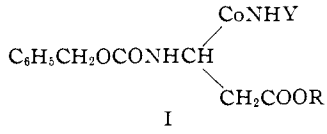
Neighboring Group Effects on Ester Hydrolysis. I. Neighboring Hydroxyl Groups

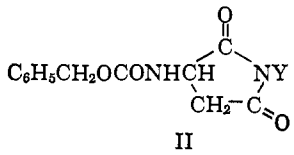
Sir:

A recent publication[1] reported the rapid hydrolysis of ester derivatives of aspartyl peptides of the general type I. Hydrolysis occurs in a two-step reaction; cy-



I

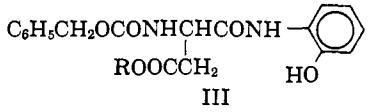
clization to the aspartoimide (II), followed by hydroly-



II

sis of the imide, the second reaction being rate-controlling. In every compound of type I studied, the over-all hydrolysis rate was much faster than that observed with simple esters. The most rapid rate of hydrolysis was found with the β-ester of N-carbobenzyloxyaspartylserylamide; the half-life in dioxane–water (1:2, v./v.) at pH 10.0 is 3 min., which is about 2000-fold faster than the hydrolysis of ethyl acetate under similar conditions.[2]

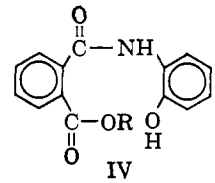
When the hydroxyl group of serine is acylated the hydrolysis rate is lowered,[3] suggesting that the OH group of serine enhances the hydrolysis of the adjacent aspartoimide due to its slightly acidic character. The pK_a of N-acetylserineamide is unusually low.[4] We have now prepared an analogous compound with a more pronounced acidic hydroxyl, namely N-carbobenzyloxy-β-benzylaspartyl-*o*-hydroxyanilide (III). The hydrolysis of this ester was followed potentiometrically, at constant pH, in a "pH-stat." Near



III

neutrality, the second-order specific rate of OH^- catalyzed hydrolysis of III is 2×10^5 M^{-1} min.$^{-1}$. The hydrolysis rate increased with a less than first-order dependence on OH^- concentration. The pH–rate profile is consistent with that anticipated for a reaction dependent on the concentration of a conjugate base with $pK \sim 9.5$, suggesting (see below) that the phenolate ion is responsible for the enhanced rate. The hydrolysis of a more rigid analog of III, namely IV, exhibited a similar pH–hydrolytic rate profile, but hydrolyzed 50% faster than III. These hydrolysis reactions are the fastest reported for ordinary esters.

The *p*-OH isomer of III is hydrolyzed at 10^{-4} times the rate of the *ortho* isomer, the hydrolysis rate being

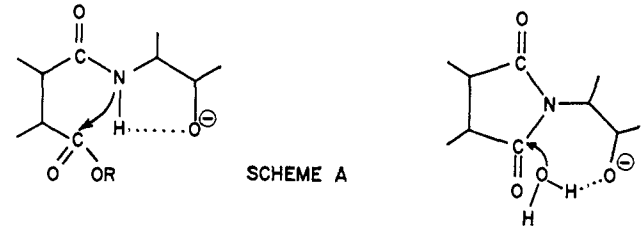


IV

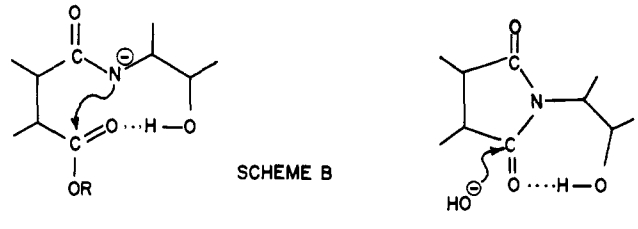
similar to that of methyl N-phenylphthalamate.[5] Moreover, since the rate of cyclization of the *para* isomer to the corresponding imide is slower than the over-all hydrolysis rate of III, the *o*-OH group must greatly enhance *both* steps in the sequential mechanism.

By examination of the ultraviolet spectrum of III in the 240–300 mμ range during the course of reaction, the hydrolytic mechanism could be clearly resolved into two consecutive steps, the first step having a specific rate ~8-fold greater than the second. The specific rate of the second step is equal to the over-all hydrolysis rate as determined from the "pH-stat" kinetics. By analogy with previous reports,[1,5] we assign the first step to the cyclization of the ester to the corresponding imide, and the second to hydrolysis of the imide intermediate.

Two mechanisms, consistent with the results presented herein, can be postulated for the rate enhancement of both cyclization and hydrolysis by an adjacent hydroxyl. (1) General base catalysis (involving alcoholate or phenolate ion), by abstraction of a proton from the attacking nucleophile, is shown in Scheme A. A similar mechanism has been proposed in related reactions.[6] (2) General acid catalysis (involving undissociated alcohol or phenol[7,8]) by enhancement of the electrophilicity of the reacting carbonyl for the anionic nucleophile is shown in Scheme B.



SCHEME A



SCHEME B

Each general base catalyzed step in the reaction sequence will follow the rate law

$$v = k[\text{phenolate}^-]$$

Since only the phenol has a measurable dissociation constant in the pH range of these experiments, each general acid catalyzed step will follow the kinetically equivalent expression

(1) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).

(2) The effect of organic solvent is to greatly increase the second-order specific rate of hydrolysis of the peptide esters, whereas ordinary esters are relatively insensitive to solvent composition. In 70% dioxane–water, the above-quoted esters differ by more than 10^6-fold in OH^- catalyzed hydrolysis rates.

(3) (a) Unpublished results from this laboratory; (b) H. Kienhuis, Ph.D. Thesis, University of Leiden, 1963. We are indebted to Dr. Kienhuis for communicating his results prior to publication.

(4) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, **1**, 7 (1962).

(5) J. A. Shafer and H. Morawetz, *J. Org. Chem.*, **28**, 1899 (1963).

(6) M. L. Bender, F. J. Kezdy, and B. Zerner, *J. Am. Chem. Soc.*, **85**, 3017 (1963).

(7) T. C. Bruice and T. H. Fife, *ibid.*, **84**, 1973 (1962).

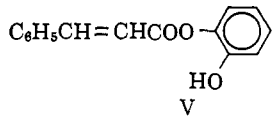
(8) S. M. Kupchan, S. P. Eriksen, and Y. T. Shen, *ibid.*, **85**, 350 (1963).

$$v = k'\alpha[\text{phenol}] = \frac{k'K_N}{K}[\text{phenolate}^-]$$

where K is the dissociation constant of the phenol, α is the fraction of the nucleophile in the anionic form, and K_N is the dissociation constant of the nucleophile, *viz.*

$$K_N = [H^+][N^-]/[HN] \cong [H^+][N^-]/[N]_{total}$$

Experiments are now in progress to distinguish between these two possible rate enhancement mechanisms. Hydroxyl groups, quite remote from a susceptible bond, when properly oriented can cooperate with a vicinal peptide group to very markedly enhance the reactivity. In some enzymes, such as pepsin[9] and carboxypeptidase,[10] where tyrosine groups are involved in the activity, the role of the phenolic group may be related to one or the other of the above-mentioned mechanisms. It should follow that the esters as well as imides of the *o*-OH phenyl derivatives should exhibit enhanced reactivity. The hydrolysis of monocinnamoylcatechol (V) was followed spectrophotometrically utilizing the change in optical density at 3000 Å. accompanying the hydrolytic reaction. The second-order specific rate of OH^- catalyzed hydrolysis is 2×10^4 M^{-1} min.$^{-1}$ near neutral pH. This is 300 times greater than the specific rate of phenyl cinnamate hydrolysis; another manifestation of the marked effect of an adjacent hydroxyl on reactivity. Similar results have been recently reported for monoacetylcatechol.[11]



V

A detailed report of these kinetic results, and of their potential pertinence to specific examples of enzymic catalysis, will be presented shortly.

Acknowledgment.—This work was supported by grants from the National Science Foundation and the Public Health Service.

(9) R. Herriott, *J. Gen. Physiol.*, **19**, 283 (1935).
(10) R. T. Simpson, J. F. Riordan, and B. L. Vallee, *Biochemistry*, **2**, 616 (1963).
(11) B. Hansen, *Acta Chem. Scand.*, **17**, 1375 (1963).
(12) On leave of absence from the Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

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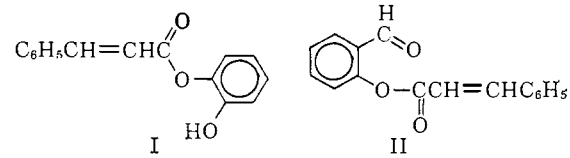
RECEIVED MARCH 30, 1964

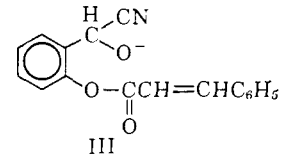
Neighboring Group Effects on Ester Hydrolysis. II. Neighboring Carbonyl Groups

Sir:

Previously,[1] and in an accompanying communication,[2] large rate enhancements in ester hydrolysis have been demonstrated in compounds containing a neighboring hydroxyl group. These results may be of pertinence to the mechanism of enzymic hydrolysis of acyl derivatives, wherever a serine hydroxyl residue is a constituent of the catalytically active site. Other neighboring functional groups may possibly exert rate enhancement effects as well. Carbonyl groups have been shown to increase the rate of hydrolysis of a neighboring ester.[3,4] Likewise, we have found that the aldehyde analog of I, cinnamoylsalicylaldehyde (II), undergoes very rapid hydrolysis. The rate is first order in OH^- concentration. The second-order specific rate is approximately 7×10^5 M^{-1} min.$^{-1}$. The activity is presumably due to the hydrated aldehyde or its conjugate base. This is indicated by the fact that in 0.002 M KCN solution, where the reactive species is probably the cyanohydrin (III), the rate of hydrolysis is increased tenfold. The catalysis may proceed similarly to either Scheme A or B of ref. 2, or *via* a nucleophilic attack on the carbonyl ester as suggested for the hydrolysis of *o*-formyl benzoate esters.[4]

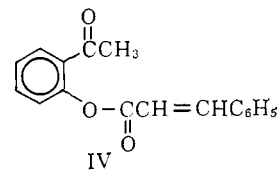


I II



III

A related ester containing a neighboring ketone, the cinnamoyl ester of *o*-hydroxyacetophenone (IV), is hydrolyzed with a specific rate of 2.6×10^3 M^{-1} min.$^{-1}$; very much slower than the hydrolysis of III,



IV

but nevertheless 40-fold faster than the hydrolysis of phenyl cinnamate. Rate enhancement by CN^- is similar to that found with II.

Carbonyl groups of aldehydes and ketones, especially the former, can have a great influence on the rate of hydrolysis of neighboring ester groups. The available evidence indicates, however, that the rate enhancement is, in reality, exerted by the hydroxylic adduct or its conjugate base.

Acknowledgment.—This work was supported by grants from the National Science Foundation and the Public Health Service.

(1) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962).
(2) Y. Shalitin and S. A. Bernhard, *ibid.*, **86**, 2291 (1964).
(3) M. S. Newman and S. Hishida, *ibid.*, **84**, 3582 (1962).
(4) M. L. Bender and M. S. Silver, *ibid.*, **84**, 4589 (1962).
(5) On leave of absence from the Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

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RECEIVED APRIL 27, 1964

Search For Accidental Degeneracy in Purines

Sir:

The strong adenine absorption at 260 mμ (38.5 kk.) appears to be a single electronic transition. Mason[1]

(1) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).