

Multifunctional Hydrolytic Catalyses. V. The Reaction of *o*-Substituted Benzohydroxamic Acids and *p*-Nitrophenyl Acetate¹⁾

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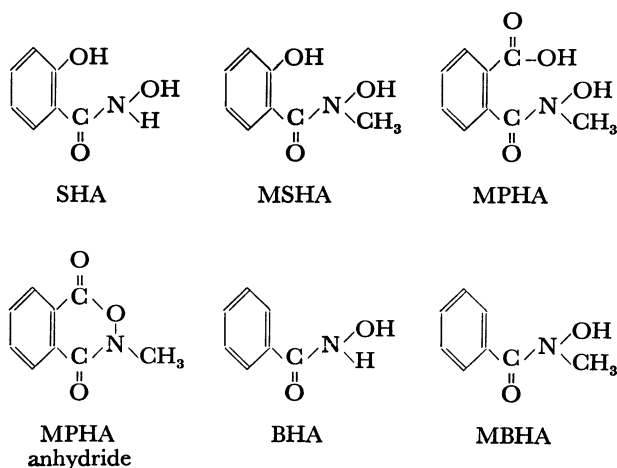
(Received March 22, 1976)

Salicylohydroxamic acid(SHA), *N*-methylsalicylohydroxamic acid(MSHA), and *N*-methylphthalohydroxamic acid(MPHA) were prepared and their acylation reactions with *p*-nitrophenyl acetate were studied in aqueous ethanol at 30 °C. Both the acidity and nucleophilicity of the hydroxamic acid group were affected by the functional *ortho*-substituents(—OH, —COOH). In particular, the undissociated hydroxamic acid group in the MPHA monoanion possessed nucleophilic reactivity. This was brought forth by the general base assistance of the adjacent carboxylate group, as confirmed by the presence of the solvent isotope effect of $k^H/k^D=2.5$. Only the hydroxamate anion acted as the nucleophile in all other cases.

At the active site of serine proteases, the nucleophilic reactivity of the seryl hydroxyl group is enhanced by hydrogen bonding with the aspartic carboxylate *via* the histidyl imidazolyl group.²⁾ We prepared several catalytic compounds which possess the hydroxamate and imidazole functions as model serine proteases, and employed them for the hydrolysis of phenyl esters.^{3–5)} In these systems, acylation by phenyl esters occurs at the hydroxamate site without assistance of the neighboring imidazolyl groups. The latter function is involved only in the deacylation process.

The juxtaposition of two functional groups at the hydrogen-bonding distance would be advantageous for realization of the simultaneous involvement of these functional groups in a single reaction step. The two functional groups at the *ortho* position of the benzene ring can form intramolecular hydrogen bonding as in salicylic acid and phthalic acid. It should be interesting to prepare benzohydroxamic acid derivatives with *ortho*-substituents having hydrogen-bonding capacity and to investigate their catalytic behavior, in connection with the multifunctional character of the acylation process of serine proteases.

The *o*-substituted benzohydroxamic acids employed in this study are salicylohydroxamic acid (SHA), *N*-methylsalicylohydroxamic acid (MSHA), and *N*-methylphthalohydroxamic acid (MPHA). The nucleophilic reactivity of these compounds was compared with those of unsubstituted counterparts, benzohydroxamic acid (BHA) and *N*-methylbenzohydroxamic acid (MBHA).



Experimental

Materials. *p*-Nitrophenyl acetate was prepared by acylation of *p*-nitrophenol with acetic anhydride and recrystallized from cyclohexane, mp 78 °C (lit.⁶⁾ mp 81–82 °C).

Salicylohydroxamic acid was prepared from salicyloyl chloride (bp 62 °C/12 mmHg) and hydroxylamine. The crude product was purified in the form of the copper chelate and then recrystallized from water, mp 161–162 °C (lit.⁷⁾ mp 169 °C).

N-Methylsalicylohydroxamic acid was similarly prepared in chloroform in the presence of triethylamine from salicyloyl chloride and *N*-methylhydroxylamine. The crude product was purified in the form of the copper chelate, and then recrystallized from water, mp 133–135 °C (lit.⁷⁾ mp 136 °C).

N-Methylphthalohydroxamic acid anhydride was synthesized as follows: 14 g (0.1 mol) of phthalic anhydride, 18 g (0.21 mol) of *N*-methylhydroxylamine hydrochloride and 23 g (0.25 mol) of triethylamine were dissolved in 300 ml of dry DMF and heated for 30 min at 100 °C. Then solvent was removed *in vacuo* and the residual oil was extracted with hot cyclohexane. Colorless flakes separated upon cooling, mp 120 °C. Yield, 40–60%. Found: C, 61.10; H, 3.98; N, 8.01%. Calcd for C₉H₇NO₃: C, 61.02, H, 3.98; N, 7.91%.

Determination of pK_a . The pK_a values for the hydroxyl and hydroxamic acid groups were determined by the potentiometric titration and the UV spectrophotometric titration at 0.1 M KCl, 30 °C in 28.9 v/v% EtOH–H₂O.

The pK_a value of the hydroxamic acid group of SHA was determined by the potentiometric titration of 0.01 M SHA with 0.1 M NaOH, using Eq. 1.

$$pK_a = \text{pH} + \log \frac{1-\alpha}{\alpha} \quad (1)$$

where α is the degree of dissociation. The pK_a value of the hydroxyl group in SHA was determined by UV spectrophotometric titration at 330 nm, using Eq. 2.

$$pK_a = \text{pH} + \log \frac{OD_1 - OD}{OD - OD_M} \quad (2)$$

where OD_M and OD_1 are absorbances at 330 nm of the undissociated and dissociated phenolic hydroxyl groups, respectively, and OD is the absorbance (330 nm) at a given pH. OD_1 was obtained by extrapolation of OD values at pH 10–12^{8,9)} and OD_M was obtained at pH 9: $OD_1=0.516$ and $OD_M=0.290$.

The two pK_a values for MSHA were similarly determined using OD_1 (330 nm)=0.506 and OD_M (330 nm)=0.480.

The pK_a of the hydroxamic acid group of MPHA was determined by the potentiometric titration (Eq. 1). The

pK_a of the carboxylic acid group could not be determined by the conventional procedure, because MPHA was readily hydrolyzed to phthalic acid at $pH < 6$. The $pK_{a,HA}$ of MPHA in the deuterated solvent was determined by potentiometric titration in 28.9 v/v% EtOD- D_2O at 30 °C, $\mu=0.1$ with 0.1 M KOH in D_2O : $pK_{a,HA}^D=9.80 \pm 0.15$. The pD values were taken as the pH meter readings plus 0.40 at 30 °C.⁹⁾

Rate Measurements. The reaction of PNPA with hydroxamic acids was carried out in the presence of large excesses of the latter, and the formation of *p*-nitrophenolate anion was followed at 401 nm. The reactions obeyed the pseudo-first-order kinetics. The rate constant k_{total} was determined from absorbances of *p*-nitrophenolate at time t , OD_t , and at the infinite time, OD_∞ . When the reaction was slower, k_{total} was obtained from Guggenheim plots¹⁰⁾ of up to 2–3 half-lives. The apparent second-order rate constant of acylation, $k_{a,obsd}$ was calculated from the rate constants of total and spontaneous hydrolyses.

$$k_{a,obsd} = \frac{k_{total} - k_{spont}}{[HA]_T} \quad (3)$$

where $[HA]_T$ is the total concentration of hydroxamic acids. The hydrolysis condition is 28.9 v/v% EtOH- H_2O , 30 °C, $\mu=0.1$ (KCl) and pH 6.5–9.5. Phosphate, Tris, and Barbitol buffers were used.

The reaction of MPHA and PNPA in the deuterated medium was studied at 30 °C, $\mu=0.1$ (KCl), pD 7.1–9.2 in 28.9 v/v% EtOD- D_2O . The buffer solution was prepared from sufficiently dried Tris and commercial 30% DCl in D_2O .

Instruments. Electronic spectra were obtained with a Hitachi 124 UV-visible spectrophotometer equipped with a thermostated cell compartment. A Toa digital pH meter (Model HM-15A) was employed for the pH measurement, and a Toa pH stat system was used for the potentiometric titration. The reaction rates were calculated using a programmable desk calculator Compet 364P. The least-squares method was used wherever possible. The correlation coefficient was better than 0.99 unless stated otherwise.

Results

pK_a Values. The pK_a value of the hydroxamic acid group is considerably influenced by the *ortho*-

substituent, as summarized in Table 1. The $pK_{a,HA}$ value of SHA, 7.90, is lower than that of benzohydroxamic acid by 1.45 pK_a units. This is attributable to the stabilization of the monoanionic species SHA^- by the intramolecular hydrogen bonding, as already considered in order to explain the pK_a difference between the carboxyl groups of salicylic acid and benzoic acid ($pK_{a,COOH}=2.98$ and 4.21 respectively, in water, 25 °C)¹¹⁾ The $pK_{a,OH}$ value of SHA, 10.3, is close to those of phenol and *m*-hydroxybenzoic acid (9.95 and 9.74, respectively, in water, 25 °C),¹²⁾ in spite of electrostatic repulsion of the neighboring hydroxamate anion. This may be explained by the stabilization of the dianionic species due to hydrogen bonding. In accord with this view, $pK_{a,OH}(=12.7)$ for MSHA which cannot form hydrogen bonding is much higher and is close to that of salicylic acid ($pK_{a,OH}=13.0$)¹³⁾ in

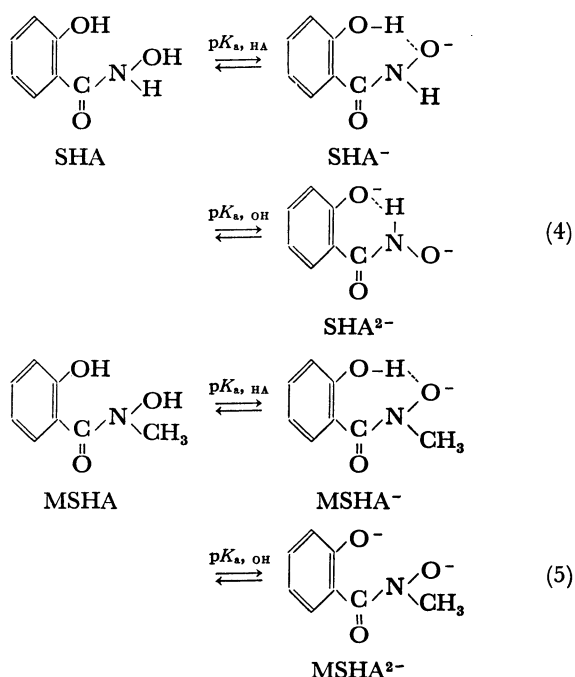
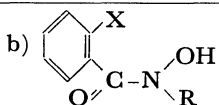


TABLE 1. ACID DISSOCIATION CONSTANTS AND RATE CONSTANTS OF ACYLATION^{a)}

Benzohydroxamic acid	Substituent ^{b)}	pK_a ^{c)}	k_a $M^{-1} s^{-1}$
BHA ^{d)}	$\begin{cases} R=H \\ X=H \end{cases}$	HA: 9.35 (PT)	50.0
SHA	$\begin{cases} R=H \\ X=OH \end{cases}$	$\begin{cases} HA: 7.90 \pm 0.07 \text{ (PT)} \\ OH: 10.3 \pm 0.1 \text{ (UV)} \end{cases}$	$\begin{cases} k_A = 4.85 \\ k_{A^2-} = 60 \end{cases}$
MBHA	$\begin{cases} R=CH_3 \\ X=H \end{cases}$	HA: 8.59 ^{e)}	16 ^{f)}
MSHA	$\begin{cases} R=CH_3 \\ X=OH \end{cases}$	$\begin{cases} HA: 6.70 \pm 0.09 \text{ (PT)} \\ OH: 12.7 \pm 0.3 \text{ (UV)} \end{cases}$	0.080
MPHA	$\begin{cases} R=CH_3 \\ X=COOH \end{cases}$	$\begin{cases} HA: 9.50 \pm 0.07 \text{ (PT)}^g) \\ COOH: < 4 \end{cases}$	$\begin{cases} k_{HA} = 0.22 \\ k_A = 10.8 \end{cases}$

a) 28.9 v/v% EtOH- H_2O , 30 °C, $\mu=0.1$ (KCl).

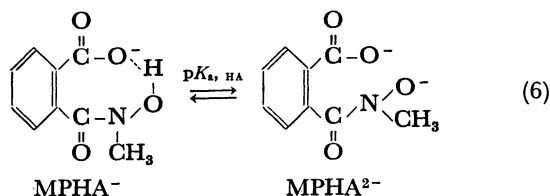


c) HA; hydroxamic acid

group. OH; phenol group. COOH; carboxylic acid group. PT; pK_a determined by potentiometric titration. UV; pK_a determined by UV spectrophotometric titration. d) from Ref. 5 e) from Ref. 14. f) Condition: 25 °C, 12 v/v% EtOH- H_2O , $\mu=0.08$. Estimated from the data of Ref. 16. g) $pK_{a,HA}^D \mu=9.80 \pm 0.15$ in 28.9 v/v% EtOD- D_2O , 30 °C, $\mu=0.1$ (KCl).

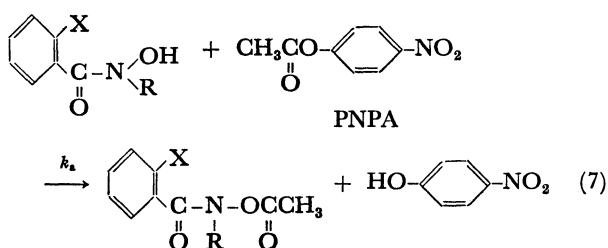
water, 25 °C). The lower $pK_{a,HA}$ value of MSHA relative to that of MBHA (6.70 and 8.59,¹⁴) respectively) again suggest the hydrogen-bonding stabilization of the monoanionic species.

The higher $pK_{a,HA}$ value of MPHA relative to that of MBHA (9.50 and 8.60, respectively) may be caused by stabilization of the monoanionic species by the intramolecular hydrogen bonding or by the electrostatic repulsion in the dianionic species.



Hydrolysis of MPHA Anhydride. MPHA anhydride is readily converted to the ring-opened derivative, MPHA, in acidic and alkaline solutions. Furthermore, MPHA is easily hydrolyzed to phthalic acid at $\text{pH} < ca. 6$ due to the general acid catalysis of the neighboring carboxyl group. This hydrolysis is complete in less than 3 h at $\text{pH} 4.5$, 30 °C. The MPHA anhydride shows an absorption maximum at 294 nm ($\epsilon=2520$) in CH_3CN . The ring-opened product gives $\epsilon=230$ at 294 nm, $\text{pH} 8.57$. The absorbance difference at 294 nm was used to follow the ring opening reaction, and the apparent first-order rate constant was calculated from the ordinary first-order plot. The experiments carried out at four concentrations of MPHA anhydride (1.0 , 2.0 , 3.0 , and 4.0×10^{-4} M) gave the following rate constant: $k_{\text{obsd}} = (1.89 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$ in 1.4 v/v% $\text{CH}_3\text{CN}-\text{H}_2\text{O}$, 30 °C, $\mu=0.1$ (KCl), $\text{pH} 8.57$, 0.1 M Tris buffer.

Reaction of PNPA with *o*-Substituted Benzohydroxamic Acids. The reactions of PNPA (10^{-4} M) with *o*-substituted benzohydroxamic acids (10^{-2} – 5×10^{-4} M) were carried out in 28.9 v/v% $\text{EtOH}-\text{H}_2\text{O}$ in the pH range of 6.5 to 9.5.



The apparent second-order rate constant $k_{a,\text{obsd}}$ was calculated according to Eq. 3. The pH-rate profiles are given in Fig. 1. If the hydroxamate anion is the only nucleophile, the profile is composed of an ascending limb (slope=1) and a plateau. However, this is not the case for most of the *o*-substituted benzohydroxamic acids. These anomalous behavior is more clearly seen in the plot of $k_{a,\text{obsd}}$ against α_{HA} (degree of dissociation of hydroxamic acid) given in Figs. 2 and 3.

In Fig. 2, the plots for BHA give a straight line which passes the origin. This indicates that the hydroxamate anion acts as the nucleophile, as have been shown for a variety of simple hydroxamic acids.^{15–18} On the other hand, the plots for SHA give a linear relation up to

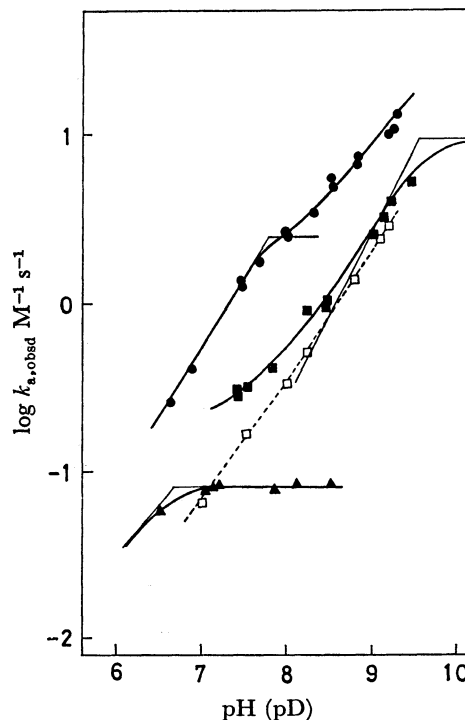


Fig. 1. pH-rate profile of the reaction of PNPA with *o*-substituted benzohydroxamic acids. Reaction Condition; 30 °C, $\mu=0.1$ (KCl) in 28.9 v/v% $\text{EtOH}-\text{H}_2\text{O}$ ●; SHA, ■; MPHA, ▲; MSHA in 28.9 v/v% $\text{EtOD}-\text{D}_2\text{O}$ □; MPHA.

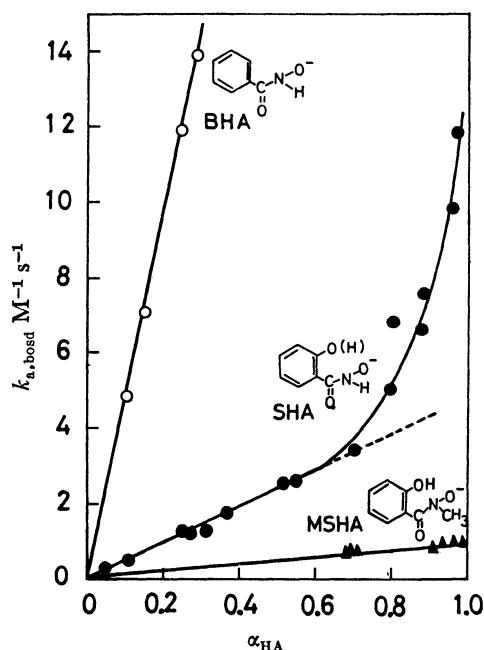


Fig. 2. Reaction of PNPA with some hydroxamic acids. Reaction condition: 28.9 v/v% $\text{EtOH}-\text{H}_2\text{O}$, 30 °C, $\mu=0.1$ (KCl), 0.15 M Barbitol buffer.

$\alpha_{\text{HA}} \approx 0.5$, and then show a steep rise. The slope of the steeper portion is close to that of BHA.

SHA can exist in three forms: neutral, monoanionic and dianionic species (Eq. 4). Since the linear portion of the $k_{a,\text{obsd}}-\alpha_{\text{HA}}$ relation passes the origin, the neutral species cannot be reactive, and the apparent

rate constant will be composed of the monoanionic and dianionic terms as follows:

$$k_{a, \text{obsd}} = k_A^- \left[\frac{\alpha_{\text{HA}}(1-\alpha_{\text{OH}})}{1-\alpha_{\text{OH}}(1-\alpha_{\text{HA}})} \right] + k_A^{2-} \left[\frac{\alpha_{\text{HA}} \cdot \alpha_{\text{OH}}}{1-\alpha_{\text{OH}}(1-\alpha_{\text{HA}})} \right] \quad (8)$$

where k_A^- and k_A^{2-} are second-order rate constants for the monoanionic and dianionic species, respectively. α_{HA} and α_{OH} are degrees of dissociation of the hydroxamic acid and phenol groups, respectively.

The solid curve for SHA was calculated from Eq. 8 using $\text{p}K_a$ values of Table 1 and by assuming appropriate k_A^- and k_A^{2-} values. The best fit with the experimental data was obtained for $k_A^- = 4.85 \text{ M}^{-1} \text{ s}^{-1}$ and $k_A^{2-} = 60 \text{ M}^{-1} \text{ s}^{-1}$.

A similar situation may as well exist for MSHA. However, the $k_{a, \text{obsd}} - \alpha_{\text{HA}}$ relation for this hydroxamic acid is simple as shown in Fig. 1. This is explained by the fact that $\text{p}K_{a, \text{OH}}$ of MSHA is rather high (12.7) and that the dianionic form is virtually nonexistent in the pH range employed (pH 6.5–8.5). The k_A^- value calculated from the slope is $0.080 \text{ M}^{-1} \text{ s}^{-1}$.

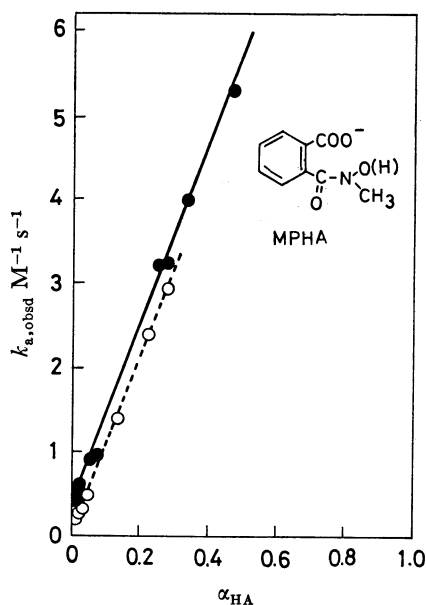


Fig. 3. Reaction of PNPA with MPHA. Reaction condition; 30 °C, $\mu = 0.1$ (KCl)

●; 28.9 v/v% EtOH-H₂O, 0.15 M Tris
○; 28.9 v/v% EtOD-D₂O, 0.1 M Tris

Figure 3 gives $k_{a, \text{obsd}} - \alpha_{\text{HA}}$ relations for MPHA in undeuterated and deuterated media. Two linear relations are obtained with different intercepts. The fact that positive intercepts were observed suggests that the undissociated hydroxamic acid possesses nucleophilic reactivity. Since the concentration of the neutral MPHA species is negligible based on the estimated $\text{p}K_{a, \text{COOH}}$ value (see Table 1), the apparent rate constant is given by

$$k_{a, \text{obsd}} = k_A^- \cdot \alpha_{\text{HA}} + k_{\text{HA}}(1-\alpha_{\text{HA}}) \quad (9)$$

where k_A^- and k_{HA} are second-order rate constants for MPHA^{2-} and MPHA^- species, respectively (Eq. 6).

The k_A^- and k_{HA} values were calculated using the slopes and intercepts of the linear relations, and the deuterium solvent kinetic isotope effects were calculated

TABLE 2. DEUTERIUM SOLVENT ISOTOPE EFFECTS IN THE REACTION OF PNPA WITH MPHA^{a)}

	Solvent		$k^{\text{H}}/k^{\text{D}}$
	28.9 v/v% EtOH-H ₂ O	28.9 v/v% EtOD-D ₂ O	
k_A^- ^{b)}	10.8 M ⁻¹ s ⁻¹	10.6 M ⁻¹ s ⁻¹	1.0
k_{HA}^- ^{b)}	0.22 M ⁻¹ s ⁻¹	0.088 M ⁻¹ s ⁻¹	2.5

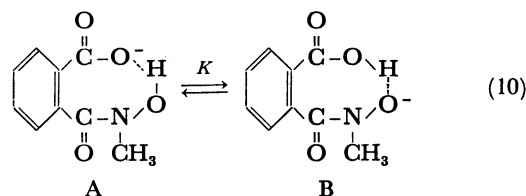
a) 30 °C, $\mu = 0.1$ (KCl), 0.1 M Tris buffer b) Calculated from Eq. 9.

as listed in Table 2.

Discussion

Acyl Transfer to Undissociated Hydroxamic Acid.

The kinetic data summarized in Table 2 indicate that the monoanionic species of phthalhydroxamic acid, MPHA^- , possess nucleophilic reactivity toward PNPA. The following monoanionic species cannot be kinetically distinguished.



The reaction rates for species A and B are given, respectively, by

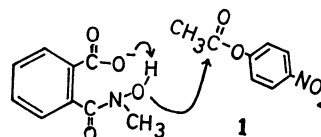
$$v_A = k_A[\text{A}][\text{PNPA}] \quad (11)$$

$$v_B = k_B[\text{B}][\text{PNPA}] = k_B K[\text{A}][\text{PNPA}] \quad (12)$$

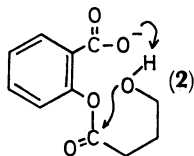
where k_A and k_B are second-order rate constants for the respective species and K is the equilibrium constant.

$\text{p}K_a$ of *N*-methylbenzohydroxamic acid is 4–5 $\text{p}K_a$ units higher than that of benzoic acid,¹²⁾ and a similar $\text{p}K_a$ difference may be expected for the first ionizations of the two functional groups of MPHA. Then, K is estimated to be 10^{-4} – 10^{-5} . If species B is solely responsible for the observed rate constant ($k_{\text{HA}} = 0.22 \text{ M}^{-1} \text{ s}^{-1}$), k_B must be as large as 2000–20000 $\text{M}^{-1} \text{ s}^{-1}$. This value is too large for a nonmicellar hydroxamate anion.¹⁹⁾ Furthermore, if species B is the effective nucleophile, the observed isotope effect must arise from the general acid catalysis of the neighboring carboxylic acid. This is unlikely for a substrate with good leaving group (PNPA).

These arguments now establish species A as the effective nucleophile. The carboxylate-catalyzed cleavage of PNPA is unlikely, since its second-order rate constant is estimated to be *ca.* $10^{-6} \text{ M}^{-1} \text{ s}^{-1}$.²¹⁾ Therefore, the observed reactivity is explained as the nucleophilic attack of the undissociated hydroxamic acid assisted by the general base catalysis of the *ortho*-carboxylate group (1).



The solvent isotope effect observed ($k_{\text{HA}}^{\text{H}}/k_{\text{HA}}^{\text{D}}=2.5$, Table 2) is consistent with this mechanism. Although the observed isotope effect is by itself consistent with the direct general base catalysis of the carboxylate anion and buffer bases, this mechanism cannot explain the observed rate constant. The magnitude of this isotope effect is similar to that ($k^{\text{H}}/k^{\text{D}}=2.28$) found by Kirby and Lloyd for the general base catalysis of the neighboring carboxylate group in the hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate (2).²²⁾



The solvent isotope effect was not observed for the reaction of the dianionic species, MPHA^{2-} , in agreement with the simple nucleophilic mechanism for the hydroxamate anion.

To our knowledge, the above mechanism (1) is the first example of the *intermolecular*, concerted nucleophilic attack on the ester carbonyl group. The reaction of simple hydroxamate anions with phenyl esters have been investigated extensively.¹⁵⁻¹⁸⁾ In all these cases, it was confirmed that only the anionic species were effective nucleophiles. This was also the case with bifunctional catalysts which contain hydroxamic acid and imidazole group^{3,4,5,23,24)} Apparently, the presence of the intramolecular hydrogen bonding as inferred from the pK_a data must render the concerted action more feasible.

Acyl Transfer to Hydroxamate Anion. It should be interesting to see how the *ortho*-substituent affects the reactivity of the hydroxamate anion. This question may be discussed in terms of the Brønsted relation. The Brønsted relation has been shown to hold true for several classes of hydroxamic acids.^{15,17,26)}

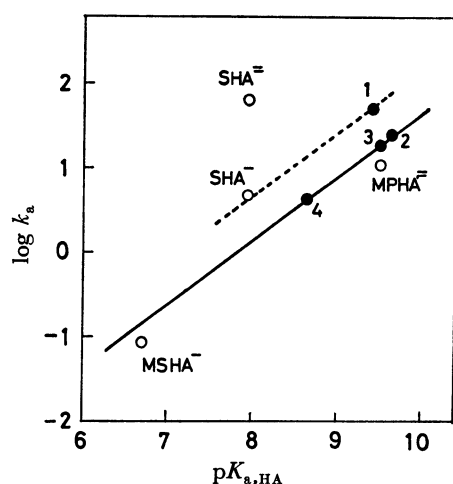


Fig. 4. Brønsted plots for the reaction of hydroxamic acids with PNPA.

Reaction condition; 30 °C, 28.9 v/v% EtOH-H₂O, $\mu=0.1$ (KCl).

1; BHA, 2; *N*-phenylisobutyrohydroxamic acid, 3; *N*-benzylbenzohydroxamic acid, 4; *N*-methyl- α -naphthohydroxamic acid.

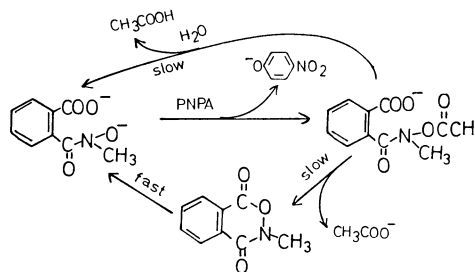
The solid line in Fig. 4 is obtained for several *N*-substituted hydroxamic acids with no additional functional groups and is given by

$$\log k_a = 0.68\text{pK}_a - 4.8 \quad (13)$$

The slope is the same as that reported by Hershfield and Bender²⁶⁾ for some other *N*-substituted hydroxamic acids under a somewhat different condition. The plots for two *N*-methylhydroxamate anions, MPHA^{2-} and MSHA^- , are located very close to this relation. The broken line is a tentative Brønsted relation for *N*-unsubstituted hydroxamic acids obtained by using the plot for BHA and the slope (0.72) found by Dessolin *et al.* for a series of *N*-unsubstituted hydroxamic acids under a different condition.¹⁷⁾ The plot for SHA^- agrees with this relation. This indicates that lowering of the nucleophilicity of SHA^- ($k_A=4.85 \text{ M}^{-1} \text{ s}^{-1}$) relative to that of BHA ($k_A=50 \text{ M}^{-1} \text{ s}^{-1}$) is accompanied by the corresponding decrease in pK_a . Thus, the hydrogen bonding of the hydroxamate anion with the *ortho* hydroxyl group brought forth decreases of both of nucleophilicity and basicity.

In contrast, the plot for the dianionic form SHA^{2-} is considerably deviated: k_A^{2-} is larger than expected from $\text{pK}_{a,\text{HA}}$. This may be explained as follows: (1) the nucleophilicity of the hydroxamate anion in SHA^{2-} is intrinsically greater than that of SHA^- or (2) the phenolate anion in SHA^{2-} is directly involved in the reaction besides the hydroxamate anion. The second-order rate constant of the reaction of PNPA and a phenoxide anion of $\text{pK}_a \approx 10$ has been estimated to be $0.97 \text{ M}^{-1} \text{ s}^{-1}$ under a comparable condition.²¹⁾ This value is too small compared with the k_A^{2-} value observed, and, consequently, the phenoxide anion cannot act as a nucleophile to any significant extent. Therefore, the increased reactivity is attributed to the increased nucleophilicity of the hydroxamate anion, which was probably caused by activation due to hydrogen bonding with the neighboring phenoxide anion (see Eq. 4).

Turnover of MPHA. MPHA is readily obtained as the cyclic anhydride. If the acyl hydroxamate intermediate is quickly converted to the cyclic compound with expulsion of the acetate ion, the catalytic cycle would be completed as below.



The first-order rate constant of the ring-opening step is $1.89 \times 10^{-2} \text{ s}^{-1}$ at pH 8.57, and the apparent rate of acylation of MPHA is $(0.86-1.8) \times 10^{-4} \text{ s}^{-1}$ with PNPA of $(0.9-1.9) \times 10^{-4} \text{ M}$ under similar conditions. Therefore, the cyclic intermediate, if formed, would be readily converted to the reactive catalyst, and the ring opening process could not be rate-determining in the catalytic cycle.

However, the turnover of MPHA was not clearly observed when it was reacted with excess PNPA. This means that the deacylation step was negligibly slow. It was expected that the deacetylation rate would be enhanced by facile cyclization or by the general base catalysis of the *ortho*-carboxylate group. Unfortunately, both of these processes were proved to be insignificant. Attempted preparation of the acetyl derivative was not successful, and the separate determination of the rate of deacylation was impossible.

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