VOLUME 114, NUMBER 13, 1992
JUNE 17, 1992


JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

# Chemistry of Phosphodiesters, DNA and Models. 2. The Hydrolysis of Bis(8-hydroxyquinoline) Phosphate in the Absence and Presence of Metal Ions

**Kenneth A. Browne and Thomas C. Bruice***

*Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106.* *Received November 20, 1991*

**Abstract:** The pH dependence of the hydrolysis of bis(8-hydroxyquinoline) phosphate and bis(6-hydroxyquinoline) phosphate has been studied in the absence and presence of varying concentrations of $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, and $Mn^{2+}$. Since both diesters are characterized by having two quinoline nitrogens as basic centers and a negative phosphate, they exist, depending upon pH, as unprotonated, mono-, di-, and triprotonated species {**IV**$^-$, **IVH**, **IVH**$_2^+$, and **IVH**$_3^{2+}$ for bis(8-hydroxyquinoline) phosphate; **V**$^-$, **VH**, **VH**$_2^+$, and **VH**$_3^{2+}$ for bis(6-hydroxyquinoline) phosphate}. The partial charges and basicity of the hydroxyl groups of 6- and 8-hydroxyquinoline are comparable. The electronic effect on the rate of hydrolysis brought about by protonation or metal ion complexing to the quinoline nitrogens should be the same for both bis(6-hydroxyquinoline) phosphate and bis(8-hydroxyquinoline) phosphate. Electronic effects upon hydrolytic rates can, therefore, be differentiated from proximity effects by comparison of the hydrolytic rate constants of the two diesters. The bis(6-hydroxyquinoline) phosphate hydrolysis is (i) quite slow ($10^{-7}$ s$^{-1}$), (ii) pH independent from slightly acidic to neutral pH, and (iii) not catalyzed by the metal ions employed. On the other hand, hydrolysis of bis(8-hydroxyquinoline) phosphate proceeds through rapid spontaneous breakdown of **IVH** and through $HO^-$ catalyzed hydrolysis of **IV**$^-$. The hydrolysis of **IVH** is not subject to buffer catalysis nor does it exhibit a deuterium solvent kinetic isotope effect. Arguments are presented in support of a mechanism for hydrolytic breakdown of **IVH** which involves quinoline nitrogen nucleophilic displacement on phosphorus with departure of N-protonated 8-hydroxyquinoline zwitterion. This mechanism provides a rate enhancement over the hydrolysis of the bis(6-hydroxyquinoline) phosphate of $1.1 \times 10^3$. Metal ion catalysis of the hydrolysis of the bis(8-hydroxyquinoline) phosphate involves a bimolecular reaction of $Ni^{2+}$, $Co^{2+}$, or $Zn^{2+}$ ($Mn^{2+}$ is not reactive) with the **IV**$^-$ species. Arguments support a mechanism of nucleophilic displacement by a quinoline nitrogen with assistance to departure of the leaving 8-hydroxyquinoline moiety by interaction of its quinoline nitrogen and the developing negative charge on its oxygen with a metal ion. This mechanism provides, at 1 M in metal ion, a rate enhancement over the spontaneous hydrolysis of **IVH** of $4.7 \times 10^4$ for $Ni^{2+}$, $1.2 \times 10^4$ for $Co^{2+}$, and $1.3 \times 10^4$ for $Zn^{2+}$. The rate enhancement brought about by the concerted nucleophilic attack of quinoline nitrogen and metal ion assistance to departure of the leaving group in the hydrolysis of **IV**$^-$ is ca. $1-5 \times 10^7$ at 1 M metal ion.

## Introduction

Considerable progress has been made during the last three decades in understanding the mechanisms of hydrolysis of phosphate esters. The most stable are the alicyclic phosphodiesters. In neutral aqueous solution at 25 °C, the pseudo-first-order rate constant for the hydrolysis of dimethyl phosphate[1a] is estimated as $2 \times 10^{-14}$ s$^{-1}$, while that for the hydrolysis of bis(2,4-dinitrophenyl) phosphate[1b] is $1.8 \times 10^{-7}$ s$^{-1}$. Although a $10^7$ acceleration of rate accompanies a change of leaving group basicity of $10^{11}$, the half-life for hydrolysis of the activated bis(2,4-dinitrophenyl) phosphate is still ~1.5 months. The stability of DNA in water, afforded by its phosphodiester linkages, poses a problem in the development of sequence specific hydrolytic agents for DNA cleavage. A number of reagent types have been advanced for the sequence specific radical oxidative cleavage of DNA.[2-6] We have

(1) (a) Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991. (b) Bunton, C. A.; Farber, S. J. *J. Org. Chem.* **1969**, *34*, 767.

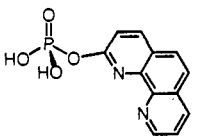
(2) Dervan, P. B. *Sci.* **1986**, *232*, 464.

0002-7863/92/1514-4951$03.00/0 

initiated studies directed toward the goal of the synthesis of reagents which hydrolyze DNA with sequence recognition.[7] The plausibility of reaching this goal is supported by the existence of restriction enzymes which carry out the facile sequence specific hydrolysis of the DNA phosphodiester bond.

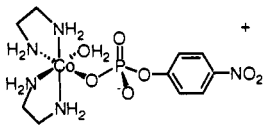
Possibly the most successful designs of molecules for the hydrolysis of phosphate diesters will be based on metal ion catalysis. Metal ions can enter into the catalysis of hydrolytic reactions by complexing the substrate. This may result in an increase in the substrate's electrophilic character and, thus, susceptibility to nucleophilic attack. Alternatively, the complexing of the substrate by a metal ion may assist the departure of a strongly basic leaving group. A divergent role for a metal ion in substrate hydrolysis is in the generation of the nucleophile. The $pK_a$ for $H_2O \rightleftarrows HO^- + H^+$ is depressed on complexation of the $H_2O$ to metal ion.[8] For this reason, higher concentrations of hydroxide ion can be obtained at a given pH in the presence of an appropriate metal ion. However, the rate constant for $HO^-$ nucleophilic attack is somewhat depressed on complexation by metal ion. This decrease in rate constant is related to the extent of bond formation in the transition state ($\beta_{nuc}$). A useful approximation for the depression in the rate constant for nucleophilic attack by $HO^-$ brought about by metal ion complexation is ca. $10^2$. Thus, the presence of a metal ion may, depending upon the metal ion and its concentration as well as the pH, greatly increase the effective concentration of hydroxide ion while only moderately decreasing the latter's nucleophilicity.[9] An additional advantage of metal ion complexed $HO^-$ over free $HO^-$ is the proximity effect that may be invoked with the former. Thus, at a catalytic site, a fixed ligand may complex a metal ion which in turn holds an $HO^-$ adjacent to the electrophilic center of a bound substrate.[9]

Fife and Pujari[10] have investigated, in the appropriately designed 2-(1,10-phenanthrolyl) phosphate monoester (I), the influence of metal ion ligation to the putative leaving group on the rates of ester hydrolysis. In these studies the leaving group is generated by cleavage of a P–OAr bond, and metal ion binding increases the stability of the phenoxide leaving group.[10] Very large rate

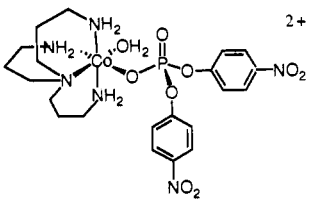


I

enhancements were obtained with $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$. The value of the rate constant for the hydrolysis of the $Cu^{2+}$ complex of the dianion of I was found to be $10^8$ greater than that for phenyl phosphate dianion. Jones, Lindoy, and Sargeson have examined the hydrolysis of the polyamine–$Co^{3+}$–4-nitrophenyl phosphate monoester complex II[11a] while Chin and co-workers studied the hydrolysis of the polyamine–$Co^{3+}$–bis(4-nitrophenyl) phosphate diester complex III.[11b] These studies established that hydrolysis of phosphate ester complexes II and III at pH 7 exceeded the rate of spontaneous hydrolysis of the corresponding 4-nitrophenyl phosphate monoester and bis(4-nitrophenyl) phosphate diester by up to $10^{10}$. De Rosch and Trogler[12] have



II

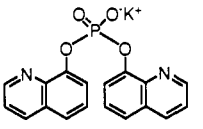


III

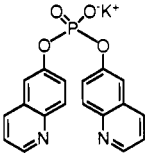
investigated the catalysis of hydrolysis of bis(4-nitrophenyl) phosphate diester in bimolecular reactions using tren and bpy complexed metal ion hydrates as nucleophiles. They report (pH 7.0) that the rate constants ($s^{-1}$) for hydrolysis of bis(4-nitrophenyl) phosphate diester in the presence of $10^{-3}$ M complexed metal exceed the pseudo-first-order rate constants for bis(4-nitrophenyl) phosphate diester hydrolysis in the absence of metal by $10^1$–$10^3$-fold.

The most effective metal ion centered catalytic system would employ metal ions both to deliver $HO^-$ as a nucleophile in a direct in-line displacement and to complex the incipient alkoxide or phenoxide leaving groups. Evidence for this is seen in enzymatic systems. Both $Mg^{2+}$ and $Zn^{2+}$ are directly involved in the 3′-to-5′ exonuclease activity of the Klenow fragment of DNA polymerase I from *Escherichia coli*. Evidence for the catalytic role of the metal ions comes from the X-ray crystallographic data of a co-crystal of DNA and the Klenow fragment.[13,14] It has been proposed that the established[15] in-line displacement of the 5′ oxygen is by $HO^-$ ligated to the $Zn^{2+}$ and that the incipient 5′ oxyanion leaving group is coordinated by $Mg^{2+}$. More recently, it has been reported that *E. coli* alkaline phosphatase[16] and phospholipase C from *Bacillus cereus*[17] also require a combination of two metal ions for activity.

We report here the investigation of the hydrolysis of bis(8-hydroxyquinoline) phosphate (IV) and bis(6-hydroxyquinoline) phosphate (V) at constant values of pH in the absence and presence of certain metal ions.



IV



V

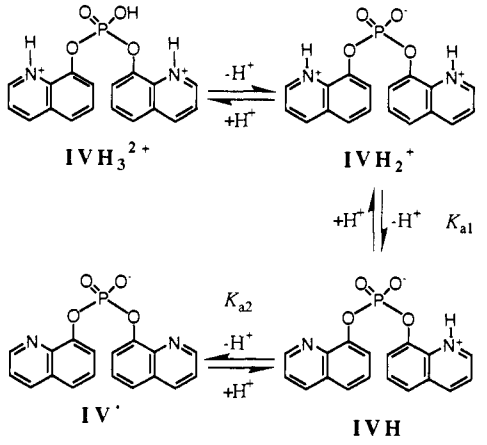
## Experimental Section

**Materials.** $POCl_3$ and 8-hydroxyquinoline were purchased from Aldrich, whereas 6-hydroxyquinoline was purchased from Pfaltz & Bauer. Buffers and metals were of the highest purity available and used as purchased as were all other chemicals with the exception of KCl. Solutions of the latter, used to maintain constant ionic strength, were demetalated by passing through a Chelex 100 resin (Bio-Rad) column. For all protium experiments, glass distilled deionized water was used. In deuterium isotope studies, 99.9% deuterium oxide (Aldrich) was used. Deuterated buffers were prepared by reduced pressure evaporation of the bases or acids of the aqueous buffers, dissolving residual salt in $D_2O$, and reevaporating the $D_2O$. This procedure was repeated two additional times prior to reconstituting the buffer to its original volume in $D_2O$. Standardized DCl and KOD from Aldrich were used with the deuterated buffers to achieve the appropriate pD.

**Preparation of phosphodiesters** followed a method analogous to that of Bunton and Farber for the preparation of diphenyl phosphate.[1b] Bis(8-hydroxyquinoline) phosphate pyridinium salt was exchanged for the potassium salt by the equimolar addition of potassium hydroxide in dry methanol. The resultant white precipitate was collected and washed with excess cold methanol. The resultant compound, IV, decomposed (>250 °C) before melting: yield 38%; $^1H$ NMR $\delta$ 8.50 (d, $J$ = 4.0 Hz,

(3) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.
(4) Barton, J. K. *Sci.* **1986**, *233*, 727.
(5) Wade, W. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1987**, *109*, 1574.
(6) (a) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961. (b) Lee, M. D.; Ellestad, G. A.; Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235.
(7) Bruice, T. C.; Mei, H.-Y.; He, G.-X.; Lopez, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1700.
(8) Buckingham, D. A.; Harrowfield, J. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 1726.
(9) Wells, M. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 5341 and references therein.
(10) Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1988**, *110*, 7790.
(11) (a) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327. (b) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186.
(12) De Rosch, M. A.; Trogler, W. C. *Inorg. Chem.* **1990**, *29*, 2409.
(13) Freemont, P. S.; Friedman, J. M.; Beese, L. S.; Sanderson, M. R.; Steitz, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8924.
(14) Beese, L. S.; Steitz, T. A. *EMBO J.* **1991**, *10*, 25.
(15) Gupta, A. P.; Benkovic, S. J. *Biochemistry* **1984**, *23*, 5874.
(16) Kim, E. E.; Wyckoff, H. W. *J. Mol. Biol.* **1991**, *218*, 449.
(17) Hough, E.; Hansen, L. K.; Birknes, B.; Jynge, K.; Hansen, S.; Hordvik, A.; Little, C.; Dodson, E.; Derewenda, Z. *Nature* **1989**, *338*, 357.

Scheme I




1 H), 8.16 (d, $J$ = 10.0 Hz, 1 H), 7.77 (d, $J$ = 7.5 Hz, 1 H), 7.58 (d, $J$ = 8.0 Hz, 1 H), 7.47 (t, $J$ = 8.0 Hz, 1 H), 7.39 (dd, $J$ = 4.5, 8.5, 1 H); IR (KBr) 1610, 1290, 1180, 915 $cm^{-1}$; UV ($H_2O$) $\lambda_{max}$ nm ($\epsilon$) 292.0 (6537), 231.5 (53295); HRMS (FAB, anion) for $C_{18}H_{12}N_2O_4P^-$ calcd 351.0535, obsd 351.0535. Compound V was prepared by a procedure analogous to that used for IV and similarly decomposed (207 °C) before melting: yield 51%; $^1H$ NMR $\delta$ 8.89 (d, $J$ = 5.0 Hz, 1 H), 8.78 (d, $J$ = 8.5 Hz, 1 H), 8.10 (d, $J$ = 9.0 Hz, 1 H), 7.88 (s, 1 H), 7.84 (d, $J$ = 8.5 Hz, 1 H), 7.83 (dd, $J$ = 2.5, 9.0 Hz, 1 H); IR (KBr) 1625, 1250, 935 $cm^{-1}$; UV ($H_2O$) $\lambda_{max}$ nm ($\epsilon$) 315.5 (9110), 235.0 (40979), 202.5 (55307); HRMS (FAB, cation) for $C_{18}H_{12}N_2O_4P^- + 2H^+$ calcd 353.0691, found 353.0702.

**Methods.** The rates of hydrolysis of IV and V were followed spectrophotometrically using thermostated Perkin Elmer 553, UVIKON 810, or OLIS modified Cary-14 recording spectrophotometers. Quartz cuvettes containing reaction solutions were maintained at either 30, 45, or 60 °C in their cell compartments. Ionic strengths were held constant at 1.0 with KCl. Constant pH from 1.0 to 3.0 was maintained with HCl, while constant pH from 12.0 to 13.0 was maintained with KOH. Formate, acetate, pyridine, cacodylate, and 2-amino-2-(hydroxymethyl)-1,3-propanediol were used as 0.1 M buffers within 1 pH unit of their $pK_a$ values. Spectrophotometric titrations were performed with a thermostated UCSB modified Cary-15 recording spectrophotometer at 30 °C ($\mu$ = 1.0 with KCl). A Radiometer Model 26 pH meter and a combined glass electrode were used for all pH measurements at the temperature of the hydrolysis experiment. Data points were fit to theoretical curves with a least-squares computer routine written in this laboratory on a Hewlett-Packard 9820A or by software written for the OLIS computerized Cary-14 spectrophotometer.

$^1H$ NMR spectra were recorded at 500 MHz on a General Electric GN-500 NMR spectrometer. The chemical shifts are reported in parts per million downfield from 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt in $D_2O$ (Aldrich). IR spectra were recorded on a Perkin-Elmer 1330 infrared spectrophotometer. High-resolution mass spectra (HRMS) were recorded using the fast atom bombardment (FAB) technique at the Midwest Center for Mass Spectrometry Laboratory at the University of Nebraska, Lincoln. Due to mass spectra matrix compatibility, bis(8-hydroxyquinoline) phosphate was submitted as the potassium salt, while the pyridinium salt was used for bis(6-hydroxyquinoline) phosphate.

## Results

**Acid dissociation constants of bis(8-hydroxyquinoline) phosphate (IV) and bis(6-hydroxyquinoline) phosphate** (V) were determined by spectrophotometric titration (Scheme I). The $pK_a$ values of 6- and 8-hydroxyquinolines were also determined. Titrations with KOH were carried out using 50-mL aliquots ~$4 \times 10^{-5}$ M in IV or V or ~$8 \times 10^{-5}$ M in the respective hydroxyquinoline. For each of these four compounds, plots of absorbance at three wavelengths (290, 313, and 330 nm; 250, 300, and 320 nm; 270, 309, and 358 nm; and 300, 340, and 370 nm, respectively) vs pH were computer least-squares fit to a theoretical equation for two $pK_a$'s. The determined $pK_a$ values are included in Table I. The $pK_a$ values for the hydroxyquinolines compare favorably with those reported in the literature.[18]

(18) Mason, S. F. *J. Chem. Soc.* **1958**, 674 and references therein.

Table I. Various $pK_a$ Values Determined via Spectrophotometric Titration and as Kinetically Apparent Constants[g]

| compound | conditions (°C) | $pK_a$ N | $pK_a$ N′ | $pK_a$ O |
|---|---|---|---|---|
| 8-hydroxyquinoline | 30[a] | | 5.03 | 9.63 |
| 6-hydroxyquinoline | 30[a] | | 5.36 | 8.77 |
| IV | 30[a] | 3.31 | 5.10 | |
| V | 30[a] | 4.13 | 5.41 | |
| IV | 30[b] | 3.28 | 5.30 | |
| IV + EDTA | 30[b,c] | 3.46 | 5.21 | |
| IV + $D_2O$ | 30[b,d] | 3.99 | 5.79 | |
| IV | 45[b] | 3.40 | 5.10 | |
| IV | 60[b] | 3.16 | 5.20 | |
| IV + $Mn^{2+}$ | 30[b,e] | 3.36 | 5.58 | |
| IV + $Ni^{2+}$ | 30[f] | 3.35 | 5.21 | |
| IV + $Co^{2+}$ | 30[f] | 3.14 | 5.17 | |
| IV + $Zn^{2+}$ | 30[f] | 3.37 | 5.07 | |

[a] Spectrophotometric titration. [b] Kinetically apparent constants obtained by the fitting of eq 1 (part A) to experimental data points. [c] In the presence of $2 \times 10^{-3}$ M EDTA. [d] Determined in $D_2O$. [e] In the presence of $2 \times 10^{-2}$ M $Mn^{2+}$. [f] Kinetically apparent constants obtained by the fitting of eq 2 to experimental data points determined in the presence of the indicated metal ion. [g] For phosphate diesters, $pK_{a1}$ (=$pK_a$ N) and $pK_{a2}$ (=$pK_a$ N′) are defined in Scheme I.

**The pH dependence of the hydrolysis of bis(8-hydroxyquinoline) phosphate** was examined at constant values of pH between pH 1 and 13. Reactions were followed spectrophotometrically by monitoring either the disappearance of the diester at 290 nm below pH 5.5, the appearance of 8-hydroxyquinoline at 330 nm above pH 5.5, or at both wavelengths around pH 5.5. Kinetic runs were initiated, at the appropriate temperature, by the dilution of a freshly prepared solution of diester ($2.4 \times 10^{-3}$ M) in water with the appropriate buffer solution to give a final concentration of $2 \times 10^{-4}$ M in ester. Except for exceedingly slow reactions (i.e., reactions below pH 2.5 and above pH 7.5 at 30 °C, and above pH 8.5 at 60 °C) which could only be followed for 1 to 2 $t_{1/2}$, reactions followed pseudo-first-order kinetics for at least 5 $t_{1/2}$. For those reactions that could be followed to completion, the final spectra were of 8-hydroxyquinoline. Repetitive scans from 400–250 nm demonstrate clean isosbestic points (Figure 1a). Buffers were employed to maintain constant pH between 3 and 9. Plots of the pseudo-first-order rate constant ($k_{obsd}$) vs buffer concentration were found to possess slopes that changed less than 10% over 10-fold changes in buffer concentration (data not shown). Thus, the hydrolysis of bis(8-hydroxyquinoline) phosphate is not catalyzed by the buffers employed (see Experimental Section). The KCl solutions used to maintain constant ionic strength, $\mu$, were demetalated by passage through a Chelex 100 column. To be certain of the absence of trace metal ions, several reactions were carried out in which EDTA ($2 \times 10^{-3}$ M) was incorporated into the reaction solutions prepared from the Chelex 100 treated KCl solution. This addition of EDTA did not change the values of $k_{obsd}$ (Table II).

The plot of the log of the pseudo-first-order rate constant ($k_{obsd}$) vs pH for the hydrolysis of bis(8-hydroxyquinoline) phosphate by lyate species at 30 °C ($\mu$ = 1.0 with KCl) can be seen in Figure 2. Examination of the best fit of the plot of log $k_{obsd}$ vs pH shows that the profile can be divided into three parts: (A) a "bell-shaped" portion from pH 1 to 8, (B) a pH independent region, and (C) a linear plot with slope of 1.0 above pH ~11. The lines which fit the points of Figure 2 were generated by use of eq 1. Values of $k_1$, $K_{a1}$, $K_{a2}$, $k_0$, and $k_{OH}$ used to generate the plots of Figure

$$k_{obsd} = \underbrace{\frac{k_1 K_{a1} a_H}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}}}_{A} + \underbrace{k_0}_{B} + \underbrace{k_{OH} K_w / a_H}_{C} \quad (1)$$

2 are recorded in Tables I and II. The constant $K_w$ represents the autoprotolysis constant for water at the appropriate temperatures.[19] The values of the constants $pK_{a1}$ and $pK_{a2}$ required to fit the experimental rate constants to the A term of eq 1 at 30

(19) *Handbook of Chemistry and Physics*; Lide, D. R., Ed.; CRC Press, Inc.; Boca Raton, Ann Arbor, Boston, 1990–1991; p 8-38.

**Table II.** Values of Rate Constants Obtained from the Fitting of Eqs 1 and 2 to the Experimental Points in Figures 2 and 4

| compound | conditions (°C) | $k_1$ ($s^{-1}$) | $k_0$ ($s^{-1}$) | $k_{OH}$ ($s^{-1}$) | $k_2$ ($M^{-1}$ $s^{-1}$) |
|---|---|---|---|---|---|
| IV | 30 | $1.51 \times 10^{-4}$ | $1.59 \times 10^{-6}$ | $2.50 \times 10^{-8}$ | |
| V | 30[a] | $1.36 \times 10^{-7}$ | | | |
| IV + EDTA | 30[b] | $1.61 \times 10^{-4}$ | | | |
| IV + $D_2O$ | 30[c] | $1.53 \times 10^{-4}$ | | | |
| IV | 45 | $8.30 \times 10^{-4}$ | | | |
| IV | 60 | $2.88 \times 10^{-3}$ | $5.00 \times 10^{-5}$ | $2.78 \times 10^{-7}$ | |
| IV + $Mn^{2+}$ | 30[d] | $1.58 \times 10^{-4}$ | | | |
| IV + $Ni^{2+}$ | 30[e] | | | | 7.04 |
| IV + $Co^{2+}$ | 30[e] | | | | 1.83 |
| IV + $Zn^{2+}$ | 30[e] | | | | 1.94 |

[a] Estimated from initial rates, $\pm 3.7 \times 10^{-8}$ $s^{-1}$. [b] Experiments conducted in the presence of $2 \times 10^{-3}$ M EDTA. [c] Determined in $D_2O$. [d] In the presence of $2 \times 10^{-2}$ M $Mn^{2+}$. [e] Determined by fitting the experimental points of plots of the log of the apparent second-order rate constants ($k_2'$) vs pH with eq 2 for each of the indicated metal ions.

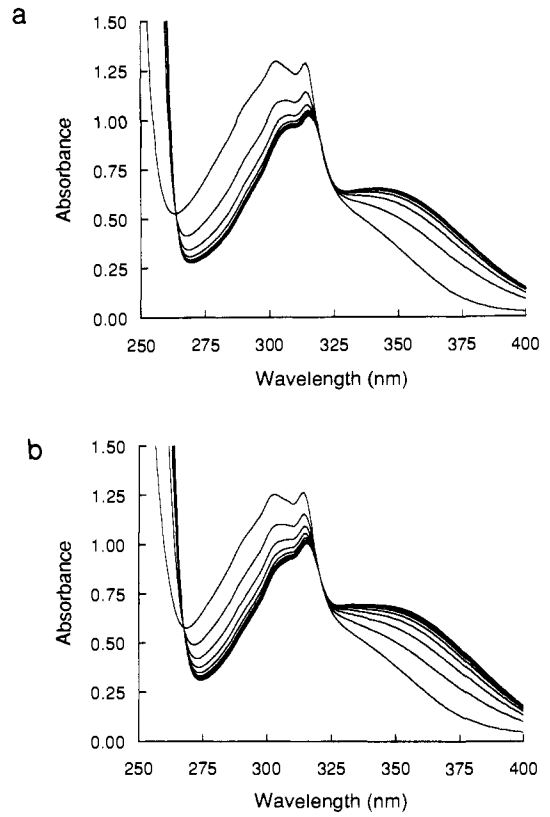



**Figure 1.** Repetitive spectral scans of the hydrolysis of bis(8-hydroxyquinoline) phosphate ($2 \times 10^{-4}$ M) at pH 4.3 ($\mu$ = 1.0 with KCl). (a) Scans taken at 100-min intervals in the absence of metal ions. The absorbance is decreasing from 264 to 319 nm, and it is increasing below 264 nm and above 324 nm. (b) Scans taken at 6-min intervals in the presence of $2 \times 10^{-3}$ M $Ni^{2+}$. The absorbance is decreasing from 268 to 319 nm, and it is increasing below 268 nm and above 322 nm.

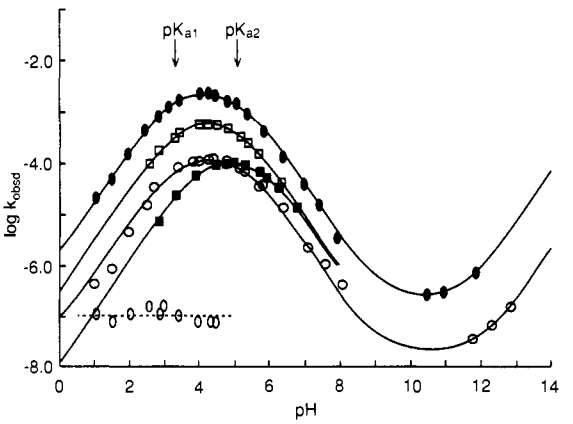



**Figure 2.** The dependence of the pseudo-first-order rate constants ($k_{obsd}$) on acidity for the hydrolysis of bis(6-hydroxyquinoline) phosphate and bis(8-hydroxyquinoline) phosphate in the absence of added metal ions ($\mu$ = 1.0 with KCl). Plots of log $k_{obsd}$ vs pH(D) for the hydrolysis of bis(8-hydroxyquinoline) phosphate ($2 \times 10^{-4}$ M) in $H_2O$ solvent at 60 °C (—●—), 45 °C (—□—), and 30 °C (—○—); hydrolysis in $D_2O$ solvent at 30 °C (—■—). Plot of log $k_{obsd}$ vs pH for the hydrolysis of bis(6-hydroxyquinoline) phosphate ($1 \times 10^{-4}$ M) in $H_2O$ solvent 30 °C (--○--). The solid lines are computer generated by iterative fitting of the experimental points by eq 1 for bis(8-hydroxyquinoline) phosphate. The dashed line indicates a mean for the values of $k_{obsd}$ for bis(6-hydroxyquinoline) phosphate. The indicated values of $pK_{a1}$ and $pK_{a2}$ for the two quinoline nitrogens of bis(8-hydroxyquinoline) phosphate were determined by spectrophotometric titration.

°C compare favorably to those obtained by spectrophotometric titration of $IVH_2^+$ to $IV^-$.

**The dependence of the rate constants for the hydrolysis of bis(8-hydroxyquinoline) phosphate on temperature** (30, 45, and 60 °C) is shown at a number of pH values in Figure 2. Activation parameters were determined from an Arrhenius plot of ln $k_1$ vs $K^{-1}$ (not shown) and Arrhenius and Eyring equations[20] using the values of $k_1$ recorded in Table II. The $\Delta S^a$ for the uncatalyzed reaction is −12.8 eu, and $\Delta H^a$ equals 19.2 kcal $mol^{-1}$ at 30 °C.

**The deuterium solvent kinetic isotope effects in the hydrolysis of bis(8-hydroxyquinoline) phosphate** were determined under an inert $N_2$ atmosphere at 30 °C. Progress of reactions at various pD values in $D_2O$ were followed under the same conditions as reactions in $H_2O$ at constant pH. Values of pD were obtained by adding 0.38 to the pH meter reading.[21] All reactions proceeded to completion and followed the first-order rate law for at least 5 half-lives. The final spectra were of 8-hydroxyquinoline. A comparison of the plots of log $k_{obsd}^H$ and log $k_{obsd}^D$ vs pH(D) is provided in Figure 2. The line which fits the points of log $k_{obsd}^D$ vs pD was generated from part A of eq 1 using the values of $k_1^D$, $K_{a1}^D$, and $K_{a2}^D$ provided in Tables I and II. The ratio of $k_1^H/k_1^D$ does not deviate appreciably from unity (1.05, from Table II) such that there is no kinetic solvent isotope effect. The increase of ca. 0.4–0.6 units in $pK_{a1}^D$ and $pK_{a2}^D$ as compared to $pK_{a1}^H$ and $pK_{a2}^H$ (Table I) is as expected for the transfer of amine from $H_2O$ to $D_2O$ solvent.[22]

**The rates of hydrolysis of bis(6-hydroxyquinoline) phosphate** were followed spectrophotometrically by following the appearance of 6-hydroxyquinoline at 355 nm below pH 5.5 and above pH 8 and at 335 nm from pH 5.5 to 8. Kinetic runs were carried out as before with aqueous buffer solutions at $1 \times 10^{-4}$ M in diester. All reactions were exceedingly slow; hence, initial rates were used to calculate all pseudo-first-order rate constants from less than one half-life of the hydrolysis.

The dependence of $k_{obsd}$ in the hydrolysis of bis(6-hydroxyquinoline) phosphate on pH is quite different from the pH dependence in the hydrolysis of bis(8-hydroxyquinoline) phosphate. An apparent plateau with $k_1 = 1.36 \times 10^{-7}$ $s^{-1}$ was calculated from initial rates from pH 1 to 4.3 at 30 °C and $\mu$ = 1.0 with KCl

(20) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row, Publishers: New York, NY, pp 203 and 209.

(21) Fife, T. H.; Bruice, T. C. *J. Phys. Chem.* **1961**, *65*, 1079.
(22) Hõgfeldt, E.; Bigeleisen, J. *J. Am. Chem. Soc.* **1960**, *82*, 15.

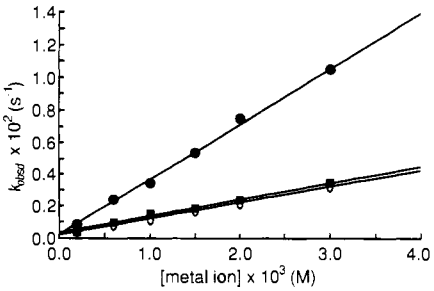



**Figure 3.** Plots of log $k_{obsd}$ vs metal ion concentration for the hydrolysis of $2 \times 10^{-4}$ M bis(8-hydroxyquinoline) phosphate at pH 5.1 and 30 °C ($\mu$ = 1.0 with KCl) in the presence of $Ni^{2+}$ (—●—), $Co^{2+}$ (—■—), and $Zn^{2+}$ (—○—). The three plots are presented as typical of the 30 "metal dilution plots" required for the construction of Figure 4. The slope of the plots represent the apparent second-order rate constant ($k_2'$) for the reaction of the given metal ion with the diester at a given pH.

(Figure 2, Table II). Rates of reactions were not calculated between pH 5 and 13 since the change in absorbance, at any wavelength, was never greater than 0.01 O.D. units over the time period of 1 week.

**The influence of divalent metal ions on the hydrolysis of bis(8-hydroxyquinoline) phosphate and bis(6-hydroxyquinoline) phosphate** was investigated at 30 °C ($\mu$ = 1.0 with KCl) using exactly the same kinetic techniques as employed in the absence of metal ions. Exploratory kinetic studies on bis(6-hydroxyquinoline) phosphate were carried out using a concentration of $1 \times 10^{-2}$ M of the $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, or $Mn^{2+}$ ions at pH 3, 4.5, and 6. *There was found to be no influence of these divalent metal ions on the values of $k_{obsd}$ for the hydrolysis of bis(6-hydroxyquinoline) phosphate* (data not shown).

The influence of $Mn^{2+}$ on the hydrolysis of bis(8-hydroxyquinoline) phosphate was assessed by comparison of the values of $k_{obsd}$ in the absence and presence of $2 \times 10^{-3}$ and $2 \times 10^{-2}$ M $Mn^{2+}$ in the pH range of 2–7. Slight if any catalysis by $Mn^{2+}$ was observed (Table II). The influence of $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ on the hydrolysis of this diester was investigated at five or six concentrations of metal ion between $2 \times 10^{-4}$ and $3 \times 10^{-3}$ M at each pH. Reactions in the presence of $Ni^{2+}$ and $Co^{2+}$ were carried out between pH 2 and 8, while reactions in the presence of $Zn^{2+}$ were studied in the pH range of 2–6. Precipitates of the metal ions or metal ion complexes were seen above pH 5.5–6.5, depending on the metal ion used. The spectrum of bis(8-hydroxyquinoline) phosphate is not appreciably changed upon addition of the metal ions used. In typical repetitive spectral scans between 400 and 250 nm, the time course for the hydrolysis of bis(8-hydroxyquinoline) phosphate in the presence of $2 \times 10^{-3}$ M $Ni^{2+}$ shows clean isosbestic points, demonstrating the absence of accumulation of any intermediate between the phosphodiester substrate and the product metal ion complex of 8-hydroxyquinoline (Figure 1b).

Plots of $k_{obsd}$ vs metal ion concentration are shown in Figure 3 for the hydrolysis of bis(8-hydroxyquinoline) phosphate in the presence of $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ at pH 5.1. The linear relationship seen in Figure 3 is typical of the $k_{obsd}$ vs [metal$^{2+}$] plots for the catalysis of the hydrolysis of bis(8-hydroxyquinoline) phosphate with $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ over the pH ranges studied. The values of the positive intercepts of such plots agree quite well with the values of $k_{obsd}$ seen in the absence of added metal ion. The slopes of the plots of $k_{obsd}$ vs [metal$^{2+}$] at constant pH represent apparent second-order rate constants ($k_2'$) for reaction of metal ion with phosphodiester which results in diester hydrolysis. In Figure 4, values of log $k_2'$ are plotted vs the pH at which they were determined. The experimental points have been fit to a curve generated from eq 2 using the values of $k_2$, $K_{a1}$, and $K_{a2}$ listed in Tables I and II. The log $k_2'$ vs pH profiles for the metal ions

$$k_2' = \frac{k_2 K_{a1} K_{a2}}{K_{a1} K_{a2} + K_{a1} a_H + a_H^2} \qquad (2)$$

$Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ do not exhibit the same "bell-shaped"

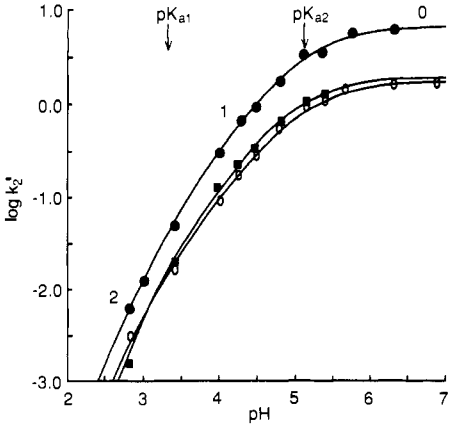



**Figure 4.** Plots of log $k_2'$ vs pH for the hydrolysis of $2 \times 10^{-4}$ M bis(8-hydroxyquinoline) phosphate at 30 °C ($\mu$ = 1.0 with KCl) in the presence of $Ni^{2+}$ (—●—), $Co^{2+}$ (—■—), and $Zn^{2+}$ (—○—). The numbers 2, 1, and 0 relate to the slopes of the plots at given pH regions as predicted by eq 2. The indicated values of $pK_{a1}$ and $pK_{a2}$ for the two quinoline nitrogens of bis(8-hydroxyquinoline) phosphate were determined by spectrophotometric titration.

structure seen in the uncatalyzed hydrolysis of bis(8-hydroxyquinoline) phosphate.

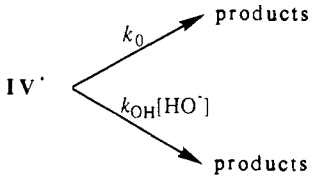
## Discussion

A comparison of the hydrolysis of the phosphodiesters bis(8-hydroxyquinoline) phosphate (IV) and bis(6-hydroxyquinoline) phosphate (V) has been made in water ($\mu$ = 1.0 with KCl) at constant values of pH in the absence and the presence of the metal ions $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, and $Mn^{2+}$. Of the various positional isomers of 8-hydroxyquinoline, $pK_a$ comparisons[18] establish that the phenolic hydroxyl group of 6-hydroxyquinoline most closely resembles that of 8-hydroxyquinoline. Thus, 8-hydroxyquinoline and 6-hydroxyquinoline should be comparable as leaving groups in the hydrolysis of bis(8-hydroxyquinoline) phosphate and bis(6-hydroxyquinoline) phosphate diesters. Comparison of the $K_a$ values for the acid dissociation of the phenolic groups of 8- and 6-hydroxyquinolines shows (Table I) an order of magnitude difference with 6-hydroxyquinoline being the stronger acid. On this basis, the hydrolysis of the 6-hydroxyquinoline diester may be expected to be slightly more facile than the hydrolysis of the isomeric 8-hydroxyquinoline diester. With bis(8-hydroxyquinoline) phosphate, the two quinoline nitrogens are adjacent to two phosphate ester oxygens such that whether free, protonated, or in loose association with metal ions, the positioning of this isomer's quinoline nitrogens allows both electronic interactions and neighboring group effects in phosphate ester hydrolysis. In the case of the 6-hydroxyquinoline diester, protonation of or loose metal ion ligation to the quinoline nitrogens could only influence diester hydrolysis through an electronic effect. Electronic effects upon the O–P ester bonds due to protonation or metal ion association should be similar for both esters. The objective in studying both bis(6-hydroxyquinoline) phosphate and bis(8-hydroxyquinoline) phosphate diesters is clear; by doing so, electronic effects can be separated from any catalytic effects due to neighboring quinoline nitrogens.[23]

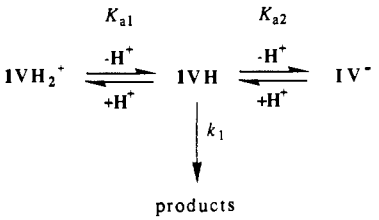
**The pH dependence of the pseudo-first-order rate constants ($k_{obsd}$) for the hydrolysis of bis(8-hydroxyquinoline) phosphate** (Figure 2) is provided by eq 1 when using the values of $k_1$, $K_{a1}$, $K_{a2}$, $k_0$, and $k_{OH}$ given in Tables I and II. The spontaneous hydrolytic term ($k_0$) and the hydroxide catalyzed hydrolytic term ($k_{OH}K_w/a_H = k_{OH}[HO^-]$) are only of importance at a pH which exceeds the $pK_a$ values of the substrate. Therefore, these two terms represent $H_2O$ and $HO^-$ catalysis of the hydrolysis of the species $IV^-$ (Scheme II). Usually the log $k_{obsd}$ vs pH profiles for the hydrolysis of phosphate diesters are characterized by proton catalysis at low pH, a pH independent region with exceedingly small

(23) Maugh, T.; Bruice, T. C. *J. Am. Chem. Soc.* **1971**, *93*, 3237.

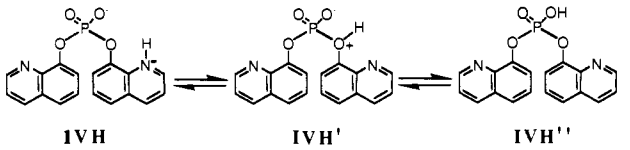
Scheme II



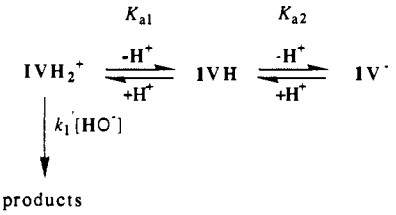

Scheme III




Scheme IV




Scheme V



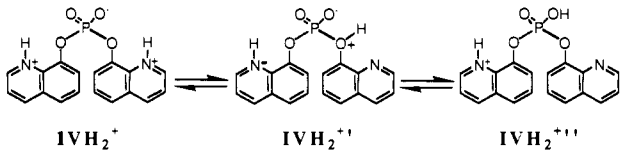

rate constants in a mid-pH range, and hydroxide catalysis at high pH.[1b,24] The hydrolysis of the $IV^-$ species shares with other phosphate diesters the pH independent ($k_0$) and $HO^-$ catalyzed ($k_{OH}[HO^-]$) pathways for hydrolysis. Of more interest is the observation (Figure 2) that the log $k_{obsd}$ vs pH profile for hydrolysis of bis(8-hydroxyquinoline) phosphate between pH 1 and 8 is "bell-shaped". Such log rate constant vs pH profiles are typically seen in the hydrolysis of monophosphate esters[10,25] but not in the hydrolysis of phosphate diesters. The "bell" is bounded between the two $pK_a$'s of the quinolinic amines with the maximum velocity of hydrolysis occurring ca. midway between the two amine $pK_a$ values. These general observations are in accord with the spontaneous or water catalyzed hydrolysis of the IVH species as shown in Scheme III. The derived rate expression for Scheme III is term A of eq 1. The values of the kinetically derived $pK_{a1}$ and $pK_{a2}$ constants compare favorably to those obtained by spectrophotometric titration of $IVH_2^+$ to $IV^-$ (Scheme I). In Scheme III, the structure IVH may be replaced by the proton isomeric species IVH' or IVH'' (Scheme IV) since the position of a dissociable proton cannot be determined by kinetic means, including heavy isotope effects. Also, the reaction sequence of Scheme III is not unique; it is kinetically equivalent to hydroxide ion hydrolysis of $IVH_2^+$ (Scheme V and the rate expression of eq 3). From eqs 1 and 3, $k_1 = k_1'K_w/K_{a1}$ and since (at 30 °C) $k_1 = 1.51 \times 10^{-4}$ s$^{-1}$, $K_w =$

$$v = \frac{k_1'K_wK_{a1}a_H}{K_{a1}\{K_{a1}K_{a2} + K_{a1}a_H + a_H^2\}} \quad (3)$$

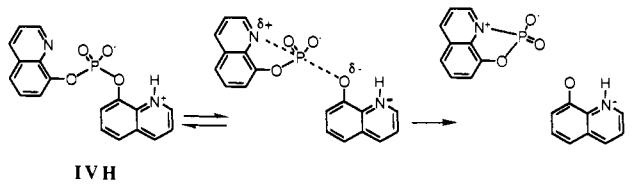
$1.47 \times 10^{-14}$, and $K_{a1} = 4.90 \times 10^{-4}$, the value of $k_1'$ (Scheme V) is equal to the allowed value of $5 \times 10^6$ s$^{-1}$. Therefore, the reaction sequence of Scheme V must be considered. Structure $IVH_2^+$ of Scheme III may be replaced by the proton isomeric species $IVH_2^{+\prime}$ and $IVH_2^{+\prime\prime}$ (Scheme VI).

(24) Kirby, A. J.; Younas, M. *J. Chem. Soc. (B)* **1970**, 510.

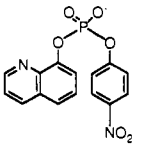
Scheme VI




Scheme VII

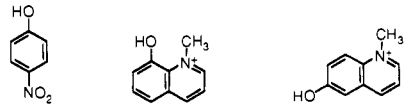



A useful analogy to the hydrolysis of bis(8-hydroxyquinoline) phosphate is the hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate (VI).[26] The rate constants for the spontaneous or water




catalyzed hydrolysis of VI ($3.5 \times 10^{-4}$ s$^{-1}$) and IV ($1.5 \times 10^{-4}$ s$^{-1}$) are comparable. This can be explained provided the $pK_a$ values for 4-nitrophenol (VII) and N-protonated 8-hydroxyquinoline are similar. If this is so, then these two species should be comparable leaving groups. Examination of the pertinent $pK_a$ values of VII and the N-methylated hydroxyquinolines[18] in Chart I leads one to believe that N-protonated hydroxyquinolines and 4-nitrophenol do indeed have $pK_a$ values that are very close. The same favorable comparison of $pK_a$ values is expected for N-protonated 6- and 8-hydroxyquinolines such that they would be comparable as leaving groups.

Chart I




| | VII | VIII | IX |
|---|---|---|---|
| $pK_a$ | 7.15 | 6.81 | 7.15 |

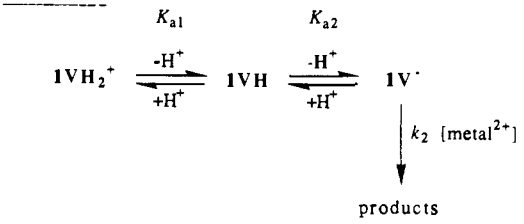
The hydrolysis of bis(6-hydroxyquinoline) phosphate is much slower than the hydrolysis of bis(8-hydroxyquinoline) phosphate at all pH values studied. Also, the values of the pseudo-first-order rate constants for hydrolysis of the 6-hydroxyquinoline ester do not exhibit the "bell-shaped" pH dependence characteristic of the hydrolysis of the 8-hydroxyquinoline ester. At pH 4.5 the values for the rate constants for hydrolysis of the 6- and 8-hydroxyquinoline esters are maximally separated. At this pH, the pseudo-first-order rate constant for hydrolysis of bis(8-hydroxyquinoline) phosphate exceeds that for hydrolysis of bis(6-hydroxyquinoline) phosphate by 1100-fold. We may conclude that this enhanced reactivity is dependent on the presence of the unprotonated neighboring quinoline nitrogen of IVH, and, thus, Scheme V may be dismissed.

A deuterium solvent kinetic isotope effect ($k_1^H/k_1^D = 1.05$, Figure 2, Table II) is not observed for the hydrolysis of bis(8-hydroxyquinoline) phosphate between pD 2.8 and 6.8 (the pD-rate maximum). Neither general-acid nor general-base catalysis of hydrolysis by added buffer species {$HCO_2^-/HCO_2H$, $CH_3CO_2^-/CH_3CO_2H$, $C_5H_5N/C_5H_5N^+H$, $(CH_3)_2AsO_2^-/(CH_3)_2AsO_2H$, $(HOCH_2)_3CNH_2/(HOCH_2)_3CNH_3^+$} is found. We may conclude that there is no proton transfer concerted with

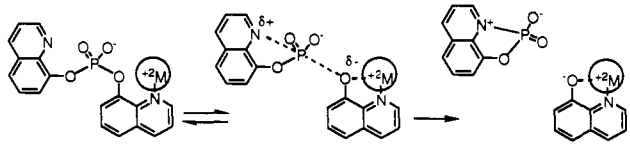
(25) Hofstetter, R.; Murakami, Y.; Mont, G.; Martell, A. E. *J. Am. Chem. Soc.* **1962**, *84*, 3041.

(26) Loran, J. S.; Williams, A. *J. Chem. Soc., Perkin Trans. II* **1977**, 64.

**Scheme VIII**




**Scheme IX**



$IV^-$

rate determining P–O bond formation or P–O bond breaking. The essential role of the neighboring quinoline nitrogen may be as a nucleophilic catalyst with the monoprotonation of the diester to give **IVH** or **IVH′** and thereby converting 8-hydroxyquinolinate into a better leaving group (Scheme VII). An intramolecular reaction, as in Scheme VII, is in accord with the low value of $\Delta S^{\ddagger} = -12.8$ eu. Loran and Williams also invoked nucleophilic catalysis in the hydrolysis of **VI** for which they observed neither a deuterium solvent kinetic isotope effect nor buffer catalysis.[26] From the ratio $k_q$ ($s^{-1}$)/$k_{pyr}$ ($M^{-1}$ $s^{-1}$) (eqs 4 and 5) they calculated an effective molarity of the unprotonated quinoline amine in **VI**

$$v = k_{pyr}[\text{methyl 2-nitrophenyl phosphate}][\text{pyridine}] \quad (4)$$

$$v = k_q[\text{4-nitrophenyl quinolin-8-yl phosphate}] \quad (5)$$

to be ca. 7000 M. Such an effective molarity would explain our observation that pyridine buffer does not behave as a catalyst for the hydrolysis of bis(8-hydroxyquinoline) phosphate. The highest molarity of pyridine used was ca. 4 orders of magnitude less than the effective molarity in the intramolecular reaction of **IVH**.

**Metal Ion Catalysis.** At pH values between 2.8 and 6.3 with $Ni^{2+}$, pH 2.8 and 6.9 with $Co^{2+}$, and pH 2.8 to 5.2 with $Zn^{2+}$, the rate of hydrolysis of bis(8-hydroxyquinoline) phosphate is dependent on the concentration of these metal species. There is no influence of $Mn^{2+}$ on bis(8-hydroxyquinoline) phosphate hydrolysis between pH 2 and 7. The rate of hydrolysis of the isomeric bis(6-hydroxyquinoline) phosphate is insensitive to the presence of $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, or $Mn^{2+}$.

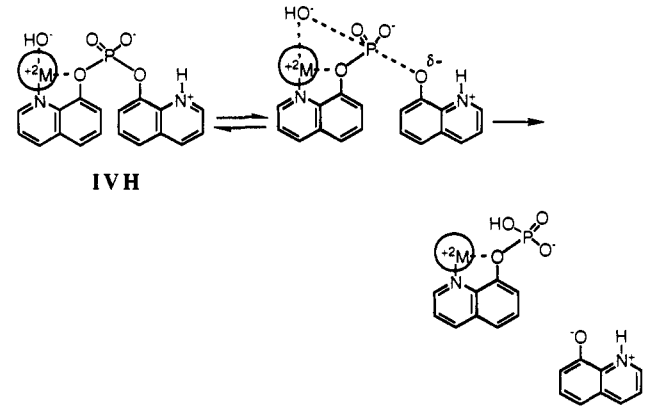
Since the hydrolysis of bis(8-hydroxyquinoline) phosphate follows the first-order rate law in the presence of catalytic concentrations of metal ion and since the pseudo-first-order rate constants ($k_{obsd}$) at constant pH are linearly dependent upon metal ion concentration, reactions are first-order in diester and first-order in metal ion (eq 6). The experimental points in the plots of log $k_2'$ vs pH (Figure 4) in the presence of $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ were

$$-\text{d[diester]}/dt = k_2'[\text{diester}][\text{metal ion}] \quad (6)$$

fit to eq 2 which can be derived from Scheme VIII. One can see that a line generated from eq 2 to fit the experimental points of the log $k_2'$ vs pH profile must have three distinct zones: (i) a region with a slope of 2, (ii) a region with a slope of 1, and (iii) a pH independent region with a slope of 0. That the experimental points fit such an expression (Figure 4) is highly supportive of the reaction sequence of Scheme VIII. Values of calculated kinetic $pK_{a1}$ and $pK_{a2}$ constants in the absence and presence of $Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$ are shown in Table I and are very close to those determined spectrophotometrically in the absence of metal ions.

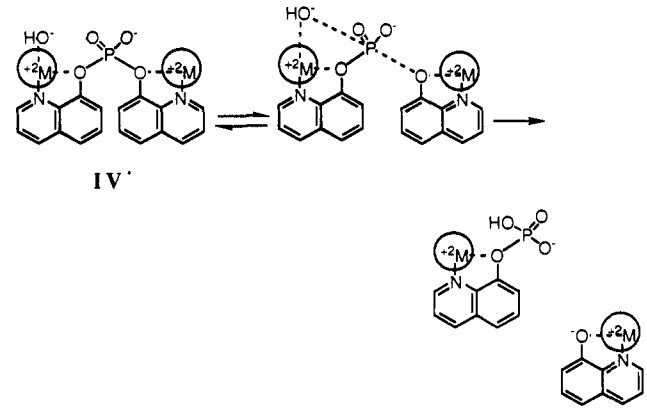
While the hydrolysis of bis(8-hydroxyquinoline) phosphate has **IVH** as the reactive species in the absence of metal ions (Scheme III), metal ion catalysis of the hydrolysis of bis(8-hydroxyquinoline) phosphate involves $IV^-$ as the reacting diester (Scheme VIII). The most likely mechanism for the metal catalyzed reaction is provided in Scheme IX. A comparison of the numerical values

**Scheme X**



**IVH**

**Scheme XI**



$IV^-$

of the rate constant for the nonmetal ion ($k_1$, $s^{-1}$) reaction and the metal ion catalyzed ($k_2$, $M^{-1}$ $s^{-1}$) hydrolysis at 1.0 M metal ion is provided by the ratio $k_2/k_1$. If bis(8-hydroxyquinoline) phosphate is such a poor ligand that its complexes with metal ions do not accumulate at 1 M metal ion, then the rate ratios at 1 M metal ion are $4.66 \times 10^4$ for $Ni^{2+}$; $1.21 \times 10^4$ for $Co^{2+}$; and $1.28 \times 10^4$ for $Zn^{2+}$. The magnitude of the rate enhancement due to a combination of metal ion catalysis (at 1 M metal ion) and intramolecular nucleophilic catalysis (Scheme IX) can be estimated to be in the range of $1-5 \times 10^7$ by comparing the value of $k_2$ to the rate constant for hydrolysis of bis(6-hydroxyquinoline) phosphate.

## Conclusions

From a comparison of the kinetics of hydrolysis of bis(8-hydroxyquinoline) phosphate, bis(6-hydroxyquinoline) phosphate, and 4-nitrophenyl quinolin-8-yl phosphate, we have reached the following conclusions. The facile hydrolysis of bis(8-hydroxyquinoline) phosphate between pH 1 and 8 is dependent upon nucleophilic attack of one quinoline nitrogen upon phosphate with concurrent protonation of the second quinoline nitrogen to create a leaving group comparable to 4-nitrophenolate (Scheme VII). The combination of nucleophilic and specific acid catalysis provides a rate enhancement of $1.1 \times 10^3$ for bis(8-hydroxyquinoline) phosphate when compared to the hydrolysis of bis(6-hydroxyquinoline) phosphate. Nucleophilic attack of a quinoline nitrogen upon phosphate is also involved in the metal ion ($Ni^{2+}$, $Co^{2+}$, and $Zn^{2+}$) catalysis of the hydrolysis of bis(8-hydroxyquinoline) phosphate. The role of the metal ion is to create a better leaving group (as shown in Scheme IX). Preassociation of a metal ion with the bis(8-hydroxyquinoline) phosphate ground state is weak because the only ligand groups available are a quinoline nitrogen and possibly a phosphate anionic oxygen. As the reaction progresses, a partial charge develops on the departing oxygen such that association of metal ion is enhanced in the transition state when compared to the ground state. The combined nucleophilic and metal ion catalysis provides, at 1 M metal ion, a rate enhancement of $10^7$ when compared to the hydrolysis of bis(6-hydroxyquinoline)

phosphate. Thus, metal ion catalysis is much more effective than specific acid catalysis via protonation of a quinoline nitrogen. This suggests that the metal must interact directly with the departing oxygen of the leaving group (Scheme IX), while the proton is much less effective in this interaction (Scheme VII). Proton transfer from N → O in the transition state is not important as shown by the absence of a deuterium solvent kinetic isotope effect in the hydrolysis of protonated (deuterated) bis(8-hydroxyquinoline) phosphate.

The following putative roles for metal ion as catalyst have not been observed in this study. The metal ion does not act to deliver $HO^-$ to the phosphorus center with specific acid catalysis of departure of the leaving group (Scheme X) or with metal ion catalysis of departure of the leaving group (Scheme XI) because the loose preassociation of metal ion does not allow these mechanisms to compete with those in which the quinoline nitrogen acts as the attacking nucleophile at the phosphorus center. Studies of models are under investigation which may exhibit the mechanisms of Schemes X and/or XI.

**Acknowledgment.** This work was supported by grants from the Office of Naval Research and the National Science Foundation.

# New Insight into the Mechanism of Base Propenal Formation during Bleomycin-Mediated DNA Degradation

**G. H. McGall,[†] Lois E. Rabow,[‡] G. W. Ashley,[§] S. H. Wu,[∥] J. W. Kozarich,*[,⊥] and J. Stubbe***

*Contribution from the Department of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, SYVA, Palo Alto, California 94303, Department of Pharmacology, Boston University Medical School, Boston, Massachusetts 02118, Department of Chemistry, Northwestern University, Evanston, Illinois 60208, Research Division, W. R. Grace and Co., Columbia, Maryland 21044, and Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742.* *Received October 21, 1991*

**Abstract:** The mechanism of interaction of the antitumor antibiotic bleomycin (BLM) and cofactors with DNA to produce base propenal and 3′-phosphoglycolate and 5′-phosphate ends has been investigated. Analytical methods have been developed that allow the isolation of glycolic acid from the modified DNA without washout of isotopic labels. Results using $^{18}O_2$, $H_2{}^{18}O$, and $H_2{}^{18}O_2$ under a variety of conditions show that 1 mol of $^{18}O$ is found per mole of glycolate in the carboxylate group and is derived from the $^{18}O_2$ not used in the formation of activated BLM. The second oxygen in the carboxylate of glycolate is derived from the 4-oxygen in the deoxyribose moiety from which it is generated. The oxygen in the aldehyde of the base propenal appears to be derived exclusively from $H_2O$. Studies using poly[dA(2′-*pro*-*R*-$^3$H)dU] and poly[dA(2′-*pro*-*S*-$^3$H)dU] demonstrate, as originally proposed by Burger et al. [Burger, R. M.; Projan, S. J.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1986**, *261*, 15855], that 2′-*pro*-*R*-hydrogen cleavage and DNA strand scission occur more rapidly than the rate of base propenal formation. An alternative mechanism to the one currently favored involving intermediate **6** (Scheme I) is proposed to accommodate all of the available data.

The bleomycins are a group of antitumor antibiotics first isolated by Umezawa in 1966.[1] These compounds are used clinically in the treatment for head and neck cancer, testicular cancer, and squamous cell carcinomas;[2] their cytotoxicity is thought to be related to their ability to bind to and degrade double-stranded DNA.[3] The DNA cleavage reaction requires two cofactors: a metal, either $Fe^{2+}$ or $Cu^+$, and $O_2$.[4,5] Extensive efforts by numerous investigators in the past decade have provided much information about the products produced during BLM-mediated degradation of DNA and the chemistry of their formation (Scheme I).[6,7]

Two monomeric products, trans base propenal **7** and nucleic acid base, have been shown to ultimately result from a putative common intermediate **1**[8-10] (Scheme I). This intermediate is produced by cleavage of the 4′ carbon–hydrogen bond of a nucleotide residue by activated BLM.[10] This reaction exhibits sequence-specificity for a pyrimidine moiety to the 3′-side of a deoxyguanosine residue.[11,12] Activated iron–BLM results from the interaction of $Fe^{2+}$, BLM, and $O_2$ and requires the presence of an additional electron.[13] The actual structure of the activated BLM species remains to be elucidated. While the pathway that leads to free nucleic acid base release has been established using a variety of methods (Scheme I, pathway B),[14-18] the pathway

* Author to whom correspondence should be addressed at the Massachusetts Institute of Technology.
[†] SYVA.
[‡] Boston University Medical School.
[§] Northwestern University.
[∥] W. R. Grace & Co. S. H. Wu was the recipient of a Damon Runyon–Walter Winchell Postdoctoral Fellowship.
[⊥] University of Maryland.

(1) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot., Ser. A* **1966**, *19*, 200.
(2) Sikik, B. I., Rozencweig, M., Carter, S. K., Eds.; *Bleomycin Chemotherapy*; Academic Press: London, 1985.
(3) Cullinan, E. B.; Gawron, L. S.; Rustum, Y. M.; Beerman, T. A. *Biochemistry* **1991**, *30*, 3055.
(4) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814.
(5) Ehrenfeld, G. M.; Shipley, J. B.; Heimbrook, D. C.; Sugiyama, H.; Long, E. C.; van Boom, H. G.; van der Marcel, G. A.; Oppenheimer, N. J.; Hecht, S. M. *Biochemistry* **1987**, *26*, 931.
(6) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.
(7) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383.
(8) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608.
(9) Burger, R. M.; Berkowitz, A. R.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832.
(10) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562.
(11) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 7983.
(12) D'Andrea, A. D.; Haseltine, N. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608.
(13) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636.
(14) Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196.

0002-7863/92/1514-4958$03.00/0 