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Polar and Hydrophobic Effects on Ester Aminolysis in Dimethyl Sulfoxide

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Aminolysis of various carboxyl-containing esters by several tetra-, tri-, and diamines was kinetically studied in dimethyl sulfoxide. Rates were measured in the presence or absence of added sulfuric acid with the amine concentration being much greater than the ester concentration. In some reactions, pseudo-first-order rate constants manifested saturation kinetic behavior with respect to the total amine concentration, indicating the formation of complexes between the amines and the esters. The complex formation was most efficient when the carboxyl group of the ester substrate was located at the *meta* position to the ester group. In addition, the complex formation was facilitated by hydrophobic amines. In order to explain the positional stereoselectivity and the hydrophobic effects observed in the kinetic study, a structure of the complex is proposed. In this structure of the complex, two nitrogen atoms of the amine are linked by an intramolecular hydrogen bond and then further interact with the two carbonyl oxygen atoms of the substrate. In addition, the hydrophobic interaction between the hydrophobic portion of the amine and the benzene ring of the substrate stabilizes the complex. Dimethyl sulfoxide accommodates both the polar and the hydrophobic interactions that are needed in the formation of the complex, mimicking the microenvironment of enzyme active sites.

INTRODUCTION

Complex formation between substrates and enzymes is essential for enzymatic reactions. For the formation of an enzyme–substrate complex, polar and hydrophobic interactions between the substrate and the active site of the enzyme play important roles. If both of these interactions are involved, the microenvironment of the active site must be suitable for both of them. Polar interactions would be facilitated by solvents of low polarity, while hydrophobic interactions by those of high polarity.

In order to gain insight into the solvent character within active sites, we investigated aminolysis of carboxyl-containing nitrophenyl esters in dimethyl sulfoxide (DMSO).[2] The esters employed in the present study are *p*-nitrophenyl acetate (**1**), *2*-carboxy-*4*-nitrophenyl acetate (**2o**), *3*-carboxy-*6*-nitrophenyl acetate (**2m**), *4*-carboxy-*2*-nitrophenyl acetate (**2p**), *p*-nitrophenyl isophthalate (**3m**), *p*-nitrophenyl terephthalate (**3p**), *3*-carboxy-*6*-nitrophenyl caproate (**4m**), and *4*-car-

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[2] Abbreviation used: DMSO, dimethyl sulfoxide.

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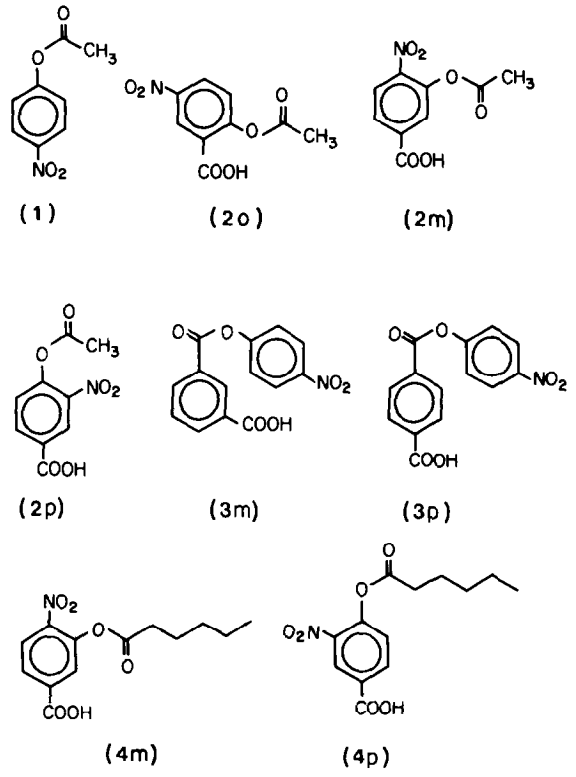
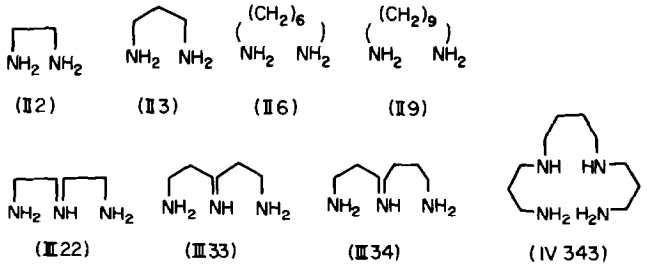


SCHEME 1

boxy-2-nitrophenyl caproate (**4p**) (Scheme 1). The amines examined in this study are 1,2-diaminoethane (**II2**), 1,3-diaminopropane (**II3**), 1,6-diaminohexane (**II6**), 1,9-diaminononane (**II9**), diethylenetriamine (**III22**), 3,3′-diaminodipropylamine (**III33**), spermidine (**III34**), and spermine (**IV343**) (Scheme 2).

The amines and the carboxyl-containing esters would form complexes in organic solvents by utilizing the polar interactions between the amino and carboxyl groups. In addition, hydrophobic interactions between the hydrophobic portions of the esters and the amines might further enhance the complex formation. DMSO



SCHEME 2

is chosen as the solvent which could accommodate both polar and hydrophobic interactions, mimicking the microenvironments of active sites.

Dipolar aprotic solvents such as DMSO lack the ability to stabilize anions through hydrogen bonding, although these solvents act as acceptors of hydrogen bonds. The properties of ions in these solvents are, therefore, often intermediate between those in water and the gas phase, providing an important bridge between these two extremes (*1*). Thus, the study of complexation in DMSO can provide information for medium effects on nonenzymatic properties and reactions, too.

MATERIALS AND METHODS

Materials. Esters, except for **3m** and **4m**, were prepared according to the literature (*2–4*), and their melting points agreed with the reported values within ±2°C. Ester **3m** was synthesized by the method (*3*) reported for **3p** and recrystallized from benzene–hexane, mp 209–210°C. Ester **4m** was prepared according to the method (*4*) reported for **4p** and recrystallized from ethanol–cyclohexane, mp 139–140°C. Amines used in this study were purchased from commercial sources and were purified by distillation or recrystallization. DMSO was purified according to the literature (*5*). Fuming sulfuric acid was used without further purification, and the titration with sodium hydroxide indicated that the acid content of the bottle used in this study was 105%. The sulfuric acid, therefore, was free of moisture and contained about 5% sulfur trioxide. The DMSO solutions of the amines were prepared and handled under a nitrogen atmosphere.

Kinetic measurements. Reaction rates were measured with a thermojacketed Beckman Model 25 spectrophotometer by following the release of nitrophenol portions of the products. Temperature was controlled to within ±0.1°C with a Haake E52 circulator. Total concentrations of the amines (N_0) were kept at least 20 times greater than the initially added concentrations of the substrates (S_0) ($0.1–1 \times 10^{-4}$ M) to ensure the pseudo-first-order conditions. The pseudo-first-order rate constants (k_0) were not affected by the changes in S_0 ($0.1–1 \times 10^{-4}$ M) as long as the N_0 and the concentration of added sulfuric acid were unchanged. The k_0 values were calculated with the infinity absorbance readings measured.

RESULTS

Aminolysis of the ester substrates was followed under the condition $N_0 \gg S_0$ at 25°C. The k_0 values were measured at several N_0. As exemplified by the aminolysis of a part of the esters by **IV343** illustrated in Fig. 1, k_0 was proportional to N_0 or manifested saturation kinetic behavior with respect to N_0.

When k_0 was proportional to N_0, k_{bi} (Eq. [1]) was taken as the average value of k_0/N_0. The k_{bi} values for the aminolysis reactions measured in the absence of added sulfuric acid (k_{bi}^0) are summarized in Table 1:

$$k_0 = k_{bi}N_0. \qquad [1]$$

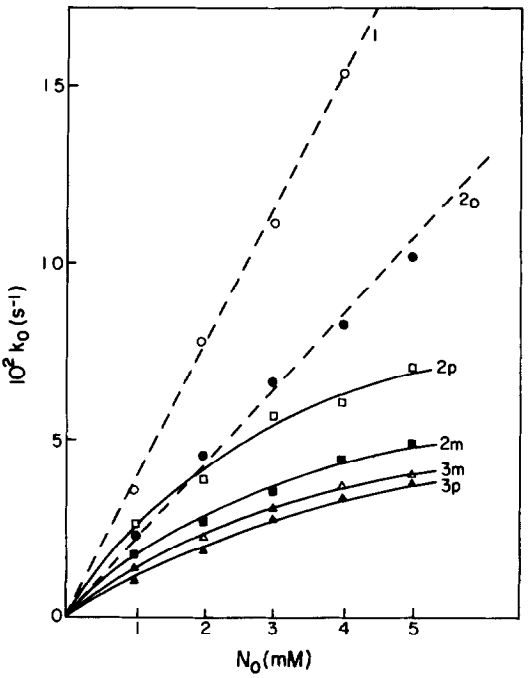


FIG. 1. Plot of k_0 against N_0 for the aminolysis of **1** (○), **2o** (●), **2m** (■), **2p** (□), **3m** (△), and **3p** (▲) by **IV343** in the absence of added sulfuric acid.

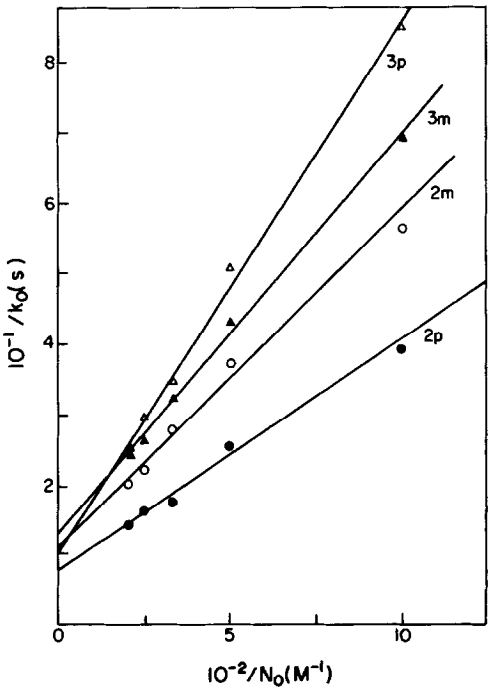


FIG. 2. Plot of $1/k_0$ against $1/N_0$ for the aminolysis of **2m** (○), **2p** (●), **3m** (▲), and **3p** (△) by **IV343** in the absence of added sulfuric acid.

TABLE 1

VALUES OF k_{bi}^0 (s^{-1} M^{-1}) AND k_{bi}^* (s^{-1} M^{-1})[a]

	II2	II3	II6	III22	III33	III34	II9	IV343
1	31 (20) [65]	40 (23) [58]	37 (32) [86]	28 (16) [57]	43 (32) [74]	38 (38) [100]	38 (29) [76]	39 (33) [85]
2o	16 (12) [75]	21 (20) [95]	27 (24) [89]	38 (—)	25 (—)	26 (—)	25 (—)	22 (—)
2m	3.4 (2.3) [68]	5.1 (3.2) [63]	— (—)	6.3 (—)	13 (—)	— (—)	— (—)	— (—)
2p	7.0 (4.2) [60]	8.5 (5.4) [64]	11 (9.2) [84]	7.7 (4.3) [56]	13 (—)	13 (15) [120]	15 (—)	— (—)
2m	3.3 (2.0) [61]	4.0 (2.6) [65]	4.7 (3.8) [81]	4.2 (—)	7.2 (—)	— (—)	— (—)	— (—)
3p	3.9 (2.6) [67]	4.8 (3.1) [65]	5.6 (4.7) [84]	3.4 (2.4) [71]	6.0 (—)	6.5 (—)	8.5 (—)	— (—)
4m	1.3 (0.76) [58]	2.3 (1.9) [83]	— (—)	1.5 (—)	4.2 (—)	— (—)	— (—)	— (—)
4p	2.8 (1.8) [64]	4.8 (3.7) [77]	3.4 (2.6) [76]	2.5 (1.9) [76]	4.2 (—)	4.1 (—)	5.7 (—)	7.4 (—)
Average of k_{bi}^*/k_{bi}^0 (%)[b,c]	65 ± 5 (8)	71 ± 12 (8)	83 ± 4 (6)	65 ± 9 (4)	74 (1)	110 ± 10 (2)	76 (1)	85 (1)

[a] k_{bi}^0 values are given followed by k_{bi}^* values within parentheses. The standard deviations of k_{bi}^0 and k_{bi}^* are 5–10% of the parameter values. In the reactions for which no k_{bi}^0 and k_{bi}^* values are listed, saturation kinetic behavior is observed. The values in brackets are the k_{bi}^*/k_{bi}^0 ratios. For N_0^{max} values, see Table 2.

[b] The numbers in parentheses represent the numbers of data available.

[c] The ratio of k_2^+/k_2^0 is given by $(3k_{bi}^* - 2k_{bi}^0)/k_{bi}^0$ (see the text). The values of k_2^+/k_2^0 thus evaluated and used in the estimation of k_c^+ are 50% for **II6**, 20% for **III33**, 130% for **III34**, 30% for **II9**, 50% for **IV343**, and 0% for the rest of the amines.

When saturation kinetic behavior was observed, rate data were analyzed in terms of

$$N + S \underset{K_m}{\rightleftharpoons} NS \xrightarrow{k_{cat}} \text{products} \qquad [2]$$

which is analogous to the Michaelis–Menten scheme:

$$K_m = N_0[S]/[NS]. \qquad [3]$$

The pseudo-first-order rate constant for this scheme is derived as

$$k_0 = k_{cat}N_0/(K_m + N_0) \qquad [4]$$

under the condition $N_0 \gg S_0$. The plot of $1/k_0$ against $1/N_0$ produced a straight line as illustrated in Fig. 2 for the aminolysis by **IV343**. From the intercept and the slope of the straight line

TABLE 2

VALUES OF k_{cat} (10^{-3} s^{-1}) AND K_m (mM) FOR THE REACTIONS IN WHICH SATURATION KINETIC BEHAVIOR IS MANIFESTED[a]

		II6		III22	III33	III34		II9		IV343	
	α:	0	⅓	⅓	⅓	0	⅓	0	⅓	0	⅓
N_0^{max} (mM)[b]:		5	5	1.1	1.5	5	1.2	1	1	5	1.2
2o	k_{cat}	—	—	49	53	—	56	—	37	—	35
	K_m	—	—	0.67	1.5	—	0.88	—	0.82	—	0.90
2m	k_{cat}	110	57	9.9	30	110	48	34	27	93	27
	K_m	8.8	3.3	0.39	0.61	4.6	0.95	1.1	0.39	4.5	0.58
2p	k_{cat}	—	—	—	53	—	—	—	22	130	56
	K_m	—	—	—	2.8	—	—	—	0.61	4.2	0.86
3m	k_{cat}	—	—	6.2	14	72	22	13	9.9	77	23
	K_m	—	—	0.44	0.57	7.3	1.0	1.2	0.37	4.4	0.46
3p	k_{cat}	—	—	—	16	—	15	—	16	100	21
	K_m	—	—	—	1.7	—	0.98	—	0.75	7.7	0.62
4m	k_{cat}	31	18	3.0	6.6	32	9.7	7.1	6.5	36	6.2
	K_m	9.2	4.3	0.29	0.48	6.9	0.82	0.87	0.41	8.4	0.52
4p	k_{cat}	—	—	—	11	—	11	—	11	—	16
	K_m	—	—	—	1.8	—	1.5	—	1.0	—	0.86

[a] Standard deviations for k_{cat} and K_m are 3–20% of the parameter values. In the reactions for which no numbers are listed, k_0 was proportional to N_0.

[b] N_0^{max} values for the amines which are not listed in this table are 5 mM when $\alpha = 0$ and 1.0 mM (**II2**) or 1.2 mM (**II3**) when $\alpha = \frac{1}{3}$.

$$1/k_0 = 1/k_{cat} + (K_m/k_{cat})(1/N_0) \quad [5]$$

the values of k_{cat} and K_m were estimated. The values of k_{cat} and K_m for the aminolysis reactions measured in the absence of added sulfuric acid are summarized in Table 2.

Rates of the aminolysis reactions were also measured in the presence of added sulfuric acid. The amount of sulfuric acid was maintained at one-third the N_0. The k_0 values measured under the condition $N_0 \gg S_0$ depended on N_0 according to either Eq. [1] or [4]. The values of k_{bi}^* (k_{bi} measured in the presence of added sulfuric acid), k_{cat}, and K_m for these reactions are summarized in Tables 1 and 2. Parameter K_m was defined as in Eq. [3], in both the presence and the absence of added sulfuric acid. When the acid is added, N_0 represents the total concentration of both unprotonated and protonated amine.

Either because the solubility of each amine was limited or because the rate was too fast at a large N_0, the largest N_0 used for each amine (N_0^{max}) was 1–5 mM (Table 2). When K_m was greater than $2N_0^{max}$, saturation kinetics were not readily distinguishable from bimolecular kinetics. Thus, an apparently bimolecular reaction may actually proceed through Eq. [2] with the K_m value being greater than $2N_0^{max}$.

Aminolysis of **1** by **III33** in the absence of added sulfuric acid was examined in various DMSO–water mixtures, and the results are illustrated in Fig. 3.

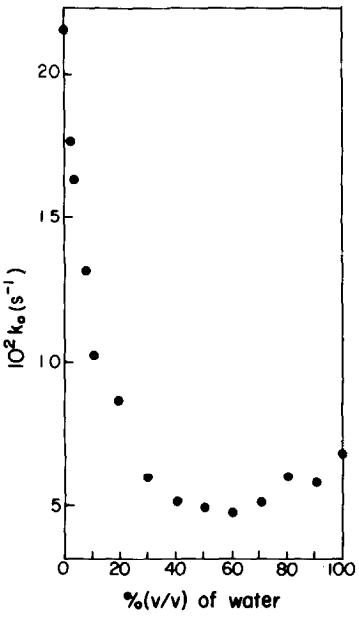


FIG. 3. The k_0 for the aminolysis of **1** by **III33** measured in various DMSO–water mixtures in the absence of added sulfuric acid.

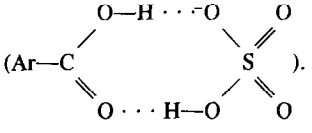
DISCUSSION

Analysis of Kinetic Data

Kinetic data obtained for the aminolysis reactions were further analyzed in terms of Scheme 3, since both bimolecular and saturation kinetics were observed in the absence or the presence of added sulfuric acid. In this scheme, the substrate (S)[3] forms complexes[4] with both unprotonated (N) and protonated (NH^+)[5] forms of the amine.

[3] The pK_a values of the carboxyl groups of the ester substrates in DMSO can be estimated by using the reported (*6*) Hammett plot for the ionization of substituted benzoic acids. Thus, pK_a values of 8.1–8.5 are calculated for the nitrobenzoic acid derivatives (**2o**, **2m**, **2p**, **4m**, and **4p**). For the phthalic acid derivatives (**3m** and **3p**), pK_a values of about 10 are calculated.

[4] It has been reported (*7*) that benzoic acid and triethylamine form an ionic complex ($C_6H_5COO^- \cdot HNEt_3^+$) in DMSO. In the presence of bisulfate ion, "heteroconjugation" (*8*) is possible between bisulfate ion and the unionized substrate



Complexation of the heteroconjugated species to the protonated amine ($ArCOOH \cdot HSO_4^- \cdot HNEt_3^+$) is also possible for the reactions investigated in this study.

[5] pK_a values of 9–11.5 have been reported for simple amines in DMSO (*9*). Although the pK_a values of the amines used in the present study are unknown, attachment of an alkyl group to the nitrogen atom leads to a lower pK_a for the amine (*9*).

$$N + S \xrightarrow{k_2^0} P$$

$$NH^+ + S \xrightarrow{k_2^+} P$$

$$N + S \underset{}{\overset{K_f^0}{\rightleftharpoons}} NS \xrightarrow{k_c^0} P$$

$$NH^+ + S \underset{}{\overset{K_f^+}{\rightleftharpoons}} NH^+S \xrightarrow{k_c^+} P$$

SCHEME 3

The expressions of k_{cat} and K_m for this scheme are

$$k_{cat} = \frac{k_2^0(1 - \alpha) + k_c^0K_f^0(1 - \alpha) + k_2^+\alpha + k_c^+K_f^+\alpha}{K_f^0(1 - \alpha) + K_f^+\alpha} \quad [6]$$

$$K_m = 1/\{K_f^0(1 - \alpha) + K_f^+\alpha\}. \quad [7]$$

Here, α represents the ratio $[NH^+]/N_0$ and is either 0 or 1/3 in the present study, since sulfuric acid is added to the amines in the molar ratio of 1 : 3.[6]

When saturation kinetic behavior is not observed ($K_m \gg N_0$), k_{bi} is given by

$$k_{bi} = k_{cat}/K_m = k_2^0(1 - \alpha) + k_c^0K_f^0(1 - \alpha) + k_2^+\alpha + k_c^+K_f^+\alpha. \quad [8]$$

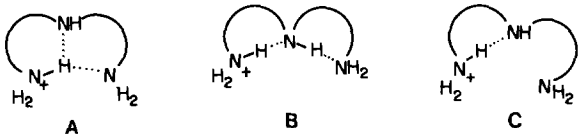
The k_{bi} values measured when $\alpha = 0$ (denoted as k_{bi}^0) and when $\alpha = 1/3$ (denoted as k_{bi}^*) are expressed by, respectively,

$$k_{bi}^0 = k_2^0 + k_c^0K_f^0 \quad [9]$$

$$k_{bi}^* = 2k_2^0/3 + 2k_c^0K_f^0/3 + k_2^+/3 + k_c^+K_f^+/3. \quad [10]$$

The ratio k_{bi}^*/k_{bi}^0 (Table 1) is close to 2/3 for aminolysis by **II2**, **II3**, and **III22**, indicating that the protonated forms of these amines are unreactive toward the esters. The k_{bi}^* values are available only for one or two esters in the aminolysis by **III33**, **III34**, **II9**, and **IV343**, and accurate analysis for these amines is difficult. However, the trend seen is that smaller diamines or triamines are deactivated by protonation while larger amines retain at least a part of the reactivity even after protonation.

Protonation of one of the nitrogens in a smaller amine could result in an intramolecular hydrogen bond that masks all of the nitrogen atoms (A or B).



This can lead to total loss of the reactivity of the amine. The results obtained with **II2**, **II3**, or **III22** can be explained in these terms. On the other hand, when a longer alkyl chain links the amine nitrogens or more amino groups are present in a molecule, the protonation may not deactivate the whole amine (C), leading to a

[6] Sulfuric acid is fully ionized to form bisulfate ion in DMSO (*10*), while the pK_a of bisulfate ion in DMSO is 14.5 (*11*).

significant value of k_2^+. This can be related to the results obtained with the larger amines.

Alternatively, the reactivity of a protonated amine may originate from the $k_c^+K_f^+/3$ term of Eq. [10]. Even if K_f^+ is too small to manifest the saturation kinetic behavior at the N_0 employed in this study, $k_c^+K_f^+/3$ can be comparable to $2k_2^0/3$. This term, however, should be much smaller for **1** than for the carboxyl-containing substrates. Since the reactivity of the protonated forms of **II6** and **III34** toward **1** is comparable to that toward the other esters (Table 1), the contribution of the $k_c^+K_f^+/3$ term is not important. Considering the magnitude of $k_c^0K_f^0$ relative to $k_c^+K_f^+$ which will be presented later in Table 3, the $2k_c^0K_f^0/3$ term of Eq. [10] is also negligible. The k_{bi}^0 and $3k_{bi}^* - 2k_{bi}^0$ values, therefore, represent k_2^0 and k_2^+, respectively.

When saturation kinetic behavior is observed, the values of the parameters listed in Scheme 3 are estimated by using the values k_{cat} and K_m values measured when $\alpha = 0$ (denoted as k_{cat}^0 and K_m^0, respectively) or when $\alpha = 1/3$ (denoted as k_{cat}^* and K_m^*, respectively).

For the reactions in which the saturation kinetic behavior is observed both in the presence ($\alpha = 1/3$) and in the absence ($\alpha = 0$) of added sulfuric acid, Eqs. [11]–[14] are used for calculation of the parameter values and the results are summarized in Table 3.

$$K_f^0 = 1/K_m^0 \qquad [11]$$

$$K_f^+ = 3/K_m^* - 2/K_m^0 \qquad [12]$$

$$k_c^0 = k_{cat}^0 - k_2^0K_m^0 \qquad [13]$$

$$k_c^+ = (3k_{cat}^*/K_m^* - 2k_{cat}^0/K_m^0 - k_2^+)/(3/K_m^* - 2/K_m^0) \qquad [14]$$

For these reactions, the values of k_2^0 and k_2^+ were not measured directly. Instead, a possible range of k_2^0 was calculated by comparing the k_{bi} values obtained in the other reactions and then was used in the estimation of k_c^0. The value of k_2^+ was estimated by using the ratio k_2^+/k_2^0 listed in Table 1 and the median value of k_2^0 estimated above. The k_2^+ thus obtained was used in the calculation of k_c^+. The values of k_2^0 and k_2^+ are only approximate. The values of k_c^0 and k_c^+, however, are not affected significantly by the large uncertainties in k_2^0 and k_2^+ as revealed by the parameter values summarized in Table 3.

For the reactions in which the saturation kinetic behavior is observed only in the presence ($\alpha = 1/3$) of added sulfuric acid, K_f^0 is much smaller than K_f^+.[7] The values of k_c^+ and K_f^+ are calculated according to Eqs. [15] and [16], while those of k_c^0 and K_f^0 cannot be evaluated. The calculated values of the kinetic parameters are summarized in Table 3.

$$k_c^+ \approx k_c^+ + 2k_c^0K_f^0/K_f^+ = k_{cat}^* - 2k_2^0K_m^*/3 - k_2^+K_m^*/3 \qquad [15]$$

$$K_f^+ = 3/K_m^* \qquad [16]$$

[7] The much greater K_f^+ values compared with K_f^0 values may be attributed to the binding to the protonated amine either of the ionized substrate or of the heteroconjugated species discussed in footnote 4.

For these reactions, k_{bi}^0 represents k_0 as discussed previously. The value of k_2^+ was estimated by using the ratio k_2^+/k_2^0 listed in Table 1, and then was used in the calculation of k_c^+. Again, k_c^+ is not significantly affected by the uncertainty in k_2^+.

For Scheme 3, the relative rate of the direct attack of the amine at the uncom-

TABLE 3

VALUES OF k_2^0 (M^{-1} s^{-1}), k_c^0 (10^{-3} s^{-1}), k_c^+ (10^{-3} s^{-1}), K_f^0 (10^3 M^{-1}), AND K_f^+ (10^3 M^{-1}) OBTAINED BY ANALYSIS OF THE PARAMETER VALUES SUMMARIZED IN TABLES 1 AND 2[a]

		II2	II3	II6	III22	III33	III34	II9	IV343
1	k_2^0	31	40	37	28	43	38	38	39
2o	k_2^0	16	21	27	38	25	26	25	22
	k_c^0	—	—	—	—	—	—	—	—
	k_c^+	—	—	—	32 (0.84)	26 (1.0) [28]	31 (1.2) [4.1]	21 (0.84) [23]	19 (0.86) [22]
	K_f^0	—	—	—	—	—	—	—	—
	K_f^+	—	—	—	4.5	2.0	3.4	3.7	3.3
2m	k_2^0	3.3	5.1	5–11	6.3	13	4–12	4–12	4–12
	k_c^0	—	—	66–13	—	—	92–55	30–21	75–39
	k_c^+	—	—	33 [39]	8.3 (1.3)	24 (1.8) [25]	34 [38]	24 [25]	20 [21]
	K_f^0	—	—	0.11	—	—	0.22	0.91	0.22
	K_f^+	—	—	0.68	7.7	4.9	2.7	5.9	4.8
2p	k_2^0	7.0	8.5	11	7.7	13	13	15	8–16
	k_c^0	—	—	—	—	—	—	—	96–63
	k_c^+	—	—	—	—	26 (2.0) [29]	—	15 (1.0) [16]	42 [44]
	K_f^0	—	—	—	—	—	—	—	0.24
	K_f^+	—	—	—	—	1.0	—	4.9	3.0
3m	k_2^0	3.3	4.0	4.7	4.2	7.2	3–8	4–10	3–13
	k_c^0	—	—	—	—	—	50–14	8–1	4–20
	k_c^+	—	—	—	5.0 (1.2)	11 (1.5) [11]	14 [17]	8.8 [9.1]	18 [19]
	K_f^0	—	—	—	—	—	0.14	0.83	0.23
	K_f^+	—	—	—	6.8	5.3	2.7	6.4	6.1
3p	k_2^0	3.9	4.8	5.6	3.4	6.0	6.5	8.5	5–12
	k_c^0	—	—	—	—	—	—	—	62–8
	k_c^+	—	—	—	—	8.5 (1.4) [9.2]	8.0 (1.2) [11]	11 (1.3) [12]	16 [17]
	K_f^0	—	—	—	—	—	—	—	0.13
	K_f^+	—	—	—	—	1.8	3.1	4.0	4.6
4m	k_2^0	1.3	2.3	1–3.3[b]	1.5	4.2	1–4	1–6	1–4.2[b]
	k_c^0	—	—	22–0	—	—	25–4	6–2	28–0
	k_c^+	—	—	10 [12]	2.7 (1.8)	5.1 (1.2) [5.3]	6.8 [7.8]	6.0 [6.2]	4.7 [4.9]
	K_f^0	—	—	0.11	—	—	0.14	1.1	0.12
	K_f^+	—	—	0.48	10	6.3	3.4	5.0	5.5

TABLE 3—*Continued*

		II2	II3	II6	III22	III33	III34	II9	IV343
4p	k_2^0	2.8	4.8	3.4	2.5	4.2	4.1	5.7	7.4
	k_c^0	—	—	—	—	—	—	—	—
	k_c^+	—	—	—	—	5.5 (1.3) [6.0]	4.2 (1.0) [6.9]	6.6 (1.2) [7.2]	11 (1.5) [12]
	K_f^0	—	—	—	—	—	—	—	—
	K_f^+	—	—	—	—	1.7	2.0	3.0	3.5

[a] The values of k_2^+ can be calculated with the k_2^0 values listed in this table and the k_2^+/k_2^0 ratio indicated for each amine in Table I. The numbers in parentheses are the effective molecularities of the amines in the NH^+S complex of Scheme 3. The numbers in brackets are the k_c^+ values obtained by neglecting the k_2^+ term ($k_2^+ = 0$) of Eqs. [14] and [15].

[b] If k_2^0 is greater than this value, k_c^0 becomes negative.

plexed ester and the intramolecular reaction within the complex is represented by

$$\frac{\text{rates of the } k_2^0 \text{ and } k_2^+ \text{ paths}}{\text{rates of the } k_c^0 \text{ and } k_c^+ \text{ paths}} = \frac{k_2^0(1-\alpha) + k_2^+\alpha}{k_c^0 K_f^0(1-\alpha) + k_c^+ K_f^+ \alpha}. \qquad [17]$$

Even if saturation kinetic behavior is manifested, a large portion of the reaction can proceed through the direct attack of the amine at the uncomplexed substrate. For example, the relative rate in Eq. [17] is about 1 for the reaction of **2o** and **III33** as calculated with the parameter values listed in Table 3. Thus, about half of the reaction occurs through the direct attack.

Structural Effects on Complex Formation

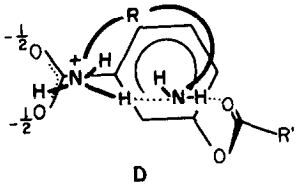
Failure to observe saturation kinetic behavior does not necessarily indicate the lack of complexation between the substrate and the amine. Even when a complex is formed, k_0 would be proportional to N_0 if the rate of the attack of an external amine at the bound substrate is comparable to that at the uncomplexed substrate. Manifestion of the saturation kinetic behavior, therefore, indicates the formation of a complex in which the bound substrate is attacked primarily by the binding amine.

Formation of such complexes was not observed in the aminolysis of **1** by any of the amines. Thus, the presence of a carboxyl group in the ester substrate is needed for the complex formation. Among the carboxyl-containing esters, the *meta* isomers (**2m**, **3m**, **4m**) are most efficient in the complex formation with either the unprotonated or the protonated amines. Between **2o** and **2p**, **2o** forms complexes with more amines than **2p**. Thus, the ability to form complexes decreases in the sequence *meta*, *ortho*, and *para* isomers. Therefore, the relative position of the ester linkage and the carboxyl group in the substrate is important for the complex formation.

Formation of complexes is not observed with **II2** and **II3** in marked contrast to more hydrophobic amines. This indicates that hydrophobic interaction between the ester substrate and the amine is involved in the complexation. Introduction of

a hydrophobic chain into the acyl portion of the ester (**4m** vs **2m**, **4p** vs **2p**) does not enhance the complex formation substantially, suggesting that the hydrophobic interaction involves the benzene ring instead of the acyl portion of the ester substrate.

Examination of space-filling models reveals that the binding mode illustrated by structure D best explains the structural effects observed for the magnitude of K_f^0 and K_f^+.[8] In this structure the nitrogens of the protonated amine are linked by hydrogen bonding. The diamine moiety then interacts with the two carbonyl oxygen atoms of the carboxylate and the ester groups. Furthermore, the methylene units that link the two amine nitrogen atoms contact the phenyl ring of the substrate in this binding mode.



D

Space-filling models indicate that the polar interaction involving the diamine moiety and the two carbonyl oxygens is geometrically accommodated best by the *meta* substrates and least by the *para* substrates. The positional stereoselectivity manifested by the isomers of the substrates is, therefore, in agreement with this structure.

In addition, the models reveal that the hydrophobic contact between the methylene units of the amine and the phenyl ring of the substrate is efficient with hydrophobic amines such as **II9** and **IV343**. The binding mode of structure D further indicates that the introduction of a hydrophobic chain into the acyl portion of the ester (**4m**, **4p**) would not affect the hydrophobic interaction significantly.

As discussed previously, the ester aminolysis involves both the reaction of the uncomplexed substrate with an external amine and the reaction of the complexed substrate. Furthermore, the bound ester of the complex is attacked primarily by the binding amine instead of an external amine. In this regard, examination of the space-filling models reveals that the complexing amine in structure D can shield the ester linkage of the complexed substrate from the attack of the external amine.

The rate data for the saturation kinetics were analyzed by assuming that the complexed ester substrate is attacked only by the complexed amine. The effective molecularity (*12*) of the complexed amine toward the bound substrate is calculated by dividing k_c values with k_2 values for the reactions whose k_c and k_2 values are accurately measured. The effective molecularity (Table 3) is 0.8–1.8 mM,

[8] Similar structures can be drawn for the phthaloyl derivatives (**4m** and **4p**). When the heteroconjugated species discussed in footnote 4 is involved in the complex, essentially the same conclusion is made concerning the structure of the complex. For the protonated forms of tetra- or triamines, Structure B has more expanded conformation compared with Structure A. The hydrophobic interaction of the protonated amine with the substrate in Structure D appears to be more efficient when the amine has the conformation of Structure A than Structure B. For the tetra- or triamines, hydrogen bonding involving only the two terminal amino groups also leads to a good fit between the amine and the substrate, according to the space-filling model.

indicating that the aminolysis within the complex is very inefficient. This is also compatible with the binding mode of structure D. The amine nitrogen located in the proximity of the ester linkage in this structure is masked by the intramolecular hydrogen bonding. Furthermore, this nitrogen has very limited access to the carbonyl carbon atom since it is hydrogen-bonded to the carbonyl oxygen.

The reactivity of **III33** toward **1** was examined in DMSO–water mixtures of various compositions. The data were collected with **1** in the absence of added sulfuric acid. The reactivity, therefore, does not involve any polar or hydrophobic interactions between the ester and the amine. As revealed by the results in Fig. 3, the intrinsic reactivity is about three times greater in pure DMSO than in pure water and the reactivity in DMSO is diminished greatly by the addition of about 10% (v/v) water.

In summary, saturation kinetic behavior is observed when the complex formed between the carboxyl-containing ester substrate and the amine has structure D. In the formation of this complex, both polar and hydrophobic interactions between the amine and the substrate are involved. DMSO accommodates both of these interactions, mimicking the microenvironment of enzyme active sites.

Many organic catalysts such as cyclodextrin derivatives (*13, 14*) or crown ether compounds (*15*) have been investigated as artificial enzymes. Major aims of these studies are to achieve tight complexation between the catalyst and the substrate as well as very close proximity between the reaction sites within the complex. In order to achieve efficient complexation, both polar and hydrophobic interactions between the catalyst and the substrate are to be employed. The present results indicate that DMSO is suitable as a solvent for such studies.

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