

Figure 7.

substituents (left-handed portion of the Hammett plot) are favoring the reaction (ρ approximately +1.0) by balancing somewhat the electronegativity of the two nitrogen atoms of the hydrazone anion, increasing the fractional negative charge at the carbon terminal, and thus favoring the C-H bond formation. As the electron-attracting character of the substituents increases further, however, a second, unfavorable factor comes into play (ρ approximately -0.8). The smaller the negative charge that remains at the N-H terminal, the stronger is the hydrogen bond which binds the first hydroxylic solvent molecule to the hydrazone anion, and the "harder" and bigger the solvation shell which is built up at the N-H terminal. Hence the base $R'-O^-$ becomes less effective in the N-H bond breaking process. Since the observed ΔS^\ddagger values take into account the highly complex over-all change in the degrees of freedom of the hydrazone anion, solvent molecules, and base, it is not surprising that one fails to note a

relationship between the enthalpy and entropy values and substituent constants. On the other hand, it seems hardly accidental that structural features which were at first thought² capable of promoting an intramolecular hydrogen transfer are present in compounds exhibiting the lowest enthalpies and the highest negative entropies of activation (see Table II). The behavior of the 2-pyridyl system, for example, can be rationalized in the light of the proposed mechanism by visualizing a hydroxylic solvent molecule "frozen" between the "ortho substituent" and the N-H terminal, with the double consequence of making it relatively easy for another hydroxylic solvent molecule to approach the carbon terminal (thus promoting the displacement of the negative charge in that direction), and also relatively easy for the base $R'-O^-$ to approach the N-H terminal. The high degree of organization which this mechanism implies (Fig. 7) explains the exceptionally negative entropy values which are observed. Similar explanations can be postulated in all cases in which an *ortho*-acceleration effect seems to operate. In accord with this mechanism, the "ortho substituent" simply aids in the fixation of a hydroxylic solvent molecule at the N-H terminal, while the previously implied² intramolecular hydrogen transfer mechanism should be sufficiently different from the more general intermolecular hydrogen transfer mechanism to cause scattering of the points corresponding to the two mechanisms on the enthalpy-entropy graph.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MISSOURI AT KANSAS CITY, KANSAS CITY 10, MO.]

Phosphate Esters. I. Catalytic Hydrolysis of *p*-Nitrophenyl Dihydrogen Phosphate by Ceric Ion Precipitates in the pH Range 2-7¹

BY FRANK MILLICH² AND ELBERT L. HAYES, JR.³

RECEIVED JANUARY 23, 1964

No catalytic influence could be observed upon the hydrolysis of the monoanion of *p*-nitrophenyl dihydrogen phosphate by hydronium, calcium, ferric, or ceric ions in monophasic aqueous solution. However, catalysis is found upon the occurrence of precipitate formation when $Ce(NH_4SO_4)_4$ is solvolyzed. Catalysis takes place in the solid phase as substrate becomes associated. The catalyst is partly defined, and two maxima, at pH 4.5 and 6.0, are revealed in a pH-rate profile of the catalyzed hydrolysis at 49°. Relation of this catalysis to enzymic acid-phosphorylase activity is briefly discussed.

Introduction

Kinetic studies have revealed that the following facts seem to be fairly general for acid hydrolysis of simple monoesters of phosphoric acid: (a) the monoanion is more susceptible to hydrolysis than the corresponding neutral or dianionic conjugate species; (b) hydrolysis of the monoanion proceeds by P-O bond cleavage; and (c) its kinetic rate depends upon the concentration of the monoanion, but the transition state does not specifically involve catalysis by hydronium ion. A brief report in the literature⁴ has indicated that these three facts obtain in the acid hydrolysis of *p*-nitrophenyl dihydrogen phosphate (PNPP) monoanion. The details of hydrolysis of dianions of simple phos-

phate monoesters have not been studied extensively because of the very small relative reactivity of these species. This paper describes results of a study undertaken to evaluate whether fact c remains unaltered in the presence of polyvalent metal ions, but which has progressed further to a study of heterophasic catalysis with ceric ion.

The catalytic activity of substrate-associated metal ions in phosphate ester hydrolysis and several other heterophasic reactions has been the subject of study by E. Bamann and his collaborators for a quarter century.⁵ They have shown these catalysts to mimic very many of the properties characteristic of enzymes. Much of the catalytic activity of metal ions and of enzymes must depend on common physical and chemical phenomena. Elucidation of the mechanism of action of the latter may profitably be approached through a study of the former without the narrow

(1) This project was partly supported by a National Science Foundation grant, NSF-G 21590, of the Undergraduate Science Education program.

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(3) Participant in National Science Foundation Undergraduate Research Program, 1962-1963.

(4) See C. A. Vernon in "Phosphoric Esters and Related Compounds," Special Publication No. 8, The Chemical Society, London, 1957, pp. 19-22.

(5) For a review, see H. Trapmann, *Arzneimittel-Forsch.*, **9**, 341, 403 (1959).

restrictions of lability of the catalyst, especially as regards stability to temperature variation; indeed, if biochemical kinetic data were more definitive, valuable quantitative comparisons would be possible. Bamann's school has shown that the characteristics of pH-rate profiles, such as that which we present, are rather sensitive to many variables; however, the importance of completely understanding such systems warrants an exhaustive investigation of their complexities.⁶

Experimental

Preparation of Materials.—PNPP was essentially prepared by previously reported procedures. Phenyl phosphorodichloridate was obtained from phenol and phosphorus oxychloride,^{7,8} then hydrolyzed⁸ and the product nitrated.⁹ Yields were about 80% at each stage. Final recrystallization from HCCl_3 - Et_2O (4:1) gave yellow-white needles (m.p. 155°).⁹

Acid Dissociation Constants of PNPP.—The first and second K_a are obtained, by potentiometric titrations, from the pH values of one-half and three-halves equivalence points. At the temperatures 30, 49, and 73 ± 1°, the values, respectively, are for $10^2 K_1$: 0.71, 1.8, and 2.6; and for $10^2 K_2$: 4.0, 8.0, and 9.5. The duration of heating at 73° and pH 3.5 was limited to 4 min. to avoid hydrolysis. The titrations were followed potentiometrically with a Coleman Model 18 pH meter. Values of titer for the 1-, $3/2$ -, and 2-fold equivalence points were determined graphically, and showed proportional correspondence, within 0.2 ml., in each case.

Measurement of Hydrolysis Rates.—Gross specific rate constants, k 's, are reported in this paper, and are based upon the amount of *p*-nitrophenol (PNP) released during hydrolysis; the k 's are not corrected for various degrees of ionization of the substrate or of association of the substrate to the heterophasic catalyst, although such calculations may be made from the accompanying data. The k 's, and the deviations, are arithmetical averages computed, in any one kinetic experiment, from k 's calculated separately for each aliquot sample taken at different periods of time, and assuming a kinetic first-order dependence on substrate concentration.

The rates of hydrolyses were followed by diluting aliquots (1 ml. × 100) with aqueous borax-boric acid solution (0.20 *M* 1.06 *M*, pH 8.20) and assaying absorption at 4080 Å. with a Beckman DU spectrophotometer. Beer's law dependence was established over the concentration range 0.977 – 8.08×10^{-5} mole/l. The arithmetic mean from 26 points gave a value of $12,100 \pm 96$ l./mole-cm. for the molar absorptivity index, a_m , while a least squares analysis also gave 12,100. Each hydrolysis run was followed for at least 30 min., corresponding to no more than 20% hydrolysis, and at least four aliquots were withdrawn at different times.

Uncatalyzed Hydrolysis.—The dependence of the rate of hydrolysis on acidity of the medium was investigated at 73° over the range of pH 2.7–5.5. Substrate, PNPP (0.025 *M*), in formic or acetic acid buffer (0.75 *M* RCOONa + appropriate amount of RCOOH) was brought to constant ionic strength, μ , by addition of NaClO₄. The results at $\mu = 1.00$ are shown in Table I. The maximum, at pH 3.5, is slightly dependent on μ at low values of μ , as shown in Table II; these k -values have a deviation no greater than $\pm 0.1 \times 10^{-3}$ min.⁻¹.

The values of k for the uncatalyzed hydrolysis at 49°, $\mu = 0.80$, and pH 3.5 and 4.5 were 4.0×10^{-4} and 2.0×10^{-4} min.⁻¹, respectively.

TABLE I

FIRST-ORDER SPECIFIC RATE CONSTANTS, $10^3 k$ (MIN. ⁻¹), AND AVERAGE DEVIATIONS AT 73° AND $\mu = 1.00$			
pH	$10^3 k$, min. ⁻¹	pH	$10^3 k$, min. ⁻¹
2.7	1.67 ± 0.08	3.7	2.36 ± 0.08
3.0	$2.28 \pm .05$	4.0	$2.22 \pm .04$
3.2	$2.39 \pm .06$	5.5	$1.56 \pm .05$
3.5	$2.57 \pm .08$		

(6) W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2420 (1955).

(7) G. Jacobsen, *Chem. Ber.*, **8**, 1521 (1875).

(8) H. F. Freeman and C. W. Colver, *J. Am. Chem. Soc.*, **60**, 750 (1938):

(9) J. M. A. Hoeflake, *Rec. trav. chim.*, **36**, 24 (1916).

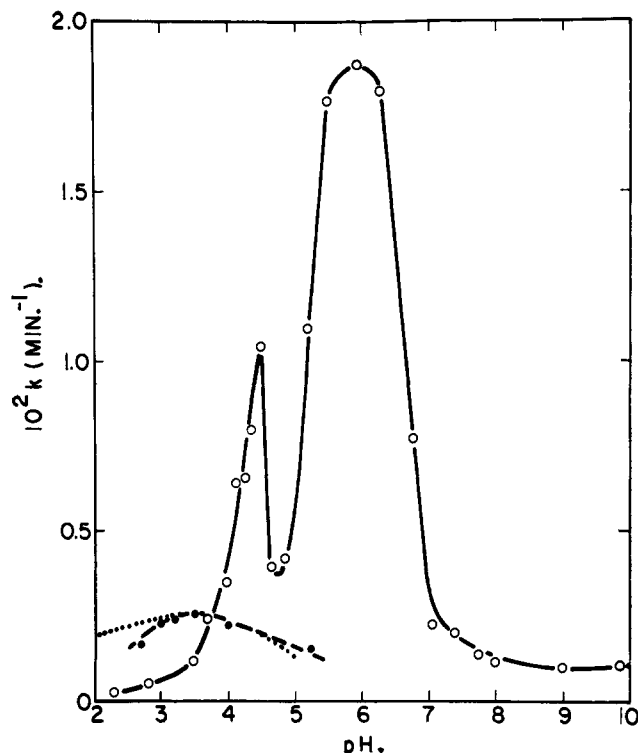


Fig. 1.—Specific rate constants, k , of hydrolysis of *p*-nitrophenyl dihydrogen phosphate (0.025 *M*) as a function of pH: —●—, uncatalyzed at 73°, $\mu = 1.0$ (NaClO₄); —○—, catalyzed at 49° in the presence of oxyhydrated ceric precipitates ($c_0 =$ of 0.025 *M*), $\mu = 0.8$; dotted curve is the calculated distribution ROPO_3H^- at 73° ($\text{p}K_1 = 1.58$). Buffers: pH 2.0–5.5, HCOONa or CH₃COONa (0.75 *M*) + x *M* RCOOH; pH 6.0–8.0, H₃BO₃ + borax (0.25–0.75 *M*); pH 7.4–9.9, H₃BO₃ (0.75 *M*) + x *M* NaOH.

TABLE II

FIRST-ORDER SPECIFIC RATE CONSTANTS, $10^3 k$ (MIN. ⁻¹), AT 73° AND pH 3.2						
μ	2.00	1.00	0.50	0.38	0.25	0.10
$10^3 k$, min. ⁻¹	3.0	3.0	3.0	3.3	3.4	3.6

Monophasic hydrolysis in the presence of polyvalent ions Ce^{+4} , Fe^{+3} , and Ca^{+2} , at concentrations of 2×10^{-6} , 3.3×10^{-6} , and 1×10^{-4} *M*, respectively, showed no catalysis at pH 3.2 and $\mu = 0.46$.

Catalyzed Hydrolysis.—A typical kinetic determination involved adding solid $\text{Ce}(\text{NH}_4\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$ (0.7908 g.) to a mechanically stirred, thermostated formate or acetate buffer solution (50 ml.). Oxyhydrated cerium(IV) precipitated immediately at concentrations above 10^{-4} *M*. After 5 min. an equimolar amount of PNPP (0.2739 g., 0.025 *M*) was added and solution was made up to 50 ml.; 5-ml. portions were withdrawn at various intervals, centrifuged, and 1-ml. aliquots of supernatant solution taken for spectrophotometric assay of PNP in the manner described above. Lower reaction temperature of 49° gave more convenient kinetic rates, and, whereas the activity of the initially formed precipitate was seen to decrease by predigestion periods of 0.5 hr. at 73°, the activity is undiminished at 49° after 1 hr.

The dependence of catalysis upon pH is shown in Fig. 1. Acetate and formate buffers were used interchangeably in the region of pH 4.3–5.0.

The PNPP content of the precipitate at various values of pH was examined by removing a hydrolysis mixture from the bath 1–3 min. after addition of substrate. The quantity of precipitate was determined gravimetrically by removing three 5-ml. aliquots of stirred mixture, filtering by suction through tared filter paper (Whatman No. 2), and oven drying at 112° to constant weight (30 min.). Triplicate samples of filtered gel were hydrolyzed at 100° for 1 hr. after resuspension in buffer medium at the original pH and volume; equivalent results were obtained in several cases at 100° after solution in aqueous HClO₄ (70%). The amount of liberated *p*-nitrophenol was assayed spectrophotometrically.

TABLE III

ANALYSIS, AT ZERO TIME, OF THE DISTRIBUTION OF PNPP BETWEEN PHASES IN THE SYSTEMS OBTAINED BY ADDING 0.2739 G. OF PNPP TO 0.7908 G. OF $\text{Ce}(\text{NH}_4\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$ AND 50 ML. OF BUFFER AT VARIOUS VALUES OF pH AT 49°^a

pH	(PNP) ₀ , ^b M	(PNPP), ^c M	(PNP) ₀ + (PNPP) _{dis} , ^d M	(PNPP) _{dis} , ^e M	(PNPP) _{gel} , ^f M	Wt. of gel., g.	PNPP pptd., ^g %	
3.0	0.0001	0.0249	0.0110	0.0109	0.0140	0.518 ± 0.014	56	
	.0004	.0246	.0111	.0107	.0139		56	
	.0003	.0247	.0111	.0108	.0139		56	
	.0003	.0247	.0111	.0108	.0139		56	
3.5	.0002	.0248	.0092	.0090	.0158	0.557 ± 0.001	64	
	.0004	.0246	.0102	.0098	.0142*		0.510	58
	.0003	.0247	.0100	.0097	.0150		61	
	.0004	.0246	.0100	.0096	.0150		61	
4.0	.0004	.0246	.0100	.0096	.0150	0.509 ± 0.002	61	
	.0005	.0245	.0085	.0080	.0165		67	
	.0002	.0248	.0100	.0098	.0150		60	
	.0002	.0248	.0102	.0100	.0148		59	
4.5	.0002	.0248	.0103	.0101	.0147	0.523 ± 0.002	59	
	.0006	.0244	.0085	.0079	.0165		67 ^h	
02500153*		.480	61
02500153*		.480	61
4.8	.0001	.0249	.0107	.0106	.0143	0.532 ± 0.002	57	
	.0001	.0249	.0107	.0106	.0143		57	
	.0002	.0248	.0107	.0105	.0143		57	
	.0009	.0241	.0086	.0077	.0164		68	
5.0	.0006	.0244	.0094	.0088	.0156	0.522 ± 0.002	67	
	.0006	.0244	.0093	.0087	.0157		67	
	.0006	.0244	.0094	.0088	.0156		67	
5.1	.0023	.0227	.0093	.0070	.0147	0.522 ± 0.002	65	

^a Buffer: 0.75 M RCOONa + x M RCOOH; R = H or CH₃. ^b Represents a blank correction at zero time; observed absorbance is interpreted in terms of molarity of *p*-nitrophenol originating in the weighed charge. Zero time is taken as time of removal of the first aliquot sample, usually occurring within 2 min. of mixing of the reagents. ^c PNPP content at time zero: 0.0250 - col. 1. ^d PNP content of solution, only, following complete hydrolysis at 100°. ^e PNPP dissolved in solution: col. 3 - col. 1. ^f Those marked with asterisk were obtained by direct analysis; all the others were calculated by difference, col. 2 - col. 4. ^g Columns 5/2. ^h One set of self-consistent analyses at pH 4.5 gave anomalous results (34%), and is rejected on the basis of the magnitude of deviation.

metrically, as described above. Blanks, receiving the same treatment, gave minor values of absorbance which amounted to no practical concentration corrections. The results are shown in Table III. Only 82 ± 5% of the weight of isolated gel can be accounted for by the data of column 5 with the assumptions that hydrolysis of $\text{Ce}(\text{NH}_4\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$ to $\text{Ce}(\text{OH})_4$ and drying are complete.

Catalytic Activity of the Solid Phase.—The catalyst preparation at pH 3.5 was filtered shortly after the addition of PNPP, and then resuspended in fresh buffer (250 ml.). The apparent specific rate constants were determined during the next 90 min. of hydrolysis at 49°, after which analysis proved the presence of only a small amount of PNPP in solution. The apparent values of 10²k, at their respective time intervals, showed the following constancy: (15 min.) 1.8, (30) 2.1, (45) 2.1, (60) 2.1, and (90) 2.1. These figures are calculated on the basis of the original substrate concentration (0.025 M) and ignored are the facts that PNPP in the filtrate was discarded, and that the volume of replaced suspension medium was increased fivefold.

Variation of Substrate-to-Catalyst Ratio.—At the pH maximum of 4.5 and at 49°, the initial concentrations of the substrate (Table IV) and the catalyst (Table V) were varied independently. Since 0.75 M RCOONa of the buffer was always present, μ may be estimated to be between 0.8–0.9 in the aqueous phase.

TABLE IV

PSEUDO-FIRST-ORDER SPECIFIC RATE CONSTANTS, 10²k (MIN.⁻¹), AND AVERAGE DEVIATIONS, AT pH 4.5, 49°, AND 0.025 M $\text{Ce}(\text{NH}_4\text{SO}_4)_4$

10 ² k, min. ⁻¹	1.44 ± 0.03	1.33 ± 0.05	1.03 ± 0.04	0.86 ± 0.02
PNPP, M	0.00625	0.0125	0.025	0.0375

TABLE V

PSEUDO-FIRST-ORDER SPECIFIC RATE CONSTANTS, 10²k (MIN.⁻¹), AND AVERAGE DEVIATIONS, AT pH 4.5, 49°, AND 0.025 M PNPP

10 ² k, min. ⁻¹	1.35 ± 0.04	1.03 ± 0.04	0.48 ± 0.02
$\text{Ce}(\text{NH}_4\text{SO}_4)_4$, M	0.05	0.025	0.012

Results and Discussion

Our results of the uncatalyzed hydrolysis of PNPP at 73° approximate those of Silber, reported briefly,⁴ and those of Holbrook and Ouellet.¹⁰ As seen in Fig. 1, the maximum at pH 3.5 agrees with that expected from the pH-concentration distribution of the monoanion, though some deviation from a quantitative correspondence of rate is indicated at lower values of pH. We also notice a dependence of rate on ionic strength of the medium, at low values of the latter.

The presence in the reaction medium of di-, tri-, and tetravalent metal ions produces no change in rate of hydrolysis at concentrations which avoid precipitate formation at pH 3.2. A similar lack of acceleration by several divalent ions of the rate of hydrolysis of the monoanion of *S-n*-butyl phosphorothioate has been reported.¹¹ These results are consistent with the reaction-intermediate structures proposed by Westheimer⁶ and by Vernon⁴ for hydrolysis of phosphatic monoester monoanions in homogeneous solution. In these the monoanion is pictured as undergoing the elimination of alcohol or phenol, alone or in concert with a molecule of water, but without the involvement of a hydronium ion.¹² The stronger electropositive

(10) K. A. Holbrook and L. Ouellet, *Can. J. Chem.*, **36**, 686 (1958).

(11) D. C. Dittmer, O. B. Ramsay, and R. E. Spalding, *J. Org. Chem.*, **28**, 1273 (1963). It was found that divalent Mg, Ni, and Mn showed no effect, while Zn caused an 18% decrease in rate.

(12) The kinetically equivalent reaction of hydroxide ion with nonionized monoester is considered less plausible [C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3574 (1958); though, see footnote 11 of D. C. Dittmer and O. B. Ramsay, *J. Org. Chem.*, **28**, 1268 (1963)]. Similarly, reaction of hydronium ion with the dianion of the monoester is a possibility; however, the insensitivity of the hydrolysis to metal ions argues against this.

field of the metal ions apparently cannot disturb the reaction intermediate, even though intermediates, such as may be expected to exist in solution, analogous to those proposed can be constructed in each case by the replacement of a hydrogen atom by that of a metal ion.

In the presence of solid phase, as produced by solvolysis of ceric ammonium sulfate, accelerated kinetic rates were observed (Fig. 1), and the optimum pH undergoes an apparent shift to a value of 4.5. Were the effect of ceric ion merely that of affecting the dissociation constant of the substrate, the shift would take place in the opposite direction toward lower values of pH.

Our data refer to an invariant method of preparation of the catalytic precipitate. Table III shows that about two-thirds of the substrate, PNPP, upon addition becomes associated with the solid phase, over the pH range 3.0–5.1. No decrease of apparent rate was observed at pH 3.5 when the filtrate from such a preparation, containing the remainder of the substrate, was discarded, and the solid phase was resuspended in five times as much buffer medium. This constancy of rate supports the conclusion that the hydrolysis is confined to the solid phase, and that the catalyst-substrate aggregate does not readily dissociate. Variation of catalyst-substrate concentration ratio at pH 4.5 and 49° influences the observed rate (Tables IV and V). A thorough examination of this factor, and others capable of affecting the hydrolytic activity, is the subject of a subsequent publication.

"Acid phosphorylase" activity by metal ion-substrate complexes were first reported in 1960.¹³ It was stated that the capacity to accelerate the hydrolysis of phosphatic monoesters in acid pH region is a special property of the tetrapositive elements of Group 4B of the periodic table, though weak activity was shown by some other elements against β -glycerophosphoric acid. The effect of thorium(IV), the most active catalyst, changes from an accelerator to an inhibitor as the molar substrate-to-catalyst ratio is increased from 0.2 to 2.0 in the hydrolysis of phenyl dihydrogen phosphate below pH 5.5. Bamann and co-workers also report that cerium(IV) is inactive in the acid region—this particular study having been performed on several aryl phosphate monoesters at 37°.¹⁴ They also showed the critical influence of temperature on activity of some metal catalysts.¹²

We have found that cerium(IV) is indeed active at 49° toward PNPP upon association of the latter. This is not surprising. In *alkaline* medium at 37°, cerium(IV) is specifically more active toward *aryl* phosphatic monoesters relative to cerium(III), whereas, toward *aliphatic* substrates the converse is true.¹⁵ Further, the phosphorolytic activity of Group 4B members in acid medium has been correlated with the strong electropositive character of these tetrapositive elements; considering the similarity of ionic charge and position in the periodic table of cerium(IV), hafnium(IV), and thorium(IV), it is not unexpected that they and some of their compounds show similar chemical and physical properties.

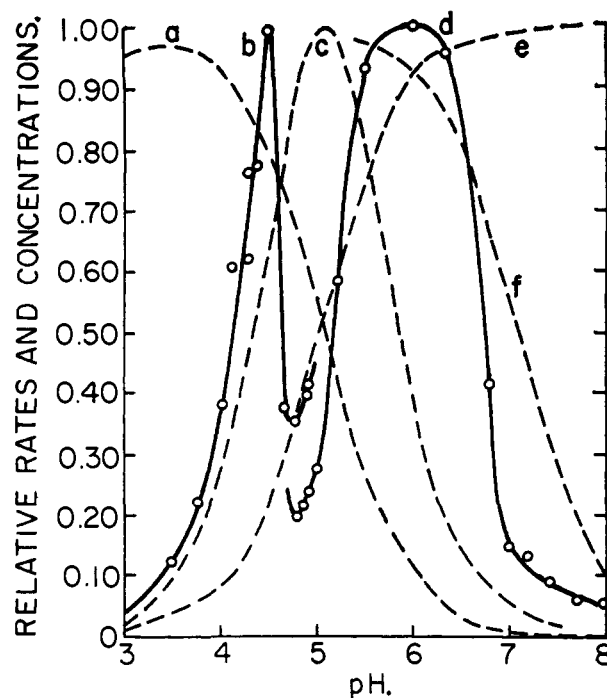


Fig. 2.—Normalized distributions of rates of hydrolysis (curves b and d) and of concentrations of conjugate acid-base species as a function of pH at 49°: a, $(\text{ROPO}_3\text{H}^-)$ and e, (ROPO_3^{2-}) , where $c_0 = 1.00$ and $pK_2 = 5.10$; c, $(\text{ROPO}_3\text{H}^-) \times (\text{ROPO}_3^{2-})$; and f, (*p*-nitrophenol), where $pK_a = 7.10$.

The pH-rate profile for cerium(IV) shows two narrow optimal peaks at pH 4.5 and 6.0 flanking a deep trough at pH 4.75. The concentrations of several components of the system undergo change over this pH range, as shown in Fig. 2. Though the positions at which these changes occur are suggestive, it can be seen from the slopes of the curves that no simple quantitative correlation of the rate of change of concentrations with that of rates of hydrolysis can be found.¹⁶ The amount of solid phase and its stoichiometric composition vary very little over the pH range 3.0–5.1 (Table III). At this time little can be said concerning changes of degrees of hydration and polymerization in the solid phase, though profound changes could obtain, especially as the PNPP portion responds to changes in pH.¹⁷

Both peaks occur in a pH range in which several acid phosphatases have been reported to exhibit optimal activity.^{18a-c} From inhibition studies of an enzyme isolated from the human prostate and evaluated against the same substrate used by us, the investigators concluded that the active site contains, or is adjacent to, a phosphate group on the enzyme.^{18c} This gives special pertinence to a supposition that a reaction complex may exist in our study system of the form $(\text{ROPO}_3\text{H}^- \cdots \text{Ce}^{+4} \cdots \text{ROPO}_3^{2-})$, and which has enzymic analogy with the active site of acid phosphorylases, whereby two of the three components of the above form are provided by the enzyme, *i.e.*, a charged center and a neighboring phosphate group.

(16) For instance, the rate of hydrolysis over the pH range 5.0–5.5 is proportional to the fourth power of the concentration of the dianion.

(17) The relatively high ionic strength of the medium, established by the substrate and the necessary use of buffer, partly determined the character of the filterable, semifiltrable precipitate, and precluded sol formation.

(18) (a) K. K. Tsuboi and P. B. Hudson, *Arch. Biochem. Biophys.*, **46**, 191 (1955); (b) E. Bamann and J. Riehl, *Naturwiss.*, **44**, 538 (1957); (c) C. A. Bunton, B. L. Silver, and C. A. Vernon, *Proc. Chem. Soc.*, 348 (1957).

(13) For a review, see E. Bamann, H. Trapmann, J. Riehl, A. Gerl, and B. Oechsner, *Arch. Pharm.*, **296**, 174 (1963).

(14) E. Bamann, H. Trapmann, and H. J. Krauss, *ibid.*, **295**, 330 (1962).

(15) E. Bamann and W. D. Mutterlein, *Chem. Ber.*, **91**, 471 (1958).

Curve c of Fig. 2 was drawn to examine such possible correspondence. We are examining this proposition more thoroughly through the use of binary mixtures of phosphatic monoesters of differing second dissociation constants, and by quantitatively evaluating the

numerous factors which determine or influence the shape of the pH-rate profile.

Acknowledgment.—The authors wish to thank Mr. C. C. Carraher, Jr., for conducting the potentiometric titrations.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, N. Y.]

Peptide Synthesis *via* Active Esters. IV. Racemization and Ring-Opening Reactions of Optically Active Oxazolones

BY MURRAY GOODMAN AND LEON LEVINE¹

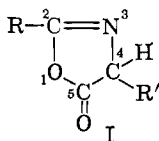
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An optically active oxazolone, 2-phenyl-L-4-benzyloxazolin-5-one, was synthesized from benzoyl-L-phenylalanine by allowing benzoylamino acid to react in an acetic anhydride-dioxane solution. The reaction was followed polarimetrically. At the point of greatest negative rotation the solvent was removed and the oxazolone isolated and purified. The rates of racemization of 2-phenyl-L-4-benzyloxazolin-5-one, using various nucleophiles, were determined. These nucleophiles were, in order of decreasing racemization rates: *p*-nitrophenylate, phenylalanine methyl ester, and pyridine. Second-order rate constants were calculated from three pseudo-first-order rate constants for each nucleophile used. Ring-opening rates were measured spectrophotometrically. The concentration of nucleophile necessary to cause ring opening must be much greater than that necessary to bring about racemization in approximately the same time. No ring-opening reactions take place over the time scale necessary for racemization. It is found that rates of reaction for ring opening follow the order: *p*-nitrophenylate > phenylalanine methyl ester >> pH "8" buffer solution > water. The equilibrium reactions of oxazolone to yield benzoylphenylalanine *p*-nitrophenyl ester, and the reverse, benzoylphenylalanine *p*-nitrophenyl ester to give oxazolone, were measured using infrared spectrophotometry. From the calculated rates, the equilibrium constant was found to favor the formation of the *p*-nitrophenyl ester. The relevance of this equilibrium racemization during peptide synthesis is discussed.

Introduction

Partial or complete racemization is observed in syntheses involving optically active N-acylamino acids and in reactions of N-protected peptides, using various condensing reagents.² Solvent, temperature, and condensing agent²⁻⁴ have been shown to be important in determining the extent of racemization.

The oxazolone I is the intermediate which most likely



undergoes racemization. These compounds were first isolated in reactions of optically active amino acids with acetic anhydride,⁵⁻⁷ ketene,^{8,9} and, more recently, with trifluoroacetic anhydride.¹⁰ However, the oxazolones obtained were optically inactive, and the reac-

tions of oxazolines which have been reported were carried out on racemized compounds.¹¹⁻¹³

Racemization of optically active peptides, such as benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester, is thought to involve the oxazolone intermediate¹⁴ but, to date, no peptide oxazolone has been isolated.

Optically active oxazolones were isolated relatively recently,¹⁵ although isolation of optically active thiohydantoin, derived from optically active oxazolones, was reported earlier.¹⁶ Since no quantitative information is available on the reactions of optically active oxazolones, and because a study of these compounds would lead to a better understanding of racemization mechanisms, we prepared optically active 2-phenyl-L-4-benzyloxazolin-5-one, and studied its racemization and ring-opening reactions with various nucleophiles.

Results and Discussion

Benzoyl-L-phenylalanine was synthesized under Schotten-Baumann conditions. A 2% solution of this compound in 1:1 acetic anhydride-dioxane was prepared, and the change in rotation followed. The point of maximum negative rotation was reached in 60 min., $[\alpha]^{25D} -40^\circ$. At this point solvent was removed by distillation and the 2-phenyl-L-4-benzyloxazolin-5-one collected. The solvent used with the acetic anhydride and the concentration of the acylamino acid have a profound effect on the value of the observed

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