"switch" position are now in progress.

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## Direct Measurement of the Rate Constant for β-Scission of the Cumyloxyl Radical by Laser Flash Photolysis with Time-Resolved IR Detection[1]

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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 alkoxyl 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 alkoxyl β-scission in solution (vis.,[9] $C_6H_5CH_2CH_2O^\bullet \rightarrow C_6H_5CH_2^\bullet + CH_2O$). 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 $k_\beta$[9-13] as well as to correlate $k_\beta$ with structure.

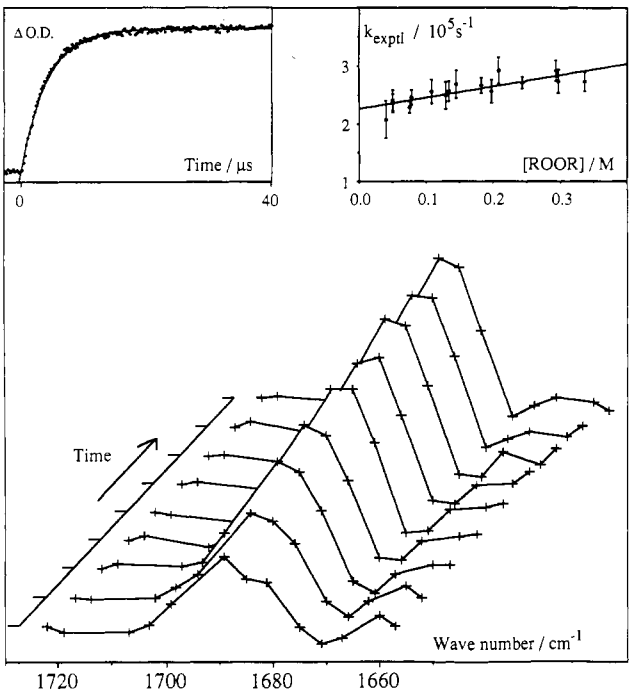


**Figure 1.** Time-resolved infrared difference absorption spectra observed following laser flash photolysis (308 nm, 2.8 $mJ/cm^2$) of dicumyl peroxide (0.16 M) in Ar-saturated $CCl_4$. The spectra are shown at 1.5-μs intervals from 0.75 to 12.75 μs. The spectra were constructed from kinetic traces 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 ΔOD = 0.0056), the solid line being a first-order fit to the points which yields $k_{exptl}$. The right-hand inset shows $k_{exptl}/(10^5\ s^{-1})$ as a function of [dicumyl peroxide]/M; points obtained in Ar- and in $O^2$-saturated solution are included.

For our initial study, we chose to measure the β-scission of the cumyloxyl radical, which was generated by 308-nm LFP of dicumyl peroxide (0.06–0.5 M) in $CCl_4$ 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).

$$[C_6H_5C(CH_3)_2O]_2 \xrightarrow[308\ nm]{h\nu} C_6H_5C(CH_3)_2O^\bullet \xrightarrow{k_\beta} C_6H_5COCH_3 + CH_3^\bullet$$

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 $mJ/cm^{-2}$ (a result that shows that cumyloxyl 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 cumyloxyl radicals can be destroyed by attack on the peroxide:

$$C_6H_5C(CH_3)_2O^\bullet + [C_6H_5C(CH_3)_2O]_2 \xrightarrow{k_p} \text{products}$$

Since $k_{exptl} = k_\beta + k_p[C_6H_5C(CH_3)_2O]_2$, the slope of this plot yields $k_p$ and the intercept $k_\beta$. 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 ($k_{exptl} = k_0 + k_a[c\text{-}C_6H_{12}]$). These rate constants ($\pm 2\sigma$) are as follows: $k_p = (1.94 \pm 0.62) \times 10^5\ M^{-1}\ s^{-1}$, $k_a = (9.53 \pm 1.35) \times 10^5\ M^{-1}\ s^{-1}$, and $k_\beta = (2.27 \pm 0.13) \times 10^5\ s^{-1}$.

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We can compare the present results with those reported by Scaiano and co-workers,[16] viz., $k_a = (2.04 \pm 0.73) \times 10^6$ $M^{-1}$ $s^{-1}$ in benzene at 27 °C and $k_\beta = 1.0 \times 10^6$ $s^{-1}$ in chlorobenzene[17] (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 $k_\beta$ 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 $k_\beta$ with Walling and Wagner's[11] values for $k_a/k_\beta$ at 25 °C, viz., 6.42 $M^{-1}$ in $CCl_4$[18] and 4.22 $M^{-1}$ in chlorobenzene, and assuming a solvent-independent $k_a$ gives $k_\beta$ in chlorobenzene = $3.45 \times 10^5$ $s^{-1}$. The most probable reason for the disagreement in the values of $k_\beta$ 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 alkoxyl radicals. Structural and solvent effects on this reaction will be explored.

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(18) Our absolute values of $k_a$ and $k_\beta$ in $CCl_4$ yield $k_a/k_\beta = (4.20 \pm 0.84)$ $M^{-1}$.

(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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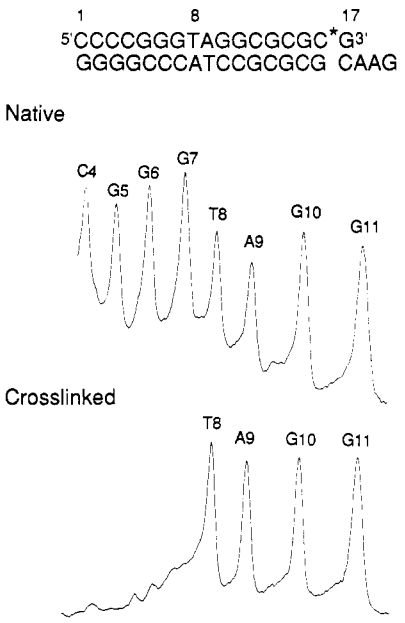
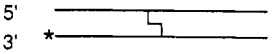



**Figure 1.** Partial fragmentation patterns for radiolabeled ((*) = $^{32}P$) native and HMT cross-linked DNA duplexes. Lettering indicates residue cleaved.

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 ($^{32}P$), 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 ($OD_{260}$) mixture of strands (see Figure 1), 17 μM in duplex DNA, 300 μM in HMT, and containing 10 mM NaCl, 10 mM $MgCl_2$, 50 mM Tris (pH 8.0), 0.1 mM EDTA, total volume 650 μL, was nutated 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-$^{32}P$]dGTP (New England Nuclear), and cross-linked DNA was isolated by denaturing PAGE (25%).[4] Iron(II) EDTA[5] cleavage reactions were carried out in 50 μM $(NH_4)_2Fe(SO_4)_2$, 100 μM EDTA, 1 mM sodium ascorbate, 10 mM $H_2O_2$, 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 Hoeffer thermojacketed 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).

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