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Imidazole Catalyses in Aqueous Systems. III. Formation of the Catalyst-Substrate Complex in the Hydrolysis of a Phenyl Ester Catalyzed by a Naphthylimidazole Derivative*1

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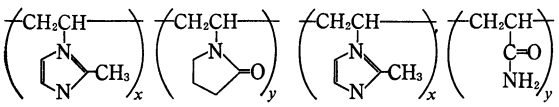
Catalytic hydrolysis of *p*-acetoxybenzoic acid by 1-[4(5)-imidazolyl]-4-aminomethylnaphthalene was carried out at 30°C in aqueous systems using a pH-stat. The catalytic rate leveled off at high substrate concentrations and increased remarkably at high pH. The data indicated that both the neutral catalytic species (AmIm) and the monoprotonated catalytic species (Am^+Im) acted as enzyme-like catalysts, the catalytic rate conforming to the Michaelis-Menten kinetics, although their catalytic efficiencies were quite different: K_m (dissociation constant of the catalyst-substrate complex) = 0.011 M and k_3 (pseudo-intramolecular rate constant of the product-formation) = 0.19 min^{-1} for AmIm, and K_m = 0.041 M and k_3 = 0.036 min^{-1} for Am^+Im. The binding function was attributed to hydrophobic interaction, on the ground that K_m was smaller for AmIm than for Am^+Im and that an increase in the ionic strength of the medium caused rate enhancement. The hydrolysate showed an inhibitory action. The wide variation of the catalytic efficiency between AmIm and Am^+Im may be related to the structural difference of the Michaelis complexes. 1-Aminomethylnaphthalene similarly catalyzed the hydrolysis and showed a substrate binding phenomenon, though the saturation phenomenon was much less pronounced as compared with the imidazole catalyst.

In the previous publications of this series, it was shown that imidazole-containing copolymers (I and II) catalyzed the hydrolysis of 3-nitro-4-acetoxybenzoic acid according to the Michaelis-Menten kinetics as in enzymatic reactions.[1)]

*1 Contribution No. 169 from this department. Presented at the 22nd Annual Meeting of the Chemical Society of Japan, March, 1969, Tokyo, Japan. A preliminary account of this work was given in C. Aso, T. Kunitake and S. Shinkai, *Chem. Commun.*, **1968**, 1483.

1) a) C. Aso, T. Kunitake and F. Shimada, *J. Polymer Sci., Part B*, **4**, 467 (1968). b) T. Kunitake, F. Shimada and C. Aso, *J. Amer. Chem. Soc.*, **91**, 2716 (1969).

$$\text{catalyst} + \text{substrate} \underset{}{\overset{K_m}{\rightleftharpoons}} \text{catalyst}\cdot\text{substrate} \xrightarrow{k_s} \text{catalyst} + \text{product} \quad (1)$$



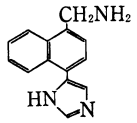
I II

The influence of the copolymer composition of I on the catalytic behavior was accounted for by assuming that the catalytic site was made of a loop of a polymer segment surrounding a substrate molecule. The binding function of the catalytic site was attributable to hydrophobic forces. It was shown that the catalytic action was inhibited competitively by several organic compounds, supporting its enzyme-like catalytic behavior.[2] In the case of catalysis by II, it was not necessary to consider loop formation. The binding function was similarly attributed to the hydrophobic interaction.[1b]

Molecular associations of organic compounds due to hydrophobic forces are frequently encountered in aqueous systems. Dye associations and the micelle formation of detergents are typical examples.

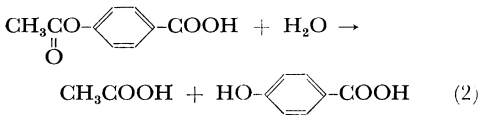
Apart from polymeric systems, there have been several investigations in which formation of catalyst-substrate complexes preceded the chemical transformation of substrates. For instance, Cramer and coworkers found that cyclodextrins catalyzed decarboxylation of substituted cyanoacetates and acetoacetates[3] and fission of pyrophosphates[4] in enzyme-like manners (Eq. (1)). Bender *et al.*[5,6] studied in detail phenyl ester cleavages catalyzed by cycloamyloses. The formation of a 1 : 1 complex between catalyst and substrate molecules with detergent-like structures of opposite charge has been proposed by Wagner *et al.*[7]

Thus, it was expected that small molecules which consist of hydrophobic and catalytic groups might show an enzyme-like catalytic behavior. The first compound we selected for this purpose was 1-[4(5)-imidazolyl]-4-aminomethylnaphthalene (III). The naphthalene ring is considered to show sufficiently strong hydrophobic interaction, since naphthalenesulfonic acid was shown by NMR spectroscopy to associate with acetone or methanol in an aqueous system,[8] and the imidazole group is known to be present in the active site of many hydrolytic enzymes.[9a]



III

In the following we describe an investigation of the enzyme-like catalysis by III for hydrolysis of 4-acetoxybenzoic acid into 4-hydroxybenzoic acid and acetic acid.



(2)

Experimental

Preparation of 1-Acetyl-4-bromonaphthalene (IV).[10,11] α-Bromonaphthalene was prepared in 70% yield from naphthalene and bromine in CCl_4,[12] and subsequently acetylated with acetyl chloride and $AlCl_3$ in CS_2 at room temperature. Yield 71%, mp 47.0—47.5°C (lit,[10] 47.0—47.5°C).

Preparation of 1-Acetyl-4-cyanonaphthalene (V). The reaction was carried out following the preparative method of 1-cyanonaphthalene.[13] Into a 300-m*l* three-necked flask equipped with a mechanical stirrer, a reflux condenser and a thermometer were added 83 g (0.33 mol) of IV, 35 g (0.38 mol) of cuprous cyanide and 65 m*l* of dimethylformamide (DMF). The reaction mixture was stirred for 6 hr with gentle refluxing at 150—160°C. A warm solution of 133 g (0.50 mol) of ferric chloride in 33 m*l* of concentrated hydrochloric acid and 200 m*l* of water was added to the reaction mixture. Some solid residues left in the flask were dissolved in warm DMF. The solutions were combined and maintained for 20 min at 60—70°C. Black precipitates were filtered and the solution was extracted with 750 m*l* (in three portions) of toluene. After distilling toluene,

2) T. Kunitake, F. Shimada and C. Aso, *Makromol. Chem.*, **126**, 276 (1969).

3) F. Cramer and W. Kampe, *J. Amer. Chem. Soc.*, **87**, 1115 (1965).

4) N. Heinrich and F. Cramer, *ibid.*, **87**, 1121 (1965).

5) M. L. Bender, R. L. VanEtten, G. A. Clowes and J. F. Sebastian, *ibid.*, **88**, 2318 (1966); R. L. VanEtten, J. F. Sebastian, G. A. Clowes and M. L. Bender, *ibid.*, **89**, 3242 (1967).

6) M. L. Bender, R. L. VanEtten and G. A. Clowes, *ibid.*, **88**, 2319 (1966); R. L. VanEtten, G. A. Clowes, J. F. Sebastian and M. L. Bender, *ibid.*, **89**, 3253 (1967).

7) T. E. Wagner, C.-J. Hsu and C. S. Pratt, *ibid.*, **89**, 6366 (1967); R. G. Shorenstein, C. S. Pratt, C.-J. Hsu and T. E. Wagner, *ibid.*, **90**, 6199 (1968).

8) E. S. Hand and T. Cohen, *ibid.*, **87**, 133 (1965).

9) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin, Inc., New York, N.Y. (1966). a) Chapter 2; b) p. 46.

10) T. L. Jacobs, S. Winstein, J. W. Ralls and J. H. Robson, *J. Org. Chem.*, **11**, 27 (1946).

11) S. Berkovic, *Israel J. Chem.*, **1**, 1 (1963).

12) H. T. Clark and M. R. Brefhen, "Organic Synthesis," Coll. Vol. I, p. 121 (1941).

13) L. Friedman and H. Shechter, *J. Org. Chem.*, **26**, 2522 (1961).

red-brown needles were obtained on cooling. Recrystallization from methanol gave 7 g (11% yield) of yellow needles: mp 130—132°C. The black precipitate was extracted with toluene in a Soxhlet extractor. Solvent removal from the extract gave red-brown needles, which were recrystallized from methanol to yield yellow needles. The combined yield amounted to 90%. Elemental analysis. Found: C, 80.30; H, 4.56; N, 7.09%. Calcd for $C_{13}H_9NO$: C, 79.98; H, 4.65; N, 7.17%. The infrared spectrum showed peaks at 2220 ($\nu_{C\equiv N}$), 1695 ($\nu_{C=O}$, conjugated), 833 (δ_{CH}, aromatic, out of plane) and 758 cm^{-1} (δ_{CH}, aromatic, out of plane).

Oxidation of V with SeO_2. The reaction was carried out by modifying the preparative method of naphthylglyoxal.[14] In a 100-m*l* three-necked flask equipped with a mechanical stirrer, a reflux condenser and a thermometer, 11.1 g (0.1 mol) of SeO_2 was dissolved in 50 m*l* of dioxane containing 2 m*l* of water at 50—55°C. 19.5 g (0.1 mol) of V was then quickly added and the reaction mixture was refluxed with stirring for 6.5 hr, and stirred at room temperature for additional 12 hr. The precipitated selenium metal and filtered and the solvent removed *in vacuo*. The residue was dissolved while still warm in 30 m*l* of acetone and the precipitate formed (Se metal) was filtered. Acetone was evaporated and the resulting brown residue (1-(4-cyanonaphthyl)-glyoxal, VI) dissolved in 200 m*l* of methanol was used for the next step without further purification. When a shorter oxidation period was employed (refluxing for 4 hr and stirring for 2 hr), the yield of the copper complex in the next step did not decrease.

Preparation of 1-[4(5)-Imidazolyl]-4-cyanonaphthalene (VII). The general procedure for preparation of imidazoles from glyoxals[15] was followed. To a 300-m*l* three-necked flask equipped with a mechanical stirrer, a reflux condenser and a thermometer was added 140 m*l* of 28% aqueous ammonia and 8 m*l* of 37% formalin. The mixture was warmed to 40°C on a water bath, and 20 g of cupric acetate and 100 m*l* of a methanol solution of VI were added with stirring. Stirring was further continued for 40 min at 55—58°C, and the copper salt of the imidazole compound was obtained as precipitates. The crude copper complex was purified by refluxing for 1 hr each in the following solvents: a. 1 : 1 aqueous methanol; b. ethyl acetate, twice; c. acetone, twice; d. chloroform, twice. The yield of the purified copper complex was 15 g (30% based on V).

The imidazole was freed from copper by treating with H_2S. The copper complex (50 g) was suspended in 2 *l* of 1 : 1 aqueous methanol and H_2S gas was introduced for 4 hr with gentle refluxing. The hot mixture was filtered, and, on cooling, 40 g of crude VII was obtained as red-brown precipitates. The crude VII was dissolved in dilute hydrochloric acid, and the hydrochloride of VII (VII-HCl) was recovered by concentration and purified by recrystallization from water with charcoal treatment. The overall yield of VII-HCl from V was approximately 10%. Colorless needles, mp 219—220°C.

Found: C, 57.68; H, 4.88; N, 13.75%. Calcd for $C_{14}H_{10}N_3Cl\cdot 2H_2O$: C, 57.63; H, 4.83; N, 14.41%.

Its infrared spectrum showed a broad peak characteristic of the imidazole group at 2800—3200 cm^{-1}, a sharp peak at 2220 cm^{-1} ($\nu_{C\equiv N}$), and peaks at 762 and 833 cm^{-1} (δ_{CH} aromatic, out of plane).

The free imidazole derivative, VII, was obtained by treating VII-HCl with concentrated aq. $NaHCO_3$: Colorless crystals, mp 216—218°C. Its infrared spectrum was consistent with what was expected for VII.

Reduction of VII with $LiAlH_4$. In a 300-m*l* three-necked flask equipped with a mechanical stirrer, a dropping funnel and a reflux condenser connected to a soda lime tube were placed 100 m*l* of carefully-purified tetrahydrofuran (THF) and 1.2 g of $LiAlH_4$. Finely powdered VII (0.6 g) was suspended in 50 m*l* of THF and slowly added over 30 min from the dropping funnel. The color of the mixture turned green. The reaction mixture was refluxed during the addition and for the subsequent 90 min. Excess $LiAlH_4$ was decomposed with 50 m*l* of water, and 70 m*l* of 10% aqueous NaOH was added. The pale yellow organic layer was separated and acidified with 10 m*l* of 4N hydrochloric acid, and THF was removed *in vacuo*. When separation of the two layers was difficult, addition of concentrated aqueous NaOH or of an aqueous solution of Rochelle salt was effective for the phase separation. Slow addition of concentrated aqueous alkali to the organic residue gave rise to white precipitates, which again went into solution on standing. When the dissolution became sluggish, an aqueous solution of excess Na_2CO_3 was added to yield 0.4 g of white precipitates (III). mp 172—175°C. Yield 70%. Reprecipitation was carried out by neutralization of an acidic aqueous solution as metioned above, and the precipitate was dried *in vacuo*.

Found: C, 75.03; H, 5.85; N, 17.75%. Calcd for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82%.

Its infrared spectrum showed the presence of a broad peak between 2900—3400 cm^{-1} (imidazole). The peak at 2220 cm^{-1} was absent, indicating disappearance of the cyano group. Peaks at 827 and 756 cm^{-1} showed that the naphthalene ring remained intact. Titration of III (10.9 mg) was carried out as described below (30°C, 1M KCl) and the alkali consumption to neutralize the protonated imidazole group was 95±3% of the calculated amount.

Preparation of 1-Aminomethylnaphthalene (VIII). Naphthalene was reacted with paraformaldehyde and concentrated hydrochloric acid to give 1-chloromethylnaphthalene[16] in 83% yield: bp 168—172°C/25 mmHg (lit,[16] 148—153°C/14 mmHg). 1-Chloromethylnaphthalene was converted to 1-aminomethylnaphthalene by treating with hexamethylenetetramine in chloroform.[17] Bp 192—198°C/25 mmHg (lit,[17] bp 200—205°C/30 mmHg). Yield 61%. 1-Aminomethylnaphthalene hydrochloride (VIII-HCl) was obtained by introducing dry HCl gas into an ether solution of VIII. Colorless plates, mp 261—263°C (lit,[17] 260—262°C).

Preparation of *p*-Acetoxybenzoic Acid. Commercial *p*-hydroxybenzoic acid, 27.6 g (0.2 mol), was dis-

14) R. C. Fuson, W. S. Emerson and H. W. Gray, *J. Amer. Chem. Soc.*, **61**, 480 (1939).

15) P. Ruggli and E. Henzi, *Helv. Chim. Acta*, **12**, 362 (1929) [*Chem. Abstr.*, **24**, 116 (1930)].

16) O. Grummit and A. Buck, "Organic Synthesis," Coll. Vol. III, p. 195 (1955).

17) F. F. Blicke and C. E. Maxwell, *J. Amer. Chem. Soc.*, **61**, 1781 (1939).

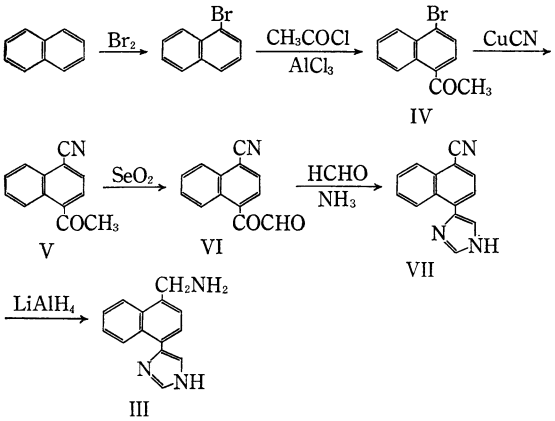
solved in 80 ml (0.8 mol) of acetic anhydride and the solution was refluxed for 3 to 4 hr. The reaction mixture was poured into ice water, and the white precipitates formed were recrystallized twice from water and dried. Additional recrystallization from benzene gave colorless flakes in 85—92% yield. Mp 192—194°C (lit,[18] 186°C).

Titration and Hydrolysis Procedures. Titration and hydrolysis were carried out at 30.0±0.05°C using a pH-stat connected with a recorder (TOA Electronics Ltd., Models HS-1B and EPR-2T, respectively). Details of the procedures have been described previously.[1b]

Miscellaneous. Imidazole and KCl, guaranteed reagents, were dried and used without further purification. Infrared spectra were obtained with a Jasco DS 301 spectrometer.

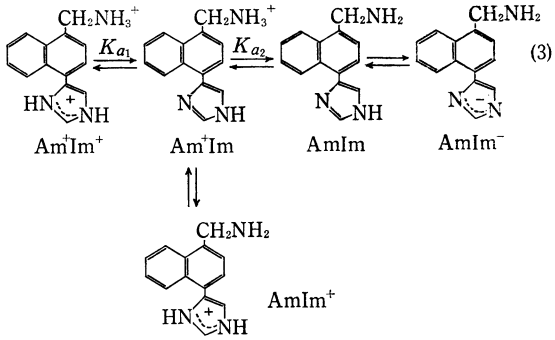
Results

Synthesis of 1-[4(5)-Imidazolyl]-4-aminomethylnaphthalene (III). III was synthesized by the following sequences.



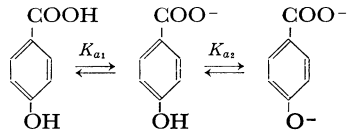
IV was obtained in good yields by acetylating 1-bromonaphthalene with acetyl chloride and $AlCl_3$ in CS_2 at 15—17°C. The conversion from IV to V proceeded smoothly with CuCN in DMF. Oxidation of V was carried out with SeO_2. Since the resulting glyoxal, VI, was unstable in air, a methanol solution of VI was used for the next step without purification. Formation of the imidazole ring was done with HCHO and NH_3 from VI. In order to obtain pure VII, extensive solvent extraction of the VII-copper complex was necessary. Otherwise, VII was accompanied by tarry materials which were difficult to remove. Reduction of the cyano group of VII was readily carried out using $LiAlH_4$. III was rather unstable in air and underwent ready discoloration, while III-HCl was sufficiently stable.

Titration. The titration behavior of the catalysts and the substrate were investigated at 30.0± 0.05°C in 1M aqueous KCl. The free naphthylimidazole catalyst (III) was dissolved in 1M aqueous KCl at 30°C and the solution was adjusted to pH 3 by adding a small amount of hydrochloric acid. Titration was carried out by adding aqueous alkali in small portions. When pH of the solution reached approximately 9.8, the catalyst started to precipitate. This compound can exist in the form of the following species:



The titration curve of this compound shown in Fig. 1 indicates the presence of two dissociation steps. Since species $AmIm^-$ need not be considered in this pH range and the amount of $AmIm^+$ appears negligible, the two dissociation steps observed were concluded to correspond to processes Am^+Im^+ to Am^+Im and Am^+Im to AmIm. pK_a values determined from the titration curve by the method of Noyes[19] are given in Table 1.

The hydrolysis product, *p*-hydroxybenzoic acid, similarly gives the following two dissociation steps.



The pK_a values of III obtained from the titration curve of Fig. 1 are shown in Table 1. The titration of imidazole and 1-aminomethylnaphthalene was carried out in the same way and the pK_a (half neutralization point) is given in Table 1. The titration curve of the latter compound (Fig. 1), however, did not show a sharp transition, and the estimation of pK_a was less precise.

The pH dependence of the fractions of each species of III present in the system was calcluted as shown in Fig. 2 from the pK_a values obtained.

Spontaneous Hydrolysis. The substrate underwent spontaneous hydrolysis at higher pH. The results of the spontaneous hydrolysis at pH 8.0 are given in Fig. 3. Its rate was proportional to the substrate concentration, *viz.*,

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

19) A. Albert and E. P. Serjeant "Ionization Constants of Acids and Bases. A Laboratory Manual," Methuen, 1962, Translated by S. Matsuura, Maruzen Co., Tokyo (1964), p. 45.

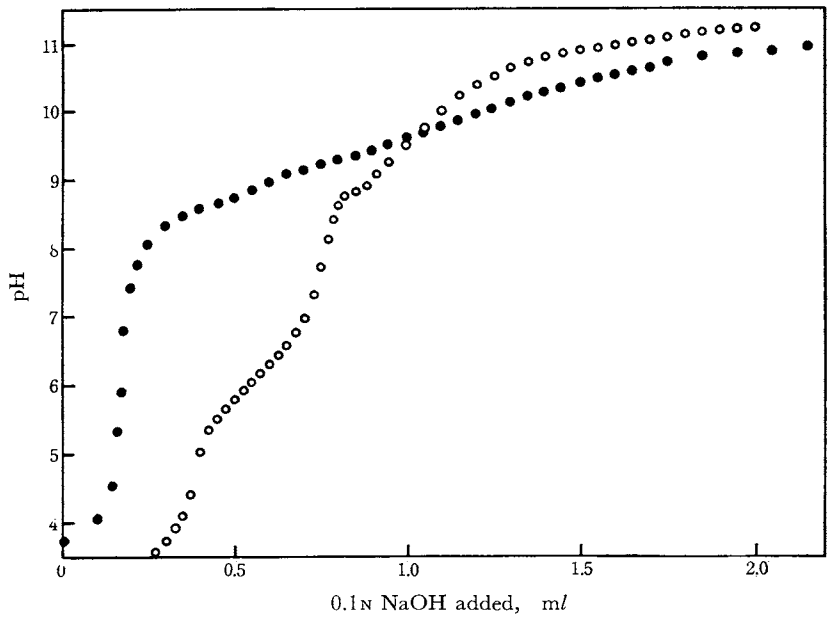


Fig. 1. Titration curves of catalysts.
30°C; μ=1.0M KCl
○: 1-[4(5)-imidazolyl]-4-aminomethylnaphthalene
●: 1-aminomethylnaphthalene

TABLE 1. pK_a VALUES[a)]

Compound	pK_{a1}	pK_{a2}
III	5.92±0.05	8.95±0.08
VIII	9.95±0.09	
Imidazole	7.13±0.03	
p-Hydroxybenzoic acid	4.44±0.04	9.17±0.04

a) 30°C, 1M aqueous KCl

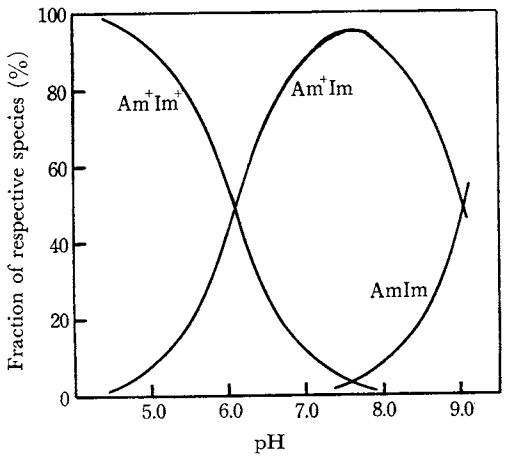


Fig. 2. pH dependence of the fractions of the species of compound III.
30°C; μ=1.0M KCl

$$v_{spont} = k_{spont} \cdot [S] \quad (5)$$

k_{spont} determined by the least square method was

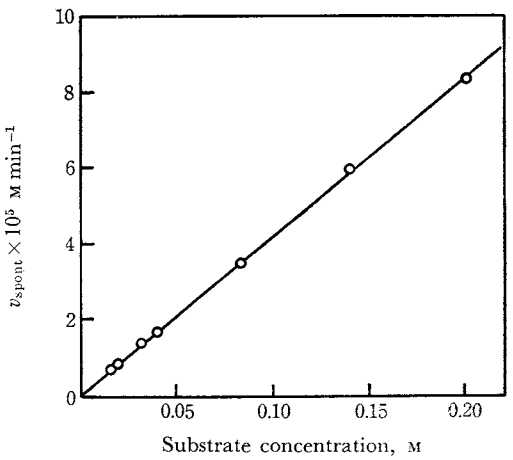


Fig. 3. Spontaneous hydrolysis.
30°C; pH 8.0; 1.0M KCl

$(4.23\pm0.02)\times10^{-4}$ min^{-1}. The spontaneous hydrolysis was not detectable at pH 7.0.

Catalytic Hydrolysis. The overall rate of hydrolysis is the sum of the rates of the catalytic hydrolysis and of the spontaneous hydrolysis, *viz.*,

$$v_{total} = v_{cat} + v_{spont} \quad (6)$$

The amount of the substrate which was spontaneously hydrolyzed prior to the addition of the catalyst solution was determined by estimating the amount of substrate and alkali used for neutralization. The extent of the spontaneous hydrolysis was 0.8% for 0.04M substrate and about 4% for 0.20M substrate

at pH 8.0. This decrease in the substrate concentration was taken into account. The inhibitory effect of the hydrolysate was rather small and neglected in the determination of the initial rate of the catalytic hydrolysis (see below).

These corrections were not necessary at pH 7.0, because spontaneous hydrolysis was not detected. The amount of alkali necessary to compensate the acid formed by hydrolysis depends upon the pH of the system and on pK_a of the substrate. For instance, 1.063 mol of alkali is required for hydrolysis of 1.000 mol of the substrate at pH 8.0.

The relationships between v_{cat} and [S] at pH 7.0 and 8.0 are shown in Fig. 4. In both cases, v_{cat} showed substrate saturation phenomena. As already shown for polymer catalysts in previous publication,s[1,2] these kinetic patterns are typical of the enzyme-catalyzed reaction, indicating formation of the Michaelis complex between catalyst and substrate (Eq. (1)). The correspondin catalytic rate is given by

$$v_{cat} = \frac{k_3 \cdot [C][S]}{K_m + [S]} \tag{7}$$

As mentioned in the previous publication,[1b] k_3 values for the imidazole catalysts studied so far are rather small, and the Michaelis constant K_m may be considered to represent the true dissociation constant in the following discussion. The kinetic constants in this equation are determined from the following equation by plotting $1/v_{cat}$ *vs.* 1/[S] (the Lineweaver-Burk plots[20]).

$$\frac{1}{v_{cat}} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \tag{8}$$

where

$$V_{max} = k_3[C].$$

Since III is present in various forms, depending on pH of the system (Eq. (3)), the large difference between v_{cat} values at pH 7.0 and 8.0 suggests that catalytic efficiencies among various species of III vary to an appreciable extent. In the pH range studied, the catalytic species can be limited to Am^+Im and AmIm. If these two species show the enzyme-like catalysis indepnedently, the overall rate consists of the two terms as follows:

$$v_{cat} = \frac{k_3[C][S]}{K_m + [S]} + \frac{k_3'[C'][S]}{K_m' + [S]} \tag{9}$$

where the first term of the right side refers to AmIm and the second term to Am^+Im.

At $[S] \gg K_m, K_m'$, the maximum rate is given by

$$V_{max} = k_3[C] + k_3'[C'] \tag{10}$$

The Lineweaver-Burk plots of the data of Fig. 4 are given in Figs. 5 and 6. The reciprocal rela-

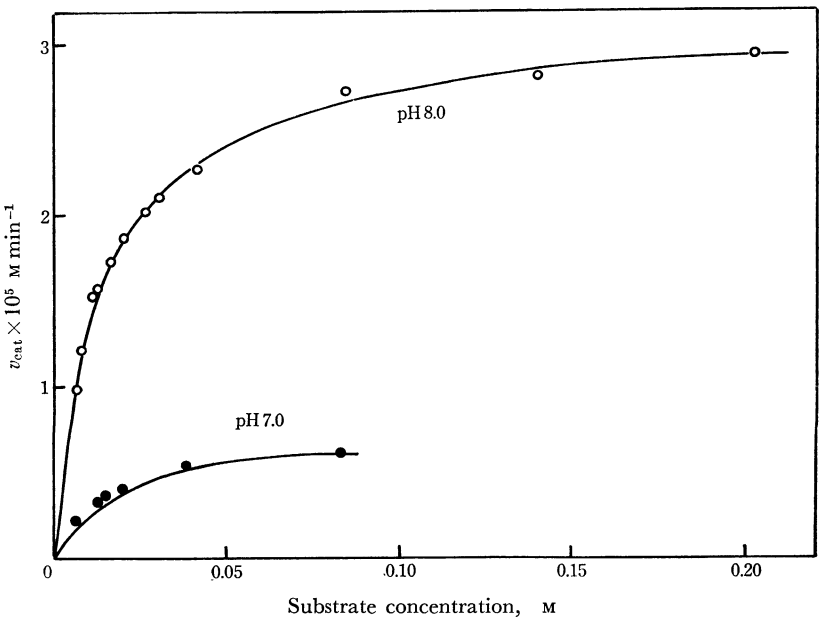


Fig. 4. Catalytic hydrolysis.
30°C; 1.0M KCl; Catalyst III 1.39×10^{-3}M; ○ at pH 8.0; ● at pH 7.0. The solid lines were obtained using K_m and k_3 values for the two catalytic species (Table 2).

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

tionships were seemingly linear,*2 and the values of V_{max} at the respective pH were determined from the intercepts. Since the fractions of each catalytic species are known from Fig. 2, k_3 and k_3' were determined to be 1.9×10^{-1} and 3.6×10^{-3} min^{-1}, respectively, by solving Eq. (10) for two values of pH.

The determination of the Michaelis constants was done by a computational trial and error as follows: K_m and K_m' were varied by an interval of 0.001M in Eq. (9) and the corresponding v_{cat} was calculated for respective substrate concentrations (v_{calcd}) from the known k_3, k_3', [C] and [C'] values. v_{calcd} was then compared with the observed rate of the catalytic hydrolysis (v_{obsd}). A set of K_m and K_m' was obtained which minimized the relative error.

$$E_{rel} = \{[\sum_{i=1}^{N}((v_{obsd})_i/(v_{calcd})_i-1)^2]/N\}^{1/2} \quad (11)$$

where N is the number of experimental runs.

When these procedures were carried out for the hydrolysis data at pH 8.0, E_{rel} was minimal (2.7%) for K_m=0.011M and K_m'=0.041M. In Table 2 are given the kinetic constants for the two catalytic species, together with the apparent kinetic constants at the two values of pH as obtained from the overall Lineweaver-Burk plots (Figs. 5 and 6). The kinetic constants obtained for catalysis of 1-aminomethylnaphthalene (see below) were also included in the table. Using these kinetic constants and the fractions of the catalytic species present, the substrate saturation curves were calculated as shown by the solid curve in Fig. 4. The agreement between v_{obsd} and v_{calcd} was satisfactory at pH 8.0 while v_{calcd} was somewhat lower than v_{obsd} at pH 7.0.

Table 2. Kinetic constants

Catalytic species	K_m (mM)	k_3 (min^{-1})	k_3/K_m (M^{-1}min^{-1})
Overall (pH 8.0)	13	0.023	1.3
Overall (pH 7.0)	16	0.053	0.32
AmIm	11	0.19	18
Am$^+$Im	41	0.0036	0.088
VIII	280	1.8	6.5

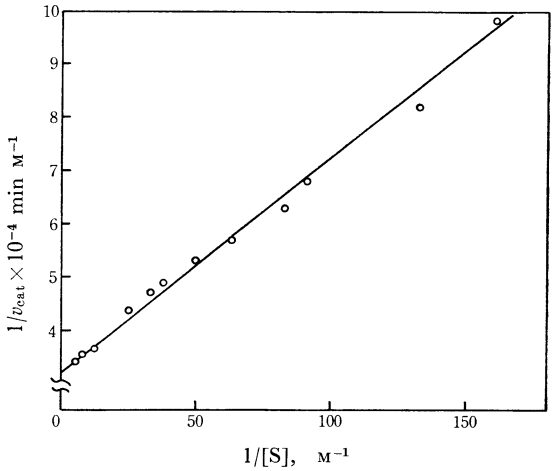


Fig. 5. Lineweaver-Burk plots of the rate data at pH 8.0.
The solid line was obtained by the least squares method.

Effect of pH and Inoic Strength on v_{cat}. The rates of catalytic hydrolysis were measured for [S]=0.010M at several values of pH (Fig. 7). The

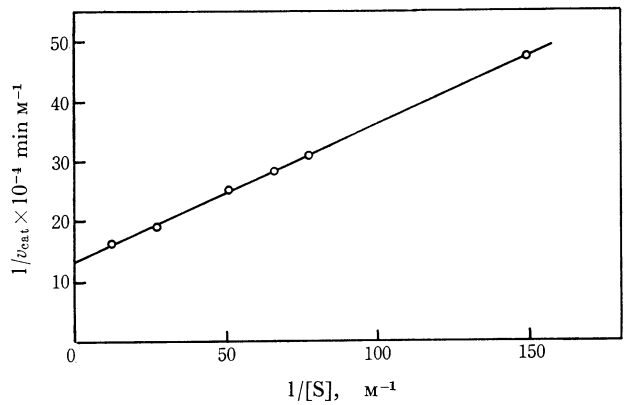


Fig. 6. Lineweaver-Burk plots of the rate data at pH 7.0.
The solid line was obtained by the least squares method.

*2 The Lineweaver-Burk plots of the overall catalytic rate are not necessarily linear, when more than one catalytic species with different K_m values are present.[1b] In the present case, K_m values for AmIm and Am$^+$Im are fairly close and the Lineweaver-Burk plotting of the overall catalytic rate did not deviate much from linearity. Thus, the linear extrapolation of the reciprocal plots to obtain k_3 values at 1/[S]=0 (Eq. (10)) may be justified.

rate was much greater at higher pH, supporting the higher catalytic efficiency of AmIm as compared with Am+Im. When v_{cat} was calculated from the kinetic constants of Table 2 for $[C]=1.39\times10^{-3}$M, the calculated rate was greater at higher pH and somewhat lower at lower pH than the observed rate. They were comparable at pH 7 to 8.

The influence of ionic strength on v_{cat} is shown in Table 3. v_{cat} decreased with decrease in the ionic stregth.

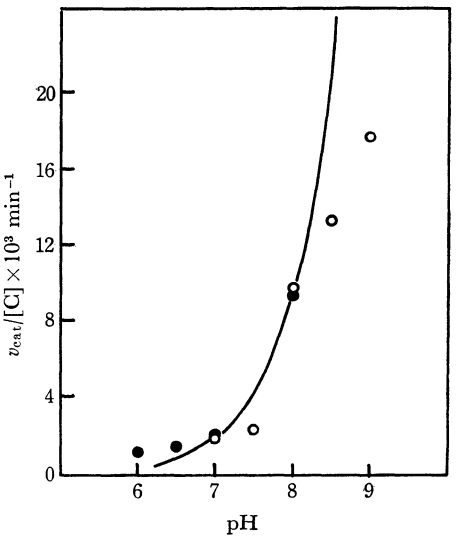


Fig. 7. pH dependence of the catalytic rate.
30°C; 1.0M KCl; substrate 0.010M
○: catalyst, III 1.98×10^{-3}M
●: catalyst, III 1.39×10^{-3}M
The solid curve was obtained by using the kinetic constants of Table 2.

TABLE 3. EFFECT OF IONIC STRENGTH ON v_{cat}[a)]

Ionic strength	$v_{cat}\times10^{6}$(M min^{-1})
0.012	8.1
0.112	10.3
1.01	14.7

a) Reaction condition: pH 8.0; 30°C; substrate 0.010M; catalyst 1.39×10^{-3}M.
b) The sum of KCl added and NaCl formed by neutralization by the time at which the initial rate was measured.

Product Inhibition. Figure 8 shows the rate-depressing effect of *p*-hydroxybenzoic acid (product of hydrolysis). v_{cat} decreased with increase in the amount of the product added, and the rate decrease was appreciable when the amount of the product was greater than 4 mM. Addition of 8 mM of the product lowered v_{cat} to about 30% of the original value. Further analysis of the inhibition was not attemped considering the kinetic complexity of this system.

It was mentioned above that the substrate underwent spontaneous hydrolysis during its dissolution process in the reaction mixture at pH 8.0. However, considering the limited extent of spontaneous hydrolysis and the small rate-depressing effect at low concentrations of *p*-hydroxybenzoic acid (Fig. 8), it was concluded that the correction of v_{cat} due to the inhibitory action of the product was not necessary.

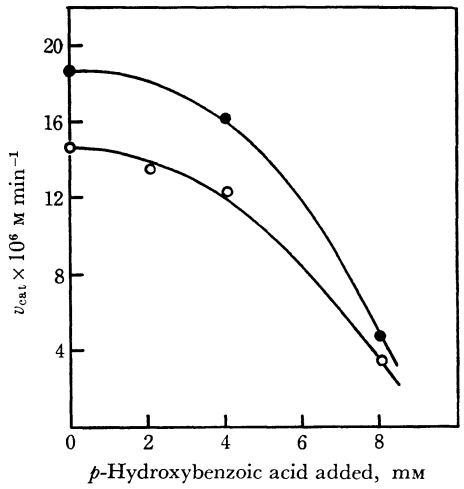


Fig. 8. Effect of *p*-hydroxybenzoic acid on v_{cat}
30°C; pH 8.0; μ=1.0M KCl; catalyst, III 1.39×10^{-3}M; ○, substrate 0.020M; ●, substrate 0.010M.

Recovery of the Catalyst. The imidazole catalyst III was precipitated from the reaction mixture by adding a few drops of 1.7N aqueous NaOH (pH>10). The precipitate was separated by centrifugation and subjected to infrared analysis. Hydrolysis was carried out under standard conditions with $[C]=1.39\times10^{-3}$M and [S]=0.010M. On an average, one mole of the catalyst had hydrolyzed 0.47 mol of the substrate when the catalyst was recovered. No amide absorption was found for the recovered catalyst, indicating that the amino group of the catalyst was not acetylated.

Catalytic Hydrolysis by 1-Aminomethylnaphthalene. The relationship between v_{cat} and [S] is shown in Fig. 9 for the catalytic hydrolysis by 1-aminomethylnaphthalene. A tendency of substrate saturation was observed, although it was much less pronounced than that observed for catalysis of III. The corresponding Lineweaver-Burk plots (Fig. 10) were linear and yielded the following data: $K_m=0.28$M and $V_{max}=5.7\times10^{-5}$M min^{-1}. Since the fraction of the non-protonated catalyst was calculated to be 2.2% at pH 8.0 from its pK_a value, k_3 for the neutral species was 1.8 min^{-1}.

In order to see the catalytic role of the amino group, hydrolysis was carried out at a higher catalyst concentration: $[C]_{total}=0.010$M, [S]=0.044M, μ=1.0M KCl, 30°C, pH 8.0. The reaction was stopped

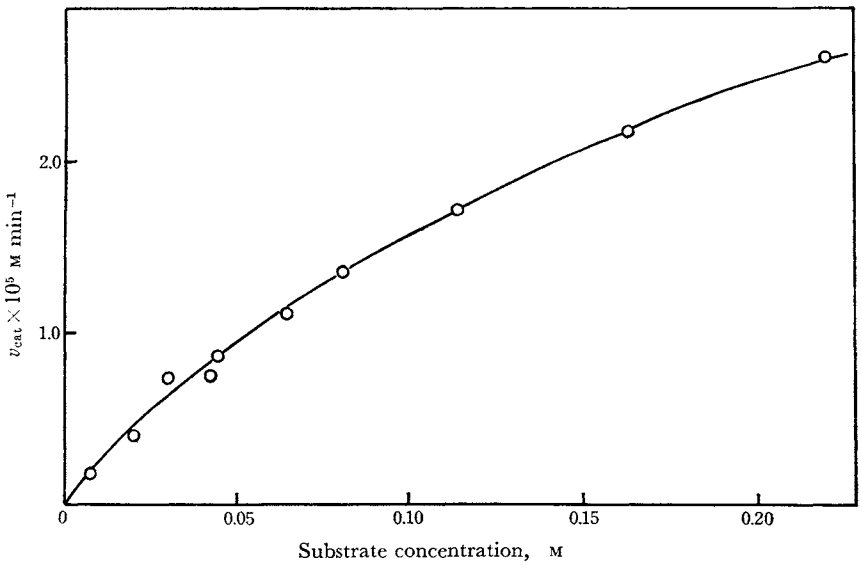


Fig. 9. Catalytic hydrolysis by 1-aminomethylnaphthalene. 30°C; pH 8.0; μ=1.0M KCl; catalyst, 1.39×10^{-3}M.
The solid line was obtained from the kinetic constants given in Table 2.

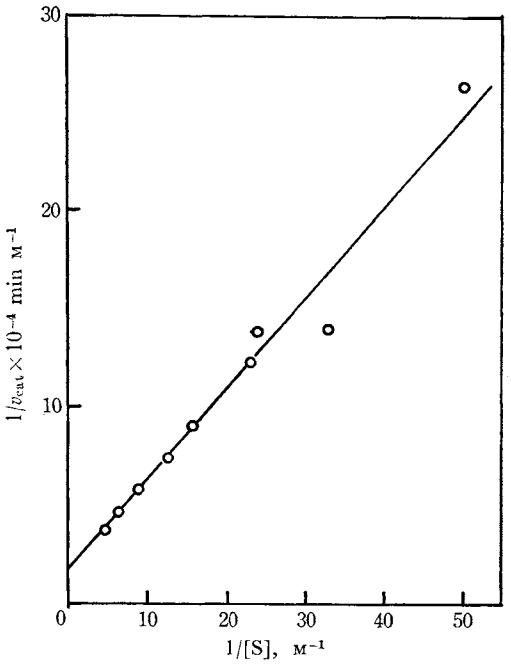


Fig. 10. Lineweaver-Burk plots of the data of Fig. 9.
The solid line was obtained by the least squares method.

when the amount of phenyl ester hydrolyzed reached 4.5×10^{-3}M (0.005M alkali consumption). The catalyst was recovered by ether extraction, drying and evaporation of ether. An IR spectrum of the recovered material (brown oil) did not show amide absorption.

Catalytic Hydrolysis by Imidazole. When imidazole was used as a catalyst, the catalytic rate of hydrolysis was first order with respect to the substrate concentration, as shown in Fig. 11. The fraction of the neutral imidazole was 88.1% at pH 8.0. The second-order rate constant obtained from the slope was 0.325M^{-1} min^{-1}.

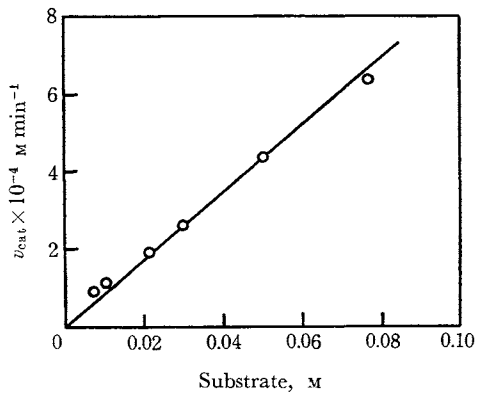


Fig. 11. Catalytic hydrolysis by imidazole.
30°C; pH 8.0; μ=1.0M KCl; imidazole, 0.0278M.
The solid line was obtained by the least squares method.

Discussion

Validity of the Enzyme-like Catalytic Mechanism. Catalytic hydrolysis with the naphthylimidazole derivative, III, showed substrate saturation phenomena, one of the important characteristics of enzyme-catalyzed reactions, as shown in Fig. 4.

The substrate saturation phenomenon, however, cannot exclude the possible coexistence of the common bimolecular catalysis by the uncomplexed imidazole catalyst (Eq. (12)) with the enzyme-like process (Eq. (1)).

$$\text{catalyst} + \text{substrate} \xrightarrow{k'} \text{catalyst} + \text{product} \qquad (12)$$

When these two catalytic processes coexist,[1b] the overall catalytic rate is given by

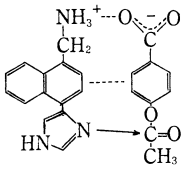
$$v_{cat} = k_3[CS] + k'[C]_{uncomplexed} \cdot [S] \qquad (13)$$

$$= (k_3 + k'K_m) \cdot \frac{[C]_{total} \cdot [S]}{K_m + [S]} \qquad (14)$$

This means that substrate saturation phenomena will be observed even in the presence of the second-order process (Eq. (12)), as long as the complexation occurs, and the relative importance of the two processes is determined from a comparison of k_3/K_m and k'. The catalytic hydrolysis of the substrate by imidazole was first-order with respect to substrate, the second-order rate constant being 0.325 $M^{-1}min^{-1}$. Since the nucleophilic reactivity of imidazole derivatives towards *p*-nitrophenyl acetate increased exponentially with the increases in pK_a,[21] the hypothetical second-order reactivity (k') of the imidazole group in III ($pK_a=5.92$) would be much smaller than that of imidazole ($pK_a=7.13$, $k'=0.325$ $M^{-1}min^{-1}$). Therefore, the overall second-order reactivity of III expressed by the k_3/K_m term (0.32 at pH 7 and 18 at pH 8) may be assumed to be much greater than the hypothetical second-order reactivity of the imidazole group in III. Thus, the enzyme-like process (Eq. (1)) is concluded to be the major pathway of the catalytic hydrolysis under the present experimental conditions.

Substrate Binding. In the hydrolysis of a phenyl ester catalyzed by imidazole-containing copolymers,[1] the binding function of the catalyst was ascribed to hydrophobic forces. The results in the present system point to the same conclusion.

There are several investigations which showed rate enhancement[22-24] and substrate saturation[25,26] due to the electrostatic interaction of catalysts and substrates. Recently, Bruice *et al.*[27] considered the possible cooperative action of electrostatic and hydrophobic forces between catalysts and substrates of the detergent-like structure. Similarly, at the biginning of the present study, we assumed that an analogous cooperative action for III, as shown by IX, would be observed. However, the Michaelis constant K_m for species AmIm was smaller than that of Am^+Im (11 mM *vs.* 41 mM), indicating that



IX

protonation of the aminomethyl group resulted not in an electrostatic attraction toward the anionic substrate but in a decrease of the binding capacity of the catalyst. Thus, the hydrophobic interaction must be responsible for formation of the Michaelis complex for both of the catalytic species (AmIm and Am^+Im) at least under the hydrolysis conditions used. This conclusion was further supported by the fact that v_{cat} decreased when the ionic strength of the reaction system decreased (Table 3). If the electrostatic interaction were responsible for the substrate binding, a decrease in the ionic strength would have caused rate enhancement.

Pseudo-Intramolecular Process. The chemical nature of the product-forming step in the present catalytic system remains to be elucidated.

Since III was recovered after hydrolysis at pH 8.0 without undergoing acetylation of its amino group, it is clear that the naphthylimidazole derivative acted as a true catalyst. The imidazole group in III may act as either a nucleophilic or general-base catalyst,[9b] and these two processes are indistinguishable from the kinetic data alone. The amino group, protonated and unprotonated, can also reinforce the catalytic action of the imidazole group as a general-acid and general-base catalyst, respectively. Involvement of the protonated amino group in catalysis, however, appears less likely, considering the much smaller k_3 for Am^+Im as compared with that for AmIm (0.0036 min^{-1} *vs.* 0.19 min^{-1}).

This large difference in k_3 is interesting, and is conceivably related to the structural variation of the Michaelis complex. The greater pseudo-intramolecular catalytic efficiency of AmIm may be explicable on the basis of either or both of the following hypotheses: a) The spacial arrangement of the imidazole and ester groups in the Michaelis

21) T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.* **80**, 148 (1958).
22) R. L. Letsinger and T. J. Savereide, *ibid.*, **84**, 114, 3122 (1962).
23) C. G. Overberger, T. St. Pierre, N. Vorchheimer, J. Lee and S. Yaroslavsky, *ibid.*, **87**, 296 (1965).
24) C. G. Overberger, R. Sitaramaiah, T. St. Pierre and S. Yaroslavsky, *ibid.*, **87**, 3270 (1965).
25) R. L. Letsinger and I. S. Klaus, *ibid.*, **87**, 3380 (1965).
26) C. G. Overberger, T. St. Pierre, N. Vorchheimer, J. Lee and S. Yaroslavsky, *Macromolecules*, **1**, 331 (1968).
27) T. C. Bruice, J. Katzhendler and L. R. Fedor, *J. Phys. Chem.*, **71**, 1961 (1967); *J. Amer. Chem. Soc.*, **90**, 1333 (1968).

complex is less favorable for catalysis when the amino group is protonated than when it is not; b) the imidazole and amino groups in AmIm catalyze the ester cleavage cooperatively, while the protonated amino group in Am^+Im cannot be involved in catalysis.

In the above discussion, the catalyst molecules were assumed to be molecularly dispersed. Considering the poor solubility of III it is possible that the catalyst molecules, AmIm in particular, were extensively associated. If the associated catalytic species show different catalytic activities from those of the molecularly dispersed species, the simple allotment of the overall catalytic activity to the two species AmIm and Am^+Im becomes questionable. In fact, as shown in Fig. 7, there was considerable discrepancy between the pH dependence of the observed and calculated catalytic rates. Since AmIm would associate more readily than Am^+Im in the case where the catalyst molecules are not molecularly dispersed, the pH dependence of v_{cat} would be correspondingly complex. Then association of the catalyst molecules could be the cause for the discrepancy. However, the fact that v_{cat} did not vary with the catalyst concentration at pH 7 and 8 (Fig. 7) speaks against this hypothesis, since association of the catalyst molecules, if any, must be concentration dependent, leading to the dependence of $v_{cat}/[C]$ on the catalyst concentration. Another possible cause for the discrepancy might be either or both of underestimation of the catalytic activity of AmIm and of overestimation of the catalytic activity of Am^+Im. At present, experimental data are not sufficient to warrant further discussion on these problems. However, the remarkable variation of the catalytic efficiency with pH (hence the nature of the catalytic species) suggests that catalytic efficiency is much more widely variable when the enzyme-like kinetics are observed than when the common second order kinetics are observed.

Catalysis of 1-Aminomethylnaphthalene. K_m for 1-aminomethylnaphthalene, VIII, was quite large (280 mM), showing the small binding capacity of this catalyst as compared with those of the imidazole derivatives. Since the substrate saturation phenomenon was not very clear in this system, it is not certain if the catalytic action consisted only of the enzyme-like process (Eq. (1)), in spite of the linear Lineweaver-Burk plots obtained. Assuming that the enzyme-like pathway is predominant, the large difference in K_m between III and VIII suggests that the imidazole group made an important contribution to the binding function. It is interesting that the primary amino group in VIII acted as a catalyst without undergoing acetylation. This was also the same with AmIm. The lack of acetylation indicates that VIII was a general-base catalyst, in marked contrast with the commonly observed nucleophilic reaction of the primary amino group with phenyl acetates. This result may be an indication of the particular geometry of the Michaelis complex, in which the nucleophilic attack of the amino group toward the ester group is not favored. The large k_3 value (1.8 min^{-1}) is probably related to the high basicity of the amino group ($pK_a=9.95$) as well as to the particular geometry of the complex. Although high K_m and k_3 values observed for VIII catalysis is in sharp contrast to those for the imidazole catalysts, it is not warranted to discuss the difference more fully, since substrate saturation with VIII was not sufficient and its catalytic behavior can be more complex than it appears.