Laser Flash Photolysis Kinetic Studies of α-Methoxy-*β*-phosphatoxyalkyl Radical Heterolysis Reactions: A Method for Alkoxyalkyl Radical Cation Detection

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Nucleotide C4′ radicals are implicated in the cleavage of DNA by antitumor antibiotics such as bleomycin.[1] Under anaerobic conditions, model C4′ radicals are thought to undergo initial heterolysis to give a radical cation and a phosphate anion.[2] Rapid proton transfer may then occur to give an allyl radical and phosphoric acid,[2,3] or the radical cation may be trapped by nucleophiles such as water. Alternatively, a direct pathway to the allyl radical involving concerted [1,3]-elimination of phosphoric acid was found computationally by Zipse.[4] Schulte-Frohlinde and co-workers followed the rates of dialkylphosphoric acid formation from *β*-phosphatoxyalkyl radicals,[5] and Giese and co-workers observed an allyl radical product from reaction of an α-alkoxy-*β*-phosphatoxyalkyl radical by ESR spectroscopy.[6] Evidence of heterolysis of C4′ DNA radicals has been reported by Giese's group,[7] and Giese, Rist and co-workers observed CIDNP effects from photolysis of a precursor to an α-alkoxy-*β*-phosphatoxyalkyl radical.[8]

A serious obstacle for direct studies of reactions of C4′ DNA radical models is the absence of a prominent UV chromophore in both the α-alkoxy-*β*-phosphatoxyalkyl radicals and the products of heterolysis. We report a probe technique that detects enol ether radical cations, thus signaling a heterolysis reaction, and permits kinetic studies. In laser flash photolysis (LFP) studies, we employ triarylamines as reporters that are oxidized by enol ether radical cations to triarylaminium cation radicals that are readily detected.

Preliminary LFP studies demonstrated that the method was viable. Irradiation of chloranil with 355 nm light gives triplet chloranil (Chl*) which is a powerful oxidant and relatively long-lived. When Chl* was produced in an acetonitrile (ACN) solution containing Ph$_3$N, the rate of decay of the signals from Chl* was comparable to that of growth of the (Ph$_3$N)$^{+\bullet}$ signals indicating an uncomplicated electron-transfer process (Figure 1A). When the reaction was repeated with dihydropyran (DHP) present, the rate of Chl* decay was accelerated due to its reaction with both DHP and Ph$_3$N. Some (Ph$_3$N)$^{+\bullet}$ was produced during the Chl* decay period, but the signal for the aminium cation radical continued to grow after Chl* was depleted (Figure 1B). These results show that the DHP radical cation produced in acetonitrile eventually oxidized Ph$_3$N (Scheme 1).

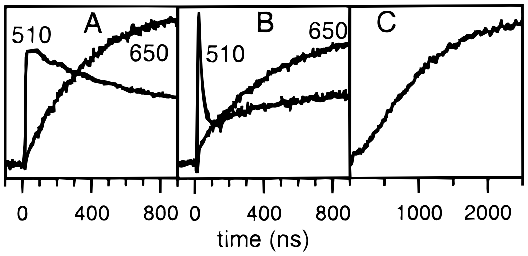
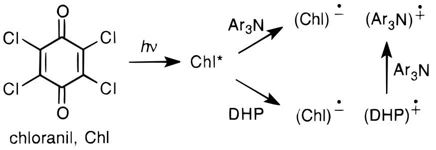


Figure 1. (A, B) Signal decay from Chl* (510 nm) and growth from (Ph$_3$N)$^{+\bullet}$ (650 nm) following 355 nm irradiation of acetonitrile solutions containing 1×10^{-3} M chloranil and 1×10^{-4} M Ph$_3$N with (A) no added dihydropyran and (B) 1×10^{-3} M dihydropyran present. The triphenylaminium cation radical absorbs at 510 nm; this results in a residual signal at 510 nm in panel A and signal growth at 510 nm following depletion of Chl* in panel B. (C) Absorbance at 650 nm following irradiation of a TFE-ACN (5:95, v:v) solution containing PTOC ester **1b** and 1×10^{-4} M Ph$_3$N.

Scheme 1



Similar results were found in acetonitrile with (p-BrC$_6$H$_4$)$_3$N and with both amines in the highly polar solvent 2,2,2-trifluoroethanol (TFE). In aqueous acetonitrile solutions, however, the Ar$_3$N reporter method cannot be used. Triplet chloranil oxidation of Ar$_3$N was observed in aqueous ACN solutions as above, but reactions conducted with added DHP showed no growth of the (Ph$_3$N)$^{+\bullet}$ signal after Chl* decay was complete. These results indicate that the DHP radical cation reacted with water rapidly in a reaction that eventually consumed this oxidant.

Reactions of α-methoxy-*β*-phosphatoxyalkyl radicals were studied by the use of the Ar$_3$N reporter method. We used PTOC esters[9] as radical precursors in LFP studies. PTOC esters are cleaved by 355 nm laser irradiation and have been employed in studies of *β*-aryl-*β*-phosphatoxyalkyl radicals.[3,10] The synthetic sequence to the PTOC esters is described in the Supporting Information.

The sequence of reactions following 355 nm laser irradiation of PTOC esters **1** is shown in Scheme 2. Photolyses gave the pyridine-2-thiyl radical (**2**) and acyloxyl radicals that rapidly decarboxylated to give the desired radicals **3**. The *tert*-butyl groups in radicals **3** preclude proton transfer to give an allyl radical and phosphoric acid. Radicals **3** reacted by heterolysis to give the enol ether radical cation **4** and phosphate anion, and radical cation **4** reacted with Ar$_3$N to give the detectable triarylaminium cation radicals. A control reaction with a simple alkyl radical generated by photolysis of a PTOC ester resulted in no signal formation

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[9] The acronym PTOC is for pyridine-2-thione-*N*-oxycarbonyl. See: Barton, D. H. R.; Crich, D.; Motherwell. W. B. *Tetrahedron* **1985**, *41*, 3901−3924.

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Scheme 2

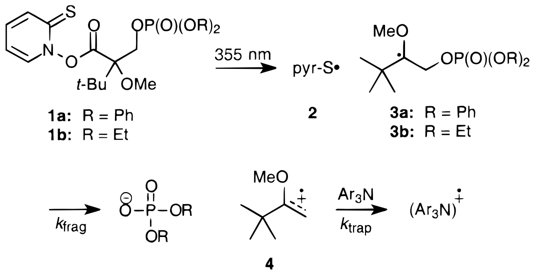

Table 1. Rate Constants for Fragmentations of Radicals **3**[a]

radical	solvent[b]	k_{frag} (s^{-1})	k_{ox} (M^{-1} s^{-1})[c]
3a	ACN[d]	(8 ± 1) × 10^6	1.2 × 10^{10}
	ACN[e]	(9 ± 1) × 10^6	1.2 × 10^{10}
	5% TFE	>2 × 10^7	1.7 × 10^{10}
3b	ACN	no reaction observed	
	4.5% TFE	(2.0 ± 0.2) × 10^6	1.7 × 10^{10}
	5% TFE[f]	(2.7 ± 0.2) × 10^6	1.6 × 10^{10}
	90% TFE	>1 × 10^7	0.7 × 10^{10}

[a] Reactions at (23 ± 2) °C conducted with 1−2 × 10^{-4} M Ph$_3$N present unless noted. [b] ACN = acetonitrile, TFE = 2,2,2-trifluoroethanol; the volume-% of TFE is listed for mixtures of TFE and ACN. [c] Second-order rate constant for electron transfer determined by dividing observed pseudo-first-order trapping rate constant (k_{trap}) by amine concentration. [d] Average results for reactions with 1 × 10^{-4}, 5 × 10^{-4}, and 1 × 10^{-3} M Ph$_3$N. [e] (p-BrC$_6$H$_4$)$_3$N used for detection. [f] Average results for 1 × 10^{-4} and 2.5 × 10^{-4} M Ph$_3$N.

from Ph$_3$N, showing that neither the pyridine-2-thiyl radical (**2**) nor the alkyl radical oxidized the amine.

Figure 1C shows a kinetic trace for formation of (Ph$_3$N)$^{+•}$ from reaction of **3b**; the shape clearly indicates a convolution of rate constants. Individual rate constants were extracted by fitting the kinetic traces to the expression for consecutive first-order reactions. The results are listed in Table 1. A precise rate constant for diphenylphosphate cleavage from **3a** could be determined in ACN. Ph$_3$N and (p-BrC$_6$H$_4$)$_3$N gave the same results, apparently because oxidations of both of the amines by radical cation **4** are diffusion-controlled. In solutions containing TFE and ACN, phosphate fragmentation from **3a** was too fast to determine the rate constants. No signal was observed from reaction of radical **3b** in ACN, apparently because this fragmentation was too slow, but kinetics could be measured in 5% TFE in ACN solution. The solvent polarity effects are as expected for heterolysis reactions.[11,12]

A detailed view of fragmentation of the β-phosphate radicals **3** is shown in Scheme 3. The heterolysis produces a contact ion pair (CIP) that can collapse back to radical **3** or solvate to give a solvent-separated ion pair (SSIP). The SSIP equilibrates with the CIP and also further solvates to give diffusively free species.

Scheme 3

$$\mathbf{3} \overset{K_{het}}{\rightleftharpoons} \text{CIP} \overset{K_{sol}}{\rightleftharpoons} \text{SSIP} \xrightarrow{k_{esc}} \text{free ions}$$

In our studies, recombination of the free ions cannot compete with reaction of radical cation **4** with Ar$_3$N. The measured rate constants for fragmentation of radicals **3** are the products of rate constants and equilibrium constants shown in Scheme 3.

We make the reasonable assumption that the diffusive escape rate constants (k_{esc}) are equal for the diphenyl- and diethylphosphate-containing SSIPs in a given solvent. If we also assume that the equilibrium constant K_{sol} is the same for both phosphates in a common solvent, then the faster reaction of radical **3a** compared to that of **3b** would reflect either the difference in the equilibrium constants for the neutral radicals and the CIPs or the difference in rate constants of the heterolysis reactions that produce the CIPs. It is noteworthy that, in reactions of β-aryl-β-phosphatoxyalkyl radicals apparently involving heterolysis and collapse of the CIP,[10] [1,2]-migrations of the diphenylphosphate group are about 100 times faster than those of the diethylphosphate group.[13,14]

This work demonstrates that the Ar$_3$N reporter method can be applied in LFP studies of DNA radical models as an indicator of heterolysis and for kinetic measurements, with the caveat that it cannot be used with aqueous solutions. The observed rate constants for fragmentations are the products of rate constants and equilibrium constants (Scheme 3), and factoring out the rate constants for heterolyses that produce the CIPs will require additional information. Intramolecular variants of the method which are under development could address these points and expand the dynamic range of the technique.

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Supporting Information Available: Synthetic methods for preparation of PTOC esters **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[11] The E_T(30) solvent polarity values (see ref 12) are as follows: ACN, 45.6; TFE, 59.8; 4.5% TFE in ACN, 55.6 (measured); methanol, 55.4.

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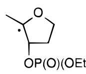
[13] The only kinetic data for phosphate fragmentation with which we can compare our results is for diethylphosphate cleavage from radical **5** in MeOH to give radical cation **6** where competition kinetic experiments gave a rate constant of >3 × 10^9 s^{-1} at 25 °C.[8] Whereas our method reports on the formation of diffusively free radical cations, the formation of MeOH-trapped products from radical cation **6** probably occurs in the SSIP and possibly in the CIP. This conclusion is based on stereoselective alcohol trapping of the radical cations produced from the diasteromeric radicals **7** where stereochemical information could be maintained in the CIP but would be lost for diffusively free species.[14] Thus, the large difference in the heterolysis rate constants for **3b** and **5** probably reflects equilibrium constants and the rate constant for diffusive escape from the SSIP that are in the kinetics for **3b** but not in those for **5**.



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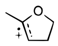

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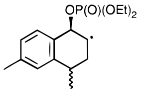

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