calculated by this method is 281,000 (Newton *et al.*, 1965).

Acknowledgment

We are indebted to Dr. V. A. Bloomfield for helpful discussions concerning the considerations of kinetic analysis.

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Decomposition of a Phosphonylated Pyridinium Aldoxime in Aqueous Solution*

George M. Steinberg and Stanley Solomon

ABSTRACT: In aqueous solution, pH 7.6, the potent anticholinesterase *O*-(isopropylmethylphosphonyl)-4-formyl-1-methylpyridinium iodide oxime (4-PPAM) decomposes, with loss of anticholinesterase activity, to the corresponding nitrile, 4-cyano-1-methylpyridinium iodide, rather than to 4-formyl-1-methylpyridinium oxime iodide (4-PAM) as anticipated on the basis of its reaction with the enzyme. Nucleophilic attack at the phosphorus atom to give 4-PAM can be achieved with hexanohydroxamate anion, imidazole, and phosphate, nucleophilic reagents of known high reactivity with organophosphonates. Thus, 4-PPAM is subject to attack by bases *via* two competitive pathways: a general base reaction at the aldehydic hydrogen atom to give Beckman elimination and nucleophilic attack on phosphorus to yield oxime.

The product ratios are consistent with the effect of these nucleophiles on the kinetics of decomposition of 4-PPAM. With effective nucleophiles such as the enzyme the latter pathway predominates.

Pyridinium aldoximes are important in the treatment of organophosphonate poisoning (Hobbiger, 1963). They function principally by reactivating the inhibited enzyme acetylcholinesterase (inhibited by phosphonylation at the active site), although in the presence of free phosphonofluoridates they may also function by reacting with the latter compounds. In both cases, the first product of reaction is a phosphonylated oxime (Hackley *et al.*, 1959; Kewitz *et al.*, 1956). The phosphonylated oximes are themselves quite toxic and a study of the kinetics of decomposition of one member of the group, *O*-(isopropylmethylphosphonyl)-4-formyl-1-methylpyridinium iodide oxime, 4-PPAM,[1] in near neutral aqueous solution has been reported (Hackley *et al.*, 1959; Lamb *et al.*, 1965). Decomposition under these conditions is comparatively slow and can be speeded appreciably by the addition of a variety of nucleophiles. In this work we have studied the pathway of decomposition in aqueous solution and the effect of accelerating nucleophiles on reaction pathway through product identification.

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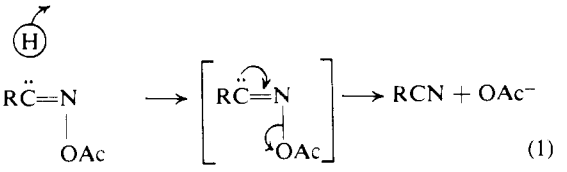
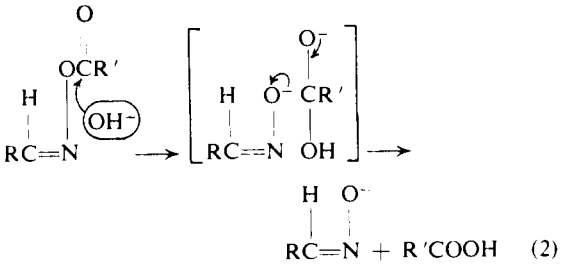
[1] Abbreviations used: 4-PPAM, *O*-(isopropylmethylphosphonyl)-4-formyl-1-methylpyridinium iodide oxime; 2-, 3-, or 4-PAM, 2-, 3-, or 4-formyl-1-methylpyridinium iodide oxime; 4-CY, 4-cyano-1-methylpyridinium iodide; GB, isopropyl methylphosphonofluoridate.

Extensive study of the alkaline decomposition of acylated aldoximes (Benger and Brady, 1950; Brady and Jarrett, 1950; Hauser and Jordan, 1935, 1936) has revealed that breakdown may occur by either of two routes, reactions 1 or 2, and that choice of route is principally a function of oxime configuration; with *anti*[2]-oximes reaction proceeds preferentially by removal of the aldehydic hydrogen atom leading to the formation of nitrile (reaction 1), and with *syn* oximes hydrolysis occurs through nucleophilic attack by hydroxyl ion on the acyl carbon atom (reaction 2). Determination of the route of alkaline decomposition of acylated aldoximes was the principal method for assignment of configuration. By analogy with the acylated aldoximes, one would expect 4-PPAM to be somewhat unstable in aqueous solution and to decompose *via* routes 1 or 2 depending on its configuration. A. L. Green (private communication) observed that in aqueous alkali 2-PPAM decomposes to 1-methyl-2-pyridone *via* the intermediate 2-cyano-1-methylpyridinium iodide. Ellin (1958) reported that 2-formyl-1-methylpyridinium iodide oxime, 2-PAM, itself will undergo this reaction sequence in aqueous alkaline solution, and Blanch and Onsager (1965) observed that 3- and 4-PAM-*O*-acetates of *syn* configuration give a mixture of oxime and nitrile upon alkaline treatment.



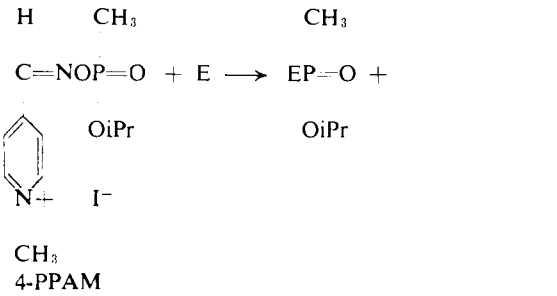
(1)



(2)

In preliminary studies, 4-PPAM was converted in aqueous alkali to 1-methyl-4-pyridone (presumably *via* the nitrile) suggesting the *anti* configuration.[3] The route of decomposition in alkali, however, is not an absolute guide to configuration. Certain *syn*-acylated aldoximes are converted principally (Benger and Brady, 1950; Brady and Jarrett, 1950; Hauser and Jordan, 1935, 1936), and *syn*-benzaldoxime arenesulfonates exclusively, to nitriles (Crawford and Woo, 1965) so that unless both isomers are available a positive assignment of configuration cannot be made.[4]

Although quaternary phosphates containing an easily hydrolyzed oxygen–phosphorus linkage, as, for example, diethyl 3-pyridyl phosphate methiodide (Burger and Hobbiger, 1951), are powerful inhibitors of acetylcholinesterase, it was surprising, initially, to discover this property in 4-PPAM since its reaction with base had led to Beckmann elimination rather than hydrolytic cleavage. Enzyme inhibition suggests the transfer of the phosphonyl group to the enzyme (reaction 3) by a reaction that is formally similar to reaction 2. Organophosphorus anticholinesterases are known to inhibit the enzyme by a direct phosphorylation reaction



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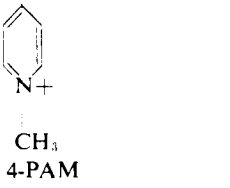


(O'Brien, 1960) and Lamb and Steinberg (1964) confirmed such a reaction in the case of 4-PPAM. They established that the product of reaction between 4-PPAM and eel cholinesterase is identical with the enzyme inhibited by isopropyl methylphosphonofluoridate, GB. Presumably the other reaction product in reaction 3 was the oxime but this had not been established. It was interesting to learn whether compounds of known high nucleophilicity toward the phosphoryl group which Lamb and co-workers found to speed the decomposition of 4-PPAM would mimic the enzyme and change the course of the decomposition reaction, and, if so, to establish the nature of the other product.

Thus, it was our purpose to (a) identify the products of the decomposition of 4-PPAM in near-neutral aqueous solution, (b) to study the effect of effective nucleophilic agents on the course of reaction, and (c) to

[2] Oxime configurations *syn* and *anti* refer to the orientation of the aldehydic hydrogen atom relative to the oximino oxygen atom.

[3] Using nuclear magnetic resonance, Poziomek and coworkers (1961) have shown that the configuration of the more readily accessible isomer of the unphosphonylated oxime,4-PAM, has the *syn* configuration. Although suggestive, their work does not define the configuration of 4-PPAM, since interconversion of oxime isomers occurs readily and the isomer isolated often depends upon small changes in experimental conditions.

[4] Configurational assignment based upon the mode of decomposition of a single isomer of 4-PPAM would be particularly doubtful because this molecule contains two features that contribute to the elimination route (reaction 1): a powerful electron-withdrawing group in the heterocyclic ring and a strong acid as the esterifying group on the oxime.

TABLE I: Composition of 4-PPAM Sample.

Run	Sample Wt g × 10² (dissolved in 1 l. of H_2O)	4-PAM Content[a] Concn (M × 10⁵)	4-PAM Content[a] Wt (g × 10³)	4-PAM Content[a] % by Wt of PAM Cation	4-PPAM Content[b] Concn (M × 10⁵)	4-PPAM Content[b] Wt (g × 10²)	4-PPAM Content[b] % by Wt of 4-PPAM Cation
141E[c]	2.31	1.71	2.35	10.02	4.87	1.25	54.1
141C[c]	2.31	1.73	2.37	10.03	4.85	1.25	54.1
141G[c]	1.48	1.06	1.45	9.79	3.05	0.772	52.1
142E[c]	2.19	1.60	2.19	10.00	4.60	1.18	53.9
142C[c]	2.19	1.60	2.19	10.00	4.60	1.18	53.9
143[d]	2.56	1.86	2.55	9.95	5.44	1.40	54.7
147[e]	1.88	1.28	1.76	9.35	3.68	0.945	50.3
149E[d]	2.73	2.02	2.76	10.01	5.58	1.43	52.4
149A[e]	2.73	1.99	2.73	10.00	5.81	1.49	54.6
				Av 9.91 ± 0.15			Av 53.4 ± 1.15

[a] Determined from absorbance at 335 mμ, pH > 13. $E_{mol} = 2.18 \times 10^4$. [b] From absorbance at 262 mμ, pH > 13. $E_{mol} = 1.15 \times 10^4$ (corrected for 4-PAM absorbance). [c] Phosphate concentration, 0.01 M. [d] Phosphate concentration, 0.1 M. [e] Contained no phosphate.

correlate the stoichiometry of the products formed with earlier kinetic results. Essentially, it was necessary to be able to distinguish between reaction paths 1 and 2 and to be able to identify products that might be produced by some other unsuspected pathway. Because the sample of 4-PPAM was not completely pure and the analytical methods using ultraviolet absorption involved some overlapping spectra and measurements that are highly sensitive to small changes in pH the results obtained are, at best, imprecise. Cross-checks, however, have been used extensively to confirm internal consistency and to minimize error; the over-all results are considered adequate to answer the questions posed definitely.

Experimental Section

Materials. All stock reagents were of CP grade. Hexanohydroxamic acid and 4-PPAM have been described in an earlier paper (Lamb *et al.*, 1965), the latter being identical with the crystalline sample referred to as *crys* 4-PPAM. 4-PPAM, 4-PAM, the methylsulfonate salt of 4-PAM, 4-CY, and the methylsulfonate salt of 4-CY were provided by Dr. B. E. Hackley, Jr., and Mr. O. O. Owens of these laboratories. The sample of 4-PPAM used for the study was partitioned into several small fractions that were stored in individual screw-capped vials in a freezer over P_2O_5. A single vial was sampled until evidence of decomposition appeared, at which time it was discarded and another vial used. In this way, the entire study could be performed with one sample.[5]

[5] With the exception of the kinetic runs, Table IV.

The composition (in weight percentage) of the 4-PPAM sample, based upon replicate elemental analyses, spectral analysis for 4-PAM, and the requirements for ionic balance, is: 4-PAM *cation*, 9.85; 4-PPAM *cation*, 54.2; iodide, 34.3; isopropyl methylphosphonate *anion*, 1.73.

Anal. Calcd for the above: C, 34.4; H, 4.6; I, 34.3; N, 7.9; P, 6.9. Found: C, 33.8; H, 4.8; I, 34.3; N, 7.5; P, 7.47; moisture (Karl Fischer) <0.04.

The results of nine independent analytical determinations of 4-PAM and 4-PPAM content given in Table I are in close conformity with the stated composition. Since the samples for these analyses were taken at widely different times, the results also indicate a satisfactory degree of homogeneity and stability for the test material.

1-Methyl-4-pyridone Hydriodide. A sample prepared by the method of Ruzicka and Fornasir (1920) was found to be quite hydroscopic. During the final drying step in which the sample was subjected to high vacuum for several hours there occurred some loss of hydriodic acid, so that the product that was obtained consisted of a mixture of the hydriodide and free base. For purposes of analysis, a weighed sample was dissolved in water, potentiometrically titrated with standard acid to pH 1, and then back-titrated with standard alkali (Beckman Model G pH meter). The sample consisted of 29.4% (by weight) of 1-methyl-4-pyridone and 70.6% of hydriodide. The pK_a, taken as the pH of half-neutralization (without correction), is 3.2. The reported value is 3.33 (Albert and Phillips, 1956). In the ultraviolet spectral region the sample in aqueous alkaline solution absorbed maximally at 262 mμ, with an E_{max} of 1.75×10^4. In aqueous acidic solution, the maximum fell

to below 240 mμ; this value was not recorded since iodide ion interferes at wavelengths below 240 mμ. Reigel and Reinhard (1926) reported maxima in alkaline and acid solution at 260 and 230 mμ, respectively; the E_{max} for the former was calculated from their data to be 1.61×10^4. More recently, Mason (1959) gave the corresponding maxima as 260 and 239 mμ, respectively, with the E_{max} for the 260 band as 1.89×10^4.

Spectra. ULTRAVIOLET. With the exception of those measured for the kinetic studies, all spectra were obtained on a Process and Instruments Co. recording spectrophotometer fitted with Beckman Model DU optics. The rate studies reported in Table II were performed in a Beckman DU spectrophotometer fitted with a thermostated cuvet holder. Measurements were made at wavelength 262 mμ, slit width 0.06 mm.

TABLE II: Rate of Decomposition of 4-PPAM, 29°, pH 8.00, 0.1 M Tris Buffer.

Run	Time (min)	Absorbance (262 mμ)	$\frac{A_0 - A_\infty}{A_t - A_\infty}$	$k \times 10^2$ min^{-1}	$t_{1/2}$ (min)
71-II	0	0.488[a]			
	2.5	0.483	1.041	1.61	
	4.5	0.479	1.076	1.63	
	10.2	0.470	1.166	1.51	
	16.2	0.460	1.282	1.53	
	24.0	0.450	1.427	1.48	
	34.7	0.433	1.764	1.64	
	46.0	0.423	2.050	1.51	
	90.0	0.389	4.53	1.68	
	105.5	0.383	5.77	1.67	
	117.5	0.378	7.47	1.71	
	130.5	0.373	10.58	1.77	
	4 hr	0.361			
			Av	1.61	43.1[b]
71-III					45[b]
75					45,[b] 43[c]

[a] Extrapolated to zero time. [b] Rate determined by absorbance measurement, 262 mμ. [c] Rate determined by measurement of anticholinesterase activity (run by Dr. H. Michel). For method see Lamb *et al.* (1965).

Reference spectra of 4-PAM, its corresponding methylsulfonate, 4-cyano-1-methylpyridinium iodide, its corresponding methylsulfonate, and 1-methyl-4-pyridone were determined at autogenous pH, in alkaline solution (pH >13) and in acidic solution (pH <1) over the required concentration range. In each instance the three spectra at each concentration were determined sequentially without removal of the sample from the cuvet. Following the initial measurement, recordings were repeated upon addition of 1 drop of 5 N sodium hydroxide (to the 3-ml sample) and after addition of 1 drop of 10 N sulfuric acid. Corrections were not made for the resulting small changes in concentration in calculating the molar absorptivity since the reaction mixtures were treated in the same manner. The E_{max} values are approximately 2 and 4% low for the alkaline and acidic solutions, respectively. In each case a good linear relationship was obtained between absorbance and concentration over a tenfold concentration range in conformity with the Beer–Lambert law.

INFRARED. A comparison of the spectrum of 1-methyl-4-pyridone with that of the alkaline decomposition products of 4-cyano-1-methylpyridinium iodide and of 4-PPAM were made on a Perkin-Elmer Infracord, Model 137. In each instance an alkaline solution of the sample was extracted with chloroform, and the chloroform layer was dried over Drierite and concentrated under vacuum. 1-Methyl-4-pyridone gave strong narrow absorption bands at 3.29, 6.09, 6.35, 7.16, 8.40, and 11.79 μ. Both of the others gave the identical absorption bands. In addition, however, each gave a narrow band at 7.95 μ and three broad bands at 9.1, 9.85, and 12.4 μ. An attempt at the direct detection of the formation of 4-cyano-1-methylpyridinium iodide as the first product of 4-PPAM decomposition through the nitrile infrared absorption band failed because of inadequate sensitivity. A 2% KBr disk of the cyano compound failed to show significant absorption in the 4–5-μ region when measured on a Perkin-Elmer Model 21 spectrophotometer, adjusted to maximum sensitivity.

Reaction Studies. A suitable aliquot of an aqueous stock solution of 4-PPAM[6] was added to 5 ml of reagent mixture that had been previously adjusted to the desired pH, and distilled water was added to bring the volume to 10 ml. Initial spectral readings could be taken within 2–3 min after mixing. Using 0.1 M phosphate buffer at pH 7.6, the pH remained perfectly constant; with 0.01 M phosphate, small changes (0.1–0.2 unit) in pH were sometimes noted.

Results

Spectra of Reference Compounds. The absorption spectra of the reference compounds 4-PAM iodide and its methylsulfonate, 4-cyano-1-methylpyridinium iodide (4-CY) and its methylsulfonate, and 1-methyl-4-pyridone are given in Figures 1–3. Table III contains a tabulation of molar absorptivities at wavelengths used for analytical purposes. 4-PAM is a weak acid; its pK_a determined as the pH of half-neutralization by potentiometric titration in 0.02 M sodium iodide is 8.50; reported as 8.6 by Ginsberg and Wilson (1957). Calculation of the pK_a from the 335-mμ absorbance at pH 7.6 gives a value of 8.4, which is in satisfactory agreement. The nitrile shows an absorption maximum at 275 mμ in aqueous solution at autogenous pH that is neither shifted nor changed in intensity at pH 7.6 or in acidic solution (pH <1). Addition of alkali causes an immediate spectral change. Reacidification, again, immediately

[6] Stock solutions of pH 5 were prepared daily. At pH 5 or below, 4-PPAM shows no detectable loss in anticholinesterase activity over a period of 16 hr (Lamb *et al.*, 1965).

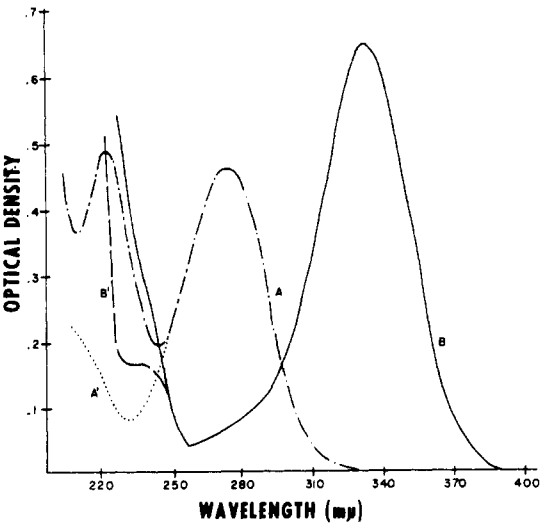


FIGURE 1: Ultraviolet absorption spectra of 4-PAM salts, 2.96×10^{-5} M: A, iodide at pH < 1 (H_2SO_4); A', methylsulfonate at pH < 1 - (H_2SO_4); B, iodide at pH 13; B', methylsulfonate at pH 13.

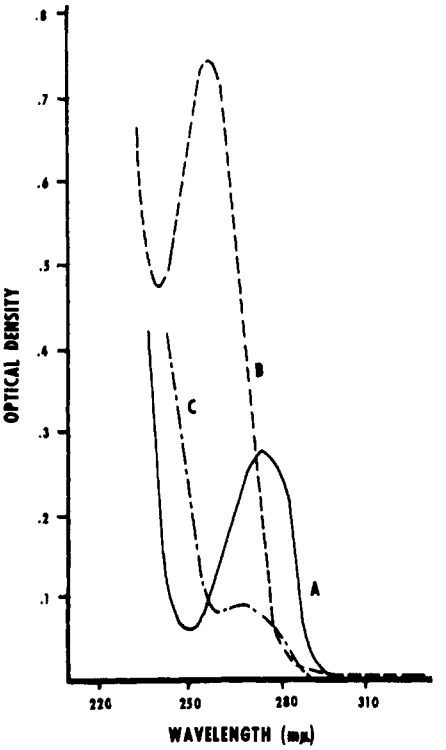


FIGURE 2: Ultraviolet absorption spectra of 4-cyano-1-methylpyridinium iodide, 6.27×10^{-5} M: A, in H_2O (or pH < 1); B, at pH > 13; C, alkaline solution (B) acidified to pH < 1 (H_2SO_4).

TABLE III: Ultraviolet Absorption of Reference Compounds.

Compound	pH	λ_{max} (mμ)	E_{mol} ($\times 10^{-4}$)
4-PAM	<1	270	1.57
	7.6	270[a]	1.26
		275	1.33
		335	0.30
	>13	262[a]	0.13
		335	2.18
		275	0.16
1-Methyl-4-pyridone	>13	262	1.75
	<1	Below 240; no maximum at 270	
4-Cyano-1-methylpyridinium iodide	Autogenous; <1	275	0.43
	0.01 M phosphate, pH 7.6	275	0.43
	>13	262	1.15[b]
	>13, then acidified	270	0.14

[a] Not an absorption maximum. [b] Sample stored overnight in 0.01 M phosphate at pH 7.6 gave, upon addition of alkali, the same λ_{max}, but the E_{mol} fell to 1.10×10^4.

changes the spectrum but not to that of the original nitrile. Repeated additions of alkali and acid cause interconversion between the species having maxima at 262 and 270 mμ, respectively. The E_{max} at 262 mμ corresponds to that of 1-methyl-4-pyridone; however, its E_{mol} is lower, and in acid the pyridone does not absorb significantly at 270 mμ. Patton (1961) has reported that 4-cyano-1-methylpyridinium iodide is hydrolyzed in aqueous alkali to a mixture of 1-methyl-4-pyridone and 4-carboxamido-1-methylpyridinium iodide; the ratio is dependent upon the reaction pH. Under the conditions of this study, the nitrile yields 65% pyridone in a constant and reproducible fashion, and hence the reaction could be used for analytical purposes.

No interactions of 4-PAM or 4-CY with each other or with iodide ion were found over the entire ultraviolet spectral range in acid solution or above 260 mμ in alkaline solution.[7]

In Figure 4, the spectrum of "pure" 4-PPAM has been constructed from that of the available 4-PPAM sample, after correction for the absorbance by the

[7] At the 220-mμ absorption maximum in alkaline solution, the absorbance of 4-PAM iodide (alone or with added iodide) failed to conform to the Beer–Lambert law.

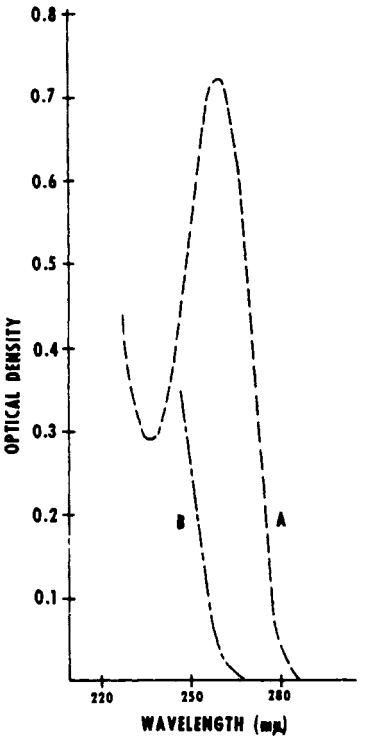


FIGURE 3: Ultraviolet absorption spectra of 1-methyl-4-pyridone, 4.08 × 10^{-5} M, containing 4 × 10^{-5} M hydriodic acid: A, at pH 13; B, pH 1 (H_2SO_4).

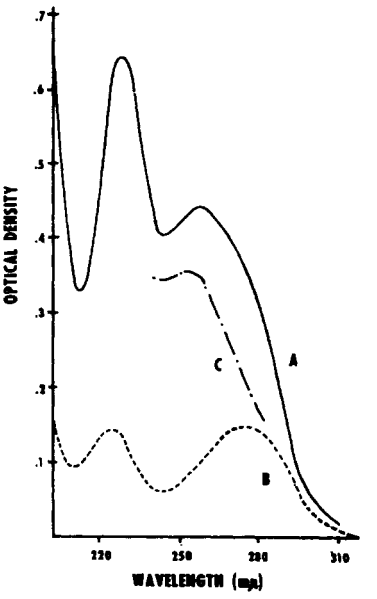


FIGURE 4: Ultraviolet absorption spectrum of 4-PPAM. A, "4-PPAM" sample containing 4-PPAM, 2.90 × 10^{-5} M, and 4-PAM, 1.00 × 10^{-5} M, pH 5; B, 4-PAM, 1.00 × 10^{-5} M, pH 5; C, 4-PPAM, 2.90 × 10^{-5} M, *by difference.*

4-PAM contained therein. The constructed spectrum gives an E_{max} for 4-PPAM at 255 mμ of 1.2 × 10^4, based upon concentrations of 4-PPAM and 4-PAM of 2.90 and 1.00 × 10^{-5} M, respectively.

The 4-PPAM Sample. 4-PAM content was determined from its absorbance at 335 mμ in alkaline solution. 4-PPAM concentration was calculated from the absorbance of the alkaline solution at 262 mμ, with correction made for the contribution of 4-PAM at that wavelength (Table I). To use this analytical scheme it was necessary to determine the extent, if any, of conversion of 4-PPAM to 4-PAM in the alkaline medium. The results given in Table IV indicate that no 4-PAM is formed when 4-PPAM is decomposed in aqueous alkali nor do the pH 7.6 4-PPAM hydrolysis products interfere with 4-PAM determination either at pH 7.6 or pH >13. Thus, the ratio of absorbance at 335 mμ for the 4-PPAM sample in pH 7.6, 0.1 M phosphate buffer to the absorbance/5 of the solution (after one-fifth dilution) at pH >13 is substantially the same as is the ratio for a sample of pure 4-PAM treated identically using another aliquot of the same buffer solution. Also, inasmuch as there was no perceptible change in pH of 0.1 M phosphate buffer over periods of at least 24 hr, the constancy of the $A_{pH7.6}:A_{pH>13}$ ratios before and after decomposition of the 4-PPAM (Table IV) indicate that the 335-mμ absorption is due only to 4-PAM. The 4-PPAM sample contained only 4-PAM

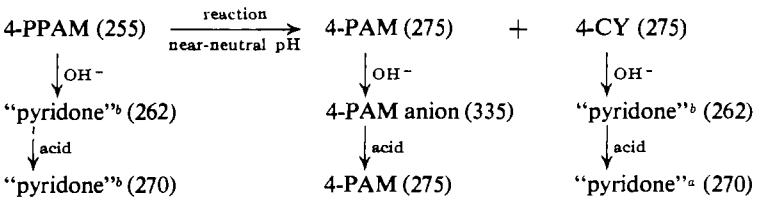
TABLE IV: 4-PAM Analysis in 4-PPAM Sample.

Compd and Run No.	Time (hr)	Absorbance at 335 mμ: pH 7.60 (0.1 M phosphate)	Absorbance at 335 mμ: After 1/5 Dilution, pH > 13	Absorbance Ratio pH 7.6/pH >13
4-PAM[a]	0	0.195	0.278	0.701
4-PPAM[a]	0	0.525	0.762	0.690
Run 143	0	0.275	0.405	0.679
	24[b]	0.515	0.751	0.685
Run 149	0	0.295	0.442	0.668
	24[b]	0.548	0.782	0.701

[a] Solutions prepared from identical buffer solution. [b] 4-PPAM completely decomposed; no measurable anticholinesterase activity.

as impurity and was quite homogeneous. Supporting data are given in Table I, in which the 4-PAM and 4-PPAM contents of aliquots of six separately prepared reaction solutions are compared, three having been run in duplicate. Both 4-PAM and 4-PPAM cation contents remained quite constant, and the average composition values of 9.91 and 53.4% by weight, respectively, compare well with the values of 9.85 and 54.2% computed from elemental composition, the

SCHEME I[a]



[a] λ_{max} (mμ) values are given in parentheses. [b] "Pyridone" refers to the mixture of 1-methyl-4-pyridone and 4-carboxamido-1-methylpyridinium iodide obtained under the conditions used for analysis in this study.

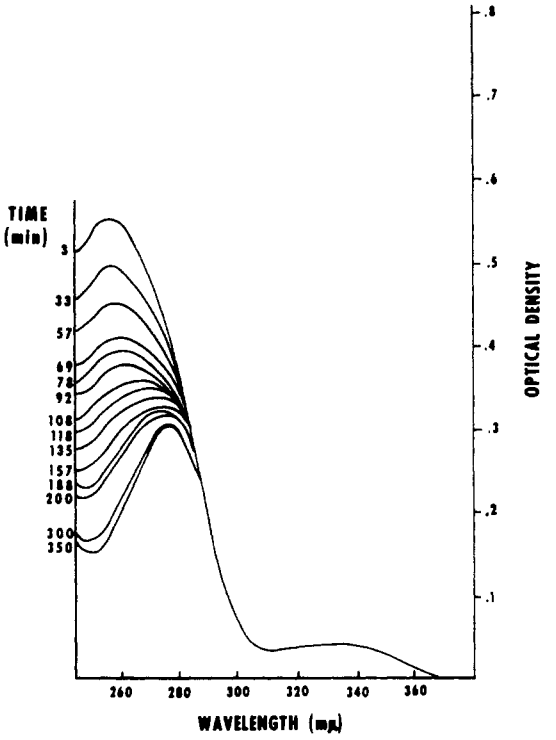


FIGURE 5: Decomposition of 4-PPAM in aqueous solution, pH *ca.* 7.6, 30°.

requirements for ionic balance, and 4-PAM content (see Experimental Section). The E_{mol} value of 1.15×10^4 at 262 mμ for 4-PPAM is based upon the assumption of its initial conversion to 4-CY followed by alkaline decomposition of the latter to 1-methyl-4-pyridone. The data strongly support this assumption. Further evidence is presented in Table II and Figure 5.

Identification and Estimation of Reactants and Products. Based upon the assumption that 4-PPAM is exclusively converted at elevated pH to "pyridone" (hydrolysis products of 4-CY) and to 4-CY and/or 4-PAM at near-neutral pH, Scheme I was established for analysis of the products of decomposition.

Decomposition of 4-PPAM. In a preliminary run of the decomposition of 4-PPAM in 0.01 M phosphate at pH approximately 7.6, 30° (Figure 5), the phosphorylated oxime was converted smoothly to a substance with absorption maximum at 275 mμ. There was no change in the 335-mμ absorbance, and the final spectrum conforms closely with that of a mixture of 4-CY and 4-PAM. The reaction followed good first-order kinetics (calculated from absorbance at 262 mμ) with a half-life ($t_{1/2}$) = 69 min. Subsequent confirmation that the change in absorbance at 262 mμ is directly related to decomposition of 4-PPAM was obtained by parallel determination of absorbance and anticholinesterase activity.[8] The results of several runs are given in Table II. The rate constant was calculated from the expression, $k = (2.303/t) \log [(A_0 - A_\infty)/(A_t - A_\infty)]$, where k is the first-order rate constant; t, time in minutes; A_0, A_t, and A_∞, the absorbance at times 0, t, and ∞, respectively.

For product analysis, the decomposition reactions were run at room temperature and allowed to reach completion by overnight storage. Analysis consisted of determination of the concentrations of 4-PPAM and 4-PAM at t = 0, and 4-PAM and 4-CY at the end of reaction from absorbance values in alkaline solution at 335 and 262 mμ. In each instance the analytical results were confirmed by separate determinations of the absorbance at 275 and 335 mμ in the original buffered solution and at 270 mμ upon acidification of the alkaline solution. The results obtained in the presence of phosphate, imidazole, and hexanohydroxamic acid are given in Table V. Spectral interference prevented determination of 4-CY in the hexanohydroxamic acid runs. Concentrations were calculated typically in the following manner.

At pH 13, [4-PAM] = $A_{335m\mu}/2.18 \times 10^4$; [4-PPAM] = $A_{262\ m\mu} - 1.3 \times 10^3$[4-PAM]$/1.15 \times 10^4$; [4-CY] = $A_{262\ m\mu} - 1.3 \times 10^3$[4-PAM]$/1.10 \times 10^4$.[9]

Confirmatory results for a typical run are given in Table VI. pH values at the end of each run were calculated from the 335-mμ absorbance using pK_a of 4-PAM = 8.4.

Discussion

This study has shown that the normal pathway of decomposition of 4-PPAM in aqueous solution yields

[8] Determined by H. Michel. For method see Lamb *et al.* (1965).

[9] See Table III. E_{mol} = 1.15×10^4 used for first hour of reaction and 1.10×10^4 is used for those having reacted overnight.

TABLE V: Decomposition of 4-PPAM to 4-PAM and 1-Methyl-4-cyanopyridinium Iodide (4-CY).

Run	pH	Phosphate (M)	Other (M)	Original Concn ($M \times 10^5$) 4-PAM	Original Concn ($M \times 10^5$) 4-PPAM	Final Concn ($M \times 10^5$) 4-PAM	Final Concn ($M \times 10^5$) Nitrile	% Conversion of 4-PPAM 4-PAM	% Conversion of 4-PPAM Nitrile	% Conversion of 4-PPAM Total
141E	7.81	0.01	—	1.71	4.87	1.87	4.73	3.3	97.2	100.5
142E	7.6	0.01	—	1.60	4.60	1.79	4.53	4.1	98.5	102.6
147E	7.6	0.01	—	1.28	3.68	1.47	3.69	5.2	100	105.2
143	7.6	0.1	—	1.86	5.44	3.45	3.16	29.2	58.1	87.3
149E	7.6	0.1	—	2.02	5.58	3.58	4.35	27.9	77.9	105.8
141C	7.8	0.01	Imidazole (1.07×10^{-2})	1.73	4.85	2.18	4.45	9.3	91.7	101.0
142C	7.6	0.01	Imidazole (9.9×10^{-3})	1.60	4.60	2.05	4.24	9.8	92.2	102.0
149F	7.6 fell to 6.5	0.01	Hexanohydroxamic acid (1.0×10^{-2})	10.1	27.9	25.4	—	55	—	—
149B	7.6	*a*	Hexanohydroxamic acid (1.0×10^{-2})	10.1	27.9	29.5	—	69.5	—	—
141F	7.6	0.01	Hexanohydroxamic acid (1.8×10^{-3})	1.06	3.05	2.60	—	50.5	—	—
142F	7.6	0.01	Hexanohydroxamic acid (2.42×10^{-3})	1.60	4.60	4.72	—	68	—	—

[a] Reaction run at constant pH in Beckman Autotitrator.

TABLE VI: Confirmation of Analytical Results in a Typical Run, 141C.

Compound	Concn[a] ($M \times 10^5$)	Molar Absorptivity, ($\times 10^{-4}$)	A_{calcd}	A_{obsd}
(a) At beginning of reaction; measured at 270 mμ at pH < 1 (of solution that had previously been raised to pH > 13).				
4-PAM	1.73	1.57	0.272	
4-PPAM	4.85	0.14	0.068	
			0.340	0.342
(b) At end of reaction; measured at 275 mμ in buffered solution, pH 7.80.				
4-PAM	2.18	1.33	0.290	
4-CY	4.45	0.43	0.192	
			0.482	0.477
(c) At end of reaction; conditions same as in a, above.				
4-PAM	2.18	1.57	0.342	
4-CY	4.45	0.14	0.062	
			0.404	0.400

[a] The concentrations of 4-PAM, 4-PPAM, and 4-CY were determined separately from absorbance measurements made at pH > 13.

the nitrile, 4-CY (reaction 1A).[10] At near-neutral pH, the rate of nitrile formation and loss of anticholinesterase activity are the same. At high pH, 4-PPAM is converted to a mixture containing 1-methyl-4-pyridone and 4-carboxamido-1-methylpyridinium iodide probably through a pathway involving initial nitrile formation. Under similar conditions of pH, 4-CY decomposes quantitatively to a spectrally identical mixture of products.

The acetylcholinesterase-mediated pathway of 4-PPAM decomposition involves phosphorus transfer by nucleophilic attack on the phosphorus atom. This reaction pathway is not unique for the enzyme, but may be entered by effective phosphate nucleophiles and results in the formation of oxime, reaction 2A,[10] Table V. Although the solvolytic reaction of 4-PPAM proceeds entirely *via* pathway 1A and the added nucleophilic reagents divert the reaction pathway to 2A, this may not be the exclusive route of the latter reaction. Lamb *et al.* (1965) observed that 4-PAM *anion* accelerates the decomposition of 4-PPAM. Such acceleration could not take place *via* route 2A, since that pathway would lead only to resynthesis. Hence, the oximate anion, a powerful nucleophilic reagent for phosphonates (Green *et al.*, 1958), contributes to the elimination reaction, 1A, suggesting that this reaction is subject to general base catalysis.

[10] The reactions of phosphonylated oxime that correspond to pathways 1 and 2 are referred to as 1A and 2A, respectively.

TABLE VII: Comparison of Observed and Calculated Values for Percentage of 4-PPAM Converted to 4-PAM, pH 7.6.

Reagent	Concentration (M)		Reaction Rate Constant[a]	Percentage 4-PAM	
	Total	Unprotonated Specie	M^{-1} min^{-1}	Obsd	Calcd
Imidazole	10^{-2}	8×10^{-3}	2.11×10^{-2}	9.3, 9.8	9
Hexanohydroxamic acid	2×10^{-3}	3.12×10^{-5}	300	50.5, 68	74
	10^{-2}	1.56×10^{-4}	300	55, 69.5	93
OH^-		3.98×10^{-7}	8.6×10^3		

[a] Lamb *et al.* (1965).

The other nucleophiles also probably make some contribution to pathway 1A. The values in Table VII support this suggestion, but they are not sufficiently precise to prove the point. In Table VII there are compared the observed percentage values for conversion of 4-PPAM to oxime with the corresponding "predicted" values calculated from the rate constants for reaction of Lamb and co-workers (1965) of 4-PPAM with hydroxide ion, imidazole, and hexanohydroxamic acid (anion). In each case the reagent was maintained at constant concentration during the course of reaction so that reaction followed first-order kinetics. Under these conditions, for parallel reactions, product ratio is proportional to reaction rate (Frost and Pearson, 1953). For purposes of calculation, the nucleophiles were assumed to react exclusively *via* pathway 2A and hydroxide *via* 1A, with correction made for the observed contribution of phosphate to pathway 2A. The calculated values represent maxima, since diversion of the nucleophilic reagents' pathway to 1A would yield lower values for percentage oxime. The agreement is reasonably satisfactory for imidazole and hexanohydroxamic acid at the lower concentration. The discrepancy for hexanohydroxamic acid at 10^{-2} M is entirely too large and is not quantitatively in keeping with the other results. It is noteworthy that the product ratios for the phosphate-mediated reaction 2A pathway are internally consistent. Using a value of 28% reaction *via* pathway 2A for 0.1 M phosphate, pH 7.6, one can calculate that in 0.01 M phosphate the conversion to 4-PAM should be 3.75%. The values given in Table V are in satisfactory agreement.

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