NMR spectra were obtained through the auspices of the Northeast Regional NSF/NMR Facility at Yale University, which was supported by NSF/Chemistry Division Grant CHE 7916210. We thank Dr. John Secrist for providing us with the authentic sample.

Oxidation and Reduction Potentials of Transient Free Radicals[1]

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Received July 8, 1985

The oxidation and reduction potentials of free radicals are fundamental quantities of particular importance. For example, reduction potentials of alkyl radicals can be combined with other thermodynamic data to give pK_a values for hydrocarbons.[2-5]

Despite their significance, only a few of these potentials have been measured. This is because most free radicals have short lifetimes and are therefore not suitable as starting materials for standard electrochemical methods.[6] As a result, they must be formed as the products of electrochemical reactions. Hence, carbonium and carbanions serve as the reagents with all the attendant experimental difficulties.

In response to these problems, we have devised a method for measuring oxidation and reduction potentials, in which the transient radicals are actually used as the starting materials for the electrochemical reaction. The apparatus (Figure 1) was built around a standard three-electrode cell, which was fitted with quartz windows and a gold mesh working electrode. Modulated photolysis was used for radical generation with phase-sensitive electrochemical detection as a device for enhancing instrumental sensitivity.[7,8]

Radicals were generated by modulated photolysis of acetonitrile solutions containing appropriate precursors (vide infra) and tetrabutylammonium perchlorate (0.1 M) as the supporting electrolyte. Samples were flowed slowly through the cell so as to avoid problems associated with sample depletion and/or product formation. The photolysis source was a 1000-W mercury-xenon lamp which was only capable of generating average radical concentrations of 10^{-7}-10^{-8} M, i.e., well below the normal level of detection for conventional electrochemical apparatus. The voltage at the working electrode was scanned slowly (20 mV/s) until the reduction or oxidation potentials of the radicals were reached, at which points small currents oscillating at the modulation frequency were obtained due to the formation of the carbanions or carbonium ions. The phase-sensitive detector gave the amplitude of the oscillating signals, which was output onto an x-y recorder. The resulting trace was a polarogram of the free radical, Figure 2.

Two chemical systems were used for radical generation: first, the photodecomposition of ketones, eq 1 and, second, photolysis

$$RC(O)R \xrightarrow{h\nu} 2R\cdot + CO \quad (1)$$

$$t\text{-BuO} - \text{OBu-}t \xrightarrow{h\nu} 2\ t\text{-BuO}\cdot \quad (2)$$

$$t\text{-BuO}\cdot + RH \rightarrow t\text{-BuOH} + R\cdot \quad (3)$$

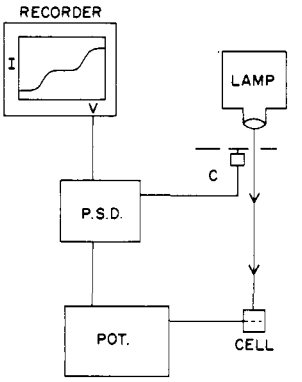

Figure 1. Diagram of apparatus. C, light chopper; POT, potentiostat; PSD, phase sensitive detector.

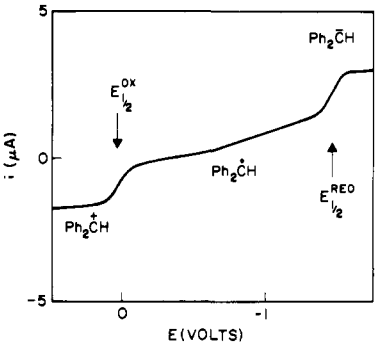

Figure 2. Polarogram of $(C_6H_5)_2\dot{C}H$ showing oxidation ($E_{1/2}^{ox}$) and reduction ($E_{1/2}^{red}$) potentials. Radical generation by modulated photolysis (43 Hz) of t-BuO-OBu-t (0.5 M) in acetonitrile containing diphenylmethane (1.0 M).

Table I. Oxidation and Reduction Potentials of Transient Free Radicals[a]

radical	$E_{1/2}^{ox}$, V	$E_{1/2}^{red}$, V	method[b]
$Ph\dot{C}H_2$	0.40 ± 0.03	−1.78 ± 0.02 (−1.76)[c]	1, 2, 3
$Ph_2\dot{C}H$	0.02 ± 0.02 (0.01)[d]	−1.47 ± 0.02 (−1.49)[c]	1, 2, 3
$Ph\dot{C}(CH_3)_2$	−0.20 ± 0.02	−2.10[e]	2

[a] In acetonitrile containing 0.1 M tetrabutylammonium perchlorate. All potentials measured with respect to $Ag/AgNO_3$ (0.1 M in acetonitrile) which has a potential of 0.334 V vs. the standard calomel electrode. [b] Method 1: see eq 1. Method 2: see eq 2, 3. Method 3: photolysis of $(C_6H_5)_2CHC(O)CH_2(C_6H_5)$. [c] Reference 2. [d] Reference 13. [e] Tentative value; limiting current of reduction wave poorly defined.

of di-*tert*-butyl peroxide (0.5 M) in the presence of hydrogen donors, eq 2, 3. Both sources led to the same oxidation and reduction potentials. The results for several radicals are reported in Table I. Clearly, radical generation by more than one well-authenticated route builds confidence in the reliability of the values reported, as does the excellent agreement with literature data in instances where they were available.

Several simple tests lend support to the measured values. No polarograms were detected by the phase-sensitive method, in the absence of photolysis or of the radical precursors. Moreover, simple dc detection of electrochemical signals due to the samples as a whole showed that little electrochemistry took place in the voltage range of interest (1.0 to −2.0 V vs. $Ag/AgNO_3$).

Analysis of the polarograms showed that the systems were, in most instances, quasi-reversible or reversible. That is, the rates of the electrochemical reactions were essentially mass transport limited. The difference between the oxidation and reduction potentials therefore represents the difference in the heats of formation, in solution, of the carbonium and carbanions derived from a given radical. For the benzyl radical in the gas phase[9-11]

(1) Issued as NRCC Publication No. 25119.
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(6) Pulse radiolysis and flash photolysis techniques have seen limited application in this context; see: Henglein, A. In "Electroanalytical Chemistry"; Bard, A. J., Ed.; Marcel Dekker: New York, 1976; Vol. 9, pp 163-244.
(7) For a related technique using optical detection, see: Griller, D. *Rev. Chem. Intermed.* **1984**, *5*, 21-36 and references cited therein.
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0002-7863/85/1507-7764$01.50/0 Published 1985 by the American Chemical Society

this difference is 186 kcal mol^{-1} while the present experiments show that, in solution, the value is ca. 50 kcal mol^{-1} [12] and point to a dramatic and preferential stabilization of one of the ions by ca. 136 kcal mol^{-1}.

In summary, modulated photochemical generation of free radicals, with phase-sensitive detection, allowed measurements of their oxidation and reduction potentials. The method was applicable to radicals which normally undergo diffusion controlled self-reaction. Under our experimental conditions, radical concentrations were typically 10^{-7}–10^{-8} M and lifetimes only ca. 10^{-3} s. As in most phase-sensitive techniques, the system may be used to measure the lifetimes of the transients under investigation and could thus provide a method for kinetic studies of carbonium and carbanion reactions.

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(12) The electrochemical oxidation of the benzyl radical is irreversible. However, the $\Delta\Delta H_f$ calculated will represent an *upper limit* since any associated overpotential will be in the anodic direction.
(13) Arnold, D. R., unpublished results.

An Efficient, Site-Specific DNA Target for Bleomycin

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The bleomycins (BLM's) are a group of structurally related antitumor antibiotics that are believed to mediate their therapeutic effect primarily via oxidative DNA strand scission.[2] By the use of supercoiled covalently closed circular DNA's (cccDNA's) and DNA fragments obtained by restriction endonuclease digestion of cccDNA's, it has been possible to demonstrate that bleomycin produces both single- and double-strand nicks in double-strand DNA, and does so with considerable sequence selectivity.[3] Also established convincingly by the use of such substrates is the requirement for an appropriate metal ion and O_2.[4,5] The use of large DNA fragments of defined structure for analysis of the chemistry of DNA strand scission has proven more difficult.[6] An alternative approach has involved extensive degradation of bulk DNA by high concentrations of bleomycin; subsequent chemical or enzymatic workup of the oligomeric product mixtures has permitted identification of some of the chemical products of BLM-mediated DNA strand scission.[7] However, this approach is poorly suited to the analysis of unstable reaction products, makes the assumption that reaction products obtained following extensive DNA degradation involve the same chemistry mediated at high

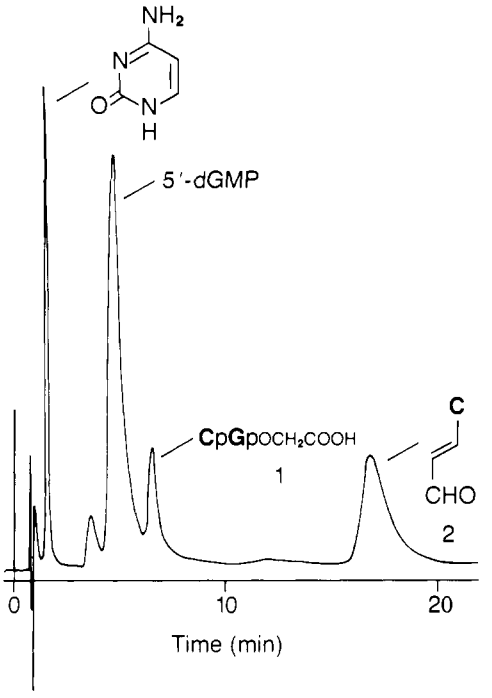
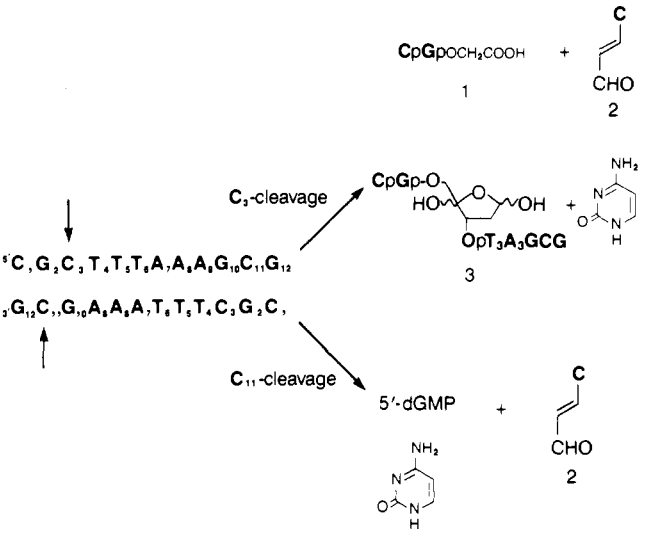


Figure 1. Hplc analysis of Fe^{II}·BLM A_2-treated dodecanucleotide. The reaction mixture (total volume 50 μL) contained 1 mM d-($CGCT_3A_3GCG$) (final nucleotide concentration), 0.2 mM BLM A_2, and 0.2 mM $Fe(II)(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate, pH 7.0. The reaction was initiated by addition of Fe(II), incubated at 0 °C for 15 min, and then analyzed promptly by HPLC on a Rainin Microsorb C_{18} column (3 μm), elution was with 0.1 M ammonium formate at a rate of 1.5 mL/min. In addition to the labeled peaks, small amounts of guanine (3.6 min) and adenine (~12 min) were observed.

Scheme I. Products Formed Concomitant with Fe^{II}·BLM-Mediated Degradation of d($CGCT_3A_3GCG$)



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(5) O_2-independent DNA degradation by Co^{III}·BLM + *hν* has also been reported. See: Chang, C. H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268.
(6) These include difficulties in (i) preparation of such species in quantity for degradation, (ii) separation of the products resulting from modification at each of several loci within a given sequence, and (iii) analysis of the resulting oligomeric products by routine spectral techniques due both to the size of the formed products and the modest extent of product formation at any given site.
(7) See, e.g.: (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608. (b) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H., Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735. (c) Uesugi, S.; Shida, T., Ikehara, M.; Kobayashi, Y.; Kyogoku, Y. *Nucleic Acids Res.* **1984**, *12*, 1581.

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