-Nitrobenzamide	0.1	60	1.53×10^{-4}	0.60	0.40	0.23
N-Methyl-*p*-nitrobenzamide	0.1	60	3.22×10^{-5}	0.62	0.40	0.34
Acetanilide	0.1	60	9.63×10^{-6}	0.43	0.25	0.11
N-Methylacetanilide	0.1	60	8.63×10^{-6}	0.85	0.72	0.58
Phenyl acetate	2.4×10^{-3}	25	$2.39\times10^{-3a)}$	1.09	1.31	2.17
Ethyl *p*-nitrobenzoate	3.3×10^{-2}	25	$1.86\times10^{-2a)}$	—	1.44	2.92
N,N-Dimethylbenzamide	0.1	60	1.03×10^{-5}	1.03	1.05	1.40
N,N-Dimethyl-*p*-nitrobenzamide	0.1	60	1.05×10^{-4}	1.17	1.85	3.07

k_0: rate constant in H_2O, k_{20}: rate constant in 20% (v/v) DMSO–H_2O, k_{40}: rate constant in 40% (v/v) DMSO–H_2O, k_{60}: rate constant in 60% (v/v) DMSO–H_2O

a) k_0 was obtained in 2% (v/v) DMSO–H_2O.



$$R_1COOR_2 + OH^- \underset{k_2}{\overset{k_1}{\rightleftarrows}} \left[R_1-\overset{O^-}{\underset{OH}{C}}-OR_2 \right] \xrightarrow{k_3} R_1COO^- + R_2OH$$

Chart 1. Mechanism of Ester Hydrolysis

according to Parker,[3] desolvation of hydroxide ion increases its activity resulting in the increase of k_{obs}. The present results of esters are explained well with this Chart.

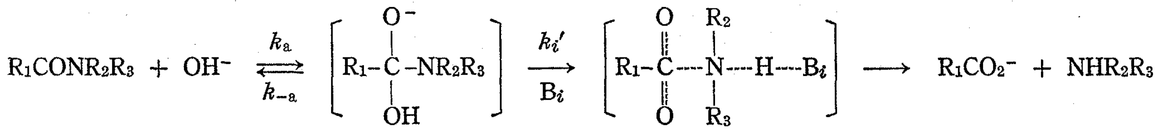
As for amide hydrolysis, Schowen[14] proposed the mechanism in Chart 2. The intermediate adduct of hydroxide ion on carbonyl carbon is hydrolyzed with specific hydroxide ion and general base catalysis, involving proton transfer step. The observed rate constant is given in a general form of Eq. (1),[14]

$$k_{obs} = \frac{k_a[OH^-]\{\sum_i k_i[B_i]\}}{k_a + \sum_i k_i[B_i]} \tag{1}$$

where B_i represents hydroxide ion, water or a general base, and $k_i = k_a k_i'/k_{-a}$ (see Chart 2). In a simple case where hydroxide ion concentration is low and the term of $\sum_i k_i[B_i]$ is neglected for k_a in the denominator of Eq. (1), the leaving of amino group from the intermediate is rate-determining, and further when only water is assumed to be acting as a general base, Eq. (1) is simplified as Eq. (2),

$$k_{obs} = k_2[OH^-][H_2O] = \frac{k_a k_2'}{k_{-a}}[OH^-][H_2O] \tag{2}$$

where k_2' is the catalytic rate constant of water.



$$R_1CONR_2R_3 + OH^- \underset{k_{-a}}{\overset{k_a}{\rightleftarrows}} \left[R_1-\overset{O^-}{\underset{OH}{C}}-NR_2R_3 \right] \xrightarrow[B_i]{k_i'} \left[R_1-\overset{O}{\underset{O}{C}}\cdots\overset{R_2}{\underset{R_3}{N}}\cdots H\cdots B_i \right] \longrightarrow R_1CO_2^- + NHR_2R_3$$

Chart 2. Mechanism of Amide Hydrolysis

In this case, DMSO effect on amide hydrolysis is explained as follows. Even when DMSO desolvates hydroxide ion and increases its activity, k_{obs} is decreased with the decrease of k_2'

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[14] R.L. Schowen, C.R. Hopper, and C.M. Bazikian, *J. Am. Chem. Soc.*, **94**, 3095 (1972).

with the solvation between DMSO and water[12c,15] and further with the decrease of water concentration.

The only exception was observed for N,N-dimethylamide compounds where DMSO increased the hydrolysis rates. Since the reactivity of the intermediate was considered to have a key for the explanation of the above phenomenon, ^{18}O-exchange reaction results and solvent isotope effects for amide and ester hydrolysis were looked up in the literature and the results were written in Table III. The ratio of exchange reaction rate constant (k_e) to hydrolysis rate constant (k_h), *i.e.*, $k_e/k_h=k_2/2k_3$[16] is greater than 1 for benzamide and N-methylbenzamide, which shows that the leaving of hydroxide ion from the intermediate is faster than that of amino group. This is well coincident with the above discussion where the leaving of amino group from the intermediate was assumed the rate determining step to explain DMSO effect which decreased the hydrolysis rates of benzamide, *p*-nitrobenzamide, *etc.* On the other hand, k_e/k_h is much less than 1, for N,N-dimethylbenzamide. This means that the leaving of amino group is much faster than that of hydroxide ion. And in the comparison of isotope effect for ester hydrolysis,[17] Bunton[8] pointed out that alkaline hydrolysis of N,N-dimethylbenzamide proceeded with the similar mechanism as ester. This is also supported with the Bender's results[18] which show that k_e/k_h is less than 1 for ester as written in Table III. From these, it can be said that desolvation of hydroxide ion with DMSO increases its activity and increases the hydrolysis rate of N,N-dimethylbenzamide, because the hydrolysis mechanism of it is similar to that of ester rather than that of usual amide. In ester, hydroxide ion addition to the intermediate is dominating the hydrolysis rate, as mentioned in the former section. To support this discussion, the further proof was investigated.

TABLE III. Deuterium Isotope Effect and ^{18}O-Exchange Reaction in Alkaline Hydrolysis of Esters and Amides

Compound	k_{H_2O}/k_{D_2O}	k_e/k_h	Lit. no.
Benzamide	1.4	4.7	8
N-Methylbenzamide	—	1.38	8
N,N-Dimethylbenzamide	0.88	0.02	8
Ethyl benzoate	—	0.21	16
Methyl *p*-toluate	—	0.09	18
Ethyl acetate	0.71	—	17

k_{H_2O}: hydrolysis rate constant in H_2O, k_{D_2O}: hydrolysis rate constant in D_2O,
k_e: ^{18}O-exchange rate constant, k_h: hydrolysis rate constant

DMSO Effect on 2,2,2-Trifluoro-N-Methylacetanilide Hydrolysis

As it has been known that the rate-determining step of 2,2,2-trifluoro-N-methylacetanilide depends on hydroxide ion concentration according to Eq. (1),[19] DMSO effect on its hydrolysis was investigated at different hydroxide ion concentrations. When hydroxide ion concentra-

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[15] J.M.G. Cowie and P.M. Toporowski, *Can. J. Chem.*, 39, 2240 (1961); R.K. Wolford, *J. Phys. Chem.*, 68, 3392 (1964); B.G. Cox and T. McTigue, *Australian J. Chem.*, 20, 1815 (1967).

[16] M.L. Bender and R.D. Ginger, *J. Am. Chem. Soc.*, 77, 348 (1955). Hydrolytic reaction is described as follows.

$$\mathrm{RCO{-}X + OH^-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{RCO^-(OH){-}X} \xrightarrow{k_3} \mathrm{RCOO^- + HX}$$

[17] K.B. Wiberg, *Chem. Rev.*, 55, 713 (1955); J.G. Pritchard and F.A. Long, *J. Am. Chem. Soc.*, 78, 6008 (1956).

[18] M.L. Bender and R.J. Thomas, *J. Am. Chem. Soc.*, 83, 4189 (1961).

[19] R.L. Schowen, H. Jayaraman, and L. Kershner, *J. Am. Chem. Soc.*, 88, 3373 (1966); R.L. Schowen, H. Jayaraman, L. Kershner, and G.W. Zuorick, *ibid.*, 88, 4008 (1966).

tion is high enough, Eq. (1) is $k_{\mathrm{obs}}=k_{\mathrm{a}}[\mathrm{OH}^-]$, and then DMSO is to increase k_{obs} with the increase of hydroxide ion activity, and on the other, DMSO is to decrease the hydrolysis rate as discussed for Eq. (2), when hydroxide ion concentration is low.

In NaOH solution (for high hydroxide ion concentration) and in NaOH-glycine buffer solutios (for low hydroxide ion concentration), the hydrolysis rate constants of the amide were obtained and the results were written in Table IV. They are in a good coincidence with the values calculated with the catalytic constants and equation reported by Schowen.[20] Accordingly, the term of each catalyst was calculated in each solution with the rate constants of Schowen, and the value of k_{a} (rate constant of OH^- addition) was compared with the value of $\sum_i k_i[\mathrm{B}_i]$ (the base catalyzed rate constant of the amino group from the intermediate) as written in Table IV. It is clearly seen that $\sum_i k_i[\mathrm{B}_i]$ is greater than k_{a} in 1.22×10^{-2}M NaOH, and that $\sum_i k_i[\mathrm{B}_i]$ is less than k_{a} in NaOH-glycine buffer solutions. This shows that the rate determining step of this amide hydrolysis is different in NaOH from in NaOH–glycine buffer solution. Consequently, DMSO effect was studied in NaOH and NaOH–glycine buffer solutions as in Table IV, to show that DMSO effect depended on the rate determining step. The results were shown in Table IV and in Fig. 2. As seen clearly, DMSO increases the hydrolysis rate in NaOH solution where hydroxide ion addition is rate-determining,[21] while it decreases the rate in NaOH-glycine buffer solution where the leaving of amino group is rate-determining.[21] This is in a fair agreement with the results predicted in the former section.

TABLE IV. Hydrolysis Rate Constants of 2,2,2-Trifluoro-N-Methylacetanilide and Dimethyl Sulfoxide (DMSO) Effect on It

	$k_{\mathrm{obs}}(\mathrm{exp})^{a)}$	$k_{\mathrm{obs}}(\mathrm{cal})^{b)}$	$k_{\mathrm{a}}^{c)}$	$\sum_i k_i[\mathrm{B}_i]^{d)}$	$k_{\mathrm{a}}/\sum_i k_i[\mathrm{B}_i]$	$k_{60}/k_0^{e)}$
1.22×10^{-2}M NaOH	8.0×10^{-2}	9.8×10^{-2}	11.5	26.4	0.44	3.24
Buffer 1	4.7×10^{-4}	5.5×10^{-4}	11.5	4.35	2.64	0.027
Buffer 2	2.9×10^{-4}	3.2×10^{-4}	11.5	4.72	2.44	0.024
Buffer 3	2.2×10^{-4}	2.5×10^{-4}	11.5	5.15	2.23	0.024

a) First order rate constant (sec^{-1}) obtained in the present report at 25°.
b) First order rate constant (sec^{-1}) calculated according to lit. 20.
c) OH^- addition rate constant ($\mathrm{M}^{-1}\mathrm{sec}^{-1}$) obtained from lit. 20. (see Chart 2).
d) Sum of the base catalyzed rate constants ($\mathrm{M}^{-1}\mathrm{sec}^{-1}$). Values of k_is were obtained from lit. 20 (see Chart 2.).
e) k_{60}: rate constant in 60% (v/v) DMSO–$\mathrm{H_2O}$, k_0: rate constant in $\mathrm{H_2O}$, same with k_{obs} (exp)
buffer 1: glycine: sodium glycinate=1: 2, $[\mathrm{OH}^-]=11.40\times10^{-5}$M
buffer 2: glycine: sodium glycinate=1: 1, $[\mathrm{OH}^-]=5.85\times10^{-5}$M
buffer 3: glycine: sodium glycinate=3: 2, $[\mathrm{OH}^-]=3.96\times10^{-5}$M
Sodium glycinate concentraton was 5×10^{-3}M in these buffer solutions.

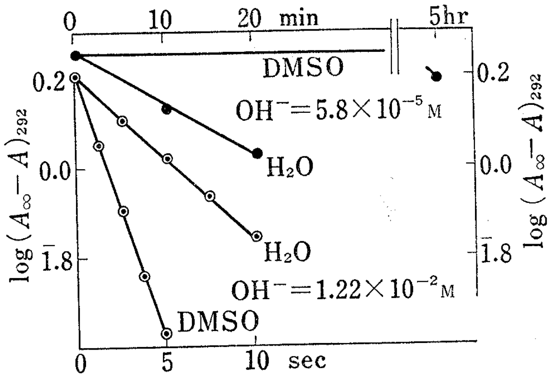

Fig. 2. DMSO Effects on Hydrolysis Rates of 2,2,2-Trifluoro-N-Methylacetanilide

DMSO–$\mathrm{H_2O}$: 60% (v/v) DMSO–water
Left coordinate is for open circles which represent results in 1.22×10^{-2}M NaOH, and right coordinate is for full circles which represent results in NaOH-glycine buffer solution ($[\mathrm{OH}^-]=5.85\times10^{-5}$M) (See Table IV).

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[20] R.L. Schowen, H. Jayaraman, and L. Kershner, *Tetrahedron Letters*, **5**, 497 (1966); R.L. Schowen and G.W. Zuorick, *J. Am. Chem. Soc.*, **88**, 1223 (1966).

[21] Strictly speaking, the difference between k_{a} and $\sum_i k_i[\mathrm{B}_i]$ is not so large enough to say which step is rate-determing definitely. The values of pH studied here was chosen for the convenience of kinetic procedure, therefore, 'rate-determining' is roughly used here for the straightforward discussion.

During the preparation of the present report, the authors have known that Gani, *et al.*[22] studied DMSO effect on alkaline hydrolysis of amide. They considered that DMSO effect depended on the necessity of proton transfer for the leaving of amino group from the OH^- addition intermediate. And they showed that for the alkaline hydrolysis of N-methylacetanilides, CH_3-CO-N(CH_3)-C_6H_4-X, DMSO increased the hydrolysis rate of a compound, X=*p*-NO_2 and that DMSO decreased the rates of compounds, X=H and *p*-OCH_3. In the former compound, C-N bond fission was considered to be occurred without proton transfer, since *p*-NO_2 group is electron attracting. The mechanism of DMSO solvent effect on amide hydrolysis proposed in the present report is very similar to theirs, although the rate-determining step is focused in the present report.

From these results, DMSO is considered to be a tool to study the rate-determining step of hydrolysis. And from this standpoint, the hydrolysis process of barbitals is considered to have the C-N bond fission as a rate-determining step, since DMSO decreases the hydrolysis rates of hexobarbital and thiamylal.

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22) V. Gani, P. Viout, and M.H. Normant, *C. R. Acad. Sc. Paris*, **274**, 1746 (1972); V. Gani and P. Viout, *Tetrahedron Letters*, **51**, 5241 (1972).