Catalytic Hydrolysis of a Phosphate Triester by Tetracoordinated Zinc Complexes[1]

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Abstract: A zinc complex of a tetraaza macrocycle catalyzes the hydrolysis of diphenyl *p*-nitrophenyl phosphate in aqueous acetonitrile. The principal products derive from loss of *p*-nitrophenol, but some alternative hydrolysis with loss of phenol is also observed. Kinetic studies show that the catalytic zinc species is a zinc hydroxide complex with a pK_a of 8.7 (or its kinetic equivalent). The greater kinetic effectiveness of this weak base than of free hydroxide ion itself, on a molar basis, indicates a bifunctional mechanism in which bound hydroxide acts as a nucleophile, while zinc acts as an electrophilic catalyst. This process is relatively strainless at phosphorus, in contrast to reactions at carbon with the same species in which the bifunctional mechanism would be strained and is not seen. A derivative of the zinc macrocycle complex carrying a long alkyl chain was examined as the catalyst for the same substrate in a Brij micelle. This lipophilic complex is even more effective, acting as a zinc hydroxide species with $pK_a = 9.1$, but in the micellar reaction there are contributions from kinetic terms higher than first order in the zinc complex. Thus, in this system, catalysis by aggregates is apparently also occurring.

The biological importance of phosphate esters and anhydrides is well-known. The significance of these classes of compounds extends beyond the wide variety of naturally occurring examples to man-made derivatives that are employed for pest control, chemical warfare, and numerous industrial tasks. The chemistry of substituent exchange, such as hydrolysis, at phosphorus(V) centers has received considerable attention because such processes occur in many crucial enzymatic reactions and are relevant to the detoxification of some pesticides and chemical weapons.

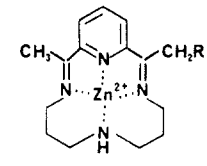
Many of the enzymes involved in nucleophilic displacement on phosphorus(V) substrates require metal cations for activity,[3] and there have been numerous reports of metal-based model systems.[4,5] Hydrolytic reactions of natural and unnatural substrates have been popular, but despite the wide range of systems studied, few have been demonstrably catalytic. Aquohydroxo(tetraamine)cobalt(III) complexes were recently found to promote hydrolysis of greater than stoichiometric quantities of *p*-nitrophenyl methylphosphonate and ethyl *p*-nitrophenyl methylphosphonate.[5d] The observed rate constant diminshed over the course of the reaction, apparently because of product inhibition.

The catalyzed hydrolysis of diphenyl *p*-nitrophenyl phosphate (DPPNPP) has been observed in nonmetallic systems. Some time ago, Bunton and co-workers found that micellar cetyltrimethylammonium bromide (CTAB) enhances the reaction of hydroxide and fluoride anions with DPPNPP.[6] Subsequent reports from that laboratory showed that even greater enhancements resulted from placing a hydroxyl group on the cationic surfactant, near the quaternary center.[7] This system was not clearly catalytic, in that no turnover was demonstrated. Two recent reports, one from Moss et al.[8] and the other from Menger and Whitesell,[9] have described micellar systems for the phosphate triester that offer more dramatic rate enhancements and rapid turnover of the reactive groups. Both systems seem to involve nucleophilic catalysis.

The common catalytic strategy of these two nonmetallic systems is reminiscent of one of the many ways that a metal cation can, in principle, facilitate phosphate ester hydrolysis.[4] Metal ions can lower the pK_a of a ligated water molecule, generating relatively large concentrations of a potent nucleophilic catalyst, the metal-bound hydroxide, near neutral pH. Sargeson and co-workers have provided strong evidence for this mechanistic path in the release of *p*-nitrophenol from the complex hydroxo(*p*-nitrophenyl phosphato)bis(ethylenediamine)cobalt(III).[10] In the catalytic cycle of alkaline phosphatase, hydrolysis of the phosphoryl-enzyme intermediate is thought to involve attack by a zinc-bound hydroxide.[11]

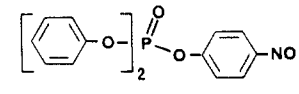
One of the problems in developing efficient, catalytic zinc-based hydrolytic agents is the precipitation of polymeric zinc hydroxide above neutral pH. In spite of a number of attempts[12,13] to develop polyimidazole ligands whose zinc complexes might imitate the zinc imidazole system in alkaline phosphatase or in carbonic anhydrase, one of the few zinc complexes so far known to hold up to alkaline conditions is the macrocyclic system **1**.[14] Woolley has shown that this stable zinc complex can catalyze the hydration of acetaldehyde and of CO_2, by analogy to carbonic anhydrase.[15] More recently, this complex has been reported to catalyze the hydrolysis of carboxylate esters.[16]

We have now found that **1** also catalyzes the hydrolysis of diphenyl *p*-nitrophenyl phosphate (DPPNPP) in a base-promoted reaction. Furthermore, we have prepared the related compound



1 R=H

2 R = $C_{16}H_{33}$



DPPNPP

2 whose long alkyl chain solubilizes it in organic media. In Brij micelles, **2** is a particularly effective catalyst for DPPNPP hy-

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drolysis. Finally, the effectiveness of these catalysts is such that one must apparently invoke a bifunctional mechanism, with both Lewis acid and nucleophilic components. Such a bifunctional mechanism may well be involved in analogous enzymic zinc-catalyzed reactions.

Experimental Section

Materials. ZnCR(Br)(ClO_4) (1) was prepared according to the reported procedure.[14] IR (protol): 3230, 1645, 1585 cm^{-1}. UV (CH_3CN, 0.025 mM): 219 nm (ϵ = 21 300), 297 (ϵ = 3320).

$(ZnCR)_2(ClO_4)_3(OH)$[14] was prepared analogously to the bromide perchlorate described above, except that $Zn(ClO_4)_2 \cdot 6H_2O$ was used in place of $ZnBr_2$. Yield, after recrystallization from water and vacuum dessication: 21%. IR (protol): 3540, 3250, 3080, 1640, 1585 cm^{-1}. UV (CH_3CN, 0.025 mM): 216 nm (ϵ = 16 700), 297 (ϵ = 3620).

2-(1-Oxoheptadecyl)-6-acetylpyridine (3). Potassium *tert*-butoxide (13.9 g, 0.12 mol) was placed in a dry 500-mL round-bottomed flask under Ar, 100 mL of THF (freshly distilled from potassium metal/benzophenone under N_2) was added, and the slurry was cooled to −60 °C. A solution of 16.3 g (0.10 mol) of 2,6-diacetylpyridine in 100 mL of distilled THF was added by syringe over 15 min. The resulting yellow–orange mixture was allowed to stir a further 30 min and then a solution of 30 mL (0.095 mol) of 1-iodohexadecane in distilled THF was added by syringe over 15 min. Considerable percipitation formed at this point. The mixture gradually turned red–brown and was allowed to warm slowly to room temperature overnight. The reaction was quenched by addition of 30 mL of 10% aqueous NH_4Cl. THF was removed by rotary evaporation, and the resulting aqueous mixture was extracted 3 times with CH_2Cl_2. Combined organic layers were dried over $MgSO_4$, filtered, and evaporated to a crude product which was further purified by flash chromatography on SiO_2 eluting with 4% ethyl acetate in petroleum ether. The off-white solid (3.2 g, 8% yield) had mp 63–65 °C; it was sufficiently pure to be used in the next step. It could be recrystallized from acetone to give mp 64–65.5 °C. IR (protol): 1700, 1580 cm^{-1}. NMR (200 MHz, $CDCl_3$): δ 8.21 (d, 2 H, J = 7.6 Hz), 7.99 (m, 1 H), 3.27 (t, 2 H, J = 7.4 Hz), 2.79 (s, 3 H), 1.76 (m, 2 H), 1.26 (m, ca. 30 H), 0.88 (m, 3 H). CI-MS (NH_3): m/e 388 (M + 1) (95%), 405 (M + 18) (60%), 250 (35%), 181 (100%), 164 (85%), 122 (40%).

ZnHCR(Br)(ClO_4) (2). To a suspension of 163 mg (0.42 mmol) of diketone **3** in 10 mL of DMF were added 230 mg (1.0 mmol) of $ZnBr_2$ and 135 mg of (1.0 mol) 3,3′-diamino-di-*n*-propylamine. Deionized water (6 mL) was added and then 10 mL of DMF. The mixture was heated to gentle reflux for 2 h. After the turbid mixture had cooled, 10 mL of 50% aqueous $NaClO_4$ was added. The resulting off-white precipitate was isolated by suction filtration, washed with deionized water, and vacuum-dessicated to give 232 mg. IR indicated that this crude product was contaminated with starting diketone. The solid was suspended in 50 mL of petroleum ether, stirred for 90 min, and then reisolated by suction filtration to give 173 mg of off-white powder (57%). IR (protol): 3250, 1645, 1590 cm^{-1}. UV (CH_3CN, 0.025 mM): 218 nm (ϵ = 20 400), 298 (ϵ = 3520). Anal. Calcd (found) for $C_{31}H_{54}N_4O_4ClBrZn$: C, 51.17 (50.79); H, 7.48 (7.65); N, 7.70 (7.65); Zn, 8.99 (8.76); Cl, 4.87 (7.07).

$(ZnHCR)_2(ClO_4)_3(OH)$ was prepared and purified in 50% yield analogously to the bromide perchlorate salt described above, except that $Zn(ClO_4)_2 \cdot 6H_2O$ was used in place of $ZnBr_2$. IR (protol): 3550, 3320, 3260, 3090, 1680, 1640, 1590 cm^{-1}. UV (CH_3CN, 0.025 mM): 198 nm (ϵ = 21 700), 282 (ϵ = 5160). Anal. Calcd (found) for $C_{62}H_{109}N_8O_{13}Cl_3Zn_2 \cdot 5H_2O$: C, 49.58 (49.75); H, 7.99 (7.85); N, 7.46 (7.63); Cl, 7.08 (8.81).

Diphenyl *p*-nitrophenyl phosphate was prepared as described,[17] mp 47–48 °C [lit.[17] mp 49–51 °C] from ethanol. NMR (200 MHz, $CDCl_3$): δ 8.35 (m, 2 H), 7.58 (m, 2 H), 7.50 (m, 4 H), 7935 (m, 6 H). CI-MS, (NH_3): m/e 389 (100%) (M + 18). EI-MIs: m/e 371 (100%), no major fragments of m/e > 100.

Diphenylphosphoric acid was recrystallized from $CHCl_3$/petroleum ether, mp 66.5–68 °C [lit.[18] mp 63–64 °C].

Phenyl(*p*-nitrophenyl)phosphoric acid was prepared by the hydrolysis of phenyl di-*p*-nitrophenyl phosphate[19] with aqueous LiOH.[20] Reverse-phase HPLC showed this material to have only trace UV-active impurities (no detectable phenol or *p*-nitrophenol); it was recrystallized from CCl_4 to give pure solid, mp 99.5–100.5 °C [lit.[21] mp 101–102 °C]. NMR (200 MHz, Me_2SO-d_6): δ 8.24 (m, 2 H), 7.44 (m, 2 H), 7.32 (m, 2 H), 7.15 (m, 3 H). CI-MS (NH_3): m/e 313 (M + 18) (100%), 330 (M + 35) (35%).

All other materials were obtained commercially and used without further purification.

Methods

Kinetics. Aminosulfonate buffers (HEPPS, MES, HEPES, CHES, and CAPS) were obtained from Sigmal Chemical Co. and used without further purification. Buffered reaction solutions in aqueous CH_3CN were prepared by mixing all water-soluble components in an appropriate volume of deionized water and adjusting the pH, as measured by a calibrated glass electrode, with 1 N NaOH. (Concentrated buffer stock solutions were adjusted so that reaction mixtures would initially be at or slightly below the desired pH.) An equivalent volume of CH_3CN (Kodak, spectral grade) was then added (containing the complex of HCR when this was used), followed by an appropriate amount of a concentrated CH_3CN solution of the phosphate triester. NaOH reaction solutions in aqueous CH_3CN were prepared by mixing appropriately diluted NaOH and CH_3CN with DPPNPP added last in concentrated CH_3CN solution.

Brij 35 was obtained from Aldrich Chemical Co. and used without further purification. Buffered reaction solutions containing ZnHCR(Br)(ClO_4) were prepared by stirring an appropriate amount of solid complex with 25 mM Brij 35 in deionized water overnight and then adding concentrated buffer and deionized water to 20 mM Brij 35 and desired final buffer and ZnHCR concentrations. The pH was adjusted by addition of 1 N NaOH. The maximum ZnHCR(Br)(ClO_4) concentration in 20 mM aqueous Brij 35 seemed to be approximately 0.5 mM. For micellar reactions involving $(ZnHCR)_2(ClO_4)_3(OH)$, the 25 mM Brij 35 solution had to be stirred for several days at room temperature before all solid had disappeared. DPPNPP hydrolysis was initiated by addition of concentrated substrate solutions in Me_2SO (final Me_2SO concentration 0.1%) followed by vigorous mixing. NaOH reaction solutions in micellar Brij 35 were prepared by adding aqueous NaOH of appropriate concentration to 25 mM Brij 35.

Reactions were followed by changes in absorbance at 400 nm (*p*-nitrophenolate absorption maximum) recorded automatically by a programmable Beckman DU-8 spectrophotometer. The cell holder was thermostated to 25 °C for all reactions. Reactions were generally followed to >90% completion and showed good first-order behavior (correlation coefficients >0.999).

HPLC. Product analysis was performed on an IBM 5-μm C_8 analytical column fitted with a hand-packed C_{18} guard column, using a Waters Assoc. M-6000 solvent delivery system and a Rheodyne 7125 injector fitted with a 0.02-mL loop. All injections were 0.2 mL. Samples eluted with 1:1 MeOH/50 mM phosphate buffer, pH 7.00, flow rate 0.6 or 0.7 mL/min. The eluent was monitored at 260 nm with a Gilson variable-wavelength detector, and peaks were recorded on a Spectra-Physics SP-4100 computing integrator.

Peak quantification was accomplished by the external standard method. Six- or seven-point calibration curves, 0.1–1.0 mM in each of the four products (*p*-nitrophenol (PNP), phenol (PhOH), diphenylphosphoric acid (DPPA), and phenyl (*p*-nitrophenyl)phosphoric acid (PPNPPA), were constructed based on a series of injections made immediately before the sample injection of interest. Sample peaks could be quantified by either peak area or peak height. The calibration solutions were always prepared by using a solvent system identical with that of the reaction solution to be analyzed (including zinc complexes, when appropriate), which was important because injection solvent had a profound effect on peak shape. The results of these analyses are reported as DPPA/PPNPPA ratios in the text because PhOH peaks were sometimes too small to allow accurate quantification, and PNP peaks were partially obscured by injection artifacts with some solvents. In general, however, measured PNP concentrations agreed well with DPPA concentrations and PhOH concentrations with PPNPPA concentrations. Reactions for product analysis were run with 1.0 mM DPPNPP. Reaction solutions were stirred at room temperature until analysis; analysis of the same sample at different long reaction times demonstrated that the reactions were complete.

Results

Kinetics in Aqueous CH_3CN. Because DPPNPP is insoluble in pure water, we selected CH_3CN as a cosolvent that would allow sufficient solubilities of potential catalysts, substrate, and buffers but not participate in the reactions. Rather than trying to measure pH directly in the aqueous CH_3CN solutions, we chose the ex-

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Table I. Apparent Second-Order Rate Constants for DPPNPP Hydrolysis in 50% Aqueous CH_3CN at pH 8.00[a]

hydrolytic agent	app k_2, M^{-1} s^{-1} [b]
$(ZnCR)_2(ClO_4)_3(OH)$ (excess)[c]	4.6×10^{-2}
$(ZnCR)_2(ClO_4)_3(OH)$ (catalytic)[d]	5.3×10^{-2}
$ZnCR(Br)(ClO_4)$ (excess)[e]	2.7×10^{-2}
NaOH[f]	2.8×10^{-2}
HEPPS[g]	8.6×10^{-5}

[a] Reaction solutions initially 0.1 mM in DPPNPP unless otherwise indicated. [b] All linear regression analyses of pseudo-first-order rate constants show correlation coefficients >0.99. [c] Based on pseudo-first-order rate constants for 0.54, 0.81, 1.08, 1.35, and 1.62 mM $(ZnCR)_2(ClO_4)_3(OH)$, 10 mM HEPPS, pH 8.00. [d] Based on pseudo-first-order rate constants for 0.054, 0.108, and 0.216 mM $(ZnCR)_2(ClO_4)_3(OH)$, 0.2 mM DPPNPP, 5 mM HEPPS, pH 8.00. [e] Based on pseudo-first-order rate constants for 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM $ZnCR(Br)(ClO_4)$, 10 mM HEPPS, pH 8.00. [f] Not at pH 8.00. True second-order rate constant based on pseudo-first-order rate constants for 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 mM NaOH. [g] Based on pseudo-first-order rate constants for 10.0, 20.0, 30.0, 40.0, and 50.0 mM HEPPS, pH 8.00.

Table II. Representative Pseudo-First-Order Rate Constants for DPPNPP Hydrolysis in 50% Aqueous CH_3CN[a]

hydrolytic agent	k_{obsd}, s^{-1} [b]
0.5 mM $(ZnCR)_2(ClO_4)_3(OH)$	5×10^{-5}
1.0 mM $ZnCR(Br)(ClO_4)$	3.9×10^{-5}
0.03 mM $(ZnCR)_2(ClO_4)_3(OH)$	3.3×10^{-6}
10 mM NaOH	6.7×10^{-4}
10 mM HEPPS, pH 8.00	9.0×10^{-7}

[a] Initial DPPNPP concentration 0.1 mM. Values for zinc complexes measured for reaction solutions containing 10 mM HEPPS, pH 8.00. [b] These values reproducible within 10%.

pedient of adjusting the pH of the aqueous portion and then adding CH_3CN, a technique that gave reproducible results. When the pH of a reaction solution is discussed below, it is always the pH of the aqueous portion *before* dilution with an equal volume of CH_3CN.

In 50% aqueous CH_3CN, the $(ZnCR)_2(ClO_4)_3(OH)$, hydroxide ion, and HEPPS buffer all promote phosphate triester hydrolysis in processes apparently first order in each component. Second-order rate constants are listed in Table I; some representative pseudo-first-order rate constants appear in Table II. When a reaction solution was prepared containing 1.0 mM $Zn(ClO_4)_2$, 10 mM HEPPS, pH 8.00, and 0.1 mM DPPNPP, a white flocculent precipitate developed within a couple of hours, and *p*-nitrophenolate release was not noticeable enhanced over the buffer rate.

The facts that the observed rate constants were increased over background for 27 and 54 mol % ZnCR (added as $(ZnCR)_2(ClO_4)_3(OH)$), i.e., when the triester was in excess, and that these reactions showed good pseudo-first-order behavior (reactions followed to 87% and 96% of DPPNPP hydrolysis, respectively; correlation coefficients >0.999) demonstrate that ZnCR is functioning catalytically in the hydrolysis.

The ZnCR-facilitated reaction was very sensitive to the presence of other ionic constituents. As shown in Figure 1, with the bromide–perchlorate complex, a small and apparently linear buffer inhibition was observed with HEPPS at pH 8.00. Since the pK_a of HEPPS in pure water at 25 °C is 8.00, a crude ionic strength adjustment was made by repeating the 10–80 mM buffer concentration series and adding NaCl to half the buffer concentration for solutions with less than 80 mM buffer. The result, shown in Figure 18 was a profound inhibition at lower buffer concentrations. The inhibitory agent was apparently chloride anion, since a similar adjustment of ionic strength with $NaClO_4$ left the observed first-order rate constant roughly unchanged. This $NaClO_4$ result suggests that the buffer inhibition is largely an ionic strength effect.

$(ZnCR)_2(ClO_4)_3(OH)$ was more active than the bromide–perchlorate version, as might be expected from the chloride inhibition effect. The perchlorate–hydroxide dimer was also more sensitive to buffer inhibition, but again, the buffer inhibition seems

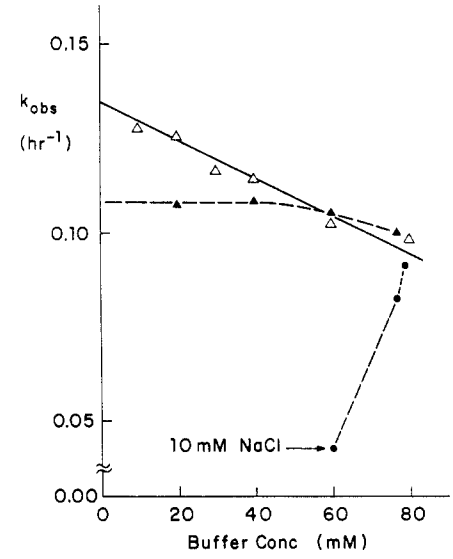


Figure 1. Rate of hydrolysis of DPPNPP by 1.0 mM $ZnCR(ClO_4)(Br)$ in 50% H_2O/CH_3CN, pH 8.00: (△) no ionic strength adjustment; (▲) ionic strength adjusted with $NaClO_4$; (●) ionic strength adjusted with NaCl, whose concentration is increased at lower buffer concentrations. Arbitrary lines connect the points.

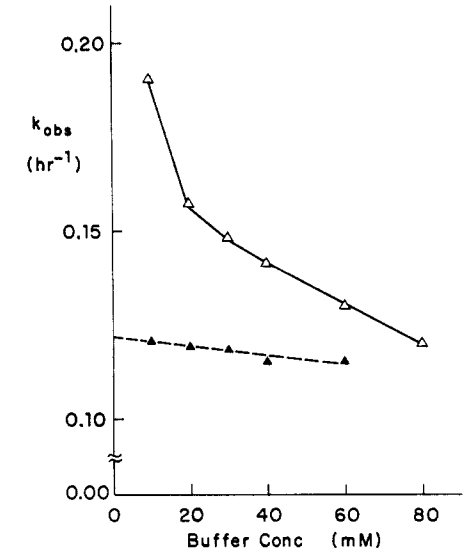


Figure 2. Rate of hydrolysis of DPPNPP by 0.54 mM $(ZnCR)_2(ClO_4)_3(OH)$ in 50% H_2O/CH_3CN, pH 8.00: (△) no ionic strength adjustment; (▲) ionic strength adjusted with $NaClO_4$. Arbitrary lines connect the points.

to be an ionic strength effect (Figure 2). The ZnCR moiety had the same catalytic properties whether added as the monomeric bromide–perchlorate or as the dimeric hydroxide–perchlorate with added bromide; the bromide–perchlorate rate constants were reproduced when equivalent amounts of hydroxide–perchlorate complex and NaBr were added to an otherwise identical reaction solution. In his studies of acetaldehyde hydration catalysis by ZnCR,[15] Woolley does not seem to have observed either ionic strength or halide effects. In fact, he does not mention whether ZnCR halide salts were used for his studies.

The pH dependence of ZnCR-mediated DPPNPP hydrolysis in 50% aqueous CH_3CN over the pH range 6.7–10.5 is shown in Figure 3. All reactions were run with 1 mM ZnCR and 10 mM buffer. The observed data points are juxtaposed with a theoretical curve (based on mechanistic assumptions presented more fully in the Discussion section). We believe that the active catalytic species has a zinc-bound hydroxide (or its kinetic equivalent), formed by dissociation of a proton on a water molecule ligated to the metal center of ZnCr (eq 1). Since the reaction of

$$[ZnCR\text{–}OH_2]^{2+} \rightleftharpoons [ZnCR\text{–}OH]^{+} + H^{+} \quad (1)$$

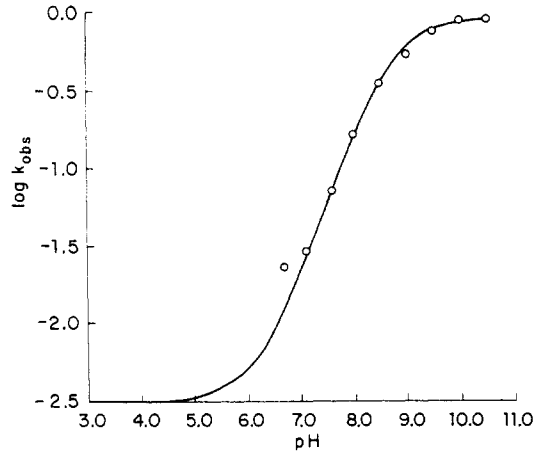


Figure 3. pH/rate profile for hydrolysis of DPPNPP by ZnCR/ClO_4. The data points are fitted by a theoretical curve with pK_a = 8.7, k_2 = 2.8 × 10^{-1} M^{-1} s^{-1}.

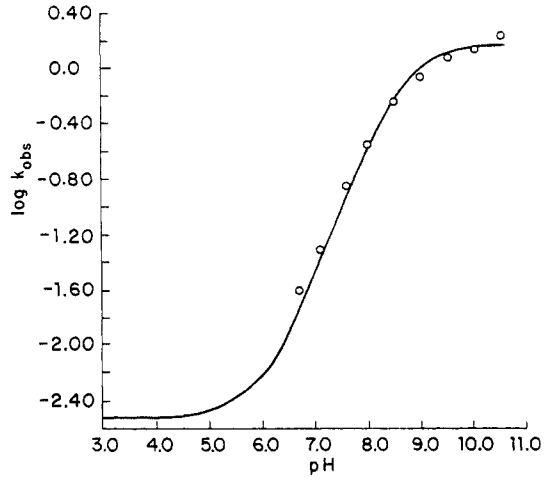


Figure 4. pH/rate profile for the hydrolysis of DPPNPP by ZnHCR/ClO_4 in H_2O/CH_3CN. The data points fit a theoretical curve with pK_a = 8.7 and k_2 = 4.4 × 10^{-1} M^{-1} s^{-1}.

ZnCR–OH with the substrate shows second-order kinetics, we can describe the rate of DPPNPP hydrolysis as the sum of three second-order terms, as shown in eq 2. k_{Zn}, k_B, and k_{OH} are, re-

$$v = k_{Zn}[\text{DPPNPP}][\text{ZnCR–OH}] + k_B[\text{DPPNPP}][\text{buffer}] + k_{OH}[\text{DPPNPP}][OH^-] \quad (2)$$

spectively, second-order rate constants for hydrolytic processes mediated by ZnCR–OH, by buffer, and by hydroxide. Based on the definition of K_a for the zinc-bound water, we can express the concentration of ZnCR–OH in terms of K_a and total zinc complex concentration, [ZnCR] (eq 3). Since $k_{obsd} = v/[\text{DPPNPP}]$, eq

$$[\text{ZnCR–OH}] = [\text{ZnCR}]/(1 + [H^+]K_a) \quad (3)$$

2 becomes eq 4. As shown in Table I, k_{OH} is 2.8 × 10^{-2} M^{-1} s^{-1}. We assume that the second-order rate constant determined for 10 mM HEPPS at pH 8.00, 8.6 × 10^{-5} M^{-1} s^{-1}, is applicable to the other aminosulfonate buffers used in this study. Since re-

$$k_{obsd} = k_{Zn}[\text{ZnCR}]/(1 + [H^+]K_a) + k_B[\text{buffer}] + k_{OH}/10^{-14}[H^+] \quad (4)$$

actions were run at constant ZnCR and buffer concentrations, 1 and 10 mM, we obtain an equation for k_{obsd} with only two variables, k_{Zn} and K_a, beside pH (eq 5). The curve in Figure

$$k_{obsd} = k_{Zn}[10^{-3}\ M)/(1 + [H^+]K_a) + (10^{-12}\ M^{-1}\ h^{-1})/[H^+] + 8.6 \times 10^{-7}\ s^{-1} \quad (5)$$

3 is for k_{Zn} = 2.8 × 10^{-1} M^{-1} s^{-1} and pK_a = 8.7. The choice of k_{Zn} was based on the high pH plateau value for k_{obsd}, and the pK_a was selected based on Woolley's report that ZnCR in water has a proton with pK_a = 8.69. The fit to the experimental points is excellent.

Table III. Apparent Second-Order Rate Constants for DPPNPP Hydrolysis in 20 mM Aqueous Brij 35 at pH 8.00[a]

hydrolytic agent	app k_2, M^{-1} s^{-1} [b]
ZnHCR(Br)(ClO_4) (excess)[c]	3.6
ZnHCR(Br)(ClO_4) (catalytic)[d]	2.4 × 10^{-1}
NaOH[d]	2.8 × 10^{-2}

[a] 0.05 mM initial DPPNPP concentration unless otherwise indicated. [b] Linear regression analyses of pseudo-first-order rate constants show correlation coefficients >0.99. [c] Based on pseudo-first-order rate constants for 0.1, 0.2, 0.3, 0.4, and 0.5 mM ZnHCR(Br)(ClO_4), 10 mM HEPPS, pH 8.00. [d] Based on pseudo-first-order rate constants for 0.01, 0.02, and 0.05 mM ZnHCR(Br)(ClO_4), 0.1 mM DPPNPP, 10 mM HEPPS, pH 8.00. [e] Not at pH 8.00. Based on pseudo-first-order rate constants for 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 mM NaOH.

Table IV. Representative Pseudo-First-Order Rate Constants for DPPNPP Hydrolysis in 20 mM Aqueous Brij 35 at pH 8.00[a]

hydrolytic agent	k_{obsd}, s^{-1} [b]
1.0 mM ZnHCR(Br)(ClO_4)	1.7 × 10^{-3}
0.01 mM ZnHCR(Br)(ClO_4)[c]	6.9 × 10^{-6}
0.5 mM $(ZnHCR)_2(ClO_4)_3(OH)$	1.0 × 10^{-3}
0.54 mM $(ZnCR)_2(ClO_4)_3(OH)$	5.2 × 10^{-6}
10 mM HEPPS, pH 8.00	2.2 × 10^{-6}
10 mM NaOH[d]	3.1 × 10^{-4}

[a] Initial DPPNPP concentration 0.05 mM unless otherwise indicated. Values for zinc complexes measured for reaction solutions containing 10 mM HEPPS, pH 8.00. [b] Reproducible within 20%. [c] Initial DPPNPP concentration 0.1 mM. [d] Not at pH 8.00.

A similar set of experiments was run with $(ZnHCR)_2(ClO_4)_3(OH)$ in buffered 50% aqueous CH_3CN. Figure 4 shows the data from these experiments and the curve generated from eq 5 for pK_a = 8.7 and k_{Zn} = 4.4 × 10^{-1} M^{-1} s^{-1}.

Kinetics in Micellar Brij 35. Cationic micelles themselves facilitate DPPNPP hydrolysis.[6] This fact and the demonstrated sensitivity of ZnCR to ionic additives led us to choose the neutral surfactant Brij 35, $C_{12}H_{25}(OCH_2CH_2)_{23}OH$, for examination of the hydrolytic activity of ZnHCR under micellar conditions. All experiments were carried out at low ionic strength and 20 mM surfactant Brij 35 concentration well above the reported[22] critical micelle concentration range of 0.06–0.09 mM.

Apparent second-order rate constants for ZnHCR- and hydroxide-mediated hydrolyses in micellar solution are shown in Table III. Some representative pseudo-first-order rate constants are shown in Table IV. The rate enhancements for hydrolysis of 0.1 mM phosphate triester by 10, 20, and 50 mol % ZnHCR(Br)(ClO_4) and the fact that all three reactions showed pseudo-first-order behavior (reactions followed to 80%, 90%, and 97% hydrolysis, respectively; correlation coefficients >0.999) are strong evidence that ZnHCR is acting catalytically. Curiously, the complex $(ZnHCR)_2(ClO_4)_3(OH)$ is less active than ZnHCR(Br)(ClO_4), in contrast to the observations with varying counteranions in aqueous CH_3CN. $(ZnHCR)_2(ClO_4)_3(OH)$ was extremely slow to dissolve in micellar Brij 35, and it is possible that some of the material actually remained undissolved as an invisible film.

Observed rate constants were determined at various pH values using 0.25 mM ZnHCR(Br)(ClO_4) and 10 mM buffer. In 20 mM Brij 35, k_{OH} remains 2.8 × 10^{-2} M^{-1} s^{-1}, but the buffer background rate rises to 2.2 × 10^{-6} s^{-1}. Accordingly, the observed rate constants should be generated by eq 6. Figure 5 shows data points for hydrolyses in the pH range 6.6–10.5 and the curve generated for k_{Zn} = 18 M^{-1} s^{-1} and pK_a = 9.1.

$$k_{obsd} = k_{Zn}(2.5 \times 10^{-4}\ M)/(1 + [H^+]K_a) + (2.7 \times 10^{-16}\ M^{-1}\ h^{-1})/[H^+] + 2.2 \times 10^{-6}\ s^{-1} \quad (6)$$

(22) Fendler, J. H.; Fendler, E. J. *Catalysis in Micellar and Macromolecular Systems*; Academic: New York, 1975; Chapter 2.

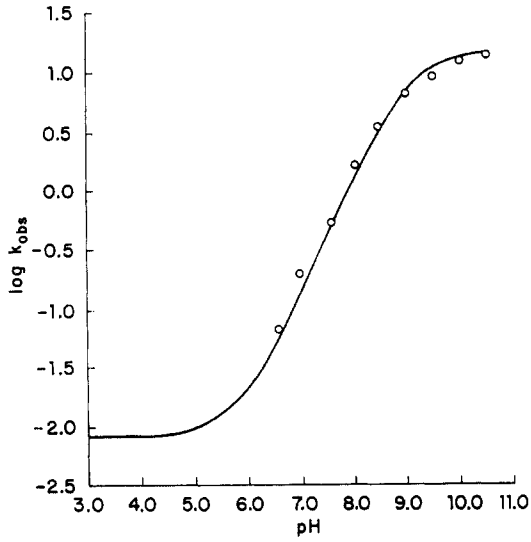


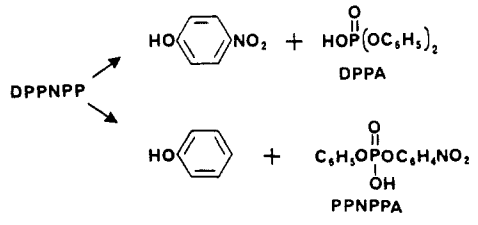
Figure 5. pH/rate profile for the hydrolysis of DPPNPP by ZnHCR/ClO_4 in a Brij micelle. The data points fit a theoretical curve with pK_a = 9.1 and k_2 = 18 M^{-1} s^{-1}.

Table V. End-Point Product Composition, Diphenyl Phosphate to Phenyl (*p*-Nitrophenyl) Phosphate Ratios[a]

hydrolytic agent	solvent	DPPA/PPNPPA[b]
$(ZnCR)_2(ClO_4)_3(OH)$[c]	50% aq CH_3CN	5.5
ZnHCR(Br)(ClO_4)[d]	20 mM aq Brij 35	3.8
NaOH[e]	50% aq CH_3CN	8.6
NaOH[e]	20 mM aq Brij 35	7.0

[a] Determined by HPLC as described under Methods. 1.0 mM initial DPPNPP concentration. [b] The error in these values is <15%. [c] Zinc complex present at 1.08 mM, 10 mM HEPPS, pH 8.00. [d] Zinc complex present at 0.5 mM concentration, 10 mM HEPPS, pH 8.00. [e] 10 mM NaOH.

End-Point Product Analysis. We had assumed that under all our reaction conditions, DPPNPP would be hydrolyzed only to *p*-nitrophenol and diphenylphosphoric acid. HPLC analysis showed the reaction to be more complex.



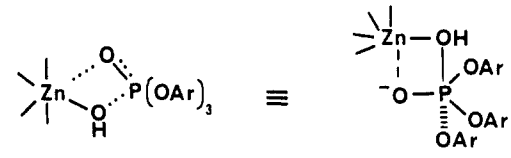
All four products were resolved and quantified by reverse-phase HPLC. Final product compositions, as expressed by the ratio of diphenylphosphoric acid to phenyl (*p*-nitrophenyl)phosphoric acid, are listed for various conditions in Table V. The ratio of *p*-nitrophenol to phenol was similar but not determined as precisely.

Discussion

It is clear from our data that both ZnCR (in 50% aqueous CH_3CN) and the lipophilic complex ZnHCR (in aqueous micellar Brij 35) are true catalysts for hydrolysis of the phosphate triester DPPNPP under neutral and mildly alkaline conditions. ZnCR's catalytic activity is sensitive to halide anions, but there is no evidence for inhibition by the anionic hydrolysis products under our conditions. The two complexes may be considered rudimentary models for zinc-containing phosphate esterases.

Reactions in Solution. The mechanism by which these zinc complexes exert their catalytic effects is of considerable interest. ZnCR in 50% aqueous CH_3CN shows the simplest behavior. At pH 8.00, the apparent second-order rate constant is essentially invariant over a wide range of complex concentrations, regardless of whether the complex is present in excess or catalytic quantity relative to the triester (Table I). The variation of the observed rate constant with pH (Figure 3) suggests that formation of the catalytically active species requires removal of a relatively acidic proton, which in turn implicates ZnCR–OH (or its kinetic equivalent, ZnCR with a free hydroxide ion) as the active species. There are two limiting mechanistic possibilities for a catalytic species involving ZnCR and hydroxide: (1) a nucleophilic process involving attack on the phosphorus atom by zinc-bound hydroxide or (2) an electrophilic activation of the phosphoryl P–O bond, by coordination to the zinc center, toward attack by free hydroxide. A "hybrid" mechanism is also possible: the zinc center delivers a coordinated hydroxide nucleophilically to a DPPNPP molecule, while simultaneously drawing electron density away from the phosphorus atom by interacting with the phosphoryl oxygen.

Some version of the hybrid mechanism seems to be operative in our system. From the pH dependence of the observed rate constant for ZnCR-mediated hydrolysis (Figure 3), the second-order rate constant for attack of fully ionized ZnCR–OH on the triester DPPNPP is approximately 2.8 × 10^{-1} M^{-1} s^{-1}. The measured second-order rate constant for hydroxide reaction with the substrate (Table I) is 2.8 × 10^{-2} M^{-1} s^{-1}, an order of magnitude *smaller*. Since free hydroxide ion is more than a million times stronger as a base than is ZnCR–OH, some remarkable polarizability effects would be required to make ZnCR–OH a better *simple* nucleophile. If, however, zinc acts also as a Lewis acid, the resulting bifunctional mechanism could well be better than simple nucleophilic attack by free hydroxide. This argument does not exclude the kinetically equivalent possibility that the substrate forms a complex with zinc which is then attacked by *free* hydroxide, but such a purely Lewis acid mechanism would require extremely strong binding and electrophilic catalysis to make up for the low concentration of free hydroxide ion. Lewis acid facilitation of nucleophilic reactions at phosphorus(V) centers has been observed,[23-25] but this mode of catalysis alone does not seem to be capable of more than modest rate enhancements.[23]



Similar bifunctional or "push–pull" mechanisms have been invoked for metal chelate-mediated hydrolyses of isopropyl methylphosphonofluoridate.[26-28] In these cases, as in ours, monohydroxo–metal complexes were implicated as active species as neutral or mildly alkaline pH, and apparent second-order rate constants for reactions of the complexes with substrate were higher than for reaction of hydroxide with substrate. None of the hydrolytic agents in these studies contained zinc.

Pure nucleophilic mechanisms have been invoked for some zinc-mediated reactions of non-phosphate substrates.[29] In his studies of ZnCR as a catalyst for acetaldehyde hydration, Woolley concluded that ZnCR–OH was acting as a simple nucleophile.[15] Free hydroxide had a much larger second-order rate constant for reaction with acetaldehyde than did the metal-bound hydroxide.

(23) The apparent second-order rate constant for hydroxide attack on trimethyl phosphate is increased 400-fold when the phosphoryl moiety is coordinated to an iridium(III) center; Hendry, P.; Sargeson, A. M. *J. Chem. Soc., Chem. Commun.* **1982**, 164.

(24) Buncel et al. have found that lithium and potassium cations enhance ethoxide reactivity toward *p*-nitrophenyl diphenylphosphinate; see ref 3c.

(25) Wadsworth has presented evidence that $ZnCl_2$-mediated methanolysis of phosphate triesters involves metal interaction with the phosphoryl oxygen and the leaving group oxygen. Wadsworth, W. S. *J. Org. Chem.* **1981**, *46*, 4080.

(26) Epstein, J.; Rosenblatt, D. H. *J. Am. Chem. Soc.* **1958**, *80*, 3596.

(27) Gustafson, R. L.; Martell, A. E. *J. Am. Chem. Soc.* **1962**, *84*, 2309.

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(29) For a general review of zinc-based enzyme model systems, see: Brown, R. S.; Huguet, J.; Curtis, N. J. *Met. Ions Biol. Syst.* **1983**, *15*, 55.

The contrast probably reflects differing geometric requirements at a trigonal-bipyramidal phosphorus and at a tetrahedral carbon. In the hybrid mechanism at phosphorus, all four atoms of the transient ring have almost normal valence angles; for such a four-membered ring containing a carbon, there would be angle strain. (Of course, the hybrid mechanism requires some geometric change in the zinc complex, so that two neighboring coordination sites are available.) Nucleophilic activity of zinc-bound hydroxide has also been inferred from the results of recent model studies of anhydride[30] and amide[31] hydrolysis. Sargeson and co-workers have reported strong evidence for intracomplex attack of a cobalt(III)-bound hydroxide on a ligated phosphate monoester.[10]

Reactions in a Micelle. Catalysis of DPPNPP hydrolysis by ZnHCR in aqueous micellar Brij 35 is probably related mechanistically to the process discussed for ZnCR in aqueous CH_3CN. The variation of k_{obsd} with pH (Figure 5) implicates ZnHCR–OH (or a kinetic equivalent) as the active species, and the second-order rate constant for ZnHCR–OH reaction with the triester DPPNPP is again larger than for hydroxide attack on the substrate. A striking difference between the two systems, however, is that at pH 8.00, the apparent second-order rate constant for ZnHCR in micellar solution increases dramatically with complex concentration, while for ZnCR in solution the apparent second-order rate constant remains essentially unchanged when the complex concentration is varied. The increase in the micellar system suggests that ZnHCR aggregates can perform hydrolysis through some higher order mechanism and that this new mechanism is more effective. A noncatalytic phosphate monoester hydrolysis involving two cobalt(III) centers has recently been described.[5b] It would be of interest to produce polynuclear zinc complexes related to ZnCR and ZnHCR in which aggregation is covalently enforced, since these substances could be even more potent catalysts.

The simple complex ZnCR shows only slight activity in the micellar medium (Table IV). This complex is sufficiently water-soluble that it is probably located largely in the aqueous phase while the substrate, which is not water-soluble, residues exclusively in the micelle. The relative inactivity of ZnCR in the micellar system is, therefore, not surprising (although it is interesting that hydroxide ion remains active). The lipophilic complex ZnHCR, on the other hand, is soluble in 50% aqueous CH_3CN and catalytically active in that medium. In fact, as indicated in Table II and Figure 4, this complex seems to be slightly more active than ZnCR itself. A 1 mM solution of ZnHCR in 50% aqueous CH_3CN forms a moderately persistent foam on vigorous mixing, indicating that the complex is a surfactant in this solvent and may self-associate. The enhanced catalytic activity relative to ZnCR may result from the aggregation effect discussed earlier for reactions in micellar Brij 35.

Product Studies. The polar end of the Brij 35 molecule is a hydroxyl group, and it was important to establish whether the process being observed in the micellar medium was hydrolysis of the triester DPPNPP or phosphorylation of a surfactant molecule. For instance, Sigman and co-workers have found that zinc complexes of multidentate ligands bearing hydroxyl groups can facilitate the decomposition of phosphate or carboxylate esters in processes that involve ligand hydroxyl phosphorylation or acylation.[32] In our case, only hydrolysis is involved. HPLC analysis of the ZnHCR-mediated micellar reaction after >10 half-lives showed that all starting phosphate triester could be accounted for in the diphenylphosphoric acid and phenyl-*p*-nitrophenylphosphoric acid produced. It is very unlikely that the surfactant could have been phosphorylated, and the resulting ester than rapidly hydrolyzed to the observed products. Phosphorylation of Brij 35 would have produced a phosphate triester carrying two phenol groups, and these should have been lost at least competitively in any subsequent hydrolysis.

In previous studies on the hydrolysis of DPPNPP, it seems to have been commonly assumed that the leaving group would be exclusively *p*-nitrophenoxide, and indeed it is the formation of this material that is normally followed in spectroscopic kinetic measurements. Our results, listed in Table V, show clearly that ejection of phenoxide in addition to *p*-nitrophenoxide occurs in both of the media we have studied, whether the hydrolytic agent is a zinc complex or NaOH. This alternate mode of cleavage has no effect on the conclusions drawn from spectroscopic kinetic experiments, since determination of a pseudo-first-order rate constant is not affected by the cooccurrence of an unseen competitive process.

The formation of the unexpected products indicates that the much greater leaving group ability of nitrophenoxide relative to phenoxide is not yet felt very strongly in the transition state for hydrolysis. Loss of the better leaving group is still preferred in all four of the cases we examined, but it is clear that in each reaction medium, hydrolysis by the zinc complex shows a smaller preference for nitrophenoxide loss than does hydrolysis by hydroxide. This effect of zinc complexes on the product ratio is consistent with the hybrid mechanism involving bifunctional catalysis we invoked above.

The normal preferential loss of nitrophenoxide probably reflects two diffferent factors. Attack by hydroxide forms a trigonal bipyramid with an equatorial O^- and one of the phenoxy groups in an apical position, in addition to the apical attacking hydroxide. Some of the selectivity for loss of the nitrophenoxide group may reflect a tendency by this group to adopt the apical position in the original trigonal-bipyramidal intermediate, but some of the selectivity probably also involves pseudorotation among the various phosphate trigonal bipyramids, with preferential forward decomposition by those intermediates with an apical nitrophenoxy group. We would expect that such pseudorotation should be hindered or completely blocked in an intermediate formed in the hybrid mechanism. If the zinc is bound both to the attacking OH and to the O^- that has developed, strain factors prevent pseudorotation using the O^- as a pivot; pseudorotation using either of the other equatorial groups would put the O^- in an apical position, which is unfavorable. The product composition from the catalyzed hydrolysis of DPPNPP by ZnCR and ZnHCR may reflect the composition of the originally formed mixture of trigonal-bipyramidal intermediates more closely than the hydroxide product composition does, and the inhibition of pseudorotation required by the hybrid mechanism would explain the observation that there is less preference for departure of nitrophenoxide in these zinc-catalyzed cases.

Numerous studies of metal complex-mediated hydrolyses of phosphorus(V) compounds have appeared in the past several decades, but ZnCR seems to be a unique example of a compound with good turnover catalysis of phosphate ester hydrolysis in aqueous/organic solution. Although micellar zinc complexes have been studied as agents for other hydrolytic reactions,[33] no such studies with phosphate substrates have been reported. As mentioned in the Introduction, two groups have recently described nonmetallic micellar systems that catalyze DPPNPP hydrolysis (and carboxylate ester hydrolysis[34]). Moss and co-workers found that DPPNPP hydrolysis in aqueous micellar CTACl was catalyzed by iodoso- and iodoxybenzene derivatives with ortho-carboxylate groups.[8] Menger and Whitesell observed a similar catalysis effected by a tetraalkylammonium surfactant containing an aldehyde group at the polar end.[9] Both systems seem to involve nucleophilic catalysis by an oxyanion. The nucleophile is present in the cyclic tautomeric forms of the iodo compounds in the former case and in the aldehyde hydrate anion in the latter. Comparison of the pseudo-first-order rate constants reported by these two groups with the values for ZnHCR in micellar Brij 35 under similar concentration and pH conditions indicates that our lipophilic zinc complex has an activity comparable to the aldehyde

(30) Breslow, R.; McClure, D. E.; Brown, R. S.; Eisenach, J. *J. Am. Chem. Soc.* **1975**, *97*, 194.
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(32) (a) Sigman, D. S.; Wahl, G. M.; Creighton, D. J. *Biochemistry* **1972**, *11*, 2236. (b) Sigman, D. S.; Jorgensen, C. T. *J. Am. Chem. Soc.* **1972**, *94*, 1724.
(33) Tagaki, W.; Ogino, K. *Top. Curr. Chem.* **1985**, *128*, 143.
(34) ZnHCR(Br)(ClO_4) facilitates carboxylic ester hydrolysis in CTAB reverse micelles; Petter, R., unpublished results.

hydrate but that both of these are outstripped by the iodine-based catalysts.

The ultimate catalysts for reference for our systems are the zinc-containing enzymes that mediate reactions at phosphorus(V) centers. It remains to be seen whether our systems will improve with the addition of other catalytic groups, mimicking some of the known enzymatic functional groups, or with construction of related di- or polynuclear complexes. In any case, our systems seem to be good first models of the zinc enzymes that catalyze phosphate ester hydrolysis.

Registry No. 1, 41546-66-1; **2**, 100946-05-2; **3**, 100928-84-5; $(ZnHCR)_2(ClO_4)_3(OH)$, 100946-07-4; $(ZnCR)_2(ClO_4)_3(OH)$, 100946-09-6; DPPA, 838-85-7; PPNPPA, 793-12-4; $Zn(ClO_4)_2$, 13637-61-1; 3,3'-diaminodi-*n*-propylamine, 56-18-8; 2,6-diacetylpyridine, 1129-30-2; 1-iodohexadecane, 40474-98-4; zinc bromide, 7699-45-8; diphenyl *p*-nitrophenyl phosphate, 10359-36-1.

Facile Heterolysis of a Carbon–Carbon Bond. Arylazodicyanomethanides as the Leaving Group Capable of Generating *tert*-Cumyl Cation and the Hydrogen-Bond-Insusceptible Behavior of the Leaving Group Anions

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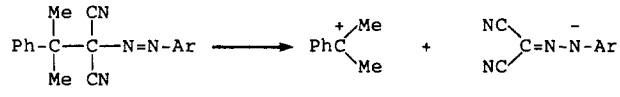
Abstract: Decompositions of (*p*-nitrophenyl)azo-*tert*-cumylmalonitrile in polar solvents were found to proceed via the heterolysis of a carbon–carbon bond to generate *tert*-cumyl cation and the conjugate base of (*p*-nitrophenyl)hydrazonomalononitrile. The major products arising from *tert*-cumyl cation are as follows: *tert*-cumyl methyl ether in methanol, the *N*-cumylpyridinium hydrazonide in pyridine, and α-methylstyrene in Me_2SO and in DMF. The reactions in MeCN and in acetone afford a rearranged product, *N*-(*tert*-cumyl)(*p*-nitrophenyl)hydrazonomalononitrile, which gradually undergoes heterolysis as well. Decompositions of the azo compound and the rearranged product are faster in Me_2SO than in methanol, being in conflict with the usual trend of solvent-ionizing power. This phenomenon is explained in terms of extensive charge dispersal of the leaving group anion which prevents hydrogen bonding with a protic solvent molecule. It is pointed out that the behavior of the conjugate base of (*p*-nitrophenyl)hydrazonomalononitrile as the leaving group anion is closely connected with the function of FCCP (the *p*-CF_3O derivative of the hydrazone) as one of the best uncouplers of oxidative phosphorylation in mitochondrial systems.

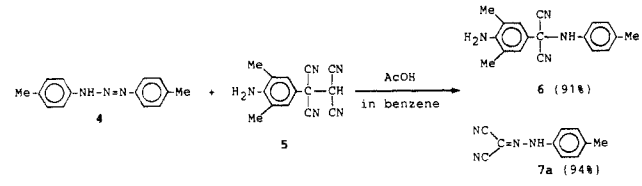
Studies of the cleavage of carbon–carbon bonds are of primary importance in organic chemistry. One of the fundamental modes of the cleavage is the heterolysis of the bonds to form cationic and anionic species; however, despite the interesting S_N1 nature of the process, very little is known about this area, the major problem being obviously the lack of efficient leaving groups which terminate in a carbon atom. The data so far reported are limited to those on the ring opening of cyclopropanes,[1] intramolecular rearrangements,[2] and the generation of extremely stable carbocations (cyclopropenium ions[3] and a tropylium ion[4]).

This paper reports the use of arylazodicyanomethanides as the novel leaving group which enables us to generate *tert*-cumyl cation **2**, a typical carbocation in solvolysis reactions. The entry into



1 **2** **3**

the study of this heterolysis was made by the observation that the metathesis products, **6** and **7a**, were formed from the reaction of 1,3-di-*p*-tolyltriazene (**4**) with the TCNE-amine adduct **5**[5] in 1:10 (v/v) acetic acid–benzene,[6] which was studied in connection with our previous investigations on the triazene chemistry.[7,8] In the



4 **5**

presence of acetic acid, the triazene **4** behaves as a mild diazonium coupling reagent because of the equilibrium involving the diazonium acetate. The result strongly suggests that the initially formed azo intermediate **8** undergoes carbon–carbon bond heterolysis and the subsequent reaction of the resulting cation **9** with *p*-toluidine leads to the formation of **6**. The azo compound **8** could not be isolated. Conceivably, the reason is that both ions **9** and **3a** generated from **8** are highly stabilized by delocalization through the electron-donating amino group and through the diaza allylic

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