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Multifunctional Hydrolytic Catalysis

VI. Catalytic Hydrolysis of *p*-Nitrophenyl Acetate by *N*-(4-Imidazolylmethyl)benzohydroxamic Acid[1]

TOYOKI KUNITAKE, YOSHIO OKAHATA, AND TOYOHIDE TAHARA

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

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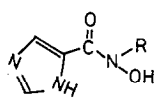
A bifunctional catalyst, *N*-(4-imidazolylmethyl)benzohydroxamic acid, was synthesized from benzohydroxamic acid and chloromethylimidazole, and used for the hydrolysis of *p*-nitrophenyl acetate. The reaction proceeded *via* the formation of the acetyl hydroxamate and its subsequent decomposition. The deacylation step was shown to be general base-catalyzed by the intramolecular imidazole group on the basis of the deuterium solvent kinetic isotope effect of 2.0. The efficiency of water attack on the acetyl hydroxamate was enhanced 130-fold by the imidazole group. The catalytic process is compared with the reactions of related monofunctional compounds, and finally its significance as a model of the charge relay system is discussed.

INTRODUCTION

One of the useful models of the charge relay system of serine proteases is a combination of a good oxygen nucleophile and an imidazole function. In previous papers of this series, we reported that *N*-alkyl(imidazole-4-carbo)hydroxamic acid possesses enhanced esterolytic activity toward PNPA due to the complementary action of the hydroxamate and imidazole functions, in nonmicellar (*1*) and micellar (*2*) systems.

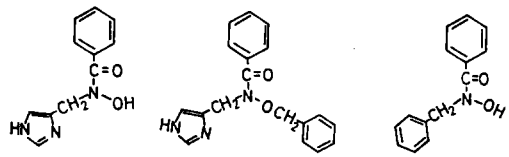


R = methyl, lauryl

Unfortunately, however, the imidazole function was not very efficient, probably because of its low pK_a value and the possibly unfavorable relative arrangement of the two functional groups. Therefore, it was considered necessary to prepare bifunctional catalysts with different functional arrangements. In addition, more detailed investigations of the catalytic process were needed for the design of highly efficient hydrolytic catalysts. Thus, a hydroxamic acid derivative that possesses a nonconjugated imidazole group was synthesized, and its catalytic action in the hydrolysis of PNPA was investigated.

[1] Contribution No. 363 from this department.

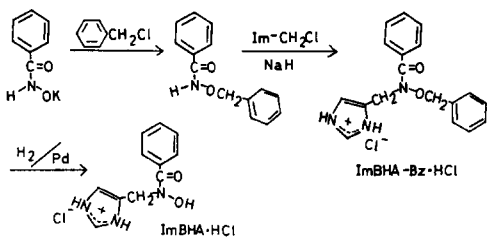
The use of complementary functional groups was briefly reported by Gruhn and Bender (*3*), and related studies with polymer catalysts have been described elsewhere (*4*, *5*).



ImBHA ImBHA-Bz BBHA

EXPERIMENTAL

N-(4-*Imidazolylmethyl*) *benzohydroxamic acid* (*ImBHA*).



Potassium benzohydroxamate as prepared from ethyl benzoate and hydroxylamine (*6*) was allowed to react with an equimolar amount of benzyl chloride in the presence of KOH in aqueous ethanol to give benzyl benzohydroxamate: colorless plates, mp 101–103°C, yield 78–84% lit. (*7*), mp 103–105°C, yield 65%.

To a carefully dried three-necked flask equipped with a stirrer, a thermometer, and a dropping funnel was added 7.20 g (0.15 mole) of 50% NaH (dispersed in oil) and 50 ml of dry THF (distilled from NaH). After purging with dry nitrogen, 38.5 g (0.17 mole) of benzyl benzohydroxamate in 200 ml of dry THF was added dropwise over 30 min with vigorous stirring at room temperature. Evolution of hydrogen, which was very vigorous in the beginning, was almost complete in 60 min. Next, 7.1 g (0.05 mole) of chloromethylimidazole hydrochloride (*8*) in 100 ml of dry DMF (distilled from NaH) was added over 60 min at 60°C. The reaction mixture was additionally stirred at 60–70°C for 2 hr. After cooling to room temperature, it was added with a small amount of methanol in order to decompose unreacted sodium hydride, and filtered. The filter solution was adjusted to pH 3–4 with concentrated hydrochloric acid, and the solvent was evaporated *in vacuo*. Acidic water (200 ml) was added to the oily residue and excess benzyl benzohydroxamate was separated as white precipitates. The solution was repeatedly washed with benzene, its pH adjusted to 3–4 and evaporated to dryness. The solid residue (ImBHA–Bz hydrochloride) was extracted by absolute ethanol and recrystallized three times from acetonitrile. mp 159–160°C, yield 65%[2], nmr 5.3 ppm (*s*, 2H), 5.5 ppm (*s*, 2H) 7.9 ppm(*m*, 11H) and 9.0 ppm (*s*, 1H).

[2] The yield varied widely (20–90%), presumably depending on the water content of the medium. In this particular case, the water contents of THF and DMF were 4 and 160 ppm, respectively, as determined by a Karl-Fischer apparatus.

Anal. Calcd for $C_{18}H_{18}O_2N_3Cl$: C, 62.88; H, 5.24; N, 12.23. Found: C, 63.05; H, 5.42; N, 12.30.

This substance (1.5 g) was dissolved in 150 ml of absolute ethanol and hydrogenated at room temperature over 0.8 g of 5% Pd on $SrCO_3$ (*9*). The theoretical amount of hydrogen was absorbed in 100 min. The oily product was crystallized from acetone: yield, 58%, pale yellow plates, mp 81–83°C, nmr: 5.5 ppm (*s*, 2H); 8.0 ppm (*m*, 6H); 9.2 ppm (*s*, 1H). *Anal.* Calcd for $C_{11}H_{12}O_2N_3Cl$: C, 52.07; H, 4.73; N, 16.57. Found: C, 52.32; H, 4.75; N, 16.65.

N-*Benzylbenzohydroxamic acid* (*BBHA*). This compound was prepared from *N*-benzylhydroxylamine (*10*) and benzoyl chloride according to Exner et al., and recrystallized from chloroform-*n*-hexane, mp 108–109°C, yield 72% lit. (*11*), 106–107°C.

Acetyl-N-*benzylbenzohydroxamate* (*Acetyl-BBHA*). *N*-Benzylbenzohydroxamic acid (13.5 g, 0.06 mole) and 7.8 g (0.08 mole) of triethylamine were dissolved in 400 ml of chloroform, and 4.5 g (0.06 mole) of acetyl chloride in 100 ml of chloroform was added with stirring at −5°C over 30 min. Stirring was continued for additional 2 hr at room temperature, and the reaction mixture washed with a dilute solution of aqueous sodium carbonate, dried, and distilled, bp 161–163°C/0.15 mm Hg, mp 66–68°C, yield 69%. *Anal.* Calcd for $C_{16}H_{15}O_3N$: C, 71.38; H, 5.58; N, 5.20. Found: C, 71.21; H, 5.68; N, 5.19.

Other materials. *p*-Nitrophenyl acetate was prepared as described before (*12*), and imidazole was recrystallized from benzene, mp 90°C.

Determination of pK_a. The pK_a values of the hydroxamic acid and imidazole groups of ImBHA, ImBHA-Bz, and BBHA were determined by potentiometric titration with 0.10 *N* KOH solution (*13*) (28–30 v/v% EtOH-H_2O, 30°C, 0.1 *M* KCl) using a Toa pH stat system (Toa Electronics Ltd). pK_a of the hydroxamic acid group in ImBHA was also determined by uv spectroscopic titration (28.9 v/v% EtOH–H_2O, 30°C, 0.1 *M* KCl), using the difference in the absorption coefficients at 300 nm between the hydroxamate anion ($\varepsilon = 1660$ as determined at pH 12) and the hydroxamic acid ($\varepsilon = 143$ as determined at pH 7). Six measurements conducted at pH 9 to 10 (0.1 *M* Tris buffer) were averaged (*14*). The phototitration of the hydroxamic acid group in BBHA was also carried out using the absorption coefficient difference at 300 nm of the anion ($\varepsilon = 2090$ as determined at pH 12) and the acid ($\varepsilon = 36$ as determined at pH 2). The pK_a data are summarized in Table 1.

Rate measurement. The hydrolysis of *p*-nitrophenyl acetate (PNPA) was conducted in 28.9 v/v% EtOH–H_2O at 30°C, 0.1 *M* KCl, and the rate was followed by appearance of *p*-nitrophenolate at 401 nm using a Hitachi 124 UV-visible spectrophotometer. One-centimeter cells were employed for the kinetic runs with low PNPA concentrations, and a spacer (0.9 cm width) was inserted in the experiments at high PNPA concentrations (burst kinetics). Detailed procedures for the burst-type experiment were as described before (*4*).

Measurements in deuterated solvents. The deuterated medium (28.9 v/v% EtOD–D_2O) was prepared from 99.5% EtOD (Stohler) and 99.8% D_2O (Merck). The buffer solution was prepared from sufficiently dried Tris and commercial 30% DCl in D_2O. The pD values were taken as the pH meter readings plus 0.4 (*15*). The pK_a^D values of the hydroxamic acid group of ImBHA and *p*-nitrophenol in 28.9 v/v% EtOD–D_2O

(10.0 ± 0.1 and 7.85 ± 0.08, respectively) were determined by the uv spectroscopic titration in the same procedure as that employed in the nondeuterated medium.

RESULTS

The reaction of PNPA with N-*Benzylbenzohydroxamic acid (BBHA).* The reaction of PNPA (9.78×10^{-5} *M*) with excess BBHA (1.61×10^{-3} *M*) was carried out at several pH's. At the high pH region the reaction was finished in 2–10 min, and the pseudo first-order rate constant k_{total} was obtained using the absorbances of *p*-nitrophenolate at time *t*, OD_t and at the infinite time, OD_∞.

$$\frac{k_{\text{total}}}{2.303} \cdot t = \log \frac{OD_\infty}{OD_\infty - OD_t}. \tag{1}$$

At the low pH region, the Guggenheim plot (*16*) was used instead, for the period of 2–3 half-lives. The correlation coefficients for the least-squares method were in the range of 0.997–0.999 in any case. The rate constant of spontaneous hydrolysis was 2–5% of k_{total}. The apparent second-order rate constant $k_{a,\text{obs}}$ was calculated as follows.

$$k_{a,\text{obs}} = \frac{k_{\text{total}} - k_{\text{sp}}}{[\text{HA}]_t}. \tag{2}$$

The plots of $k_{a,\text{obs}}$ against α_{HA} (degree of dissociation of the hydroxamic acid group) gave a satisfactorily linear relation which passed the origin ($r = 0.999$). The true second-order rate constant of the hydroxamate anion was calculated from the slope to be 19.5 M^{-1} sec^{-1} (Table 1).

TABLE 1

DISSOCIATION CONSTANTS AND ACYLATION RATE CONSTANTS BY PNPA[a]

Catalyst		pK_a	k_a (M^{-1} sec^{-1})
ImBHA	HA[b] group	9.50 ± 0.15[d] (9.45 ± 0.08)[e]	17.6[f]
	Im[c] group	5.45 ± 0.10[e]	16.8[g]
ImBHA–Bz	Im[c] group	5.45 ± 0.15[e]	$k_{\text{Im}} = 0.040$[g]
BBHA	HA[b] group	9.55 ± 0.15[d] (9.50 ± 0.10)[e]	19.5[g]
Imidazole		6.70 ± 0.06[e]	$k_{\text{Im}} = 0.203$[g]

[a] 30°C, 28.9 v/v% EtOH–H_2O, $\mu = 0.1$ (KCl).
[b] Hydroxamic acid.
[c] Imidazole.
[d] Ultraviolet spectroscopic titration.
[e] Potentiometric titration, 28–30 v/v% EtOH–H_2O.
[f] Obtained by burst kinetics, Eq. (5).
[g] Obtained by pseudo first-order kinetics, Eqs. (1) and (2).

The hydrolysis of PNPA by N-(*4-imidazolylmethyl*)*benzohydroxamic acid* (*ImBHA*). The value k_{total} was determined under pseudo first-order conditions ([ImBHA] = 5.04×10^{-4} *M*, [PNPA] = 4.55×10^{-5} *M*) using Eq. (1) or by the Guggenheim procedure. k_{sp} was negligibly small, compared with k_{total}. The acylation reaction may occur at the two nucleophilic sites (hydroxamate and imidazole) of this bifunctional compound. When $k_{a,obs}$ was plotted against α_{HA}, a linear relation which passed the origin was obtained as shown in Fig. 1 ($r = 0.999$). This suggests that the imidazole and undissociated hydroxamic acid groups do not contribute detectably to the reaction

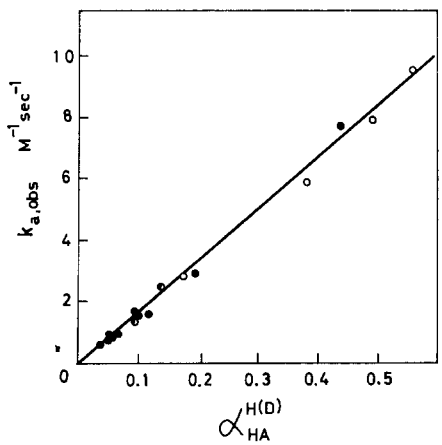


FIG. 1. Acylation of ImBHA by PNPA. 30°C, $\mu = 0.1$ (KCl), 0.1 *M* Tris. ○, from pseudo first-order kinetics in 28.9 v/v% EtOH–H_2O; ●, from burst kinetics in 28.9 v/v% EtOH–H_2O; ◐, from burst kinetics in 28.9 v/v% EtOD–D_2O, α^{D}_{HA} were calculated by using $pK^{D}_{a,HA} = 10.0 \pm 0.1$.

under the condition employed (pH 8–9.5). The k_a value calculated from the slope was 16.8 M^{-1} sec^{-1} (Table 1).

Typical burst kinetics were observed when ImBHA was allowed to react with excess substrate ([ImBHA] = $2.79 - 7.39 \times 10^{-4}$ *M*, [PNPA] = $4.55 - 18.2 \times 10^{-3}$ *M*): fast *p*-nitrophenol release followed by slower, steady release. In contrast, the steady release of *p*-nitrophenol was not observed for BBHA after *p*-nitrophenol equimolar to BBHA was released.

The nucleophilic catalytic process of the hydrolysis of PNPA is expressed by

$$C + S \xrightarrow{k_a} CS' \xrightarrow{k_d} C + P_2, \qquad (3)$$
$$\quad + \quad P_1$$

where *C*, *S*, *CS'* denote catalyst (hydroxamate anion in the present case), substrate, and acyl intermediate, respectively. The analysis of the burst kinetics was first reported by Bender et al. (*17*, *18*) and its application to the bifunctional catalytic process was described previously (*1*, *2*, *4*, *5*). The formation of *p*-nitrophenol (P_1) is given by

$$[P_1] = At + B(1 - e^{-bt}) \qquad (4)$$

where

$$A = \frac{k_a \cdot k_d \cdot [C]_0 \cdot [S]_0}{k_a \cdot [S]_0 + k_d}, \qquad B = \frac{k_a^2 \cdot [S]_0^2 \cdot [C]_0}{(k_a \cdot [S]_0 + k_d)^2},$$

$$b = k_a \cdot [S]_0 + k_d.$$

The k_a and k_d values can be determined in several ways, but the following equations were used exclusively in this study because they were more accurate than other procedures.

$$k_a = \frac{b}{[S]_0} \cdot \left(\frac{B}{[C]_0}\right)^{1/2}, \tag{5}$$

$$k_d = b - k_a \cdot [S]_0. \tag{6}$$

The $k_{a,\,obs}$ and $k_{d,\,obs}$ values were determined at several pH's by using Eqs. (5) and (6) as shown in Table 2. The $k_{a,\,obs}$ values thus obtained were plotted against α_{HA} (Fig. 1) to give a linear relation ($r = 0.999$). The k_a value calculated from the slope was

TABLE 2

BURST KINETICS, HYDROLYSIS OF PNPA BY IMBHA[a]

pH	[ImBHA] ($\times 10^4$ M)	[PNPA] ($\times 10^2$ M)	$k_{a,\,obs}$[b] (M^{-1} sec^{-1})	$k_{d,\,obs}$[c] ($\times 10^3$ sec^{-1})
8.08	7.39	1.82	0.522	1.18
8.08	7.39	1.82	0.500	1.13
8.15	7.39	1.82	0.564	1.56
8.25	6.93	1.82	0.667	1.23
8.35	6.46	1.82	0.799	1.35
8.56	6.46	1.36	1.47	1.36
8.63	5.54	1.36	1.45	1.54
8.88	2.79	0.910	2.90	1.29
8.97	3.69	0.910	2.47	1.43
9.38	3.69	0.455	7.85	1.56
pD = 9.03[d]	4.62	1.44	1.29[e]	0.662[f]
pD = 9.03[d]	4.62	1.92	1.52[e]	0.733[f]
pD = 9.20[d]	2.46	0.479	2.68[e]	0.708[f]

[a] 30°C, 28.9 v/v% EtOH–H_2O, $\mu = 0.1$ (KCl), 0.1 M Tris.
[b] Calculated from Eq. (5).
[c] Calculated from Eq. (6).
[d] 28.9 v/v% EtOD–D_2O, 30°C, $\mu = 0.1$ (KCl), 0.1 M Tris.
[e] $k^D_{a,\,obs}$.
[f] $k^D_{d,\,obs}$.

17.6 M^{-1} sec^{-1} in satisfactory agreement with that obtained under the pseudo first-order condition ($k_a = 16.8$ M^{-1} sec^{-1}). Therefore, the determination of the kinetic constant from burst kinetics was shown to be of sufficient reliability.

The log $k_{a,\,obs}$ values were plotted against pH as shown in Fig. 2, and log $k_{d,\,obs}$ values were similarly plotted against pH in Fig. 3.

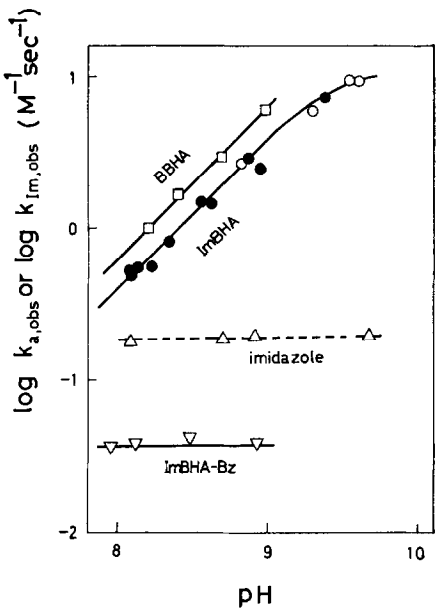


FIG. 2. pH-dependence of acylation by PNPA. 30°C, 28.9 v/v% EtOH–H_2O, $\mu = 0.1$ (KCl), 0.1 *M* Tris or Barbital. ○, □, △, ▽, obtained by pseudo first-order kinetics; ●, obtained by burst kinetics.

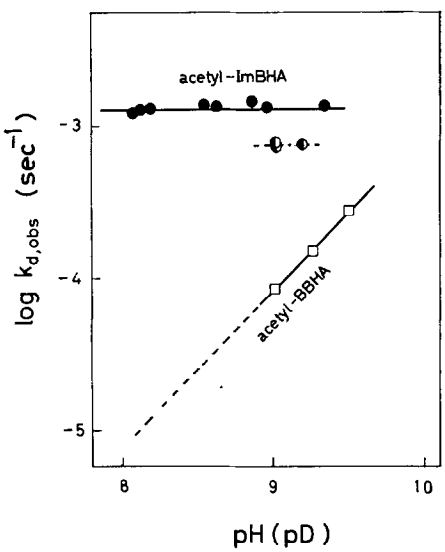
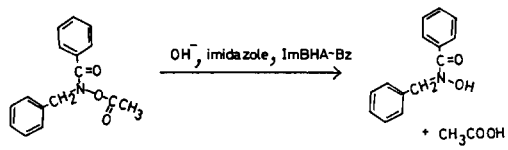


FIG. 3. pH(pD)-dependence of deacylation step. 30°C, $\mu = 0.1$ (KCl). ●, from burst kinetics in 28.9 v/v% EtOH–H_2O; □, from pseudo first-order kinetics in 28.9 v/v% EtOH–H_2O; ◐, from burst kinetics in 28.9 v/v% EtOD–D_2O. The plots for acetyl-BBHA were obtained by extrapolation to the zero buffer concentration. The others were obtained at 0.1 *M* Tris.

The reaction of ImBHA with excess PNPA was also carried out in deuterated solvent (28.9 v/v% EtOD–D_2O) and $k^D_{a,obs}$, and $k^D_{d,obs}$ values were similarly determined. The difference in the pK_a value of *p*-nitrophenol in the ordinary medium (7.33) and in the

deuterated medium (7.85) was taken into account in calculating the rate constants. These acylation data agreed with those in the nondeuterated medium ($k_a^H/k_a^D = 1.0 \pm 0.1$, see also Fig. 1). Therefore, a deuterium solvent kinetic isotope effect was not observed in the acylation step. On the other hand, the deacylation step showed a definite isotope effect, as can be seen from Table 2 and Fig. 3 ($k_d^H/k_d^D = 2.0 \pm 0.1$).

Hydrolysis of acetyl N*-benzylbenzohydroxamate (acetyl-BBHA) and PNPA by lyate species, imidazole and the imidazole group of ImBHA-Bz.*



(7)

The spontaneous hydrolysis rate of acetyl-BBHA was determined from appearance of the absorption of the hydroxamate anion at 300 nm. The ester does not absorb at 300 nm, while the absorption coefficients of *N*-benzylbenzohydroxamic acid and its anion are 36(ε_{HA}) and 2090(ε_{A^-}), respectively. Therefore, the apparent rate constant of hydrolysis $k'_{d,\,obs}$ is given by

$$\frac{k'_{d,\,obs}}{2{,}303} \cdot t = \log \frac{[\text{acetyl–BBHA}]_0}{[\text{acetyl–BBHA}]_0 - \dfrac{OD_t}{\varepsilon_{A^-} \cdot \alpha_{HA} + \varepsilon_{HA}(1 - \alpha_{HA})}}, \tag{8}$$

where OD_t is the absorbance of the reaction mixture at time t. The experiment was performed at pH 9.04, 9.30, and 9.50, and results were extrapolated to the zero buffer concentration (Fig. 3). The Tris buffer accelerated the hydrolysis, but the barbital buffer showed no or a negative effect. The $k_{d,\,obs}$ values thus obtained conformed to

$$k_{d,\,obs} = k_{OH}[OH^-] + k_0 \tag{9}$$

and $k_{OH} = 5.0\ M^{-1}\ sec^{-1}$ and $k_0 = 1 \times 10^{-5}\ sec^{-1}$ as determined from the $k_{d,\,obs} - [OH^-]$ plots ($r = 0.989$).

The spontaneous hydrolysis of PNPA was similarly carried out at pH 8.6–9.6 with Tris buffer and extrapolated to zero buffer concentration. From the pH dependence of k_{sp} were obtained $k_{OH} = 10.9\ M^{-1}\ sec^{-1}$ and $k_0 = 1 \times 10^{-5}\ sec^{-1}$ using Eq. (9).

The catalytic hydrolyses of acetyl-BBHA by imidazole and ImBHA–Bz were conducted under pseudo first-order conditions ($[C]_0 \gg [S]_0$), and the apparent rate constants were calculated from Eqs. (1) and (2) using the absorption of the hydroxamate anion at 300 nm. At pH 8–9.5, where the experiments were done, the imidazole groups exist in the neutral form almost exclusively ($pK_a = 5.45$ for ImBHA–Bz and 6.70 for imidazole). Therefore, the reaction rates were independent of pH for both catalysts. The true second-order rate constants were $0.007 \pm 0.001\ M^{-1}\ sec^{-1}$ with imidazole and $0.006 \pm 0.002\ M^{-1}\ sec^{-1}$ with ImBHA–Bz. The hydrolysis rates of acetyl-BBHA are shown in Table 3.

Also, the second-order rate constants for the catalytic hydrolysis of PNPA were $0.203\ M^{-1}\ sec^{-1}$ with imidazole and $0.040\ M^{-1}\ sec^{-1}$ with ImBHA–Bz, as shown in Table 1 and Fig. 2.

TABLE 3

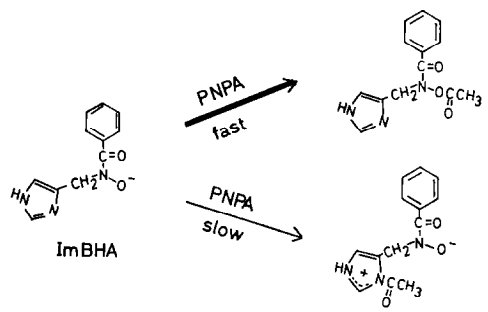
DEACYLATION OF ACETYL HYDROXAMATES[a]

	k_{Im} (M^{-1} sec^{-1})	k_{OH^-} (M^{-1} sec^{-1})	k_0 (sec^{-1})
Acetyl-ImBHA			$k_{d,obs} = (1.3 \pm 0.2) \times 10^{-3}$ [b]
Acetyl-BBHA	$(7 \pm 1) \times 10^{-3}$ [c] $(6 \pm 2) \times 10^{-3}$ [d]	5.0	1×10^{-5}
Acetyl *N*-methylaceto-hydroxamate[e]	0.070[c]	14	$<3 \times 10^{-4}$
(PNPA	0.203[c]	10.9	1×10^{-5})

[a] 30°C, 28.9 v/v% EtOH–H_2O, $\mu = 0.1$ (KCl).
[b] Intramolecular general base catalysis by imidazole group in 0.1 *M* Tris, see also Table 2.
[c] Catalyzed by imidazole.
[d] Catalyzed by the imidazole group of ImBHA–Bz.
[e] 25°C, $\mu = 1.0$ (*20*).

DISCUSSION

Acylation step. The bifunctional catalyst ImBHA contains two nucleophilic sites, and therefore the reaction with PNPA may occur at the hydroxamate and/or the imidazole sites. The acylation data shown in Fig. 1 clearly indicate that only the hydroxamate anion is the effective nucleophile. The nucleophilicity of the imidazole group in ImBHA is supposedly comparable to that of ImBHA–Bz (imidazole nucleophile), since their pK_a values are identical within experimental error (Table 1). The $k_{Im,obs}$ value for ImBHA–Bz is constant (0.040 M^{-1} sec^{-1}) over the pH range of 8 to 9. The $k_{a,obs}$ value for the hydroxamate anion is thus 10 to 100 times larger than that of the imidazole group in this pH region (Fig. 2).

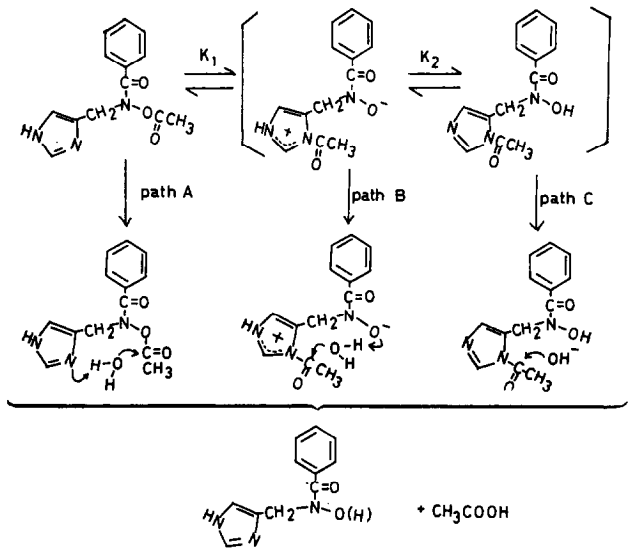


(10)

(11)

The acylation process is a simple nucleophilic attack of the hydroxamate anion, as shown by the relation of Fig. 1. This is consistent with the lack of a deuterium solvent kinetic isotope effect ($k^H_{a,obs}/k^D_{a,obs} = 1.0 \pm 0.1$) given in Table 2 and Fig. 1. The reaction of imidazole and PNPA (a nucleophilic reaction) was reported not to show the isotope effect (*19*, *20*).

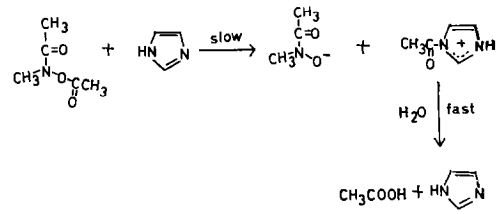
Deacylation step. The deacylation rate of acetyl-ImBHA (obtained by burst kinetics) is much larger than that of acetyl-BBHA, as shown in Fig. 3. Furthermore, deacylation of acetyl-ImBHA was pH-independent in the pH range studied, in contrast to the pH-dependent (hydroxide catalyzed) hydrolysis observed for acetyl-BBHA. These results clearly indicate that the deacylation mostly occurs with the help of the *intramolecular* imidazole group. There is no involvement of buffer catalysis, since $k_{d,\,obs}$ is pH-independent. Therefore, possible mechanisms for the process are given by the following scheme.



Path A is the general base (imidazole)-catalyzed hydrolysis of the acetyl hydroxamate. On the other hand, the imidazole group acts as a nucleophile in path B and C to form the acetylimidazole intermediate, which, in turn, is hydrolyzed through general base catalysis of the hydroxamate anion or by the hydroxide catalysis. Among these possibilities, path A is most favored on the following grounds: (1) $k_{d,\,obs}$ was pH-independent, in consistence with the fact that the imidazole group is almost completely in the neutral form in the pH range studied. Assuming that the acetyl transfer from the hydroxamate to imidazole group is fast enough, log $k_{d,\,obs}$ should increase proportionately with pH in the case of paths B and C. The water-catalyzed hydrolysis of the acetylimidazole in path C would be much less efficient than the hydroxide-catalyzed reaction. (2) The deuterium solvent kinetic isotope effect ($k^{H}_{d,\,obs}/k^{D}_{d,\,obs} = 2.0$) was observed for deacylation, supporting the general base mechanism. 3; The pK_a difference of the hydroxamate and imidazole groups ($\Delta pK_a \cong 4$) suggests that the acy group stays overwhelmingly at the hydroxamate site in the equilibrium (if it is) of the acetyl transfer.

Intermolecular imidazole catalysis of the hydrolysis of acetyl hydroxamate proceeds via the nucleophilic mechanism (*21*).

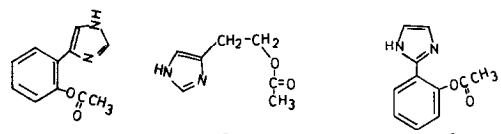
In general, formation of the acetylimidazole intermediate (nucleophilic catalysis) in the imidazole catalyzed hydrolysis of phenyl esters is detected when pK_a of the



(12)

leaving group is not greater than that of imidazole by more than 3–5 pK_a units (*21*, *22*). The hydrolysis of acetyl hydroxamate by imidazole (Eq. (12)), and the hydrolysis of acetyl-BBHA by imidazole and by the imidazole group of ImBHA–Bz are all included in this category ($\Delta pK_a < 5$).

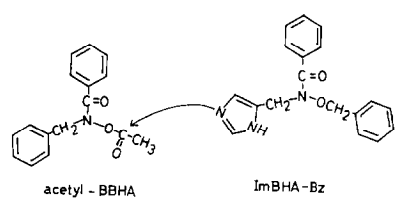
The nucleophilic mechanism occurs less readily during intramolecular catalysis. Bruice and co-workers (*22–25*) found that hydrolysis of phenyl esters by the intramolecular imidazole group occurred by way of a general base mechanism, in spite of the pK_a difference of only 3 to 5. The deuterium solvent kinetic isotope effects for the



1 **2** **3**

hydrolysis of **1** and **3** were 3.23 (*25*) and 3.91 (*22*), respectively. The predominance of the general base mechanism in the intramolecular catalysis is supposedly attributable to the fact that the acetylimidazole intermediate, if formed, would readily revert to the starting material because of the much higher nucleophilicity of the phenoxide group. This problem has been very lucidly discussed in the case of the intramolecular carboxylate catalysis (*26*).

An intermolecular version of the deacylation process of acetyl-ImBHA ($k_{d,\,obs} = (1.3 \pm 0.2) \times 10^{-3}$ sec^{-1} at pH 8–9) would be the hydrolysis of acetyl-BBHA by ImBHA–Bz ($k_{Im} = (6 \pm 2) \times 10^{-3}\ M^{-1}$ sec^{-1}).



(13)

The effective concentration of the imidazole group in ImBHA is given by the ratio of the intramolecular and intermolecular rate constants: $k_{d,\,obs}(\text{intra})/k_{Im}(\text{inter}) = 0.2$ M, not a remarkable value. However, this comparison may not be sound, since the reaction mechanisms are different. A more appropriate evaluation of the efficiency of the intramolecular catalysis is given by the relative deacylation rate of acetyl-ImBHA and acetyl-BBHA. The water-catalyzed hydrolysis (k_0 term in Eq. (9)) of acetyl-BBHA

is 1×10^{-5} sec^{-1} (see Table 3). Thus, the k_d value of acetyl-ImBHA is larger than k_0 by 130-fold. This means that the water attack on the acetyl hydroxamate is accelerated by more than 100-fold in the presence of the intramolecular imidazole group. This acceleration is smaller than the enhanced water attack (max 1000 times) by the intramolecular imidazole **1**, but larger than the *ca.* 50 times acceleration of water attack by the neighboring carboxylate group in the hydrolysis of aspirin (*25*).

Catalytic efficiency of ImBHA. The true efficiency of a catalyst is related to its turnover rate. In the case of the nucleophilic catalysis of PNPA (Eq. (3)), the rate constant for turnover is expressed by (*2*, *5*)

$$k_{\text{turnover}} = \frac{k_a \cdot [S]_0 \cdot k_d}{k_a \cdot [S]_0 + k_d}. \tag{14}$$

The k_{turnover} value can be calculated at appropriate substrate concentrations by using $k_{a,\text{obs}}$ and $k_{d,\text{obs}}$ values given in Table 2. At 10^{-2} *M* PNPA, $k_{a,\text{obs}} \cdot [S]_0 \gg k_{d,\text{obs}}$ and k_{turnover} is nearly equal to k_d, in consistence with the observation of the burst kinetics On the contrary, $k_{a,\text{obs}} \cdot [S]_0 \ll k_{d,\text{obs}}$ at 10^{-4} *M* PNPA and the acylation process is rate-limiting, giving pseudo first-order kinetics.

Finally, it should be pointed out that ImBHA is a better model of the charge relay system found in serine proteases. In the charge relay system, acylation and deacylation at the seryl hydroxyl site are general base-catalyzed by the histidyl imidazole group (*27*). The acylation reaction with ImBHA is a simple nucleophilic process, unlike that in the enzyme system, but the deacylation processes have a common mechanism (general base catalysis). Unfortunately, however, k_{turnover} in the present system is in the range of 10^{-4} to 10^{-3} sec^{-1}. This value is much smaller than that of α-chymotrypsin (1.6×10^{-2} sec^{-1}) obtained in the hydrolysis of PNPA (*28*). Further efforts are needed to attain greater catalytic efficiencies in the synthetic systems.

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Note added in proof. Gruhn and Bender (*29*) recently discussed the deacylation mechanism of a related bifunctional catalyst in detail. According to their procedure, k_{H_2O} in our system is estimated to be 1.1×10^{-7} sec^{-1} and the water attack is accelerated by a factor of 1.2×10^4 by the intramolecular imidazole group.

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