

"switch" position are now in progress.

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Direct Measurement of the Rate Constant for β -Scission of the Cumyloxy Radical by Laser Flash Photolysis with Time-Resolved IR Detection¹

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The combination of laser flash photolysis (LFP) with time-resolved infrared (TRIR) detection provides a new and potentially extremely powerful tool for the measurement of absolute rate constants for fast reactions and for structural studies on reactive intermediates. However, IR bands generally have small extinction coefficients relative to UV bands, and this has restricted the application of the LFP/TRIR technique almost entirely to the study of metal carbonyl systems.⁴⁻⁷ The single exception in organic chemistry is Schuster and co-workers' investigation of 1,2-didehydroazepines.⁸

It seemed important to us to widen the organic applications of this valuable new technique. The β -scission of alkoxy radicals in solution should be an ideal reaction to study since ketones (and aldehydes) have rather strong IR absorptions within the 1600–2000-cm⁻¹ range of line-tunable carbon monoxide lasers. There has been only one direct, time-resolved measurement of an alkoxy β -scission in solution (vis.,⁹ C₆H₅CH₂CH₂O* → C₆H₅CH₂* + CH₂O). Reliable β -scission rate data for other systems are required in order to understand why solvents would appear to have such a profound effect on k_{β} ⁹⁻¹³ as well as to correlate k_{β} with structure.

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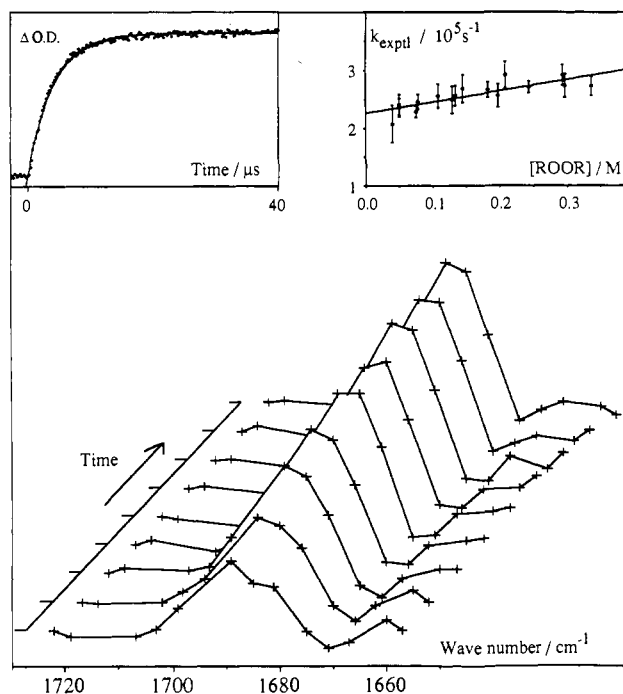
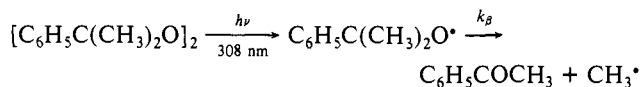
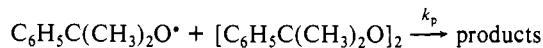


Figure 1. Time-resolved infrared difference absorption spectra observed following laser flash photolysis (308 nm, 2.8 mJ/cm²) of dicumyl peroxide (0.16 M) in Ar-saturated CCl₄. The spectra are shown at 1.5- μ s intervals from 0.75 to 12.75 μ s. The spectra were constructed from kinetic traces taken at CO laser frequencies indicated by the + symbols. The left-hand inset shows transient absorbance at 1689 cm⁻¹ as a function of time (top Δ OD = 0.0056), the solid line being a first-order fit to the points which yields k_{exptl} . The right-hand inset shows $k_{\text{exptl}}/(10^5 \text{ s}^{-1})$ as a function of [dicumyl peroxide]/M; points obtained in Ar- and in O₂-saturated solution are included.

For our initial study, we chose to measure the β -scission of the cumyloxy radical, which was generated by 308-nm LFP of dicumyl peroxide (0.06–0.5 M) in CCl₄ at room temperature (23 °C) in a flow system (1.3 mL/min) within a calcium fluoride cell (path length, 2 mm). The apparatus has been described.^{14,15} TRIR spectra derived from kinetic traces show first-order growth of a band at 1689 cm⁻¹, as expected for acetophenone (see Figure 1).



The experimental rate constant for growth, k_{exptl} , was identical for argon- and oxygen-saturated solutions (a result consistent with the formation of acetophenone from an oxygen-centered precursor) and for laser doses varying from 2 to 6 mJ/cm² (a result that shows that cumyloxy radicals are not destroyed by radical-radical reactions). However, k_{exptl} does decrease slightly as the peroxide concentration is decreased (see right-hand inset in Figure 1) because the cumyloxy radicals can be destroyed by attack on the peroxide:



Since $k_{\text{exptl}} = k_{\beta} + k_p[\text{C}_6\text{H}_5\text{C}(\text{CH}_3)_2\text{O}]_2$, the slope of this plot yields k_p and the intercept k_{β} . We also measured the rate constant for hydrogen abstraction from cyclohexane, k_a , by the addition of various concentrations of cyclohexane with a fixed concentration of peroxide ($k_{\text{exptl}} = k_0 + k_a[\text{c-C}_6\text{H}_{12}]$). These rate constants ($\pm 2\sigma$) are as follows: $k_p = (1.94 \pm 0.62) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $k_a = (9.53 \pm 1.35) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, and $k_{\beta} = (2.27 \pm 0.13) \times 10^5 \text{ s}^{-1}$.

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We can compare the present results with those reported by Scaiano and co-workers,¹⁶ viz., $k_a = (2.04 \pm 0.73) \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ in benzene at 27 °C and $k_\beta = 1.0 \times 10^6 \text{ s}^{-1}$ in chlorobenzene¹⁷ (as calculated from their Arrhenius equation). Walling^{10,11} was the first to suggest that solvents will influence bimolecular reactions to a lesser extent than unimolecular reactions. Taken together, our results and Scaiano's lend support to this idea. However, the difference between our direct measurement of k_β and Scaiano's value cannot be accounted for if the usual assumption^{10,11} is made that k_a is solvent independent. That is, combining our value of k_β with Walling and Wagner's¹¹ values for k_a/k_β at 25 °C, viz., 6.42 M⁻¹ in CCl₄¹⁸ and 4.22 M⁻¹ in chlorobenzene, and assuming a solvent-independent k_a gives k_β in chlorobenzene = $3.45 \times 10^5 \text{ s}^{-1}$. The most probable reason for the disagreement in the values of k_β is the indirect method used by Scaiano and co-workers to measure this quantity.¹⁹

We conclude that the LFP/TRIR technique provides a direct and reliable method for measuring the rate constants for β -scission of alkoxy radicals. Structural and solvent effects on this reaction will be explored.

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(17) It proved difficult to study the β -scission of cumyloxy in chlorobenzene because of this solvent's absorption at 1690 cm⁻¹. Preliminary experiments indicate that $k_{\text{expl}} \sim 3 \times 10^5 \text{ s}^{-1}$, which gives an upper limit for k_β in chlorobenzene.

(18) Our absolute values of k_a and k_β in CCl₄ yield $k_a/k_\beta = (4.20 \pm 0.84) \text{ M}^{-1}$.

(19) These workers relied on product studies to partition the rate constant for β -scission from the rate constants for competing bimolecular reactions.

Determination at Single-Nucleotide Resolution of the Sequence Specificity of DNA Interstrand Cross-Linking Agents in DNA Fragments

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Interstrand cross-linking of DNA is believed to account for the acute cytotoxicity of many bifunctional alkylating agents, including important antitumor substances.¹ Determining the structural details of cross-linked DNAs is complicated by both molecular size and the inefficiency of cross-linking, with monofunctional binding to DNA usually greatly exceeding the bifunctional, cross-linking mode. One strategy, exhaustive hydrolysis of the phosphodiester backbone of cross-linked DNA followed by conjugate isolation and structure elucidation, can effectively define sites of covalent linkage between drug and DNA (e.g., guanine N-7), but is not generally useful for determining preferred base sequence at the cross-link site. We describe herein a simple and general chemical method for determining the base sequence preferences of DNA interstrand cross-linking drugs at single-nucleotide resolution. The method is demonstrated by using DNAs crosslinked with a psoralen, mitomycin C, and an analogue of a pyrrolizidine alkaloid metabolite.

The sequence dependence of noncovalent protein-DNA^{2a} and drug-DNA^{2b} interactions has been studied by "footprinting", in which the complexed DNA is randomly cleaved and the fragment size distribution analyzed.^{2c} Analogous treatment of a cross-linked DNA might be expected to define cross-link location. Single-hit, random cleavage to the radiolabeled (*) 3'-side of the cross-link

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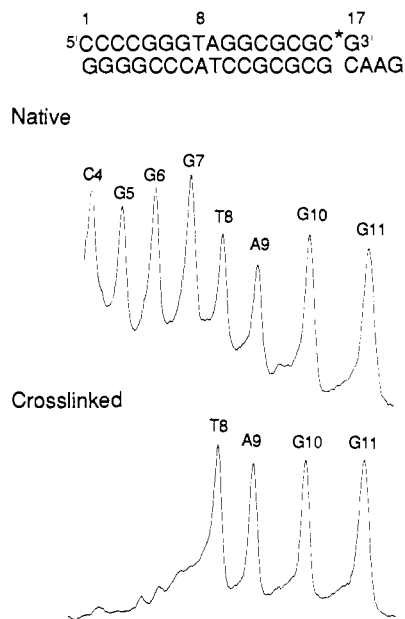
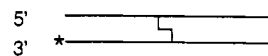


Figure 1. Partial fragmentation patterns for radiolabeled (**) = ³²P native and HMT cross-linked DNA duplexes. Lettering indicates residue cleaved.

in the DNA shown schematically below should provide short radiolabeled fragments. Cleavage at any other site on either strand should afford a much larger radiolabeled fragment. Electrophoretic separation of the fragment mixture should provide a discontinuity diagnostic for the cross-link site.



A DNA duplex containing a single 5'-d(TA) cross-linkable sequence was cross-linked by using 4'-(hydroxymethyl)-4,5',8-trimethylpsoralen (HMT), a substance known to bridge covalently two thymine moieties.³ The product was selectively end radiolabeled (³²P), and the cross-linked DNA was separated from residual single-stranded DNA by denaturing polyacrylamide gel electrophoresis (PAGE).⁴ Radiolabeled native and cross-linked materials were subjected sequentially to iron(II)/EDTA cleavage,⁵ single-base-resolution PAGE,⁶ autoradiography, and one-dimensional scanning densitometry.⁷ As predicted by the above analysis,

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(7) DNA was synthesized (Applied Biosystems Model 380A) and was purified by denaturing PAGE (20%, 95:5 acrylamide/bis(acrylamide)). A 1:1 (OD₂₆₀) mixture of strands (see Figure 1), 17 μM in duplex DNA, 300 μM in HMT, and containing 10 mM NaCl, 10 mM MgCl₂, 50 mM Tris (pH 8.0), 0.1 mM EDTA, total volume 650 μL, was nated for 1.5 h at 25 °C and then irradiated at 4 °C in a silanized Pyrex test tube at 351 nm (100 mW, Spectra-Physics argon laser model 2025-05) for 0.5 h. An aliquot was 3'-end labeled by using Klenow fragment (Boehringer Mannheim)/[α-³²P]dGTP (New England Nuclear), and cross-linked DNA was isolated by denaturing PAGE (25%).⁴ Iron(II) EDTA⁵ cleavage reactions were carried out in 50 μM (NH₄)₂Fe(SO₄)₂, 100 μM EDTA, 1 mM sodium ascorbate, 10 mM H₂O₂, 10 mM NaCl, 10 mM Tris (pH 8.0), 1 min, 25 °C, and were stopped with excess thiourea. Samples were lyophilized, suspended in 90% formamide, 10 mM Tris (pH 8.0), 0.1% xylene cyanol, 0.1 mM EDTA, heat denatured at 90 °C for 1 min, cooled to 0 °C, and subjected to electrophoresis on a 25% polyacrylamide gel (95:5 acrylamide/bis(acrylamide), 50% urea, 0.35-mm thick, 41 × 37 cm) using a Hoefer thermo jacketed Poker Face gel stand at ca. 1500 V and 70 °C until the dye had traveled 19 cm. The gel was dried (Bio-Rad Model 583) onto Whatman 3MM paper and autoradiographed on Kodak XAR-5 film. Cleavage site was assigned by reference to a Maxam-Gilbert G-lane.^{6a} The autoradiogram was scanned and plotted by using the program Spectra Calc (Galactic Industries Corporation, Salem, NH).