AD-A270 799

JMENTATION PAGE

Form Approved
OMB No 0704-0188

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
1993	Open literature publication

4. TITLE AND SUBTITLE
Degradation of three related bis(pyridinium) aldoximes in aqueous solutions at high concentrations: examples of unexpectedly rapid amide group hydrolysis

5. FUNDING NUMBERS
62787A
3M162787A875

6. AUTHOR(S)
Korte, WK, and Shih, ML

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
US Army Medical Research Institute of Chemical Defense
ATTN: SGRD-UV- VA
Aberdeen Proving Ground, MD 21010-5425

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAMRICD
ATTN: SGRD-UV-RC
APG, MD 21010-5425

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
USAMRICD-P90-005

11. SUPPLEMENTARY NOTES
Appeared in Journal of Pharmaceutical Sciences, 82(8), 782-786, 1993

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Abstract □ The principal initial degradation products of two bis(pyridinium)aldoxime organophosphate-inhibited acetylcholinesterase reactivators, **1** (HI-6) and **3** (HS-6), in concentrated nonbuffered aqueous solutions approximating potential therapeutic dosage concentrations were found to be the carboxylic acid derivatives **2** and **4** formed from the hydrolysis of the amide functional group. Compounds **2** and **4** were prepared by heating **1** and **3** in the presence of high concentrations of hydroxylamine hydrochloride and characterized by ^{1}H and ^{13}C NMR, IR, and UV analyses. Estimates of the rates of hydrolysis of the amide groups in **1** and **3** and in model compounds **5**, **7**, and **8** under similar conditions were determined. The unexpectedly rapid hydrolysis of the amide groups in **1** and **3** was attributed to both the hydrogen ion catalysis of the concentrated aqueous solutions of the unusually acidic bis(pyridinium)aldoximes **1** and **3** and general acid catalysis by the aldoxime group.

14. SUBJECT TERMS
HI-6, HS-6, bis(pyridinium)aldoximes, antidotes, nerver agents

15. NUMBER OF PAGES
5

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	None

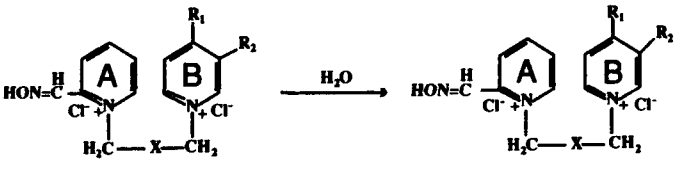
Degradation of Three Related Bis(Pyridinium)Aldoximes in Aqueous Solutions at High Concentrations: Examples of Unexpectedly Rapid Amide Group Hydrolysis

WILLIAM D. KORTE*‡x AND MING L. SHIH*

Received September 20, 1991, from the *U.S. Army Medical Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.
Accepted for publication December 2, 1992. ‡Present address: Department of Chemistry, California State University at Chico, Chico, CA 95929.

Abstract □ The principal initial degradation products of two bis(pyridinium)aldoxime organophosphate-inhibited acetylcholinesterase reactivators, **1** (HI-6) and **3** (HS-6), in concentrated nonbuffered aqueous solutions approximating potential therapeutic dosage concentrations were found to be the carboxylic acid derivatives **2** and **4** formed from the hydrolysis of the amide functional group. Compounds **2** and **4** were prepared by heating **1** and **3** in the presence of high concentrations of hydroxylamine hydrochloride and characterized by ^1H and ^{13}C NMR, IR, and UV analyses. Estimates of the rates of hydrolysis of the amide groups in **1** and **3** and in model compounds **5**, **7**, and **8** under similar conditions were determined. The unexpectedly rapid hydrolysis of the amide groups in **1** and **3** was attributed to both the hydrogen ion catalysis of the concentrated aqueous solutions of the unusually acidic bis(pyridinium)aldoximes **1** and **3** and general acid catalysis by the aldoxime group.

Among the numerous bis(pyridinium)aldoximes that have been synthesized and evaluated as potential antidotes to the toxic effects of organophosphorus pesticides and nerve agents, **1** [HI-6; 1-[[[4-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride] is currently considered one of the most effective and least toxic.[1–5] Previous stability studies of **1** have indicated a highly labile compound in aqueous solution degrading by multiple pathways.[6–12] At pH <4, the two principal initial pathways were reported to be the acid-catalyzed cleavage of the aminal-acetal bridge between the two pyridinium rings aided by intramolecular catalysis by the acidic aldoxime group and the acid-catalyzed hydrolysis of the aldoxime group to aldehyde and hydroxylamine.[6–9] At pH >4, initial degradation primarily occurred either by base-promoted aminal-acetal bridge cleavage or by elimination of water from the aldoxime group.[9–12] Both low and high pH pathways led to subsequent cascades of reactions and products. The amide group in Ring B (Scheme I) was expected to hydrolyze only slowly under both acidic and basic conditions, and the degradation product **2** (1-[[[4-carboxypyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride) was observed in trace amounts.[9]



	X	R_1	R_2		X	R_1	R_2
1	O	$CONH_2$	H	**2**	O	COOH	H
3	O	H	$CONH_2$	**4**	O	H	COOH
5	CH_2	$CONH_2$	H	**6**	CH_2	COOH	H

Scheme I—Reactants and major initial products of the bis(pyridinium)-aldoxime hydrolysis reactions.

However, in this study, initially aimed at methodology to stabilize **1** for use in an autoinjector, HPLC analysis of the degrading samples of **1** at high concentrations indicated that **2**, the carboxylic acid derivative formed by hydrolysis of the amide group in ring B, was the principal initial degradation product in nonbuffered aqueous solution.[13] Therefore, the amide hydrolysis reaction was unexpectedly more rapid than the aminal-acetal bridge cleavage reaction.[6–9] To test whether this relatively rapid amide hydrolysis was unique for **1**, the major initial products of the degradation reactions of two similar bis(pyridinium)aldoximes, **3** (HS-6; 1-[[[3-(aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride) and **5** (1-[3-[4-(aminocarbonyl)pyridinio]propyl]-2-[(hydroxyimino)-methyl]pyridinium dichloride) were determined. In addition, the rates of hydroxysis of the amide group in **1** and **3** were estimated and compared with amide hydrolysis rates for **5** and two pyridinium salts that do not contain an oxime group, **7** (4-(aminocarbonyl)-1-methylpyridinium chloride or 1-methylisonicotinamide chloride) and **8** (3-(aminocarbonyl)methylpyridinium chloride or 1-methylnicotinamide chloride). This report also includes details concerning the isolation and characterization of the hydrolysis products **2**, **4** (1-[[[3-carboxypyridinio]-methoxy]methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride), and **6** (1-[3-[4-carboxypyridinio]-propyl]-2-[(hydroxyimino)methyl]pyridinium dichloride).

Experimental Section

Materials—**1**, **3**, and **5** were obtained from the Walter Reed Army Institute of Research, Washington, D.C. 2-[(Hydroxyimino)-methyl]-1-methylpyridinium chloride (2-PAM Cl or pralidoxime chloride) was obtained from Ayerst Labs. The four pyridiniumaldoximes were determined to be quite pure (>98% purity by HPLC analysis) and used without further purification. Compounds **7** (mp 252–259 °C, lit.[14] 255–260 °C), **8** (mp 238–240 °C, lit.[14] 238–240 °C), 4-carboxy-1-methylpyridinium chloride (mp 255–260 °C dec., lit.[15] 265 °C dec.), and 3-carboxy-1-methylpyridinium chloride (mp 255–257 °C dec., lit.[15] 258–259 °C dec.) were synthesized according to standard procedures.[15] Hydroxylamine hydrochloride, vanillin, and *N*-hydroxynicotinamide were purified by recrystallization prior to use, and the other chemicals obtained from commercial sources were used without purification.

Instrumentation—NMR studies were performed on a Varian XL-400 NMR spectrometer with D_2O solvent and sodium 3-(trimethylsilyl)propionate as an external standard. IR spectra were obtained on a Nicolet SX-60 FTIR spectrometer. UV spectra were obtained with a Carey model 219 Spectrophotometer. The liquid chromatography (LC) system included a Waters 6000A pump. WISP

710B autoinjector, Lambda-Max 481 spectrophotometer, model 730 data module, and a Hewlett-Packard diode-array detector.

Degradation Studies—Initial studies were performed with 0.8-mL samples of **1**, **3**, and **5** placed in sealed 1-mL ampules; however, placing duplicate 0.8-mL samples in tightly sealed (with a silicone septum) 1-mL reaction vials gave satisfactory reproducibility, so sealed vials were used while collecting the data for subsequent reactions. After sealing, the ampules or vials were heated in ovens controlled to ±2 °C. Either ampules were opened or aliquots were removed from the sealed vials at timed intervals and diluted for HPLC analysis. Selected samples were also analyzed for hydroxamic acid and hydroxylamine content.

Phosphate buffers made with NaH_2PO_4 and H_3PO_4 were used in the pH-adjusted reactions because those species were shown to have only a slight catalytic effect on the hydrolysis of nicotinamide[16] and led to only small changes of acidity (±0.2 pH units) with changes in the reaction temperature.[17]

HPLC Assay—Separations were performed with either a Waters C_{18} μBondapak 25 cm (10μ) or an Altex Ultrasphere 12.5 cm octyldecylsilane (5 μm) reversed-phase column. The mobile phase was adjusted by either changing the quantity of the organic modifier (5 or 10% acetonitrile) and/or the pH (3.5 or 5.5) to obtain optimal separation of the products from the two types of reaction mixtures generated by either the degradation of **1**, **3**, and **5** or the hydrolysis of the single ring pyridinium amides **7** and **8**. The mobile phase was prepared by taking acetonitrile (50 or 100 mL), 1.0 M H_3PO_4 (2 mL), 1.0 M tetramethylammonium chloride (0.5 mL), and 1-heptanesulfonic acid sodium salt (120 mg) and mixing them with water to make 1 L of solution. The pH was then adjusted with 25% methanolic tetramethylammonium hydroxide.

UV signals were usually monitored at 254 nm. Confirmation of previously identified products of the degradations at pH <4 was accomplished by measuring the UV absorption spectrum with the diode-array detector set from 220 to 360 nm, comparing retention times, and spiking with authentic samples. All the compounds identified gave absorption signals that were linear with respect to concentration for the range of concentrations monitored.

Hydroxamic Acid and Hydroxylamine Tests—The presence of hydroxamic acids in the samples was determined by treating portions with 10% ferric chloride in 0.3 M HCl and measuring the absorbance at 540 nm.[18] *N*-Hydroxynicotinamide was used as the reference standard. The concentration of hydroxylamine was determined by an HPLC procedure with vanillin as a derivatizing agent.[19]

Preparation and Identification of the Carboxylic Derivatives 2, 4, 6—The bis(pyridinium)aldoximes **1**, **3**, or **5** (1.0 g, 0.0027 mol) and hydroxylamine hydrochloride (1.0 g, 0.014 mol) were mixed with water (3 mL), placed in a reaction vial (5 mL), and heated in a 60 °C oven for 4 days. After each 24-h heating period, the cap was loosened for a moment to allow for the release of a gaseous mixture assumed to be hydroxylamine degradation products generated during the reaction. After completion of the 4-day heating period, the entire vial was placed in a freezer at −10 °C while the crude carboxylic acid derivative crystallized. The crystals were filtered cold and then recrystallized from water/ethanol by a similar procedure. Yields after recrystallization ranged from 40 to 65%. HPLC analysis of the products after one recrystallization indicated that 1–3% of the unreacted starting material was mixed with the carboxylic acid derivative. Further recrystallization did not remove the residual starting material. Compound **4** formed from the slower reacting **3** contained the greatest amount (3%) of starting material. Melting points were as follows: **2**, 148–150 °C (dec.); **4**, 135–138 °C (dec.); **6**, 207–209 °C (dec.). Selected spectroscopic information confirming the structures of **2**, **4**, and **6** are found in Tables I–III. Both ^1H and ^{13}C NMR of the three products were essentially identical to the reactants. NMR data for **1** have been published.[9,11,12] The pK_a (K_a is the acid dissociation constant) values for the carboxylic acid group in **2**, **4**, **6**, and 3-carboxy-1-methylpyridinium chloride were determined to be 2.4 ± 0.1 by a potentiometric titration method with 0.01 M carboxylic acid and 0.1 M sodium hydroxide at 25 °C.[20]

Results

The major degradation products of concentrated aqueous solutions of **1** (HI-6), **3** (HS-6), and **5** under accelerated conditions summarized in Table IV were found to be the carboxylic acids **2**, **4**, and **6**, respectively (see Scheme I) for at least the first half-life of the degradation reactions. The yields of the bis(pyridinium)carboxylic acids from **1** and **5** were greater than the yield from **3**. Figure 1 displays curves representing the typical changes in the concentrations of the bis(pyridinium)aldoxime and the carboxylic acid derivative with time. In the specific case shown in Figure 1 of the degradation of **1** (0.77 M) at 45 °C, the concentration of **2** near the half-life ($t_{1/2}$, 13 days) was >80% of the total degradation products and was approximately four times greater than the

Table I—IR Carbonyl Band Stretching Frequencies

Compound	Frequency, cm^{-1}	Shift, cm^{-1} [a]
1	1685	
2	1715	30
3	1680	
4	1718	38
5	1688	
6	1725	37

[a] The difference between carbamide carbonyl and carboxylic acid carbonyl stretching bands.

Table II—Selected ^1H and ^{13}C NMR Shifts[a]

Position of Carbon Atom or Proton	Chemical Shift in ppm for:			
	1	2	4	6
Aldoxime C	144.6	144.7	144.6	145.0
Carbonyl C	169.0	168.6	166.9	167.9
Bridge Cs				
1	88.2	88.4	88.3	58.1 or 60.0
2	—	—	—	33.9
3	89.5	89.6	89.7	58.1 or 60.0
Oxime H on C	8.6	8.6	8.6	8.6

[a] Sodium 3-trimethylsilylpropionate was used as the external standard.

Table III—UV Maxima (λ_{max}) and Molar Absorptivity (ϵ_{max}),

Compound	λ_{max}, nm[a]	ϵ_{max}, $M^{-1}cm^{-1}$	λ_{max}, nm[b]	ϵ_{max}, $M^{-1}cm^{-1}$
1	302	11 900	355	16 100
2	301	11 900	355	16 500
3	302	10 700	355	13 500
4	302	10 400	355	14 000
5	295	11 300	343	15 800
6	295	11 100	343	15 800

[a] pH 6. [b] pH 10.

Table IV—Reactant and Carboxylic Acid Derivative Compositions of Selected Degradation Reactions Near the First $t_{1/2}$[a]

Compound	Concentration, M	Temperatures °C	k_d, day^{-1}[b]	$t_{1/2}$, day	Composition, %	
					Reactant	Carboxylic Acid Derivative
1	.25	60	.09	7.6	50	35
1	.50	60	.14	5.0	49	40
1	.50	80	.79	0.9	56	25
1	.77	45	.06	12.	47	43
3	.50	60	.08	9.0	53	21
3	.50	80	.66	1.0	57	15
5	.50	60	.02	37.	52	27
5[c]	.50	60	.16	4.3	62	32

[a] The composition data is based on the HPLC analysis of the sample taken closest to the first $t_{1/2}$ of each reaction. [b] First-order rate constants, defined as k_d, for the degradation reactions. [c] This reaction was buffered with NaH_2PO_4 and H_3PO_4 to pH 2.4.

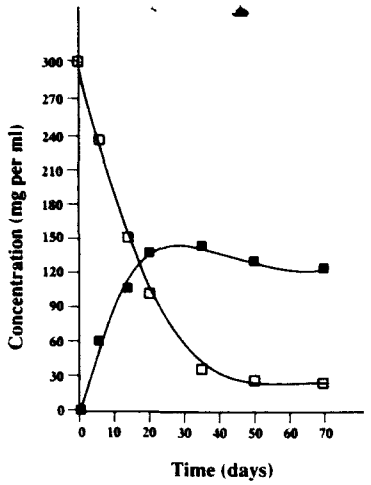


Figure 1—The changes in concentration of reactant **1** (□) and degradation product **2** (■) with time at 45 °C.

concentration of isonicotinamide, the major product from cleavage of the aminal–acetal bonds. Therefore, the rate of amide hydrolysis was approximately four times greater than rate of aminal–acetal cleavage. The concentration of **2** reached a maximum at about two $t_{1/2}$s and then decreased as the rate of aminal–acetal bridge cleavage in **2** became greater than the rate of formation from **1**. During the course of the reaction, a green-black water-insoluble material accumulated. The polymeric material presumably was formed from 2-[(hydroxyimino)methyl]pyridine, another major product from the aminal–acetal cleavage of both **1** and **2**.[9]

With the comparable reaction of **3**, the ratio of the concentration of carboxylic acid **4** to the aminal–acetal cleavage product, nicotinamide, near $t_{1/2}$ was ~2/1, indicating a similar 2/1 ratio for the relative rates of amide hydrolysis and aminal–acetal bridge cleavage. The amide group in the 3-position of the B pyridinium ring in **3** reacted at approximately one-half the rate of the amide group in the 4-position of the B pyridinium ring in **1**.

Degradation of the model compound, **5**, which does not contain an aminal–acetal bridge but a trimethylene bridge that should be relatively stable to cleavage,[7] gave primarily the carboxylic acid derivative **6** for the first $t_{1/2}$. Some smaller molecular weight substances were observed in the HPLC chromatograms of the degrading **5**, indicating bridge cleavage.

The first seven reactions listed in Table IV were run in nonbuffered water. Under these conditions, the pH of the solutions decreased by 0.2 to 0.4 pH units during the course of the reactions, presumably due to the formation of the highly acidic carboxylic acid derivatives. However, first-order rate analysis gave plots linear with respect to the concentration of aldoxime reactants for at least the first two $t_{1/2}$s of the reactions. This observation was consistent with a previous analysis of the nonbuffered degradation of **1**.[21] The rates of degradation were also consistent with those previously reported. For example, the degradation of **1** (0.77 M) at 45 °C had a $t_{1/2}$ of 12 days in this study, whereas degradation of **1** (1.0 M) at 50 °C was reported to have a $t_{1/2}$ of 7.5 days (180 h).[21] Analysis of samples of **1** and **3** for the presence of hydroxylamine due to the hydrolysis of the oxime group under acidic conditions indicated that the concentrations of hydroxylamine were <2% for the first two $t_{1/2}$s of the reactions.

Compounds **2**, **4**, and **6** were prepared in reasonable yields and with only small quantities of impurities (HPLC and ^{13}C NMR analysis) by performing the hydrolysis reactions with a large excess of hydroxylamine hydrochloride. The addition of hydroxylamine not only accelerated the hydrolysis of the amide group, because the $t_{1/2}$ for the reactions with **1**, **3**, and **5** was decreased to <1 day, but it also appeared to minimize the cleavage of the aminal–acetal bridge in the reactions of **1** and **3**.

The presence of a carboxylic acid group in **2**, **4**, and **6** was indicated by the strong acidity, a pK_a value of 2.4 for the first dissociation constant, of each compound. A comparable pK_a value of 2.4 was obtained for the acidity of the model carboxylic acid, 3-carboxy-1-methylpyridinium chloride. Additional support for the presence of the carboxylic acid group came from the observation of the characteristic IR shifts (Table I) of the carbonyl stretching absorption band near 1700 cm^{-1} of 30 to 37 cm^{-1}, which are typical of differences between carboxamide and carboxylic acid carbonyl groups.[22] The appearance of a new IR band near 1300 cm^{-1} that could be attributed to the stretching vibration of the carbon–oxygen single bond formed during hydrolysis was also observed in the spectrum of each compound. Usually the disappearance of the nitrogen–hydrogen stretching bands near 2.5 cm^{-1} can be followed during amide hydrolysis; but the broad oxygen–hydrogen stretching band of the oxime group masked that region of the IR spectra. The carboxylic acid ^{13}C signals (Table II) from 166.9 to 168.6 ppm were essentially indistinguishable from the carboxamide carbon signal near 169 ppm.

Because hydroxylamine reacts readily with amides to form hydroxamic acids,[18] major contamination with a hydroxamic acid derivative was expected. However, ^{13}C NMR did not display a second peak for the hydroxamic acid carbonyl group. The hydroxamic acid color test showed a trace (<1%) of the hydroxamic acid derivative in the purified material.

The UV bathochromic shift of ~50 nm with a corresponding hyperchromic effect associated with a change from acidic to basic pH, summarized in Table III, confirmed the presence of the unaltered oxime group in the major degradation products.[23,24] Additional evidence came from the almost identical ^{13}C NMR signals for the aldoxime carbon found at 144.6 ppm in **1** (HI-6), at 144.7 ppm in **2**, at 144.6 in **4**, and at 145.0 ppm in **6**. The ^{1}H NMR signal at 8.6 ppm for the aldoxime hydrogen-on-carbon was identical in starting material and products and was consistent with an unaltered *syn* stereochemistry associated with the aldoxime group.[25]

In an attempt to duplicate the conditions for the degradation of the bis(pyridinium)oximes with simple model compounds containing an amide group but not the oxime group, pyridinium carboxamides **7** and **8** were hydrolyzed in phosphate-buffered solutions at comparable initial acidities and ionic strengths. Hydrolysis $t_{1/2}$ values an pseudo-first-order rate constants (k_a) for the reactions of **7** and **8** in buffered solutions as well as in the presence of 2-PAM Cl, an alternate source of an aldoxime group, are listed in Table V. Concentration measurements were made over a period of one-half to two $t_{1/2}$s depending on the rate of the reaction. First-order rate analysis gave linear plots with respect to amide concentration during the measured time period. Initial pH values are listed in Table V to illustrate relative rates of the reactions with large changes in acidity. Relatively small pH changes (0.1 to 0.2 pH units) observed during the course of the reactions from the formation of strongly acidic products and small pH variations due to temperature effects[16] on the various solutions were assumed to make only a minor contribution to the differences in relative rates. Because it was necessary to perform the reactions at very high concentration so that a comparison can be made with the degradation reactions of the bis(pyridinium)aldoximes, the exact nature of the reacting medium could not be clearly described as would be ideal in kinetic measurements. Therefore, the absolute rates and relative rates of reaction should be viewed with appropriate caution when compared with values obtained from reactions

Table V—Relative Rates of Selected Hydrolysis Reactions[a]

Reaction	Reactants	pH[b]	k_a, day^{-1}[c]	$t_{1/2}$, day	Relative Rate[d]
A	1 + H_2O	2.4	—[e]	5[f]	16.0
B	5 + H_2O	4.0	—[e]	41[f]	2.0
C	5 + buffer[g]	2.4	—[e]	6[f]	14.0
D	7 + buffer[h]	2.4	0.017	40	2.0
E	7 + 2-PAM[i]	2.4	0.030	23	3.5
F	7 + buffer[h]	4.0	0.009	81	1.0
G	3 + H_2O	2.8	—[e]	9[f]	9.0
H	8 + buffer[h]	2.8	0.014	49	1.7
I	8 + 2-PAM[i]	2.8	0.020	35	2.3
J	7 + H_3PO_4[j]	1.9	0.036	19	4.3
K	8 + H_3PO_4[j]	1.9	0.025	28	2.9

[a] All reactions were 0.5 M in substrate and heated to 60 °C. [b] Initial pH at room temperature. [c] First-order rate constants, defined as k_a, for acid-catalyzed hydrolysis. [d] The $t_{1/2}$ (81) for reaction F divided by the $t_{1/2}$ for the specific reaction. [e] Not determined. [f] Estimate based on the $t_{1/2}$ for the degradation reaction in Table IV. [g] Buffer was prepared from H_3PO_4 (0.5 M) and NaH_2PO_4. [h] Buffer was prepared from NaH_2PO_4 (0.5 M) and H_3PO_4. [i] The 2-PAM concentration was 0.5 M and the pH was adjusted with H_3PO_4. [j] 1.0 M.

in dilute solutions.[16] Hydrolysis $t_{1/2}$ values for **1**, **3**, and **5** (Table V) were estimated from the degradation $t_{1/2}$ values experimentally measured and reported in Table IV. Adjustments were made for the competing reactions in the degradation process such as bridge cleavage reactions.

Discussion

The observations that the amide group in ring B of **1** hydrolyzed to the carboxylic acid group in a concentrated aqueous solution at a faster rate than the cleavage of the aminal–acetal linkage between rings and that **2** was the principal degradation product for the first $t_{1/2}$ of the reaction differed from previous reports where **2** was either not observed[6–8] or found in very low concentrations.[9] However, the isolation and characterization of **2** by UV, IR, and NMR analyses enabled the confirmation of the presence of **2** among the numerous products in the HPLC analysis of the degrading **1**. Finding a similar degradation pattern of hydrolysis of the amide group in **3** and **5** indicated that the degradation of **1** was not a unique reaction process. The use of the diode-array detector was an additional aid for identifying components of the complex mixture of degradation products for **1** and **3** during HPLC analysis because it disclosed the UV maximum at 302 nm of the bis(pyridinium)carboxylic acid derivatives still containing the oxime group during the actual HPLC assay. Aminal–acetal cleavage products containing only ring A or B components do not show UV maxima above 290 nm. Mass spectroscopic detection had limited application in identifying **2** and **4** because the fragmentation pattern of the bis(pyridinium)aldoximes **1** and **3** and the derivatives **2** and **4** (parent peak at 123 amu, etc.) were remarkably similar to the patterns of the products from the cleavage of the aminal–acetal linkage.[13]

The recognition of an additional major product and process in the degradation reactions of **1** and **3** does not require the reassessment of previously reported kinetic data indicating a first-order rate for the degradation of **1**[9,21,26] or **3**.[27] Acid-catalyzed hydrolysis of nicotinamide has been shown to be a first-order reaction[28] and the hydrolysis of concentrated solutions of both amides **7** and **8**, reactions D and H at pH 2.4 (Table V), were first-order with respect to amide for at least the initial $t_{1/2}$ of the reaction.

The unexpectedly rapid rate of hydrolysis of the amide group in the concentrated unbuffered solutions of **1** and **3** appeared to be associated with two major factors: **1** and **3** had enhanced acidity at high concentrations and the aldoxime group catalyzed the hydrolysis reaction.

Prior measurements indicated pK_a values of 7.28 and 7.30 for **1** and **3**, respectively.[28] When dissolved at 0.5 M concentrations, solutions of **1** and **3** had pH values of 2.4 and 2.8, respectively. Simple calculations give estimated pK_a values, −4.5 for **1** and 5.3 for **3**, significantly different from the reported values that were measured at lower concentrations. However, the pH value of 4.0 for 0.5 M model **5** was consistent with that expected for a typical aldoxime with a pK_a in the 7–8 range. The difference in pH between solutions of **1** and **5** accounted for the major portion of the eightfold difference in rates of amide hydrolysis for the nonbuffered reactions A and B (Table V). When the pH of a solution of **5** was adjusted to 2.4, the rate of hydrolysis of **5** (reaction C) is almost identical to the rate of hydrolysis of **1** (reaction A).

The large increase in effective acidity of **1** and **3** at high concentrations would imply significantly increased stabilization of the oximato ion due to proximity to other bis(pyridinium)aldoxime molecules. Because the solution of **5**, which contains a trimethylene bridge, displayed a normal acidity at high concentration, the stability of the oximato ion in concentrated solutions of **1** and **3** may involve increased oxime proton participation with the oxygen atom in the aminal–acetal bridge and simultaneous ion pair formation with the quaternary pyridinium ion of an adjacent molecule.

Aldoxime participation in the catalysis of the degradation of **1** has been reported;[21] however, the catalysis was suggested to be associated with the cleavage of the aminal–acetal bridge by the oximato ion. Added aldoxime in the form of 2-PAM chloride to either **7** (reaction E) or to **8** (reaction I) led to increased rates of amide hydrolysis relative to buffered reactions D and H, respectively. The role of the aldoxime group as a general acid catalyst in the hydrolysis of the amide groups in **1**, **3**, and **5** would also be consistent with the previously reported general acid catalysis by oximes[29] and the use of hydroxylamine hydrochloride as a acid catalyst in the preparation of **2**, **4**, and **6** for this study.

The increased rate of amide hydrolysis of **1** compared with **3** (reactions A and G in Table V) was attributed to both an increase in the acidity (pH 2.4) of the solution of **1** compared with the solution of **3** (pH 2.8) and the decrease in charge density of the carbonyl group in the 4 position relative to the 3 position of ring B. The latter effect is demonstrated in the relative rates of hydrolysis of **7** to **8** under identical conditions (reactions J and K). The 4-carboxamido pyridinium species **7** reacted 1.5 times faster than the 3-carboxamido pyridinium species **8**.

In a preliminary report[13] we suggested that a major contributor to general acid catalysis might be the hydroxylammonium ion generated in the rapidly degrading samples of **1** from hydrolysis of the aldoxime group at pH 2.4 rather than the aldoxime group itself. That perspective was based on the catalytic role of the relatively small hydroxylammonium ion in the formation of hydroxamic acids from amides[18] and esters.[30] However, determinations of hydroxylamine concentrations for **1** and **3** at various times during the first $t_{1/2}$ of the degradation reactions A and B showed hydroxylamine levels to be <2% of the concentration of the undegraded oxime. Therefore, the hydroxylammonium ion would have made only a small contribution to the general acid catalysis observed.

Conclusions

The hydrolysis of the ring B amide group was the major initial cause of degradation of three related amide-containing bis(pyridinium)aldoximes in concentrated aqueous solutions. Compounds **1** and **3**, which contain aminal–acetal bridges, were particularly vulnerable to amide hydrolysis due to the

unusual acidity of the oxime group at high concentrations. The reaction was both hydrogen ion and general acid catalyzed primarily by the oxime group. These insights may be of value during the evaluation of the stability of new antidotes for organophosphate poisoning that contain a similar carboxamide moiety such as the compound 1-[[[4-(aminocarbonyl)-pyridinio]methoxy]methyl]-2,4-bis[(hydroxyimino)methyl] diiodide (LÖ-7).[31]

References and Notes

1. Clement, J. G. *Archives Toxicol.* **1992**, *66*, 143–144.
2. Hamilton, M. G.; Lundy, P. M. *Arch. Toxocol.* **1989**, *63*, 144–149.
3. Kušić, R.; Bošković, B.; Vojvodić, V.; Javanović, D. *Fundam. Appl. Toxicol.* **1985**, *5*, S89–S97.
4. Clement, J. G. *Fundam. Appl. Toxicol.* **1983**, *3*, 533–555.
5. Kepner, L. A.; Wolthuis, O. L. *Eur. J. Pharmacol.* **1978**, *49*, 415–425.
6. Brown, N. D.; Kazyak, L.; Doctor, B. P.; Hagedorn, I. *J. Chromatogr.* **1986**, *351*, 599–603.
7. Brown, N. D.; Stermer-Cox, M. G.; Doctor, B. P.; Hagedorn, I. *J. Chromatogr.* **1984**, *292*, 444–450.
8. Eyer, P.; Hell, W. *Arch Pharm.* **1985**, *318*, 938–946.
9. Eyer, P.; Hell, W.; Kawan, A.; Klehr, H. *Arch. Toxicol.* **1986**, *59*, 266–271.
10. Ligtenstein, D. A.; Wils, E. R. J.; Kossen, S. P.; Hulst, A. G. *J. Pharm. Pharmacol.* **1987**, *39*, 17–23.
11. Lin, A. J.; Klayman, D. L. *J. Pharm. Sci.* **1986**, *75*, 797–799.
12. Mdachi, R. E.; Marshall, W. D.; Ecobichon, D. J.; Fouad, F. M.; Connolley-Mendoza, C. E. *Chem. Res. Toxicol.* **1990**, *3*, 413–422.
13. Korte, W. D.; Shih, M. L. *Proceedings of the 1989 Medical Defense Review*, Columbia, MD, August 15–17, 1989, USAMRICD Contract Office, Aberdeen Proving Ground, MD, 1989, 655–659.
14. Sakurai, T.; Hosoya, H. *Biochim. Biophys. Acta* **1966**, *112*, 459–468.
15. Smissman, E. E.; Hite, G. *J. Am. Chem. Soc.* **1959**, *81*, 1201–1203.
16. Finholt, P.; Higuchi, T. *J. Pharm. Sci.* **1962**, *51*, 655–661.
17. Bates, Roger G. *Determination of pH*; Wiley: New York, 1973; pp 122–126.
18. Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1964**, *86*, 5616–5620.
19. Korte, W. D. *J. Chromatogr.* **1992**, *603*, 145–150.
20. Albert, Adrien; Serjeant, E. P. *Ionization Constants of Acids and Bases*; Methuen: London, 1962; pp 16–34.
21. Eyer, P.; Hagedorn, I.; Ladstetter, B. *Arch. Toxicol.* **1988**, *62*, 224–226.
22. Socrates, G. *Infrared Characteristic Group Frequencies*, Wiley: New York, 1980; pp 65, 74–75.
23. Ellin, R. I. *J. Am. Chem. Soc.* **1958**, *80*, 6588–6589.
24. Ellin, R. I.; Easterday, D. E.; Zvirblis, P.; Kondritzer, A. A. *J. Pharm. Sci.* **1966**, *55*, 1263–1267.
25. Poziomek, E. J.; Kramer, D. N.; Mosher, W. A.; Michel, H. O. *J. Am. Chem. Soc.* **1961**, *83*, 3916–3917.
26. Fyhr, P.; Nicklasson, M.; Gunnvald, K.; Brodin, A. *Int. J. Pharm.* **1987**, *40*, 193–200.
27. Christenson, I. *Acta Pharm. Sueccia* **1972**, *9*, 323–330.
28. Hagedorn, I.; Stark, I.; Lorenz, H. P. *Angew. Chem.* **1972**, *84*, 354–356.
29. Bell, R. P.; Higginson, W. C. E. *Proc. Roy. Soc. A* **1949**, *197*, 141–159.
30. Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778–1786.
31. Eyer, P.; Ladstetter, B.; Schafer, W.; Sonnenbichler, J. *Arch. Toxocol.* **1989**, *63*, 59–67.

Acknowledgments

We greatly appreciate the assistance of L. Szafraniec and W. Beaudry of the Chemical Research, Development, and Engineering Center, Aberdeen Proving Ground, Edgewood, MD, in obtaining and interpreting the NMR spectra, and the technical assistance of R. Smith and G. Salem of the United States Army Medical Research Institute of Chemical Defense (USAMRICD). W. D. Korte thanks the USAMRICD for generous support under the Intergovernmental Personnel Act. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.