1304 BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48(4), 1304—1309 (1975) [Vol. 48, No. 4

Multifunctional Hydrolytic Catalyses. III. Catalytic Hydrolysis of *p*-Nitrophenyl Acetate by Imidazole-4-carbohydroxamic Acids[1)]

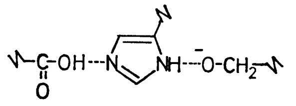
Toyoki Kunitake and Seiji Horie

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812

(Received November 5, 1974)

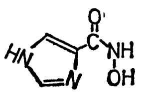
Imidazole-4-carbohydroxamic acids (I) and its *N*-methylhydroxamic acid derivative (II) were synthesized from imidazolecarboxylic acid and hydroxylamines, and the catalytic hydrolysis of *p*-nitrophenyl acetate was studied. The reaction was analyzed by the pseudo-first-order kinetics and the burst kinetics. The hydrolysis proceeds *via* the formation and the subsequent decomposition of the acetyl hydroxamate intermediate. The acylation rate with these catalysts were comparable to those observed with other simple hydroxamic acids. On the other hand, the deacylation of the acetyl intermediate of II was greater than expected and increased with pH. This suggests that the intramolecular imidazole group (neutral and/or anionic species) assists deacylation, thus increasing the overall efficiency of catalysis.

Multifunctional catalysis is one of the major characteristics of the enzyme reaction. A typical example is the charge relay system which is present at the active site of several serine proteases.[2)] This system is composed of a series of hydrogen bonding connecting the aspartyl carboxylate, histidyl imidazole and seryl hydroxyl groups, and acylation by specific ester and amide substrates occurs at the seryl hydroxyl group. Therefore, a combination of the imidazole function and a good oxygen nucleophile like the hydroxamate anion would be considered as a model charge relay system.

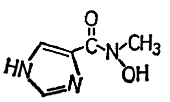


charge relay system

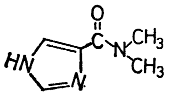
The present paper is an account of our initial efforts toward preparations of such catalysts in small-molecule systems. The catalyst compounds employed are imidazole-4-carbohydroxamic acid (I) and its *N*-methyl derivative (II). Similar attempts by using polymer catalysts have been described elsewhere.[3,4)]



I



II



III

Experimental

Melting points are uncorrected. UV-visible spectroscopic measurements were made with a Hitachi 124 spectrophotometer. NMR spectra were obtained with a Varian A 60 instrument.

Materials. *p*-Nitrophenyl acetate was prepared by reaction of *p*-nitrophenol with acetic anhydride and recrystallized from cyclohexane, mp 78 °C (lit[5)], 81—82 °C).

Imidazole-4-carboxylic acid was prepared essentially by the procedure of Pyman[6)] by oxidation of 4-hydroxymethylimidazole[7)] with concentrated nitric acid (d=1.38) at 80 °C for 3 hr. After the reaction was over, solvent was removed *in vacuo*. Water was removed completely as an azeotrope with benzene. The remaining colorless granules were dissolved in a smallest amount of water, and the solution was neutralized to pH 6 by slow addition of a cold saturated aqueous solution of Na_2CO_3. Colorless precipitates were recrystallized repeatedly from hot water. Mp 285—287 °C (dec) (lit[6)] mp 281 °C (dec)).

According to the procedure of Wegner and Weidenhagen,[8)] imidazole-4-carboxylic acid was converted by PCl_5 to the corresponding acid chloride. The pale yellow, hygroscopic solid was used as such for the subsequent experiment.

N,N-*Dimethylimidazole-4-carboxamide (III)*. The acid chloride was prepared from 1.0 g (0.0089 mol) of imidazolecarboxylic acid and 1.78 g (0.0089 mol) of PCl_5. Dimethylamine hydrochloride (1.4 g, 0.0178 mol) was mixed with 3.6 g (0.036 mol) of triethylamine in 30 m*l* of DMF. Triethylamine hydrochloride was filtered and the solution was added to the solid acid chloride with cooling. After standing overnight, precipitates were filtered and the solvent removed *in vacuo*. The dark brown, viscous residue was carefully dissolved in concentrated hydrochloric acid and, then solvent was removed *in vacuo* from the resulting solution. The white solid formed was recrystallized from a mixture of ethanol and ethyl acetate and from ethanol to give pale yellow needles: yield 30—40%, mp 196—198 °C. IR and NMR spectra were consistent with the structure. Found: C, 39.05; H, 5.98; N, 22.81%. Calcd for $C_6H_9N_3O\cdot 1/2H_2O$: C, 39.12; H, 5.96; N, 22.76%.

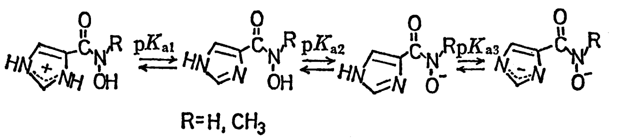
Imidazole-4-carbohydroxamic Acid (I). Hydroxylamine hydrochloride (10.0 g, 0.144 mol) was reacted with 29 g (0.287 mol) of triethylamine in DMF, and triethylamine hydrochloride was filtered. The solution was added to the solid acid chloride which had been prepared from 2.8 g (0.025 mol) of imidazolecarboxylic acid. After standing overnight, triethylamine hydrochloride was filtered and the solvent removed *in vacuo*. The pale red residue was dissolved in hydrochloric acid and the solvent evaporated to dryness. Recrystallization from 1 : 2 H_2O-CH_3OH gave 1.5 g (40% yield) of colorless needles. Mp 202—206 °C. IR: 3420 cm^{-1} (ν_{NH}, hydroxamic acid), 1650 cm^{-1} ($\nu_{C=O}$, hydroxamic acid). Found: C, 26.57; H, 4.35; N, 23.18%. Calcd for $C_4H_5N_3O_2\cdot HCl\cdot H_2O$; C, 26.44; H, 4.41; N, 23.14%.

N-*Methylimidazole-4-carbohydroxamic Acid (II)*. *N*-Methylhydroxylamine hydrochloride (4.0 g, 0.042 mol) which was obtained by zinc reduction of nitromethane was mixed with 8.0 g (0.080 mol) of triethylamine in dry DMF, and triethylamine hydrochloride formed was filtered. The DMF solution was added to the acid chloride which had been prepared from 2.24 g (0.020 mol) of imidazolecarboxylic acid. The work-up was the same as that described above. Pale yellow crystals were obtained as the hydrochloride in 40% yield and recrystallized from methanol or from methanol-acetonitrile: mp 196—197 °C (dec). IR: hydroxamic acid, 3220 cm^{-1} (ν_{OH}), 1640 cm^{-1} ($\nu_{C=O}$); imidazole, 3080, 2800, 860 cm^{-1}. Found: C, 33.85; H, 4.50; N, 23.58%. Calcd for $C_5H_7N_3O_2\cdot HCl$: C, 33.80; H, 4.51; N, 23.66%.

Rate Measurements. The rate of reaction of hydroxamic acids with PNPA was determined by following the formation of the *p*-nitrophenolate anion at 401 nm. A 1-cm cell was employed for the hydrolysis of PNPA at low concentrations. On the other hand, when high PNPA concentrations were used in order to observe the burst kinetics, a 0.9-cm spacer was inserted into the 1-cm cell.

Results

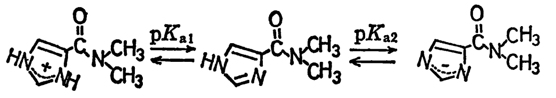
pK_a *Determination.* Imidazolecarbohydroxamic acids I and II possess the following three dissociation processes:



(1)

The pK_a values for the first two processes can be readily determined by the potentiometric titration. The titration was carried out by using a Toa pH stat system, using a jacketed titration vessel. Water was circulated from a constant temperature bath and 0.1 N alkali was added in 0.05-m*l* portions from an autoburet. The pK_{a3} value was difficult to determine by this method.

Two dissociation processes are present for imidazole derivative III, and its pK_{a1} was determined by the potentiometric titration.



(2)

The pK_a values for the dissociation of the imidazole proton were difficult to determine by the potentiometric titration, because they are above 10.

The UV titration can be employed for the pK_a determination by using the characteristic absorption of each species.[9)]

$$\mathrm{p}K_a = \mathrm{pH} + \log\frac{\mathrm{OD_I}-\mathrm{OD}}{\mathrm{OD}-\mathrm{OD_M}} \tag{3}$$

where $\mathrm{OD_I}$: absorbance of the ionic species at a given wavelength, $\mathrm{OD_M}$: absorbance of the neutral species and OD: absorbance of species mixtures. The above equation is transformed to

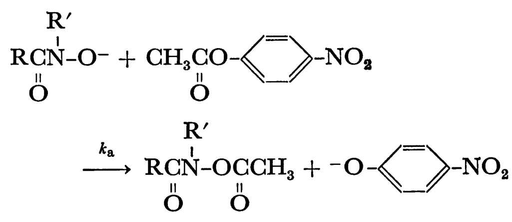
$$\mathrm{OD} = \mathrm{OD_I} - \frac{1}{K_a}[\mathrm{H^+}](\mathrm{OD}-\mathrm{OD_M}) \tag{4}$$

The hydroxamate anion possesses a characteristic UV absorption maximum at *ca.* 270 nm. In the case of hydroxamic acid I, UV spectra showed a complex variation with pH and an attempt to determine pK_a was unsuccessful. An anionic species in which proton on the hydroxamic acid nitrogen is dissociated may exist at higher pH's, in addition to those shown in Eq. (1).

The dissociation behavior of hydroxamic acid II is simpler than that of I. Assuming that only the monoanionic species exist at pH 12, $\mathrm{OD_I}$ (300 nm) = 0.740 and $\mathrm{OD_M}$ (300 nm) = 0.009 were obtained at pH 12 and pH 2, respectively (30 °C, 28.9 v/v% EtOH–H_2O, [I] = 1.13×10^{-4} M). The OD values were determined in the pH range of 8.3 to 9.3, and pK_{a2} was obtained from those data, using Eq. (3). The pK_{a3} value was not obtainable by this method.

The determination of the pK_{a2} value of III from Eq. (3) was not possible, because the accurate determination of $\mathrm{OD_I}$ was difficult. Eq. (4) is useful in this case. Thus, OD (280 nm) was measured at pH 10 to 13, and plotted against $[\mathrm{H^+}](\mathrm{OD}-\mathrm{OD_M})$ ($\mathrm{OD_M}$=0.005, [III] = 2.06×10^{-4} M). A good linear relationship was obtained (correlation coefficient = 0.9998), and $\mathrm{OD_I}$ and pK_a were determined to be 0.300 and 12.09, respectively. These results are summarized in Table 1.

Reaction of I with PNPA. The hydroxamate anion reacts with PNPA quite readily to yield acetyl hydroxamate and *p*-nitrophenol.[10–13)]



(5)

The reaction can be followed very readily by the release of the *p*-nitrophenolate anion. Since PNPA hydrolyzes spontaneously, the overall rate *v* is composed of the catalytic and spontaneous terms.

$$v_{\mathrm{total}} = v_{\mathrm{cat}} + v_{\mathrm{spont}} \tag{6}$$

When hydroxamic acid I (1.98×10^{-4} M) was reacted with an excess of PNPA (3.86×10^{-3} M) at 30 °C, pH 8.92, *p*-nitrophenol (2.28×10^{-4} M) was released within one minute and further release was not observed, apart from the spontaneous hydrolysis. The amount of *p*-nitrophenol released approximately corresponded to that of the hydroxamic acid. This means that the acetyl transfer occurred quantitatively according to Eq. (1), and that the acetyl hydroxamate formed is too stable to regenerate hydroxamic acid.

The acetylation reaction was further investigated under the pseudo-first-order condition ([HA]$\gg$[PNPA]), and $k_{\mathrm{a,obs}}$ was determined by

$$k_{\mathrm{total}} = k_{\mathrm{a,obs}}[\mathrm{HA}]_0 + k_{\mathrm{spont}} \tag{7}$$

$$\log\frac{\mathrm{OD}_\infty}{\mathrm{OD}_\infty-\mathrm{OD}_t} = \frac{k_{\mathrm{total}}\cdot t}{2.303} \tag{8}$$

TABLE 1. pK_a VALUES[a)]

Compound	pK_{a1}	pK_{a2}	pK_{a3}
I	3.52	9.43	>12[c)]
II	4.50	8.90, 8.81[b)]	>12[c)]
III	4.30	12.1[b)]	—
Benzohydroxamic acid	9.36	—	—

a) Determined by the potentiometric titration. 30 °C, aqueous ethanol (ethanol content: 20—30 v/v%). b) Determined by UV titration. The pK_{a2} value of II from the UV titration is less reliable than that obtained by the potentiometric titration and, therefore, not used for the calculation of rate data. c) Estimated from potentiometric titration.

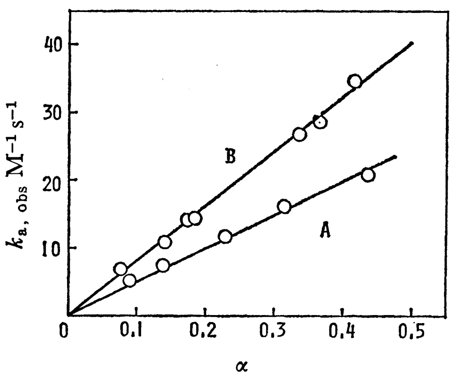


Fig. 1. Dependence of the rate constant of reaction between PNPA and hydroxamic acid upon the degree of dissociation of hydroxamic acids (α).
Reaction conditions: 30 °C, 28.9 vol% EtOH-H_2O, 0.1 M KCl, 0.15 M Barbital buffer.
A: benzohydroxamic acid, 1.88×10^{-3} M. PNPA, 4.07×10^{-5} M.
B: I, 4.44×10^{-4} M, 13.9×10^{-4} M or 17.3×10^{-4} M. PNPA, 3.85×10^{-5} M.

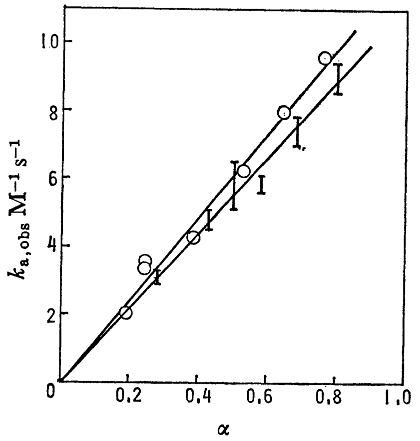


Fig. 2. Dependence of the rate constant of acylation of II by PNPA upon the degree of dissociation of the hydroxamic acid group (α).
Reaction condition: 30 °C, 28.9 vol%EtOH-H_2O, 0.1 M KCl, 0.15 M Barbital buffer.
The circle is the rate constant determined under the pseudo first-order condition. The vertical line indicates the range of $k_{a,obs}$ estimated from the burst kinetics (see Table 2).

where OD_∞ is the absorbance of *p*-nitrophenol at the infinite reaction time and OD_t is the absorbance at time *t*.

The reaction was carried out in the pH range of 8.3 to 9.3, and $k_{a,obs}$ was determined by using Eqs. (7) and (8). Figure 1 shows the relationship of $k_{a,obs}$ thus obtained and α (degree of dissociation of hydroxamic acid). A linear relationship was obtained (correlation coefficient=0.9987). Similar results were obtained for the reaction of benzohydroxamic acid and PNPA.

The k_a values (true second-order rate constant) determined from the slope of Fig. 1 were 80.4 and 50.0 $M^{-1}s^{-1}$ for I and benzohydroxamic acid, respectively.

Reaction of II with PNPA. In the first place, the reaction of PNPA was carried out with large excesses of hydroxamic acid II: *i.e.*, under the pseudo-first-order condition. And $k_{a,obs}$ was determined by the use of Eqs. (7) and (8). The $k_{a,obs}$ value showed a linear dependence on α, as shown in Fig. 2. The true second-order rate constant k_a was determined to be 12.1 $M^{-1}s^{-1}$ from the slope by the least squares method (correlation coefficient=0.9996).

Subsequently, when the reaction was carried out in the presence of large excesses of PNPA relative to II, typical burst reactions were observed: the initial rapid release of *p*-nitrophenol followed by the slower, steady release. This result suggests that the acetyl hydroxamate formed is hydrolyzed to a measurable degree and that the hydroxamate anion is regenerated.

The burst kinetics were analyzed according to the procedure of Bender *et al.*[14] The nucleophilic catalysis of the ester hydrolysis is expressed by

$$C + S \xrightarrow{k_a} \underset{\displaystyle P_1}{\underset{+}{CS'}} \xrightarrow{k_d} C + P_2 \tag{9}$$

where C, S and CS′ denote catalyst, ester substrate and acyl intermediate, respectively.

The formation of *p*-nitrophenol (P_1) is given by

$$[P_1] = At + B(1-e^{-bt}) \tag{10}$$

where $A=a-(a/b)k_a[S]_0$, $B=(a/b^2)\cdot k_a\cdot[S]_0$, $a=k_a[C]_0\text{-}[S]_0$, and $b=k_a[S]_0+k_d$.

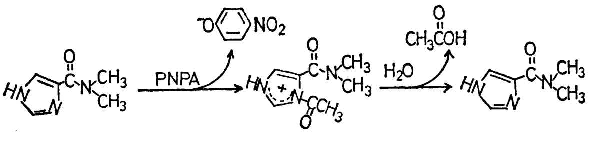
The rate constants may be determined by more than one method. However, the slope of the linear portion was rather small in the present system, and the following equations were employed for calculating rate constants in order to minimize the arithmetic erorr.

$$k_a = \frac{b}{[S]_0}\sqrt{\frac{B}{[C]_0}} \tag{11}$$

$$k_d = b - k_a[S]_0 \tag{12}$$

Since determination of the k_a and k_d values can sometimes involve large errors, the reaction was carried out many times at a given pH, and the undoubtedly erroneous data were discarded. The approximate agreement of the k_a values thus determined with those determined under the pseudo-first-order condition was a measure of the reliability of data. The resulting k_a and k_d values are summarized in Table 2. The k_a value for the hydroxamate anion was determined from the slope of $k_{a,obs}$ *vs.* α (Fig. 2) by the least-squares method to be 11.1 $M^{-1}s^{-1}$.

Reaction of III with PNPA. The hydrolysis of PNPA is catalyzed by imidazole compound III.



(13)

Supposedly, the formation of the acetylimidazole intermediate is rate-limiting, and the decomposition of acetylimidazoles is fast. The reaction was conducted under the pseudo-first-order condition ([III]≫

Table 2. Hydrolysis of PNPA catalyzed by II[a)]

pH	Degree of dissociation of hydroxamic acid (α)	$k_{a,obs}$ $M^{-1}s^{-1}$	$10^4 \cdot k_{d,obs}$ s^{-1}
8.48	0.275	3.24, 3.11 3.35, (3.08)	5.55, 5.04 5.38, (5.92)
8.77	0.426	(5.48), 5.06, 5.09, 4.95, 4.64	(9.08), 7.06, 6.58, 8.75, 6.98
8.93	0.503	5.56, 5.17, 6.30, 6.45, 6.48	8.07, 8.74, 8.29, 7.51, (9.17)
9.04	0.580	5.60, 6.05, 5.80, 5.78	10.43, 10.53, 10.56, 10.00
9.24	0.686	7.05, 7.35, 7.64, 7.80, (8.57)	13.53, (8.87), 12.81, 13.98, 15.31, 16.08
9.50	0.799	9.02, 8.71, 8.56, 9.38, 9.25	17.72, 19.66, 18.14, (23.79), 17.96

a) 30 °C, 0.1 M KCl, 0.15 M Barbital, 28.9 vol% $EtOH-H_2O$, $[HA]_0=(6-8)\times 10^{-4}M$, $[PNPA]=(5-14)\times 10^{-3}M$. The data in parenthesis are less reliable.

Table 3. Reaction of III with PNPA[a)]

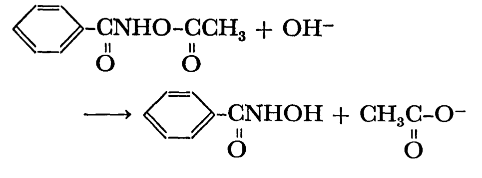
Reaction pH	$10^6 \cdot k_{total}$ s^{-1}	$10^6 \cdot k_{spont}$ s^{-1}	$10^2 \cdot k_{a,obs}$ $M^{-1}s^{-1}$
7.06	2.76	0.86	0.43[b)]
7.84	3.11	1.92	0.26
8.43	7.40	4.82	0.58
9.08	22.0	15.2[c)]	1.51
9.68	82.0	55.7	5.89

a) 30 °C, 0.1 M KCl, 0.15 M Barbital, 28.9 v/v% $EtOH-H_2O$, $[III]=4.46\times 10^{-3}M$, $[PNPA]=1.20\times 10^{-4}M$. b) Phosphate buffer, 0.15 M. c) Obtained at pH 9.04.

[PNPA]). The release of p-nitrophenol was quite slow, and, therefore, the rate constants (k_{total} and k_{spont}) were determined from the initial portion of the first-order plot. The results are summarized in Table 3. The $k_{a,obs}$ value increases rapidly above pH 9.

Hydrolysis of O-Acetyl Benzohydroxamate. O-acetyl benzohydroxamate was dissolved in aqueous alkali (pH 12) and the solution kept at 30 °C. Figure 3 shows the change of the UV spectra with time. An isosbetic point is found at 265 nm. After 24 hr, the spectrum became identical with that of the benzohydroxamate anion. The final concentration of the anion was calculated to be 3.38×10^{-5} M by using $\varepsilon=5150$ at $\lambda_{max}=268$ nm. The initial concentration of O-acetyl benzohydroxamate was 3.16×10^{-5} M.

These UV results imply that the following hydrolysis occurred.



(14)

The half-life of the hydrolysis was estimated to be approximately 4 hr from the UV spectral change. The corresponding rate constant was *ca.* $10^{-5}\,s^{-1}$ at pH 12. The k_a value of acetyl isobutyro-N-methylhydroxamate is estimated to be $0.24\,s^{-1}$ at pH 12.[13)] The difference amounts to more than 10^4 fold.

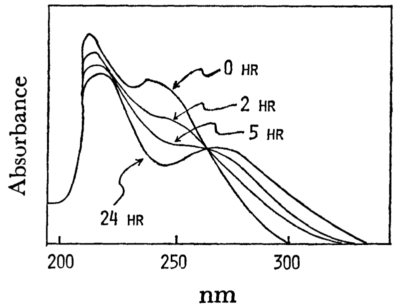
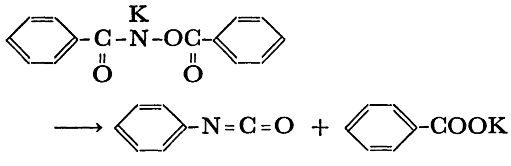


Fig. 3. Spectral change of aqueous acetyl benzohydroxamate.
pH 12, 30 °C.
Acetyl benzohydroxamate; 3.16×10^{-5} M.

The smooth hydrolysis observed for O-acetyl benzohydroxamate is not necessarily consistent with the literature. Dougherty and Jones[15)] reported the occurrence of the Lossen rearrangement along with hydrolysis, and Hauser *et al.*[16,17)] made a kinetic study for potassium N,O-dibenzoylhydroxylamine derivatives in 0.1 N aqueous ammonia.



(15)

In contrast, the exclusive hydrolysis was observed in the present study. It is possible that the O-acetyl derivative is less prone to rearrange than the O-benzoyl counterpart.

Discussion

The object of the present study is two-fold. The hydroxamate anion is a very good nucleophile toward esters. However, the hydrolysis of acyl hydroxamate intermediates is very slow under ordinary reaction conditions, and the use of hydroxamic acids as catalyst for ester hydrolysis is limited. It was hoped that introduction of the neighboring imidazole group would facilitate the deacylation process. Secondly, the combination of a good oxygen nucleophile and the

Table 4. Rate constants of acetylation[a)]

Hydroxamic acid	pK_a of hydroxamic acid group	k_a $M^{-1}s^{-1}$
I	9.43	80.4
II	8.90	12.1[b)], 11.1[c)]
Benzhydroxamic acid	9.36	50.0
Imidazole	6.70[d)]	0.47

a) 30 °C, 28.9 vol% $EtOH-H_2O$, 0.1 M KCl, (0.15 M Barbital). b) Determined under the pseudo-first-order condition. c) Estimated from the burst kinetics. d) pK_a of imidazole.

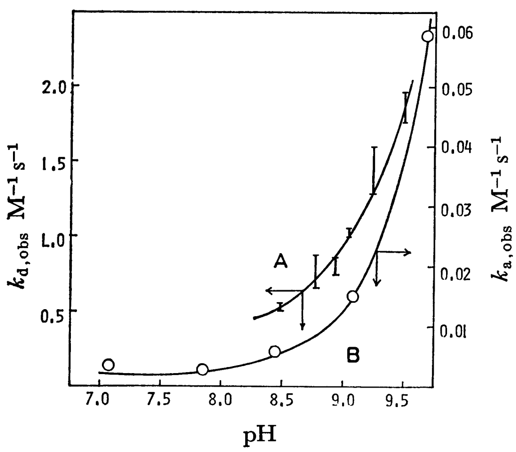


Fig. 4. pH Dependences of deacylation of the acetyl derivative of II(A) and acylation of III with PNPA (B).

Reaction condition: 30 °C, 28.9 vol%EtOH-H_2O, 0.1 M KCl, 0.15 M Barbital buffer.

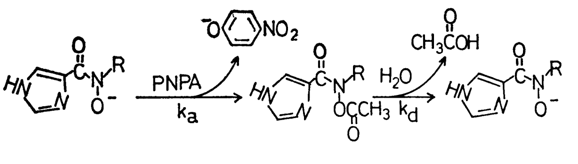
The vertical line indicate the range of $k_{d,obs}$ estimated from the burst kinetics (see Table 2).

imidazole function resembles the charge relay system found at the active site of serine esterases, and an examination of this combination as a model enzyme system is important.

The powerful nucleophilicity of the hydroxamic acids employed are readily seen from the rate data of Table 4. The k_a value of hydroxamic acid I is greater than that of benzohydroxamic acid by 60%. This may be a reflection of the pK_a difference between the two hydroxamic acids (ΔpK_a=0.07), in consistence with a good Brønsted relationship (β=0.72) found by Dessolin *et al.*[11] for ring-substituted benzohydroxamic acids.

The smaller k_a value for II relative to that of I is not unexpected. The nucleophilic reactivity of *N*-alkylhydroxamic acids is several times lower than that of the corresponding *N*-unsubstituted hydroxamic acids, and the reason for this difference has been discussed previously.[12,18] The observed difference in nucleophilicity between I and II is a reasonable one, when compared with other examples.

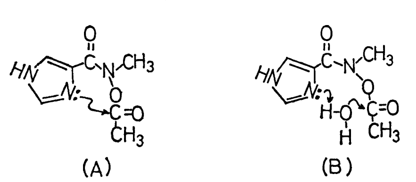
The deacylation reaction of the acetyl derivative of I was very slow. In fact, the occurrence of deacylation could not be detected in the burst kinetics at pH 8 to 9. On the other hand, the deacylation step was clearly observed in the burst kinetics of the *N*-methylhydroxamic acid II.



(16)

Figure 4 shows the pH dependence of the apparent rate constant of deacylation. The observed rate of deacylation is estimated to be much greater than those of simple hydroxamic acids. For example, $k_{d,obs}$ was 2.8×10^{-5} s^{-1} at pH 9 for acetyl *N*-phenylisobutyrohydroxamic acid.[13] The corresponding rate constant for acetylated II is 1×10^{-3} s^{-1}. This difference of almost 40 fold may suggest that deacylation is assisted by the intramolecular imidazole group, as we had conceived.

The intramolecular imidazole group can assist the hydrolysis of the acetyl intermediate by nucleophillic catalysis (A) and/or by general-base catalysis (B).



In general, the general-base mechanism will be of greater significance compared with the nucleophilic mechanism in the hydrolysis of esters whose leaving groups are stronger bases than the imidazole catalyst.[19] The pK_a values of II are 8.9 for the hydroxamic acid group and 4.5 for the imidazole group. Therefore, the pK_a difference is 4.4, and the general-base mechanism B may be the preferred course of deacylation in the pH region where the neutral imidazole species plays a greater role. However, the anionic imidazole species (p$K_a\cong$12.5) is more important above pH 8 (see below) and the intramolecular attack of this species appears to be difinitely nucleophilic.

Bruice and Schmir found in the hydrolysis of PNPA with a large number of imidazole derivatives that imidazoles with values of pK_{a1} of 4.0 or lower owed their catalytic activity partially or primarily to the presence of their anionic species.[20] The increase of $k_{d,obs}$ at higher pH's (Figure 4) suggests the increasing involvement of the anionic imidazole group in the deacylation process. Unfortunately, the pK_{a3} value of II was not possible to determine by titration, and the efficiencies of intramolecular catalysis of the neutral and anionic imidazole species cannot be compared directly. However, pK_{a3} for II in 28.9% EtOH-H_2O may be estimated to be *ca.* 12.5 on the basis of the pK_a data shown in Table 1, by considering the difference of pK_a of the first and second deprotonations of III and the solvent change.

The following Brønsted rules have been reported for the nucleophilic attack of the neutral and anionic imidazole species.[20]

$$\log k_N = 0.8\ pK_{a1} - 4.30 \qquad (17)$$

$$\log k_A = 0.15\ pK_{a2} + 1.35 \qquad (18)$$

where k_N and k_A are rate constants for neutral and anionic imidazoles, respectively.

By substituting pK_a values (pK_{a1}=4.50, pK_{a2}=12.5), the relative rate constant (k_A/k_N) becomes *ca.* 10^4. The fraction of the anionic imidazole is about 10^{-3} at pH 9.5, the rest being the neutral species. Therefore, the anionic imidazole species is ten times more effective than the neutral species in the deacylation at pH 9.5. On the other hand, the anionic imidazole fraction is 4×10^{-5} at pH 8, and the contribution of the anionic imidazole species toward deacylation can be considered negligible below pH 8.

Quite the same conclusion is obtained for the reaction of PNPA with imidazole-4-carboxamide (III).

This reaction is a model reaction of deacylation of the acyl intermediate (compare Eqs. (13) and (16)).

As shown in Fig. 4, the $k_{a,obs}$ value does not change from pH 7 to 8.5 and then increases with pH rather rapidly. This increase is again considered to be a reflection of the involvement of the anionic imidazole species. The parallel tendency observed in Fig. 4 is not unexpected, since the pK_a values for the imidazole proton are very close in these imidazole compounds.

In conclusion, it was shown that a bifunctional catalyst containing the hydroxamate and imidazole functions, II, was effective in the hydrolysis of PNPA. The neighboring imidazole group appears to assist the decomposition of the acetyl hydroxamate intermediate. Unfortunately, the acceleration of deacylation is not remarkable. This may be attributed to the lower nucleophilicity of the imidazole group and/or to the possibly unfavorable arrangement of the two functional groups. Hopefully, superior hydrolytic catalyst systems will evolve from the study of compounds possessing various arrangements of the hydroxamate and imidazole functions.

The authors wish to express their appreciation to Mr. Y. Okahata and Miss R. Ando for their cooperation during the course of this study.

References

1) Contribution No. 328 from this department. Presented in part at the 29th meeting of the Chemical Society of Japan, Oct 1973, Hiroshima.

2) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, **221**, 337 (1969).

3) T. Kunitake, Y. Okahata, and R. Ando, *Macromolecules*, **7**, 140 (1974).

4) T. Kunitake and Y. Okahata, *Chem. Lett.*, **1974**, 1059.

5) I. Heilbron and H. M. Bunbury, "Dictionary of Organic Compounds," Oxford Univ. Press, New York, N.Y. (1953).

6) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).

7) J. R. Tolter and W. D. Darby, "Organic Syntheses," Coll. Vol. 3, p. 460 (1955).

8) H. Wegner and R. Weidenhagen, *Ber.*, **70**, 7309 (1937).

9) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases. A Laboratory Manual," Methuen & Co. (1962), Chapt. 4.

10) M. Dessolin and M. Laloi-Diard, *Bull. Soc. Chem. Fr.*, **1971**, 2946.

11) M. Dessolin, M. Laloi-Diard, and M. Vilkas, *ibid.*, **1970**, 2573.

12) M. Dessolin, *Tetrahedron Lett.*, **1972**, 4585.

13) T. Kunitake, Y. Okahata, and R. Ando, This Bulletin, **47**, 1509 (1974).

14) M. L. Bender and T. H. Marshall, *J. Amer. Chem. Soc.*, **90**, 201 (1968).

15) G. Dougherty and L. W. Jones, *ibid.*, **46**, 1535 (1924).

16) W. B. Renfrow and C. R. Hauser, *ibid.*, **59**, 2308 (1937).

17) R. D. Bright and C. R. Hauser, *ibid.*, **61**, 618 (1939).

18) J. D. Aubort and R. F. Hudson, *Chem. Commun.*, **1970**, 938.

19) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1., Benjamin, N.Y. (1966), p. 58.

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