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R. A. Abramovitch,* S. R. Challand, E. F. V. Scriven
Department of Chemistry, University of Alabama University, Alabama 35486
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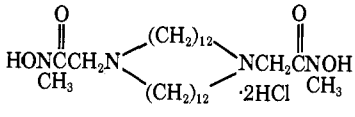
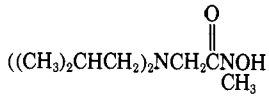
Nucleophilic and Metal Ion Acceleration of Ester Hydrolysis in a Hydrophobic Complex. A Reactive Enzyme Model System

Sir:

We wish to report the synthesis and preliminary characterization of macrocyclic *N*-methylhydroxamic acid (I), the first homolog of a projected series of simple macrocyclic and macrobicyclic amines with appended catalytic functionalities, *i.e.*, binding sites with proximate active sites.[1] The ease in incremental variation of ring size and rigidity, charge, and functional group together with its steric relationship to the binding site suggests that an unambiguous explanation for the large rate acceleration, described below, is possible. It is hoped that the adaptability of this system will enable a better assessment of the relative importance of the factors involved in the complexation and acylation steps of proteolytic enzymes.



I



II

In order to separate the influence of binding on reaction rates from any intrinsic functional group specificity, a specificity constant, k_r, was defined as the ratio of k_I, the apparent second-order rate constant for the reaction of I with a series of *p*-nitrophenyl carboxylates, to k_{II}, the second-order rate constant for the reaction of II with the respective carboxylate. For this purpose k_r will be a valid indicator if compounds I and II are similar in physical properties and if II represents a normally reactive hydroxamic acid. Both requirements are confirmed by the following observations. (1) The λ_{max} of their infrared carbonyl absorption is the same and the *N*-methyl and α-methylene hydrogens of the hydroxamate group have identical chemical shifts. (2) In a 5% $FeCl_3$–0.1 *N* HCl solution the ϵ at 540 nm of I is twice that of II and approximately equal to that of α-aminohydroxamic acids under similar conditions.[2] (3) The first pK_a of I is 6.8 ± 0.1 while that for II is 7.0 ± 0.2, determined spectrophotometrically. (4) A Brønsted plot for the reaction of *N*-methylacetohydroxamic acid,[3,4] *N*-methyl-1-methoxyacetohydroxamic acid,[4] and II with *p*-nitrophenyl acetate (*p*-NPA) affords an excellent linear correlation with a slope of 0.68, close to the value found for the reaction of moderately basic oxygen anions with *p*-NPA.[5] (5) A linear correlation exists between the logarithm of the rate constants for the hydroxide-accelerated hydrolysis of the ethyl carboxylates of Table I and the reaction of II with the *p*-nitrophenyl esters of these acids.[6] (6) Consistent with previous studies on the reactivity of *N*-methylhydroxamic acids, at ester concentrations greater than "catalyst" concentration, both I and II displayed kinetically biphasic reactivity—burst kinetics[7] —with all *p*-nitrophenyl esters except that of chloroacetic acid. Titration of I by measurement of the burst size indicates that only one hydroxamate group per molecule of I is active.[8] (7) The release of *p*-nitrophenol from *p*-nitrophenyl hexanoate in the presence of I showed a dependence on one group with pK_a = 6.7 ± 0.1. As a consequence of these observations, any departure of the reactions accelerated by I from the corresponding acceleration by II must arise from some unique property of compound I's aliphatic portion, not its functional groups, or the special reactivity of II.

Table I. Comparison of the Effects of I and II on *p*-Nitrophenol Release from *p*-Nitrophenyl Carboxylates at pH 6.80[a]

p-Nitrophenyl ester	k_I, M^{-1} sec^{-1} [b]	k_{II}, M^{-1} sec^{-1}	k_r
Acetate	1.18	0.693	1.7
Propionate	1.32	0.527	2.5
Butyrate	4.00[g]	0.420	9.0
Isobutyrate	2.29	0.230	10
Valerate	3.31	0.340	9.8
Hexanoate	6.35	0.350	15
Octanoate	34.2	0.190	150
Dodecanoate[c]	152[d]	0.02[f]	7600
Chloroacetate[e]	240		

[a] 25°, in aqueous phosphate buffer, μ = 0.088, containing 10.55% methanol–1.75% acetonitrile (v/v). [b] 9.5 × 10^{-6} *M* I employed in all determinations with substrate in excess, except as indicated. [c] 6 × 10^{-6} *M* dodecanoate. [d] 1.9 × 10^{-5} *M* I. [e] pH 6.37, [I] = 8.7–17 × 10^{-6} *M*. [f] Measured at pH 7.98 (Tris, μ = 0.088, 10.55% methanol, 1.75% acetonitrile (v/v)), and corrected to pH 6.80. [g] Obtained from the inverse of the slope of a Lineweaver–Burk plot.

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(8) For the general scheme

$$I + S \underset{}{\overset{K_s}{\rightleftharpoons}} I \cdot S \xrightarrow{k_2} I' \xrightarrow{k_3} I + P_2$$
$$\qquad\qquad\qquad\qquad\quad +$$
$$\qquad\qquad\qquad\qquad\quad P_1$$

it has also been shown that

$$B = \frac{I_0(k_2/(k_2 + k_3))^2}{(1 + K_m/S_0)^2}$$

where B is the burst size, I_0 and S_0 are initial I and ester concentrations, respectively, and $K_m = [k_3/(k_2 + k_3)]K_s$. In the case of PNPB where k_2 and K_s have been separated, k_2 is greater than $200k_3$ as judged from the slope of the linear portion of the progress curve. Consequently, $K_m < 5 \times 10^{-6}$ *M* for this substrate, and presumably much less for longer chain acids, so that $B = I_0$.

Table I records values of k_r for the release of *p*-nitrophenol from a number of *p*-nitrophenyl carboxylates. With one exception (discussed below) a linear dependence on ester concentration was observed in reactions with compound I. Menger and Portnoy[9] have shown that the rate of alkaline hydrolysis of *p*-nitrophenyl dodecanoate decreases as the ester concentration is increased and have attributed this phenomenon to complexation. As indicated by the ratio of k_{II} for the acetate to k_{II} for the dodecanoate which is consistent with similar data in analogous studies,[1a,k] and by the fact that semilogarithmic plots were linear to greater than 2 half-lives for the reaction of I with the dodecanoate ([I] > [ester]), aggregation phenomenon is probably unimportant in this study because of the low ester and high organic solvent concentrations employed; the small k_{II} value is probably an intramolecular steric effect.[1a,k,10]

It is difficult to account for the irregular increase in k_r with acyl chain length by other than stereospecific productive binding on the path to products. If hydrophobic association between substrate and I does exist, as Table I implies, it should be possible to observe Michaelis–Menten kinetics. In fact saturation of I by *p*-nitrophenyl butyrate (*p*NPB) does indeed occur.

Considering the high methanol–acetonitrile concentration present, binding between I and *p*-NPB is very strong ($K_s = 9.9 \pm 0.2 \times 10^{-4}$ *M*). For example, an increase in acetonitrile concentration from 0.5 to 10% causes a tenfold increase in K_s for cyclodextrin substrates.[11] The aliphatic portion of *p*-NPB must be primarily responsible for binding, since fourfold higher concentrations of *p*-NPA were linear in ester concentration.

The evidence for a discrete binding site is further substantiated by the effect of potassium iodide on the kinetics of phenol release from *p*-nitrophenyl hexanoate. With II present addition of KI causes a small rate enhancement. In contrast, though hydrophobic binding normally increases with increasing ionic strength,[12] KI inhibits the I-accelerated reaction in excellent accord with $v/v_i = 1 + i/K_i$.[12] Simmons and Park[14] have reported evidence consistent with encapsulation of iodide within the cavity of an *in,in*-[10.10.10]diazabicycloalkane with an association constant greater than 10, comparable to the K_i of 7×10^{-2} *M* measured for this system. The qualitative agreement of this inhibition data with Simmons' findings, the X-ray structural determinations of Dunitz[15] on cyclic amines, the conformational predictions of Dale[16] based on space-filling molecular models and physical constants of cyclic alkanes, and the observation that 1,8,15,22-tetraazacyclooctacosane crystallizes from aqueous solution with a molecule of water included in its cavity[17] all support the hypothesis that the productive binding site for I is a cavity approximately 5.6 Å in diameter formed by the aliphatic chains of I. The possibility that binding outside the cavity of I leads to rate acceleration cannot, however, be discounted.[1a]

Table II. Effect of Cupric Chloride on *p*-Nitrophenol Release from *p*-Nitrophenyl Carboxylates in the Presence of I and II[a]

Ester	Ester concn $\times 10^4$, *M*	$CuCl_2$ concn $\times 10^5$, *M*	$k_{obsd} \times 10^4$, sec^{-1} [b]
	[I] = 9.5×10^{-6} *M*		
Propionate	2.77	0	3.8
Propionate	2.77	1.08	32
Hexanoate	0.755	0	4.8
Hexanoate	0.755	1.08	59
	[II] = 1.35×10^{-3} *M*		
Hexanoate	0.755	0	29
Hexanoate	0.755	13.5[c]	32

[a] 25°, in aqueous phosphate buffer, $\mu = 0.088$, containing 10.55% methanol–1.75% acetonitrile. [b] Observed pseudo-first-order rate constant. [c] Limited by buffer precipitation at higher $CuCl_2$ concentration.

Finally, the preliminary data collected in Table II indicate that an equivalent of $CuCl_2$ accelerates the reaction of I with *p*-nitrophenyl carboxylates about tenfold, but has no effect of the reactivity of II.[18] Consequently, k_r for *p*-nitrophenyl hexanoate is about 150 while that for the dodecanoate is approximately 60,000 with $CuCl_2$ present.

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(18) The reaction of *N*-methylacetohydroxamic acid with *p*-nitrophenyl acetate is not affected by the presence of cupric ion.[4]

(19) NIH Predoctoral Fellow, 1966–1969.

Robert Hershfield,[19] Myron L. Bender*
Department of Chemistry, Northwestern University
Evanston, Illinois 60201
Received October 28, 1971

Remote Secondary Deuterium Isotope Effects. III.[1] β-Arylalkyl Systems

Sir:

Based on an analysis of curved Hammett plots, it has been proposed[2] that the acetolysis of secondary β-arylalkyl derivatives involves rate-determining competition between two discrete strongly assisted pathways: k_S (solvent assisted) and k_Δ (aryl assisted).[5] In this analy-

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(5) The k_S, k_Δ scheme was first proposed by Winstein to explain the solvolytic behavior of primary β-arylalkyl derivatives.[6] For such primary systems, the k_S pathway is considered to involve direct SN2 displacement by solvent on covalent substrate.[6c,d,g] For secondary systems, the role of the solvent has been less precisely defined.

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