BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2581—2588 (1970)

Imidazole Catalyses in Aqueous Systems. IV. Bimolecular and Michaelis-Menten-Type Catalyses of a Phenyl Ester Hydrolysis by Some Hydrophobic Imidazole Derivatives*1

Toyoki KUNITAKE and Seiji SHINKAI

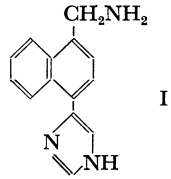
Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka

(Received February 23, 1970)

Catalytic hydrolyses of *p*-acetoxybenzoic acid by several imidazole derivatives were conducted at 15—40°C in aqueous systems using a pH-stat. Imidazole compounds containing the naphthalene ring catalyzed the hydrolysis following the Michaelis-Menten kinetics as in enzymatic reactions, while catalysis by less hydrophobic imidazoles followed the second-order kinetics. Enhancement of the overall catalytic efficiency in the Michaelis-Menten pathway was estimated to be ten times at most as compared with the bimolecular pathway. The thermodynamic and activation parameters were obtained at 15—40°C for the Michaelis-Menten pathway. The binding of catalyst and substrate was characterized by small positive enthalpy changes ($\Delta H \cong +3.5$ kcal/ mol) and large positive entropy changes ($\Delta S_u \cong +28$ eu), indicating that the complex formation was ascribable to hydrophobic interactions. The intra-complex product formation was characterized by not unreasonable $\Delta H^{\neq}$ values and extraordinarily large negative $\Delta S^{\neq}$ ($\cong -50$ eu) values. The unexpectedly small rate enhancement in the Michaelis-Menten pathway was attributed to the unfavorable $\Delta S^{\neq}$ term, which suggested that the structure of the Michaelis complex was quite different from that of the transition state of the product formation.

In a previous publication of this series, we showed that a naphthylimidazole derivative (I) catalyzed the hydrolysis of a phenyl ester by the Michaelis-Menten kinetic as in enzymatic reactions.[1]

$$\text{catalyst} + \text{substrate} \underset{K_m}{\rightleftharpoons} \text{catalyst}\cdot\text{substrate} \xrightarrow{k_{cat}} \text{catalyst} + \text{product}$$



I

The substrate binding was attributed to hydrophobic interactions between catalyst and substrate. Apparently the naphthalene ring was hydrophobic enough to bind the substrate, *p*-acetoxybenzoic acid, under the hydrolytic conditions. In fact, naphthalenesulfonic acid and other water-soluble naphthalene derivatives were shown by NMR spectroscopy to associate with methanol or acetone in an aqueous system.[2] Occurrence of the substrate saturation in the hydrolysis of phenyl esters due to the hydrophobic interaction

*1 Contribution No. 203 from this department.

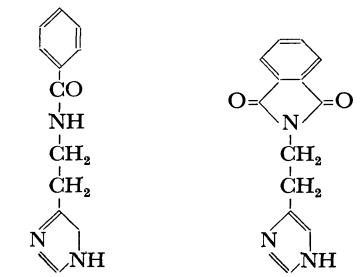
1) a) C. Aso, T. Kunitake and S. Shinkai, *Chem. Commun.*, **1968**, 1483. b) T. Kunitake, S. Shinkai and C. Aso, This Bulletin, **43**, 1109 (1970).

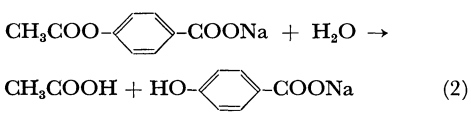
2) E. S. Hand and T. Cohen, *J. Amer. Chem. Soc.*, **87**, 133 (1965).

have been observed increasingly in polymeric[3-6] and non-polymeric[7,8] systems. Interest in these studies arises from the fact that the active site of many hydrolytic enzymes comprises hydrophobic regions and the hydrophobic interaction with substrate is intrinsic in determining the specificity and efficiency of the active site.[9]

We showed that the catalytic efficiency was quite different in the neutral and monoprotonated spcies of I, indicating that the structural difference of the catalyst affected its catalytic activity appreciably. On the other hand, remarkable rate enhancement which might be expected from formation of the Michaelis complex was not observed because of rather small k_{cat} values.

Thus it was felt necessary to study in more detail the influence of hydrophobicity on the catalytic behavior of imidazoles. In the present investigation thermodynamic and activation parameters were estimated for the catalytic process in the hydrolysis of *p*-acetoxybenzoic acid (Eq. (2)) by several imidazole derivatives (Bz-Im, Ph-Im, N-oyl-Im, and N-yl-Im), in the hope that the results would be useful for designing more efficient catalytic systems.





(2)

Experimental

***N*-Benzoylhistamine (Bz-Im)** was prepared by reaction of histamine and benzoyl chloride in the presence of triethylamine,[10] mp 148.5—149.5°C (lit,[10] 148°C).

***N*-[4(5)-Imidazolylethyl]-phthalimide (Ph-Im).** *o*-Phthaloyl chloride was prepared from phthalic anhydride and phosphorus pentachloride in 90% yield, bp 142—146°C/15 mmHg (lit,[11] 131—133°C/9—10 mmHg). Histamine dihydrochloride (1.8 g, 0.01 mol) was dissolved in 60 m*l* of 4 N NaOH in a three-necked flask equipped with a mechanical stirrer. *o*-Phthaloyl chloride (1.0 g, 0.005 mol) in 30 m*l* of chloroform and 3.0 m*l* of 4 N NaOH were separately added dropwise over a 15 min period of ice cooling. The solution was confirmed to be alkaline and then stirred for 1 hr. Sodium chloride was added and the chloroform layer was separated. The aqueous layer was extracted with 30 m*l* of chloroform, and the combined chloroform solution was washed with dil. aqueous alkali and with a small amount of water. Chloroform was evaporated and the residue was recrystallized from water, colorless plates, mp 221—225°C (dec.), yield 45%. IR (KBr): $\nu_{C=O}$ 1700, 1772 cm^{-1}. Found: C, 64.59; H, 4.60; N, 15.99%; Calcd for $C_{13}H_{11}O_2N_3$: C, 64.72; H, 4.60; N, 17.43%; mol wt, 241.24. The equivalent weight determined from titration of the imidazole group was 244.

***N*-α-Naphthoylhistamine (N-oyl-Im).** Histamine dihydrochloride (1.8 g, 0.01 mol) was dissolved in a mixture of methanol (150 m*l*) and water (20 m*l*), containing 7.2 g (0.072 mol) of triethylamine. To this solution was added at 0—4°C 2.1 g (0.01 mol) of *N,N'*-dicyclohexylcarbodiimide in 40 m*l* of acetonitrile and 1.7 g (0.01 mol) of naphthalene-α-carboxylic acid in 20 m*l* of methanol in succession. The reaction mixture was kept with occasional shaking at 0—4°C for three days and at room temperature for four days. The unreacted carbodiimide was converted to *N,N'*-dicyclohexylurea by addition of 5 m*l* of glacial acetic acid. Upon addition of 3 m*l* of concentrated hydrochloric acid, the mixture was concentrated to about 20 m*l*, separated from dicyclohexylurea formed, added to saturated aqueous sodium carbonate and extracted with chloroform. Evaporation of chloroform and recrystallization of the residue from water yielded colorless needles

3) a) C. Aso, T. Kunitake and F. Shimada, *J. Polym. Sci., Part B*, **6**, 467 (1968). b) T. Kunitake, F. Shimada and C. Aso, *J. Amer. Chem. Soc.*, **91**, 2716 (1969). c) T. Kunitake, F. Shimada and C. Aso, *Makromol. Chem.*, **126**, 276 (1969).

4) R. L. Letsinger and I. S. Klaus, *J. Amer. Chem. Soc.*, **87**, 3380 (1965).

5) Yu. E. Kirsh, V. A. Kabanov and V. A. Kargin, *Vysokomol. Soedin.*, **A10**, 349 (1968).

6) a) C. G. Overberger, R. Corett, J. C. Salamone and S. Yaroslavsky, *Macromolecules*, **1**, 331 (1968). b) C. G. Overberger, M. Morimoto, I. Cho and J. C. Salamone, *ibid.*, **2**, 553 (1969).

7) a) R. L. VanEtten, J. F. Sebastian, G. A. Clowes and M. L. Bender, *J. Amer. Chem. Soc.*, **89**, 3242 (1967). b) R. L. VanEtten, G. A. Clowes, J. F. Sebastian and M. L. Bender, *ibid.*, **89**, 3253 (1967).

8) R. G. Shorenstein, C. S. Pratt, C.-J. Hsu and T. E. Wagner, *ibid.*, **90**, 6199 (1968).

9) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, Inc., New York (1969), Chapter 8.

10) G. Gerngross, *Z. physiol. Chem.*, **108**, 58 (1915).

11) E. Ott, "Organic Syntheses," Coll. Vol. II, p. 528 (1943).

in 67% yield, mp 112—114°C (dec.). Found: C, 72.15; H, 5.85; N, 14.45%. Calcd for $C_{16}H_{15}ON_3$: C, 72.43; H, 5.70; N, 15.84%; mol wt, 265.32. The equivalent weight determined by titration of the imidazole group was 270.5.

1-Acetamidomethyl-4-[4(5)-imidazolyl]naphthalene (N-yl-Im). 265 mg of 1-aminomethyl-4-[4(5)-imidazolyl]naphthalene[1b] (10^{-3} mol) was reacted with 79 mg (10^{-3} mol) of acetyl chloride in a mixture of 10 *ml* of triethylamine and 100 *ml* of acetonitrile in an ice bath. Upon stirring for 1 hr solvents were evaporated, and the solid residue taken up in 0.1 N hydrochloric acid was reprecipitated by addition of saturated aqueous sodium carbonate. The precipitate was recrystallized from 3 : 2 water - ethanol, mp 144—150°C (dec.), 60% yield. IR (KBr): $\nu_{C=O}$, 1640 cm^{-1} (broad). Found: C, 72.04; H, 5.99; N, 15.35%. Calcd for $C_{16}H_{15}ON_3$: C, 72.43; H, 5.70; N, 15.84%; mol wt, 265.32. The equivalent weight determined by titration of the imidazole was 272.1.

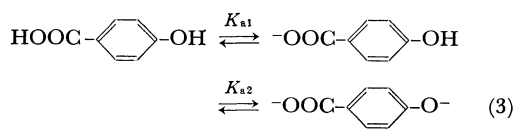
Substrate. *p*-Acetoxybenzoic acid was prepared as described before.[1b,12]

Titration and Hydrolysis Procedures. Titration and hydrolyses were carried out at several temperatures using a pH-stat connected with a recorder (TOA Electronics, Ltd., Models HS-1B and EPR-2T, respectively). The water-jacket holding the reaction vessel was maintained at given temperatures by circulating water from a constant temperature bath. The pH meter was calibrated at two pH values at the respective temperatures. Further details of the procedures have been described previously.[3b]

NMR Measurements. The imidazole derivative N-oyl-Im was saturated at room temperature in D_2O containing a trace of ethanol, and its NMR spectrum was obtained with a Varian A-60 spectrometer. Acetonitrile and contaminating water were used as internal standards. The imidazole protons at the 2 and 4(5) positions appeared at 7.56 and 6.80 ppm, respectively. Histamine dihydrochloride was dissolved in D_2O (30 mg/*ml*) and neutralized to pH 7—8. At this pH range, only the amino group is protonated. The imidazole protons at the 2 and 4(5) positions appeared at 8.11 and 7.39 ppm, respectively.

Results

Spontaneous Hydrolysis. Table 1 gives the rate of spontaneous hydrolysis of the substrate and pK_a of the resulting *p*-hydroxybenzoic acid at several temperatures. Spontaneous hydrolysis was undetectably slow at 15°C and increased with temperature. Dissociation of *p*-hydroxybenzoic acid is as follows.



That the phenol group was partially dissociated at pH 8 was taken into consideration in calculating the rate of hydrolysis from alkali consumption.

12) E. R. Marshall, J. A. Kuck and R. C. Elderfield, *J. Org. Chem.*, **7**, 450 (1942).

Table 1. Spontaneous hydrolysis of substrate and pK_a of *p*-hydroxybenzoic acid

Temp. (°C)	k_{spont} (10^{-4} M^{-1}min^{-1})[a]	pK_{a1}[b]	pK_{a2}[b]
15	~0	—	—
20	0.78±0.05	4.51	9.05
30	4.23±0.02	4.44	9.17
40	14.7 ±0.4	4.34	8.94

a) pH 8.0, 1 M KCl.
b) 1 M KCl. Experimental error, ±0.05.

Catalytic Hydrolysis by Bimolecular Mechanisms. The total rate of hydrolysis in the presence of the catalyst consists of the two terms—spontaneous and catalytic hydrolyses.

$$v = v_{cat} + v_{spont} \quad (4)$$

Throughout the present investigation v_{cat} represents the initial rate of catalytic hydrolysis. Spontaneous hydrolysis prior to the addition of catalyst was small and necessary corrections were made for substrate concentration. Figure 1 shows the relation between v_{cat} and substrate concentration with Bz-Im catalyst. In the temperature range from 15°C to 40°C, v_{cat} values are reasonably proportional to substrate concentration, and the catalytic hydrolysis with Bz-Im was concluded to proceed *via* the common second-order kinetics:

$$\text{catalyst} + \text{substrate} \xrightarrow{k'} \text{catalyst} + \text{product} \quad (5)$$

$$v_{cat} = k'[C][S] \quad (6)$$

where C and S denote catalyst and substrate, respectively. In Fig. 2 is given v_{cat} *vs.* [S] with Ph-Im as a catalyst. Similarly, a linear relationship was obtained. The second-order rate con-

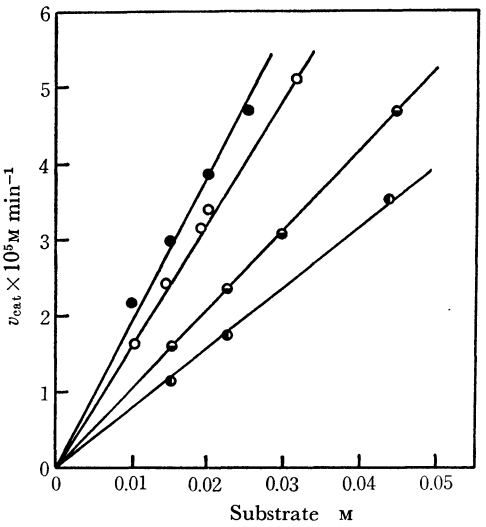


Fig. 1. Catalytic hydrolysis.
catalyst, Bz-Im 3.22×10^{-3}M; pH 8.0; 1.0M KCl.
●, 40°C; ○, 30°C; ◒, 20°C; ◐, 15°C.

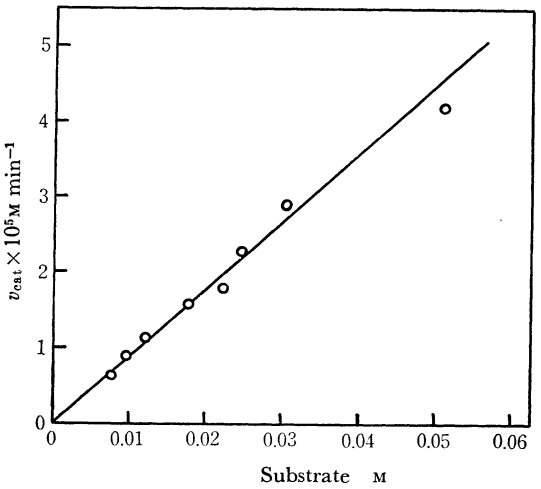


Fig. 2. Catalytic hydrolysis. catalyst, Ph-Im 2.16×10^{-3}M; pH 8.0; 1.0M KCl; 30°C.

TABLE 2. SECOND ORDER RATE CONSTANT OF CATALYTIC HYDROLYSIS

Catalyst	Temp. (°C)	pK_a[a]	k'(M^{-1}min^{-1})[b]
Bz-Im	15	7.35	0.24_6
Bz-Im	20	7.24	0.33_6
Bz-Im	30	7.21	0.50_7, 0.49[c]
Bz-Im	40	7.20	0.64_1
Ph-Im	30	6.75	0.40_5, 0.39[c]
Iimidazole[d]	30	7.13±0.03	0.32_5

a) 1M KCl. Experimental error: ± 0.05 unless stated otherwise.
b) pH 8.0, 1M KCl.
c) Catalyst concentration was varied instead of substrate concentration.
d) from Ref. 1b.

stants k' were obtained from these data and are given in Table 2, together with the data with imidazole catalyst. When the catalyst concentration was varied ($(1—10) \times 10^{-3}$M) at a constant substrate concentration ([C]=0.020M, 30°C, 1.0M KCl), the relationships between v_{cat} and [S] were similarly linear, with the second-order rate constant of 0.49M^{-1}min^{-1} and 0.39M^{-1}min^{-1} for Bz-Im and Ph-Im, respectively. Since the imidazole groups are partially protonated at pH 8.0, the effective catalyst concentrations ([C]$_{eff}$) were calculated from the corresponding pK_a values

$$[C]_{eff} = [C]_{total} \cdot \frac{K_a}{K_a + a_H} \quad (7)$$

where K_a is the acid dissociation constant of the conjugate acid and a_H is the hydrogen ion activity. Bz-Im and imidazole show similar catalytic activities, whereas Ph-Im is somewhat more efficient.

Catalytic Hydrolysis by the Michaelis-Menten Mechanism. The relationships between v_{cat} and [S] at four temperatures are given for imi-

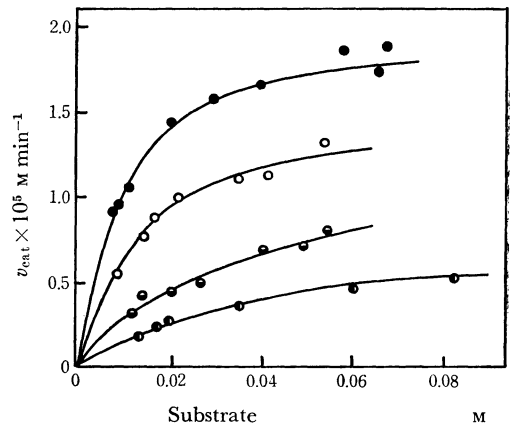


Fig. 3. Catalytic hydrolysis. catalyst, N-oyl-Im 0.82×10^{-3}M; pH 8.0; 1.0M KCl.
●, 40°C; ○, 30°C; ◒, 20°C; ◐, 15°C.

dazole derivative N-oyl-Im in Fig. 3. Undoubtedly, v_{cat} showed substrate saturation which was particularly clear at high temperature. A similar catalytic behavior was previously observed for a related imidazole derivative[1] and some imidazole-containing polymers.[3] These kinetic patterns, typical of the enzyme-catalyzed reaction, can be described by Eq. (1) and the corresponding catalytic rate is expressed by

$$v_{cat} = \frac{k_{cat}[C][S]}{K_m + [S]} \quad (8)$$

Since k_{cat} values for the imidazole catalysts used are sufficiently small, the Michaelis constant K_m is considered to represent the true dissociation constant.[3b] The kinetic constants in the equation are determined from the Lineweaver-Burk plotting[13]

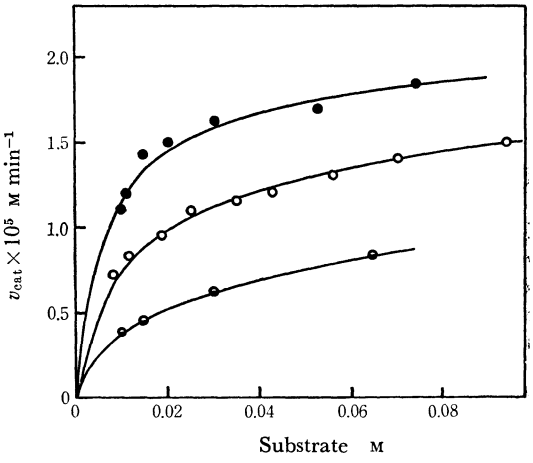


Fig. 4. Catalytic hydrolysis. catalyst, N-yl-Im 0.31×10^{-3}M; pH 8.0; 1.0M KCl.
●, 40°C; ○, 30°C; ◒, 20°C.

13) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).

Table 3. Kinetic parameters of Michaelis-Menten-type hydrolysis

Catalyst	Temp. (°C)	pK_a[a]	K_m(mM)[b]	k_{cat}(min^{-1})[b]	k_{cat}/K_m($min^{-1}M^{-1}$)
N-oyl-Im	15	6.88	40.0	0.0095	0.24
N-oyl-Im	20	6.80	20.4	0.012	0.59
N-oyl-Im	30	6.75	16.4	0.021	1.3
N-oyl-Im	30	—	14.9[c]	0.017[c]	1.2
N-oyl-Im	40	6.58	13.7	0.029	2.1
N-yl-Im	20	6.06	16.7	0.033	2.0
N-yl-Im	30	6.00	13.9	0.054	3.9
N-yl-Im	40	5.86	11.1	0.071	6.4

a) 1M KCl. Experimental error, ±0.05.
b) pH 8.0, 1M KCl.
c) pH 7.0, 1M KCl.

of $1/v_{cat}$ *vs.* $1/[S]$. The plots were sufficiently linear, showing the validity of the Michaelis-Menten expression of the rate data. Substrate saturation curves were similarly observed for the catalytic hydrolysis with N-yl-Im as a catalyst (Fig. 4). The corresponding Lineweaver-Burk plots were sufficiently linear. The kinetic constants for the enzyme-like catalysis are summarized in Table 3. Also included are kinetic constants obtained from hydrolysis at pH 7.0 with N-oyl-Im at 30°C.

The effect of ionic strength of the reaction medium was studied for the substrate-binding catalysts as shown in Fig. 5. v_{cat} increased with increase in ionic strength for both systems. Catalysis with N-oyl-Im was more sensitive to ionic strength as compared with N-yl-Im.

Thermodynamic and Activation Parameters of Catalytic Hydrolysis. From these kinetic data are obtained the activation parameter of the bimolecular catalysis by Bz–Im and the thermodynamic and activation parameters of the Michaelis-Menten-type catalysis by N-oyl-Im and N-yl-Im. The free energy of activation $\Delta G^{\ddagger}$ was calculated from k_{cat} or k' from the relationship $\Delta G^{\ddagger}=RT\ln(kT/hk_r)$,[14] and plotted against T. Satisfactorily linear relationships were obtained as shown in

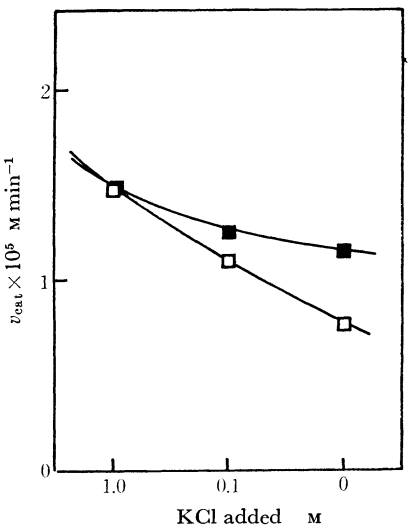


Fig. 5. Effect of ionic strength on catalytic rate. pH 8.0 : 30°C
□: catalyst, N-oyl-Im 0.82×10^{-3}M; substrate, 0.022M.
■: catalyst, N-yl-Im 0.31×10^{-3}M; substrate, 0.020M.

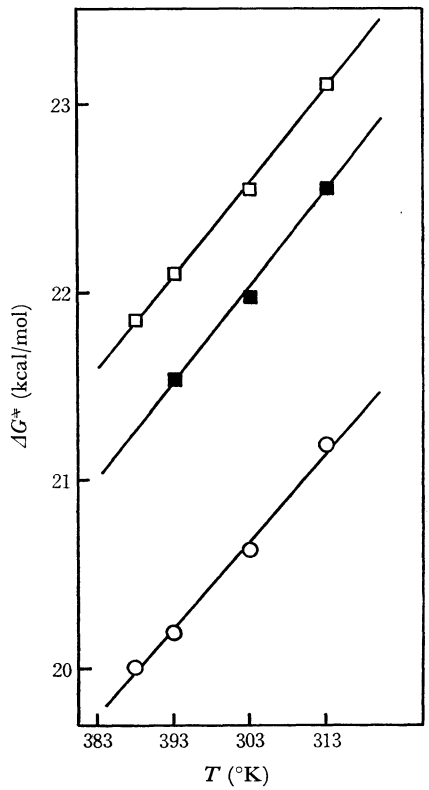


Fig. 6. Free energy of activation *vs.* absolute temperature.
catalyst: ○, Bz–Im; □, N-oyl-Im; ■, N-yl-Im.

14) A. A. Frost and R. G. Pearson "Kinetics and Mechanisms," 2nd. Ed. John Wiley & Sons, Inc., New York (1961), p. 98.

TABLE 4. THERMODYNAMIC AND ACTIVATION PARAMETERS OF CATALYTIC HYDROLYSIS

Catalyst	Substrate Binding			Bimolecular and Intra-complex Processes	
	ΔH (kcal/mol)	ΔS (eu)	ΔS_u (eu)	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (eu)
Bz-Im[a)]	—	—	—	6.43	−47.0
N-oyl-Im[b)]	3.74	20.5	28.5	7.31	−50.4
N-yl-Im[b)]	3.44	19.9	27.9	6.26	−52.0

a) Bimolecular pathway
b) Michaelis-Menten pathway

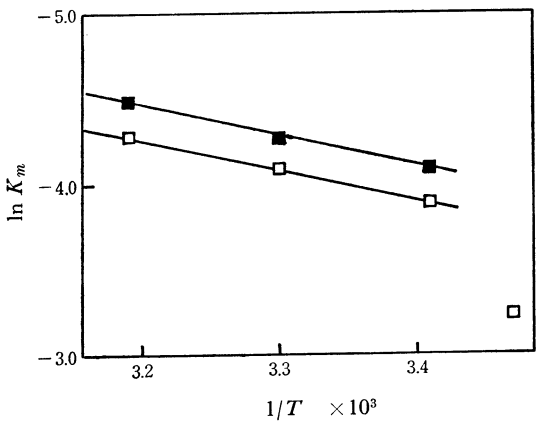


Fig. 7. Temperature dependence of the Michaelis constant. catalyst: □, N-oyl-Im; ■, N-yl-Im.

Fig. 6. $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were obtained from intercept and slope, respectively, from the relationship $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$, and are given in Table 4. Particularly noteworthy are exceptionally large negative entropies of activation obtained for the pseudo-intramolecular process.

The thermodynamic parameters of the dissociation process of the Michaelis complex were obtained from plots of $\ln K_m$ *vs.* $1/T$ (Fig. 7). Linear relationships are obtained for the respective catalysts except for a plot at 15°C for N-oyl-Im catalyst. The deviation may indicate that the bimolecular process is involved at this temperature. The enthalpy and entropy changes of the binding process are obtained from the relationship using the equation $\ln K_m = \Delta H/RT - \Delta S/R$. The thermodynamic parameters of binding are very similar for these two catalysts and characterized by small positive enthalpies and large positive entropies (see Table 4). The unitary entropy change was obtained from the relation $\Delta S_u = \Delta S + 7.98$.[15)]

Discussion

The results given above clearly demonstrate the different catalyst behavior between imidazole derivatives containing the naphthalene ring and less hydrophobic imidazole compounds. Thus, v_{cat} increased linearly with substrate concentration with the latter catalysts, whereas the Michaelis-Menten kinetics were observed for the former catalysts. The kinetic difference can be attributed to a large hydrophobicity of the naphthalene ring sufficient to bind a substrate molecule prior to the catalytic process. The benzene ring in Bz–Im or Ph–Im was not sufficiently hydrophobic to bind a substrate molecule under hydrolysis conditions and the formation of the Michaelis complex, if any, was not significant for these catalysts.

On the other hand, a bimolecular catalytic process by uncomplexed catalyst molecules might coexist in the overal Michaelis-Menten kinetics as long as the binding of catalyst and substrate occurs.[3b)] In this case the relative importance of the two processes is determined from a comparison of k_{cat}/K_m and k'. The second-order rate constants k' for three non-binding catalysts are 0.3—0.5 $min^{-1}M^{-1}$ at 30°C. Therefore, the hypothetical k' values for N-oyl-Im and N-yl-Im will certainly not be greater than 0.3—0.5 $min^{-1}M^{-1}$, considering their lower pk_a values. Since k_{cat}/K_m at 30°C is 1.3 and 3.9 for N-oyl-Im and N-yl-Im, respectively, the enzyme-like catalytic pathways are considered to be preferred to with these catalysts. At lower temperatures, however, the dissociation constant increases and the overall rate enhancement due to complexation is less pronounced. In fact, substrate saturation was not very clear for the catalytic hydrolysis at 15°C (Fig. 3). Deviation of the corresponding $\ln K_m$ plot from linearity in Fig. 7 and comparable k_{cat}/K_m and k' values (0.24 *vs.* 0.25 $min^{-1}M^{-1}$) obtained for closely related catalysts (Bz-Im and N-oyl-Im) suggest that the significance of the two catalytic processes becomes comparable at this temperature.

The tendency of non-polar groups to adhere to one another in aqueous environments has been referred to as hydrophobic bonding,[15)] and this process is usually accompanied by a large positive entropy change and a small enthalpy change. The thermodynamic parameters obtained (large positive ΔS_u and small ΔH for the binding process) clearly indicate that hydrophobic forces are responsible for substrate binding. Very close values obtained for both ΔH and ΔS_u with catalysts N-oyl-Im and N-yl-Im seemingly reflect the fact that the naphthalene

15) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

ring is the major site of binding in these catalysts.

The pseudo-intramolecular rate constant k_{cat} was greater (about 2.5 times at 30°C) for imidazole N-yl-Im than for N-oyl-Im in spite of the lower pK_a value for the former. Since the nucleophilicity of simple imidazoles in the hydrolysis of *p*-nitrophenyl acetate was proportional to pK_a of the conjugate acid,[16] reversal in the intra-complex rate constant may be attributed to the structural difference of the Michaelis complexes. It was found that k_{cat} differed considerably between catalysts I and its protonated form (0.19 *vs.* 0.036 min^{-1}),[1b] and we anticipated a much greater difference in catalytic activity between N-oyl-Im and N-yl-Im, because k_{cat} was expected to be particularly sensitive to the spacial disposition of the imidazole group relative to the bound substrate. However, the difference in k_{cat} was rather small, although it would be greater if the difference in pK_a is to be corrected. Appearance of the imidazole proton peaks in N-oyl-Im at higher magnetic fields than the corresponding peaks in histamine by about 0.6 ppm can be ascribed to the paramagnetic shielding of the former proton by the naphthalene ring. Thus, the imidazole group in N-oyl-Im is conceivably located at a position fairly close to the plane of the naphthalene ring. The difference in pK_a between Bz-Im and N-oyl-Im also supports this supposition. Klotz and Lyndrup[17] noted that the charged state of an N-ethylimidazole residue placed in a polyvinylpyrrolidone matrix was destabilized relative to the uncharged state, because of changes in the local polymer or solvent environment, causing lowering of pK_a by about 1.15 pK units. Similarly we found that pK_a of the benzimidazole unit in polymers decreased with increasing hydrophobicity of the polymer in aqueous systems.[18] Thus the decreased pK_a of N-oyl-Im relative to that of Bz-Im can be considered to arise from destabilization of the charged state of the imidazole group due to the nearby hydrophobic naphthalene ring. The greater sensitivity to the ionic strength of the medium of the catalytic efficiency of N-oyl-Im as compared with that of N-yl-Im (Fig. 5) may be also ascribed to the conformational flexibility of N-oyl-Im molecules. Thus, a compact conformation of N-oyl-Im at $\mu=1.0$ will tend to lessen the difference in catalytic activity between N-oyl-Im and N-yl-Im, since the location of the imidazole group relative to the naphthalene ring will not be very different in these catalysts.

The overall enhancement in the catalytic efficiency due to substrate binding can be estimated from k_{cat}/K_m *vs.* k'. The rate enhancement observed in the present study was at most ten times (Table 2 *vs.* 3). A similar extent of rate enhancement was observed for related polymer catalysts, and some discussions were made concerning small k_{cat} values.[3b] These results are in a sharp contrast with much greater rate enhancement observed in imidazole-catalyzed hydrolyses of phenyl esters[8,19,20] and acylation of amines[21,22] where the hydrophobic interaction of long hydrocarbon chains significantly contributed to rate enhancement. The small rate enhancement in the present system is associated with a small k_{cat}. Bruice and Benkovic showed that intramolecular catalysis was characterized by favorable entropy terms (*ca.* 15 eu) compared with those of bimolecular catalyses.[23] The remarkable efficiency of enzyme-catalyzed reactions is commonly attributed to formation of the Michaelis complex and to the facile intra-complex reaction. However, the activation parameters shown in Table 4 indicate that the activation entropies are extremely unfavorable for the intra-complex process. The large negative $\Delta S^{\neq}$ observed betrays our expectation that the intra-complex product formation would be facilitated by the favorable entropy term. In fact $\Delta S^{\neq}$ for k_{cat} was more negative than that for k'.

The unfavorable entropy factor for k_{cat} may be explained by either or both of the following assumptions: (1) The relative disposition of catalyst and substrate in the Michaelis complex may be very different from the structure of the transition state, and extensive reorganization of the structure of the complex becomes necessary for the action of the imidazole group on the ester group to occur. In this case, k_{cat} will increase greatly if suitable combinations of catalyst and substrate are selected. Connors and coworkers[24] made extensive studies on the modification of reaction rates by complex formation in aqueous solutions. An analogous study may be necessary in order to establish the structure-reactivity relationship in the complex. Also interesting is the finding by Gitler and Ochoa-Solano that elongation of the hydrocarbon chain in substrate decreased the activation energy of the imidazole-catalyzed ester hydrolysis in micelles,[19]

16) T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, **80**, 148 (1958).

17) I. M. Klotz and M. L. Lyndrup, *Biopolymers*, **6**, 1405 (1968).

18) T. Kunitake, S. Shinkai and C. Aso, to be published.

19) a) A. Ochoa-Solano, G. Romero, and C. Gitler, *Science*, **156**, 1243 (1967); b) C. Gitler and Ochoa-Solano, *J. Amer. Chem. Soc.*, **90**, 5004 (1968).

20) J. R. Knowles and C. A. Parsons, *Nature*, **221**, 53 (1969).

21) J. R. Knowles and C. A. Parsons, *Chem. Commun.*, **1967**, 755.

22) a) I. M. Klotz and V. H. Stryker, *J. Amer. Chem. Soc.*, **90**, 2717 (1968). b) G. P. Royer and I. M. Klotz, *ibid.*, **91**, 5885 (1969).

23) T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963).

24) a) J. A. Mollica, Jr., and K. A. Connors, *ibid.*, **89**, 308 (1967). b) P. A. Kramer and K. A. Connors, *ibid.*, **91**. 2600 (1969). c) K. A. Connors, M. H. Infeld, and B. J. Kline, *ibid.*, **91**, 3597 (1969).

since their results suggest that the disposition of substrate relative to catalyst possibly affects the activation energy.[25] (2) If it is assumed that acetylimidazoles are intermediates of the pseudo-intramolecular transformation, the transition state for the acyl transfer is considered to be rather polar. The polar transition state will not be favored on the surface of the hydrophobic naphthalene ring. Thus, the hydrophobic interaction between catalyst and substrate may have to be largely destroyed for the intra-complex reaction to occur, resulting in the large negative entropy of activation.

25) Recently this problem was discussed also by Cordes and Dunlop: E. H. Cordes and R. B. Dunlop, *Accounts Chem. Res.*, **2**, 329 (1969).

In conclusion, both of the bimolecular and Michaelis-Menten-type catalyses were observed for the imidazole-catalyzed hydrolysis of *p*-acetoxybenzoic acid in aqueous systems. Unfortunately, however, the rate acceleration expected from formation of the catalyst-substrate complex was not remarkable because of the highly unfavorable entropy of activation. We are now attempting syntheses of related catalytic systems which presumably possess different organizations of the catalytic site from those of the present one.

The authors are deeply grateful to Professor C. Aso of this department for his unfailing encouragement and advice.