Participation of a Neighbouring Oxime Group in Phosphonate Ester Hydrolysis

By CLAIRE N. LIESKE, JOSEPH W. HOVANEC, GEORGE M. STEINBERG,*† and PETER BLUMBERGS

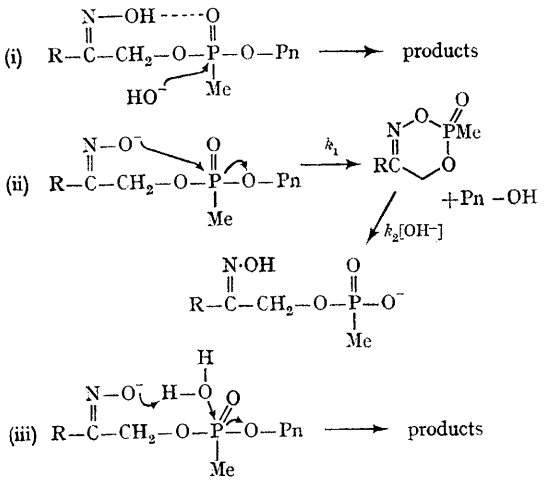
(†*Biochemistry Branch, Medical Research Laboratory, Edgewood Arsenal, Maryland,* 21010 *and Ash-Stevens, Inc. Detroit, Michigan,* 48202)

A variety of neighbouring groups has been shown to participate as intramolecular catalysts in the hydrolysis of carboxylic esters[1] and in the hydrolysis of phosphates.[2] These compounds are convenient models for studying hydrolysis reactions that may reflect conditions at the active sites of enzymes. This relationship has been discussed by numerous investigators.[3]

We have already reported on the participation of the neighbouring ketonic carbonyl group in the hydrolysis of *p*-nitrophenyl phenacyl methylphosphonate.[4] Participation of the carbonyl group accelerates the hydrolysis reaction by a factor of 9000. We have also studied the hydrolysis of the corresponding oxime, *p*-nitrophenyl phenacyl methylphosphonate oxime,‡ and report a *further* acceleration of the hydrolysis reaction by a factor of *ca.* 2×10^3. The oxime is hydrolyzed§ with $k(OH^-) = 6{\cdot}56 \times 10^8$ M^{-1} min.$^{-1}$ in 0·1 M-KCl, 2% acetonitrile, 25·0°, over the pH range 3·49 to 4·90. The reaction is first-order each in phosphonate and in hydroxide ion. The production of *p*-nitrophenol is stoicheiometric.¶ One mole of acid is produced concurrently.** At pH 4·90 t = 1·34 min. ±0·7%. In deuterium oxide, *p*-nitrophenyl phenacyl methylphosphonate oxime is hydrolyzed†† with $k_{(OD^-)} = 8{\cdot}11 \times 10^8$ M^{-1} min.$^{-1}$ in 0·1 M-KCl, 2% acetonitrile, 25·0°, over the pD range[5] 4·20 to 5·70. At pD 5·12, $t_{\frac{1}{2}}$ = 4·76 min. ± 3·1%. The resulting deuterium isotope effect is $k(OD^-)/k(OH^-) = 1{\cdot}24$.

Hydrolysis studies with the radiometer titration apparatus showed no detectable salt effect as the KCl solution was varied from 0·1 M to 1·0 M. No acceleration in hydrolysis was produced by a variety of bases including the highly nucleophilic hydroxamate anions.

The observed experimental results suggest three possible mechanisms. These include: (i) direct attack by hydroxide ion on phosphorus facilitated by hydrogen-bonding assistance; (ii) direct attack by the oxime anion on phosphorus; and (iii) a water-mediated attack involving the oxime anion.



Mechanism (i) is consistent with the observed deuterium isotope effect. However, one might have expected acceleration by other nucleophiles,[6] which was not observed. Mechanism (ii) demands that $k_2 > k_1$ by a factor of at least 10, since the rates of acid and *p*-nitrophenol production are the same. Therefore $k_2 \geqslant 6 \times 10^9$ M^{-1} min.$^{-1}$. It is highly unlikely that the cyclic phosphonate would be subject to such very rapid hydrolysis. The most probable mechanism for the hydrolysis of

‡ Prepared by Dr. C. B. Thanawalla, Ash-Stevens, Inc. from phenacyl bromide oxime and silver *p*-nitrophenyl methylphosphonate. The compound has m.p. 115—117°, with acceptable C, H, and N analyses and u.v. and n.m.r. spectrograms. Studies are in progress to establish the configuration of the oxime.

§ Radiometer automatic titration unit used, equipped with a PHA 630T scale expander. Calculations done by the method of Guggenheim with a Univac Solid State 90 Computer.

¶ *p*-Nitrophenyl methylphosphonic acid is quite stable under these conditions. Hydrolysis, which is speeded in acid, is <1% in 22 hours at pH 1.

** Since the pK_a of *p*-nitrophenol is 7·15, its formation over the pH range of this study would not be detected as liberated acid. From the hydrolysis solution, pH 5, there was isolated a white, water-soluble salt which corresponds in weight to approximately 100% yield of the sodium salt of phenacyl methylphosphonic acid oxime, γ_{max} 232·5. In 0·6 M-hydrochloric acid, this was converted during 2 hr. to a new stable compounds with γ_{max} 246·7. The u.v. spectrum of the latter is identical with that of phenacyl methylphosphonic acid, which is stable under these conditions.

†† Same procedure as §. Titration with NaOD.

p-nitrophenyl phenyl methylphosphonate oxime in aqueous solution is, therefore, an oximate anion-catalyzed water-mediated reaction [mechanism (iii)].

Hydrolysis of *p*-nitrophenyl phenacyl methylphosphonate oxime proceeds *via* an entirely different mechanism than that of the closely related ethyl α-hydroxyamino-*p*-nitrobenzyl methylphosphonate.[2d] Hydrolysis rate of the latter is pH independent over the range of 2 to 3·5.

In addition to the large rate enhancement by a neighbouring oxime group in phosphonate ester hydrolysis, it is *particularly* noteworthy that the intramolecular reaction between oximate anion and phosphonate differs in mechanism from the corresponding intermolecular reactions. The latter involves nucleophilic attack by the oximate ion to yield a phosphonylated oxime.[7] It has been widely assumed that the reactivation of organophosphonate-inhibited cholinesterase by oximes proceeds by a nucleophilic displacement (to yield free enzyme and phosphonylated oxime).[8] Since reactions on the surfaces of enzymes bear a closer relationship to intramolecular reactions than intermolecular reactions, this assumption bears reconsideration.

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