J. Am. Chem. Soc. **2001,** *123,* 6445−6446

Direct Measurement of Enol Ether Radical Cation Reaction Kinetics

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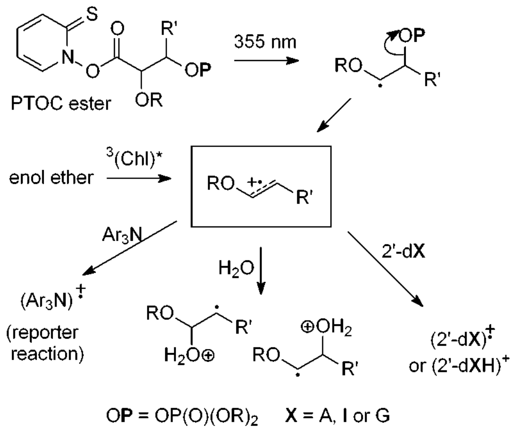
Received January 29, 2001
Revised Manuscript Received May 8, 2001

Radicals produced at the C4′ position of a DNA sugar are implicated in the degradation of DNA by antitumor antibiotics such as bleomycin.[1] Under anaerobic conditions, C4′ DNA radicals and models fragment heterolytically to enol ether radical cations and phosphate anions.[2,3] Rate constants for heterolyses of DNA model radicals[3] and DNA radicals[4] were determined by indirect kinetic methods, and heterolysis rate constants for DNA model radicals were measured directly.[5] A sugar-centered enol ether radical cation produced by heterolysis of a DNA C4′ radical can oxidize a guanosine base in DNA, and Giese's group has employed this entry to DNA radical cations in their elegant studies of electron (or hole) transfers in DNA.[4,6] Despite the importance of enol ether radical cations in DNA oxidations, kinetic studies of these intermediates are limited, although the kinetics of electron-transfer reactions to guanosine derivatives have been reported.[4,7] We report here direct kinetic studies of enol ether radical cation reactions with water and with purine 2′-deoxynucleosides using laser flash photolysis (LFP) methods.

We described direct measurements of the rate constants for *formation* of enol ether radical cations via radical heterolyses using an LFP reporter method for detecting enol ether radical cations that lack useful chromophores.[5] A variation of that method was used here to measure rate constants for enol ether radical cation reactions with water and with purine nucleosides (Scheme 1). Enol ether radical cations were produced rapidly either from an α-alkoxy-*β*-phosphatoxyalkyl radical (from a PTOC ester[8]) that fragmented heterolytically or by oxidation of an enol ether with chloranil triplet, Chl*.

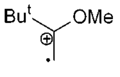
Scheme 1



The enol ether radical cations oxidized triarylamines in diffusion-controlled reactions to give triarylaminium cation radicals that absorb in the long wavelength visible region. When another agent was present that reacted with the radical cation in competition with the amine, the observed rate constant (k_{obs}) for formation of triarylaminium cation radical was given by eq 1 ,

$$k_{obs} = k_0 + k_T[\text{Trap}] \qquad (1)$$

where k_0 is the sum of the pseudo-first-order rate constants for all background reactions consuming the radical cation (including reactions with Ar_3N), k_T is the second-order rate constant for the competing reaction of interest, and [Trap] is the concentration of the agent of interest. Multiple reactions were conducted with varying concentrations of "Trap" to give plots with slopes of k_T and intercepts of k_0. Typical data are shown in Figure 1.



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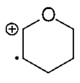

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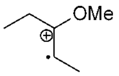

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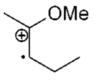

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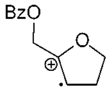

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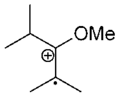

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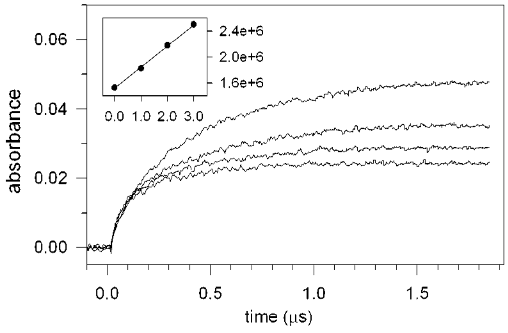

Figure 1. Kinetic traces at 650 nm from reactions of radical cation **3** with Ph_3N in the presence of (from the top) 0, 1 × 10^{-3}, 2 × 10^{-3}, and 3 × 10^{-3} M dA. The inset shows the observed rate constants (right axis) plotted against the mM concentration of dA (bottom axis).

The series of enol ether radical cations **1**−**6** was studied. Reactions were conducted in mixed solvents of acetonitrile (ACN) and 2,2,2-trifluoroethanol (TFE) or mixtures of ACN and TFE containing water. Radical cations **2**, **3**, and **6** were produced by oxidation of the corresponding enol ethers with Chl* in reactions that were complete within 100 ns. Radical cations **4** and **5** were formed by heterolysis of the corresponding α-alkoxy-*β*-(phosphatoxy)alkyl radicals that were produced by 355 nm irradiation

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(8) The acronym PTOC is for pyridine-2-thioneoxycarbonyl. PTOC esters were originally designed as radical precursors for synthetic applications. See: Barton, D. H. R.; Crich, D.; Motherwell, W. B. *Tetrahedron* **1985**, *41*, 3901−3924.

10.1021/ja015602c CCC: $20.00

Table 1. Rate Constants for Reactions of Enol Ether Radical Cations[a]

radical cation	water	dA	dI	dG
1[b]	10×10^5	15×10^8	17×10^8	87×10^8
1[c]	8×10^5	10×10^8	14×10^8	
2[c]	10×10^5	5×10^8	26×10^8	92×10^8
3[c]	6×10^5	3.4×10^8	11×10^8	48×10^8
4[b]	0.9×10^5	0.8×10^8	1.8×10^8	8×10^8
5[b]	2.5×10^5	0.9×10^8		10×10^8
6[c]	1.4×10^5	1.1×10^8	4.6×10^8	38×10^8

[a] Second-order rate constants in units of M^{-1} s^{-1}. Experimental details and error limits are given in the Supporting Information. [b] From the phosphatoxy radical. [c] From triplet chloranil oxidation of the enol ether.

of PTOC ester precursors; these reactions were "instant" on the nanosecond time scale.[5] Radical cation **1** was produced by both methods to test for a large systematic difference in the two methods. Triphenylamine was the reporter for **1**−**5**, and tris-(*p*-methoxyphenyl)amine was used as the reporter for **6**.

Rate constants for reactions of the enol ether radical cations with water are listed in Table 1. In these reactions, water acts as a nucleophile to give a distonic radical cation that is then deprotonated and/or as a base that deprotonates the radical cation to give an allyl radical. Addition reactions should occur with low regioselectivity.[2b] In general, an expected decrease in rate constants with increasing alkyl substitution on the radical cation was observed. The rate constants for reactions of the "dialkyl" radical cations **3**−**5** with water are somewhat smaller than that determined indirectly for reaction of water with a duplex DNA radical cation centered on a T residue ($k = 2 \times 10^6$ M^{-1} s^{-1}).[4,9] They are similar in magnitude to the rate constants for reactions of alcohols with styrene radical cations.[10]

Preliminary work demonstrated the feasibility of kinetic studies of enol ether radical cation reactions with purine nucleosides. Specifically, oxidations of 2′-deoxyadenosine (dA), 2′-deoxyinosine (dI), and 2′-deoxyguanosine (dG) by Chl* gave the corresponding radical cations that did not oxidize Ph_3N. This is a necessary condition for application of the reporter kinetic method. For tris(*p*-methoxyphenyl)amine, used as the reporter for radical cation **6**, no oxidation of the amine by the dG radical cation was observed, and a slow growth of signal from the aminium radical cation was observed from reaction of the amine with the radical cation from dA. The radical cation produced by oxidation of thymidine by Chl* oxidized Ph_3N.

Rate constants for reactions of dA, dI, and dG with the enol ether radical cations were measured in the same manner as those for reactions of the radical cations with water (Table 1). The most easily oxidized species, dG, apparently reacted in diffusion-controlled processes with radical cations **1** and **2**, but not in diffusion-controlled reactions with the other radical cations. None of the dI or dA reactions was diffusion-controlled.

The purines can react with the enol ether radical cations by electron transfer or as bases or nucleophiles. For dG, the major reaction pathway appears to be electron transfer on the basis of the agreement between the rate constants for reactions of the "dialkyl" enol ether radical cations **3**−**5** and the results reported by Giese and co-workers.[4] They observed that rate constants for oxidations of dG positions in polymeric duplex DNA containing radical cation sites on T-based sugars fit a logarithmic distance-dependent function, and extrapolation of that function to a distance of 3−4 Å gives ET rate constants in the range of $k = (1-4) \times 10^9$ s^{-1}.

The relative rate constants for oxidations of the purines can be evaluated with Marcus theory by using the simplified form in eq 2 (because one reactant is not charged), where $\Delta G^‡$ is the free

$$\Delta G^‡ = \Delta G^‡_{\text{int}} + \Delta G^0/2 + (\Delta G^0)^2/16\,\Delta G^‡_{\text{int}} \qquad (2)$$

energy of activation, $\Delta G^‡_{\text{int}}$ is the intrinsic barrier, and ΔG^0 is the free energy change of the electron-transfer reaction.[11] The intrinsic barrier is not known, but we assume that it is similar for the different purines. Oxidation potentials for G, I, and A are available,[12] and the $E^0_{1/2}$ values for oxidations of simple enol ethers[13] can be used as approximations of the oxidation potentials.

The oxidation potential of G is about 150 mV lower than that of I, and one calculates that a given enol ether radical cation should oxidize dG about an order of magnitude faster than it oxidizes dI. Thus, the results for dI in Table 1 might be consistent with electron transfer as a major pathway for reactions of dI. They are not consistent with predominant oxidation of dA, however, which is calculated to be a factor of 2000 less rapid than oxidation of dG.[14]

The enol ether radical cations most likely react with dA in acid−base reactions or as acceptors in nucleophilic addition reactions. A recent study[15] suggested that the diethyl phosphate anion rapidly deprotonated an enol ether radical cation structurally similar to **5** within an ion pair with an estimated rate constant of 1×10^9 s^{-1}, and dA is a stronger base than diethyl phosphate. It seems likely, therefore, that a major reaction pathway for dA is the acid−base reaction.

In summary, we have demonstrated a direct kinetic method for studies of reactions of enol ether radical cations that can be employed in the presence of a number of chromophores. The method should also be useful for intramolecular enol ether radical cation reactions including complex enol ether radical cations such as those produced in fragmentations of pyrimidine nucleoside radicals.

Acknowledgment. We thank the National Institutes of Health (GM56511 to M.N. and CA60500 to D.C.) for support.

Supporting Information Available: Details of the syntheses of new compounds, details of kinetic studies, and kinetic results with standard deviations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA015602C

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(9) The pseudo-first-order rate constant for reaction of the DNA enol ether radical cation with water was determined to be 1.1×10^8 s^{-1} from competition between reaction of water and KI with the radical cation and an assumption that the electron-transfer reaction with KI was diffusion-controlled with a rate constant of ca. 5×10^9 M^{-1} s^{-1} (ref 4).

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