Chemiluminescence Mechanism and Quantum Yield of Synthetic Vinylpyrene Analogues of Benzo[a]pyrene-7,8-dihydrodiol†

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Received October 22, 1985

Abstract: We have previously reported that the dioxetane chemiluminescence (CL) of the proximate carcinogenic metabolite (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8-Diol) could be produced by free singlet oxygen ($^1O_2$) in solution. We now report that the *trans*-1-(2-methoxyvinyl)pyrene (*t*-MVP) model chemical analogue, in which an ene reaction is prevented, has a CL quantum yield ϕ_{CL} (*t*-MVP) of 0.003, 180 times that of 7,8-diol. The CL emission spectrum of *t*-MVP was identical with the fluorescence emission spectrum of the dioxetane decomposition product, pyrene-1-carboxaldehyde (**6** in Scheme II), which was isolated in 10% yield in $^1O_2$ reactions. The solvent dependencies of ϕ_{CL} *t*-MVP of dioxetanes parallel the fluorescence quantum yield (ϕ_{Fluor}) of **6** and the CL yield from dioxetanes of 7,8-Diol in these same solvents. This dioxetane CL of the *t*-MVP analogue supports the originally proposed dioxetane CL mechanism of 7,8-Diol. The limiting factor in 7,8-Diol CL appears to be the low chemical yield for formation of the 9,10-dioxetane.

I. Introduction

Anderson[1] proposed that metabolic oxidation of polycyclic aromatic hydrocarbons (PAH) resulted in the release of photons whose absorption by "sensitive" molecules within the cell resulted in malignant transformation. He demonstrated that a weak chemiluminescence (CL) could be produced nonenzymatically for all PAH's tested, using strongly oxidizing chemical conditions. On the basis of the dioxetane mechanism in bioluminescent reactions, Seliger[2] proposed that oxygenase reactions of detoxification in mammalian organisms might also result in dioxetane intermediates and that the most likely candidates for observable CL would be the oxidized planar fluorescent residues of PAH's. The first experimental evidence of metabolic PAH CL and of the use of CL as a noninvasive assay for the rate of detoxification of PAH's was by Seliger and Hamman.[3,4] In these same papers it was shown that the intensity of CL correlated with the *carcinogenicity* of the parent PAH and with the *inducibility* of the benzo[*a*]pyrene (B[a]P) cytochrome P-450 system, using microsomes extracted from rat liver. The low quantum yields of CL implied that the proposed dioxetane pathway was a *minor* parallel pathway concomitant with the production of dihydrodiol epoxide carcinogenic metabolites and that the CL was a *tracer* for the rate of production of carcinogenic dihydrodiol epoxides. The ubiquitous carcinogen B[a]P was chosen as a model compound for the study of this metabolic CL, since an extensive literature on B[a]P metabolism already existed and synthesized metabolic products of B[a]P were available from the National Cancer Institute Chemical Repository. In subsequent microsomal chemiluminescence studies,[5,6] it was shown that the microsomal metabolite of B[a]P with the highest specific microsomal CL yield was the proximate carcinogen, (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8-Diol) (**1** in Scheme I). We therefore proposed 7,8-Diol-9,10-dioxetane (**2**) as the metabolic intermediate producing 7,8-Diol microsomal CL and that, to produce CL, all other metabolites including the parent B[a]P must proceed through stepwise metabolism to produce the saturated 7,8-Diol. In order to demonstrate this, all of the B[a]P metabolites were reacted in SDS solution with singlet oxygen ($^1O_2$) produced chemically by the injection of NaOCl into H_2O_2, a standard synthetic method for the chemical synthesis of dioxetanes.[7-10]

Owing to the short lifetime of $^1O_2$ in aqueous solution (~2 μs), a pulse of $^1O_2$ was predicted to produce a chemiluminescent dioxetane only from the 7,8-Diol metabolite. Seliger et al.[11] demonstrated that not only was ϕ_{CL} for the 7,8-Diol 10^2–10^5 times higher than for all of the other metabolites, but ϕ_{CL} for $^1O_2$ initiated CL was 10^4 times higher than for microsome-initiated CL. The former result was used to develop a $^1O_2$ reaction assay specific for 7,8-Diol in complex mixtures of microsomal metabolites of B[a]P.[12]

Efforts to accumulate and to isolate **2** or the predicted fluorescent dialdehyde product **3** by long-term, low-temperature exposure of 7,8-Diol to $^1O_2$ produced by a rose bengal photosensitization were unsuccessful. However, we did note that the $^1O_2$-initiated CL of 7,8-dihydrobenzo[*a*]pyrene (unpublished results) was equally as efficient as that of 7,8-Diol; therefore, it appeared that the hydroxyl groups on the saturated 7,8-distal bond were not essential for CL. Since (a) 2 + 2 cycloaddition of $^1O_2$ to electron-rich heteroatom-substituted C=C double bonds had been reported,[13-17] (b) methoxy groups were known to activate methoxyvinyl aromatic systems toward 2 + 2 cycloaddition with $^1O_2$,[18] and (c) monoolefins which do not possess allylic hydrogen atoms tended to undergo 2 + 2 cycloaddition reactions. Therefore,

† Supported under research Grant NIEHS-1-PO1-ES-02300.
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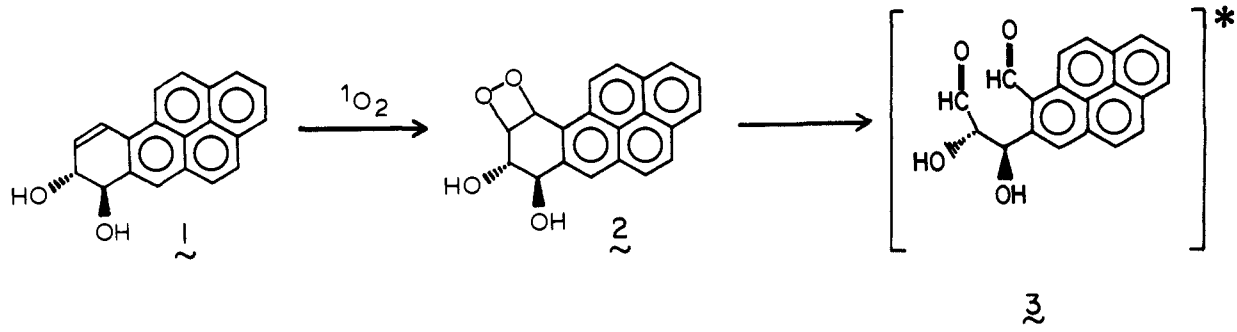
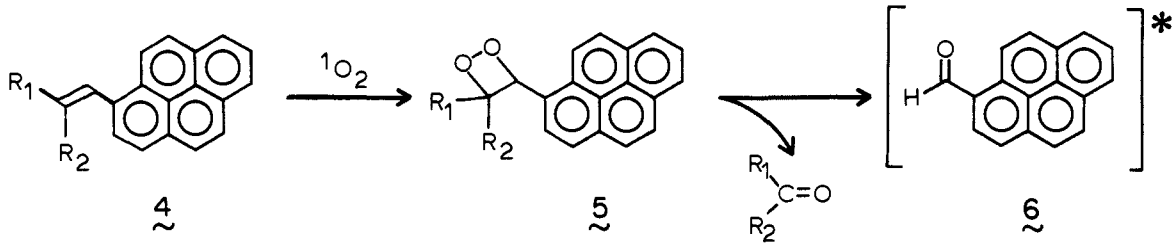
0002-7863/86/1508-4498$01.50/0

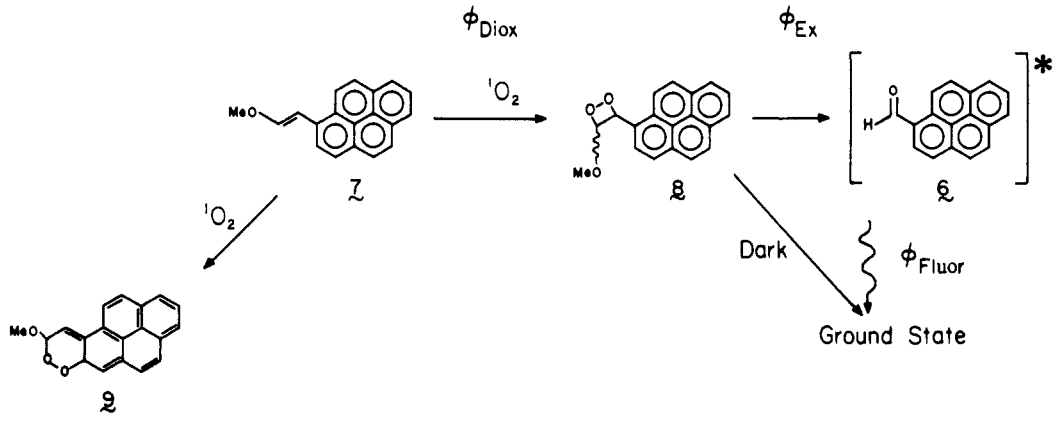
Scheme I



Scheme II



Scheme III



we synthesized a series of substituted vinylpyrene analogues (**4** in Scheme II) including *cis*- and *trans*-1-(2-methoxyvinyl)pyrenes (*c*- and *t*-MVP) in order to model the dioxetane CL of 7,8-Diol and to eliminate competing ene reactions of $^1O_2$. The product in the singlet electronic excited state resulting from the decomposition of the pyrenyldioxetane **5** is pyrene-1-carboxaldehyde (**6**). Structure **6** (Scheme II) is analogous to the proposed dialdehyde product (**3**) from 7,8-Diol (Scheme I), differing only by the dihydroxypropionaldehyde substituent, ortho to the carboxaldehyde substituent.

In this paper we report the synthesis and characterization of these vinylpyrene analogues and the CL kinetics, emission spectra, quantum yields, and product isolations from the *trans*-1-(2-methoxyvinyl)pyrene (*t*-MVP) (**7** in Scheme III) analogue compared directly with similar experiments using 7,8-Diol.

These results indicate that the limiting step in $^1O_2$ 7,8-Diol CL appears to be the chemical efficiency for the production of a dioxetane and lead us to propose *t*-MVP as a highly efficient chemiluminescent probe for $^1O_2$.

II. Experimental Section

CL Detection Methods and Spectra. Photon detection methods were either single photon counting[6] or DC measurement,[19] both calibrated for absolute photon sensitivity by the luminol chemiluminescent reaction.[20] Day-to-day calibrations were checked using either a quenched ^{3}H-scintillation solution as a secondary single photon standard[21] or a ^{14}C-activated luminous epoxy resin sealed in a small test tube.[19] The CL emission spectra were measured with a 1-m *f*/3 Fastie-Ebert grating spectrometer[22] calibrated for spectral sensitivity with an NBS standard lamp.[23]

Photosensitization Reactions. Photosensitization reactions were carried out by using a modified Kodak slide projector with either a tungsten DFD 1000 W or a CZX 500W lamp to irradiate a sample containing the hydrocarbon substrates and either free rose bengal or Sensitox II (Hydrion Laboratories) as the sensitizer. The light beam from the projector was passed through 2 cm of water and a Corning 3-71 glass filter to absorb the infrared and blue radiation, respectively.[11] Concentrations of $^1O_2$ were determined by measuring the effective fractional absorption of rose bengal in our geometry with a thermopile. The amount of $^1O_2$ produced was then calculated using the quantum yield for the production of $^1O_2$ (0.76) by free rose bengal in methanol.[24] All photosensitization reactions were carried out in methanol to keep the lifetime and amount of $^1O_2$ produced constant for all reactions.

Typical photosensitizations at ambient temperatures entailed the irradiation of a sample in a 1-cm quartz cuvette containing 10 μM rose bengal ($A_{555} \cong 1$) and micromolar concentrations of the CL substrate for varying periods of time, ≤6 min. During these short irradiation times, the bleaching of rose bengal was <5%. Aliquots of the irradiated samples were then transferred to a vial containing the solvents of interest and placed in the photometer. This operation required only 10–15 s. The

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long lifetimes (minutes) of the chemiluminescent intermediates permitted precise extrapolation of measured CL to zero time (CL_0). The decays of CL were first order over 4 half-lives of decay in all solvents studied. The total light emitted by these first-order reactions is given by CL_0/k, where k is the first-order rate constant.

Determination of ϕ_{CL} for $^1O_2$-Initiated CL of *t*-MVP. There are three parts to the quantum yield ϕ_{CL} from the *t*-MVP substrate. These are shown in eq 1 where α is the chemical yield of dioxetanes produced by $^1O_2$ reaction with the substrate, ϕ_{EX} is the yield of excited-state product

$$\phi_{CL}(t\text{-MVP}) = \alpha\phi_{EX}\phi_{FL} \quad (1)$$

molecules due to decomposition of the dioxetane, and ϕ_{FL} is the chemiexcited fluorescence yield of the excited-state product. The interplay of α, ϕ_{EX}, and ϕ_{FL} in the $^1O_2$ reaction with *t*-MVP is indicated in Scheme III. The overall quantum yield for CL is operationally defined as eq 2.

$$\phi_{CL}(t\text{-MVP}) = \frac{\text{no. of photons emitted}}{\text{no. of substrate molecules reacted}} \quad (2)$$

The number of dioxetanes produced by the $^1O_2$ reaction can be conservatively estimated by HPLC, as the amount of pyrene-1-carboxaldehyde **6** produced. Determination of **6** is independent of ϕ_{FL} and ϕ_{EX}, regardless of whether all dioxetane decompositions lead to excited states of product aldehydes. Therefore from (1) comes (3) and from (2) comes (4).

$$\phi_{CL}(\text{dioxetane}) = \phi_{EX}\phi_{FL} \quad (3)$$

$$\phi_{CL}(\text{dioxetane}) = \frac{\text{no. of photons emitted}}{\text{no. of } \mathbf{6} \text{ produced}} \quad (4)$$

A small percentage of the dioxetanes produced may undergo C-heteroatom cleavage[10] without 1,2-dioxetane C–C bond scission, but no evidence for this reaction was obtained.

The photophysical fluorescence quantum yield (ϕ_{Fluor}) of **6** was measured in each solvent in which ϕ_{CL}(*t*-MVP) was determined and was assumed to be equal to the chemiexcited fluorescence yield, ϕ_{FL}. This may not always be true and will be discussed later.

For determinations of both ϕ_{CL}(*t*-MVP) and ϕ_{CL}(dioxetane), samples containing 10 nmol of rose bengal and 9.5 nmol of *t*-MVP, in 1 mL of methanol, were irradiated for 2 min. Aliquots of the irradiated solution were transferred to a vial containing the solvent of interest such that the final concentration of the solvent was >95%. This vial was placed in a photometer, and the total light emission was determined as CL_0/k. Aliquots were also analyzed by HPLC to quantitate the amounts of *t*-MVP that were lost to the reaction and the amounts of **6** produced. An isocratic 80% methanol/water mobile phase was used for this separation with a Whatman 5-μm particle ODS-3 C_{18} reverse-phase column. A Kratos FS-950 fluorescence detector and an ISCO UA-5 absorption monitor (254 nm) were used to quantitate the HPLC separation.

Photosensitization Reactions at −78 °C. Low-temperature irradiations were carried out by irradiating 0.15 g of Sensitox II and either 1.85 μmol of 7,8-Diol or 2.19 μmol of *t*-MVP in 5 mL of methanol in a test tube immersed in a methanol/dry ice slurry contained in a clear Dewar. Solutions were kept stirred and oxygenated by bubbling dry O_2 through them. At various times the relative amount of dioxetane accumulated was assayed by transferring 50-μL aliquots to vials containing 1.0 mL of 0.1 M SDS at room temperature and measuring the resultant CL. Sensitox II was Soxhlet extracted for 24 h with methanol and for 14 h with methylene chloride before use.

ϕ_{Fluor} and Fluorescence Emission Spectra of Pyrene-1-carboxaldehyde. Fluorescence spectra of **6** ($A_{347nm} \leq 0.010$) in different solvents were measured on a SLM 8000 photon-counting spectrofluorometer calibrated for spectral sensitivity with an NBS standard quartz–halogen lamp. Fluorescence quantum yields for **6** in different solvents were determined relative to quinine sulfate (ϕ_{Fluor} = 0.60) in 0.1 M percloric acid[25] with excitation at 347 nm.

Calculations of ϕ_{Fluor} for **6** were according to the methods of Seliger[23] and Parker.[26] UV absorbances of pyrene-1-carboxaldehyde and quinine sulfate were measured in stoppered quartz cuvettes on a Cary 219 recording spectrophotometer immediately prior to fluorescence measurements. Pyrene-1-carboxaldehyde was purchased from Aldrich and recrystallized from methylene chloride/methanol. HPLC analysis showed >99.7% purity. Quinine sulfate (Ultrex grade) was purchased from Baker and used without further purification.

Determination of Activation Energies for Dioxetane Decomposition of 7,8-Diol and *t*-MVP. Either 19.1 nmol of 7,8-Diol or 16.8 nmol of *t*-MVP in 1.5 mL was irradiated as described for 2 min at ambient temperatures. After the irradiation period, samples were transferred to a jacketed quartz cuvette at a series of temperatures and placed in front of a 1P21 phototube. The CL was recorded as a function of time. The temperature of the solution in the cuvette was measured with a Keithley digital thermometer before and after the measurement of CL intensity. Temperature was varied in seven steps ranging from 19.2 to 52.5 °C. The decay of CL in all cases was first order for *greater* than 6 half-lives. Activation energy was calculated from an Arrhenius plot. E_A of 7,8-dihydrodiol dioxetane was determined in both 0.1 M SDS and methanol. E_A of *t*-MVP dioxetane was measured in methanol.

Synthesis, Purification, and Characterization of Substituted Vinylpyrenes and 2-Methylpyrenes. Preparative thin-layer chromatography (TLC) was carried out with Analtech silica gel GF plates. Visualization of developed plates was accomplished via fluorescence quenching (254 nm). Proton nuclear magnetic resonance (^1H NMR) spectra were obtained at 80 MHz (Varian CFT-20) or 300 MHz (Bruker WM-300). Chemical shifts are reported in parts per million (δ) relative to internal tetramethylsilane (δ_{Me_4Si} 0.000) in deuteriochloroform. Melting points were measured on a Mel-Temp apparatus and are uncorrected. Low-resolution mass spectra were determined with a Kratos MS-50 spectrometer through the NSF Regional Instrumentation Facility, Middle Atlantic Mass Spectrometry Laboratory. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

Tetrahydrofuran (THF), diethyl ether, and benzene were purified by distillation from the sodium ketyl of benzophenone. Methylene chloride, dimethylformamide (DMF), dimethyl sulfoxide (Me_2SO), chlorobenzene, and *N,N,N'*-trimethylethylenediamine were distilled from calcium hydride. Chloroform was distilled from phosphorous pentoxide. Commercial *n*-butyllithium in hexanes (Aldrich, ~1.6 M) was standardized by titration using diphenylacetic acid as the indicator.

Vinylpyrene was from Aldrich. (±)-*trans*-7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene was received from the NCI Chemical Carcinogen Reference Standard Repository, NIH, Bethesda, MD. Concentrations of 7,8-Diol were determined by UV absorbance in methanol using a molar extinction coefficient at 367 nm of 50 500.

***c*- and *t*-MVP.** To a stirred suspension of (methyoxymethyl)triphenylphosphonium chloride (380 mg, 1.10 mmol) in dry THF (5.0 mL) at −25 °C under argon was added *n*-butyllithium (1.10 mmol) in hexane. The red solution which resulted was stirred 20 min and then treated with a solution of pyrene-1-carboxaldehyde (231 mg, 1.00 mmol) in dry THF (3.0 mL) upon which the color was discharged. After an additional 20 min, the mixture was allowed to warm to ambient temperature, stirred for 15 h, and then partitioned between saturated ammonium chloride and diethyl ether. The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the residue which resulted was chromatographed on silica gel (hexane:diethyl ether, 95:5) to provide 131 mg (53%) of MVP as a mixture of isomers (*c*:*t* = 2:3; ^1H NMR). Anal. Calcd for $C_{19}H_{14}O$: C, 88.34; H, 5.46. Found: C, 88.18; H, 5.46.

Preparative TLC or HPLC afforded pure samples of isomers. *t*-MVP: ^1H NMR ($CDCl_3$) δ 3.89 (s, 3 H), 6.79 (d, 1 H, J = 12.9 Hz), 7.19 (d, 1 H, J = 12.9 Hz), 7.90–8.20 (m, 7 H), 8.33 (d, 1 H, J = 9.2 Hz); UV (MeOH) λ_{max} (ϵ_{max}) 352 nm (27 600), 284 (23 400), 243 (28 000). *c*-MVP: ^1H NMR ($CDCl_3$) δ 3.88 (s, 3 H), 6.20 (d, 1 H, J = 7.4 Hz), 6.50 (d, 1 H, J = 7.4 Hz), 7.90–8.25 (m, 7 H), 8.53 (d, 1 H, J = 8.8 Hz); UV (MeOH) λ_{max} (ϵ_{max}) 367 nm (30 400), 352 (331 800), 287 (29 900), 276 (24 700), 243 (31 900).

2-Pyrenylidene-1,3-dithiane. To a stirred solution of 2-(trimethylsilyl)-1,3-dithiane (197 mg, 1.02 mmol) in dry THF (91.0 mL) at 0 °C under nitrogen was added *n*-butyllithium (1.02 mmol) in hexane. After 15 min, a solution of pyrene-1-carboxaldehyde (230 mg, 1.00 mmol) in THF (2.0 mL) was added, and stirring continued for an additional 15 min. The mixture was allowed to warm to ambient temperature, stirred 2 h, and then partitioned between diethyl ether and saturated ammonium chloride. The organic layer was dried (K_2CO_3) and concentrated in vacuo. Preparative TLC on silica gel (hexane:diethyl ether, 85:15) afforded 222 mg (67%) of the dithiane as a yellow solid which was recrystallized (methylene chloride/hexane): mp 138.5–140.0 °C (soften 110 °C); ^1H NMR ($CDCl_3$) δ 2.00–2.50 (m, 2 H), 2.80–3.30 (m, 4 H), 7.65 (s, 1 H), 7.80–8.50 (m, 9 H); UV (MeOH) λ_{max} (ϵ_{max}) 363 nm (27 200), 277 (23 800), 267 (19 300), 236 (51 400). Anal. Calcd for $C_{20}H_{16}S_2$: C, 75.86; H, 4.85; S, 19.29. Found: C, 75.93; H, 4.90; S, 19.24.

***trans*-1-(3,3-Dimethyl-1-butenyl)pyrene.** To a stirred solution of neopentyltriphenylphosphonium iodide[27] (230 mg, 5.00 mmol) in dry

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THF (10.0 mL) at 0 °C under N_2 was added *n*-butyllithium (5.00 mmol) in hexane. After 20 min, the ice bath was removed and the reaction was warmed to ambient temperature and stirred for an additional 45 min. The orange slurry which resulted was treated with a solution of pyrene-1-carboxaldehyde (115 mg, 5.00 mmol) in THF (2.00 mL) upon which the orange color was discharged. The reaction was then refluxed for 5 h and cooled to ambient temperature. The mixture was partitioned between saturated ammonium chloride and diethyl ether. The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the remaining residue was chromatographed on silica gel (hexane:diethyl ether, 5:1) to yield 68 mg (95%) of 1-(3,3-dimethyl-1-butenyl)pyrene which was further purified by recrystallization from hexane to give 5 mg of *trans*-1-(3,3-dimethyl-1-butenyl)pyrene as a yellow crystalline solid: mp 98–99 °C; ^1H NMR ($CDCl_3$) δ 1.29 (s, 9 H), 6.48 (d, 1 H, J = 16.2 Hz), 7.36 (d, 1 H, J = 15.8 Hz), 7.39–8.41 (m, 9 H); UV (MeOH) λ_{max} (ϵ_{max}) 359 nm (33 300), 343 (31 700), 283 (29 800), 258 (38 300), 254 (38 700); IR (CCl_4) 840, 710 cm^{-1} (C–H out-of-plane bending); MS, *m/e* 284 ($M^{+\cdot}$).

2-Methylpyrene-1-carboxaldehyde. To a stirred solution of *N,N,N'*-trimethylethylenediamine (0.14 mL, 1.1 mmol) in THF (3.0 mL) at −20 °C under argon was added *n*-BuLi (1.05 mmol) in hexane.[28] We thank Prof. Comins for a preprint of this paper and for some helpful comments. After 20 min, a solution of pyrene-1-carboxaldehyde (230 mg, 1.00 mmol) in THF (0.15 mL) was added which resulted in the formation of a white precipitate. The precipitate dissolved (~5 min), and after 30 min *n*-BuLi (3.00 mmol) in hexane was added dropwise over a 10-min period. The deep-red solution was allowed to stand at −20 °C for 24 h, treated with methyl iodide (0.75 mL, 12 mmol), and then allowed to stand at −20 °C for 120 h. The mixture was partitioned between saturated ammonium chloride and benzene. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (diethyl ether:hexane, 1:3). Recrystallization (methylene chloride/hexane) provided 38 mg (15%) of 2-methylpyrene-1-carboxaldehyde as a yellow solid: mp 141–144 °C; IR ($CHCl_3$) 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.99 (d, 3 H, J = 1.2 Hz), 7.25–8.20 (m, 7 H), 9.19 (d, 1 H; J = 9.1 Hz, H-10), 10.99 (s, 1 H); MS, *m/e* 244 (M^+); UV (MeOH) λ_{max} (ϵ_{max}) 397 nm (11 300), 364 (18 800), 292 (22 200), 237 (42 500). Anal. Calcd for $C_{18}H_{12}O$: C, 88.50; H, 4.95. Found: C, 88.53; H, 5.02.

1-(2-Methoxyvinyl)-2-methylpyrene. To a stirred solution of 2-methylpyrene-1-carboxaldehyde (16.0 mg, 0.066 mmol) in dry THF (0.50 mL) at −10 °C under argon was added via syringe a stock solution of (methoxymethylene)triphenylphosphorane (~0.2 M, prepared as described above) until the red color of the ylide persisted. The mixture was then allowed to warm to ambient temperature, stirred 24 h, and then partitioned between saturated ammonium chloride and diethyl ether. The organic layer was dried (K_2CO_3), filtered through a plug of silica gel, and concentrated in vacuo. Preparative TLC on silica gel (hexane:diethyl ether:triethylamine, 95:4.5:0.5) afforded 6.0 mg (33%) of 1-(2-methoxyvinyl)-2-methylpyrene as a mixture of geometrical isomers (c:t, 2:1). Further preparative TLC on silica gel afforded samples of the isomers. trans: ^1H NMR ($CDCl_3$) δ 2.73 (s, 3 H), 3.88 (s, 3 H), 6.16 (d, 1 H, J = 13.0 Hz), 6.68 (d, 1 H, J = 13.0 Hz), 7.75–8.20 (m, 7 H), 8.45 (d, 1 H, J = 9.4 Hz); MS, *m/e* 272 (M^+); UV (EtOH) λ_{max} (ϵ_{max}) 354 nm (26 000), 282 (26 100), 246 (46 631). cis: ^1H NMR ($CDCl_3$) δ 2.72 (s, 3 H), 3.66 (s, 3 H), 5.80 (d, 1 H, J = 7.0 Hz), 6.43 (d, 1 H, J = 7.0 Hz), 7.50–8.15 (m, 7 H), 8.25 (d, 1 H, J = 9.3 Hz); UV (EtOH) λ_{max} (ϵ_{max}) 343 nm (22 300), 280 (24 500), 245 (45 000).

Caution: Preliminary results indicate that *c*- and *t*-MVP are highly mutagenic in the *Salmonella* azaguanine resistance assay both with and without metabolic activation (John Seed, private communication). Therefore, extreme care was taken in the synthesis and handling of these compounds, and minimal quantities were used in order to reduce risk.

III. Results

Relative CL Yields of Substituted Vinylpyrenes. A number of substituted vinylpyrene derivatives were synthesized to study which would yield significant $^1O_2$-initiated CL (Table I). The $^1O_2$-induced CL of *t*-MVP was the most efficient of the substituted vinylpyrene derivatives and, presumably due to the elimination of the competing ene reaction and higher efficiency of reaction with $^1O_2$, was 180 times higher than that of 7,8-Diol under the same irradiation conditions. The two products isolated and identified from the reaction of *t*-MVP (**7**) with $^1O_2$ were pyrene-1-carboxaldehyde (**6**) in 10% yield and what appeared to be the endo peroxide (**9**) in 87% yield (Scheme III). The mean lifetime of *c*-MVP CL in 0.1 M SDS (2.3 min) was significantly shorter than that of *t*-MVP CL (5.1 min) or that of 7,8-Diol CL (5.8 min). The lifetime of the 1-(2-methoxyvinyl)naphthalene CL (21 min) was much longer than that of *t*-MVP. The relative CL quantum yields of the cis isomers of both MVP and (methoxyvinyl)-2-methylpyrene were both lower than for the trans isomers.

Table I. Relative CL Yields from a Number of Substituted Vinylpyrenes and (Methoxyvinyl)-2-methylpyrenes[a]

substrate			
R_1	R_2	τ, min	rel CL yield[b]
MeO	H	5.1	180
H	MeO	2.3	13
MeC[b]	H	1.3	6.7
chloro	H	2.3	2.7
tert-Butyl	H	5.3	1.7
S—$(CH_2)_3$—S		7.6	0.31
H	H	2.6	0.081
H[c]	MeO	1.3	0.077

[a] Light yields are relative to that of 7,8-Diol under the same experimental conditions. Reactions contained micromolar concentrations of the pyrenyl substrates and 10 nmol of rose bengal in 1.0 mL of methanol. Samples were irradiated for 2 min. Aliquots (50 μL) were transferred to a vial containing 1 mL of 0.1 M SDS and placed in a photometer. CL light yields were normalized to initial substrate concentrations. Mean lifetimes (τ) of the pyrenyldioxetanes are also shown. R_1 and R_2 positions are indicated by **4** in Scheme II. [b] Relative to CL yield of 7,8-Diol under identical experimental conditions (5.5 × 10^{-5} photons/nmol). [c] Pyrenyl substituent was 2-methylpyrene.

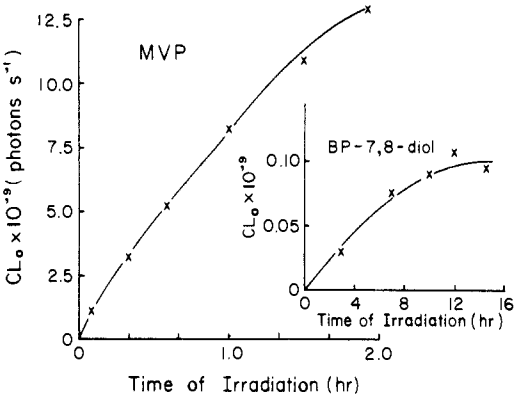

Figure 1. Low-temperature photosensitization (−78 °C) of 7,8-Diol and *t*-MVP in methanol. Low-temperature irradiations were carried out by irradiating a test tube containing 0.15 g of Sensitox and either 1.85 μmol of 7,8-Diol (inset) or 2.19 μmol of *t*-MVP in 5 mL of methanol, immersed in a clear Dewar containing a methanol/dry ice slurry. At various times the concentration of dioxetane was assayed by transferring 0.05-mL aliquots to a vial containing 1.0 mL of 0.1 M SDS and measuring the resultant CL. The quantity, CL_0, is the extrapolated initial CL intensity at zero time.

Photosensitization of 7,8-Diol and *t*-MVP at −78 °C. Low-temperature photosensitization of 7,8-Diol and *t*-MVP was monitored by CL and HPLC techniques. These data are shown in Figure 1. With *t*-MVP we were able to accumulate significant quantities of chemiluminescent intermediate, in contrast to 7,8-Diol. Under the irradiation conditions, the loss of *t*-MVP occurred with k = 0.255 h^{-1}, whereas the loss of 7,8-Diol occurred with k = 0.013 h^{-1}. The CL yields from 7,8-Diol under these conditions were <2% of the CL yield from *t*-MVP.

Despite the measurable loss of 7,8-Diol (18%) under these irradiation conditions (up to 15 h), we were unable to identify any HPLC product whose fluorescence emission corresponded with that of the CL. In addition, unlike the accumulation results for *t*-MVP, *less* CL was observed for low-temperature irradiation of 7,8-Diol than for room-temperature irradiation. A similar absence of accumulation of dioxetanes at low-temperature irradiation was observed for *trans*-1-(methoxyvinyl)-2-methylpyrene.

Dependence of CL_0 of 7,8-Diol on $^1O_2$ Concentration. CL_0 was directly proportional to the amount of $^1O_2$ produced (Figure 2)

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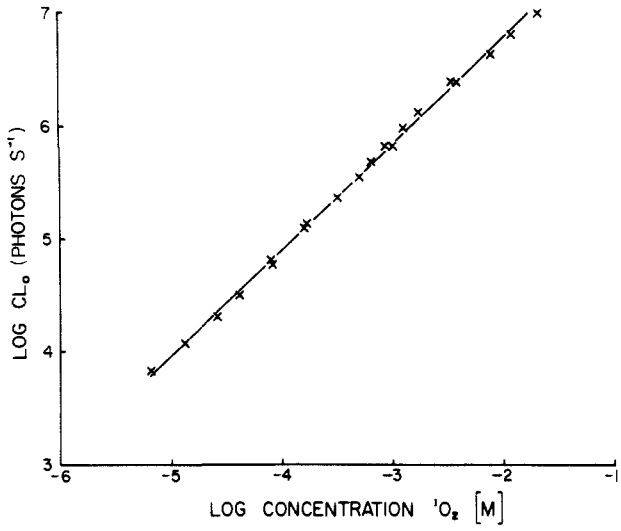

Figure 2. Dependence of the initial CL intensity (CL_0) from 7,8-Diol at room temperature on photochemically produced $^1O_2$. Two-milliliter solutions of methanol containing 20 nmol of rose bengal and 50 nmol of 7,8-Diol were irradiated with a projector using either a 500-W or 1000-W tungsten lamp. The amount of $^1O_2$ produced during the irradiation was varied by decreasing the lamp intensity using neutral density filters in the lamp beam and/or varying the time of irradiation (0.25–6.0 min).

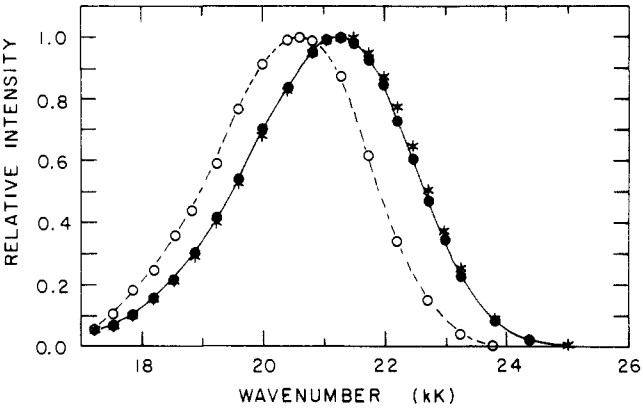

Figure 3. $^1O_2$-initiated CL emission spectra from the *cis*- (*) and *trans*-MVP (●) and 7,8-Diol (○) in 0.1 M SDS. For the CL emission spectra of *c*- and *t*-MVP, 100 nmol of each isomer were reacted separately with $^1O_2$ by injecting 50 μmol of NaOCl into 5 mL of 0.1 M SDS containing 300 μmol of H_2O_2. For the CL emission spectrum of 7,8-Diol, 85.4 nmol of 7,8-Diol was reacted with $NaOCl/H_2O_2$ in 3 mL of 0.1 M SDS. The fluorescence emission spectrum of 18.0 μM pyrene-1-carboxaldehyde in 0.1 M SDS is shown as the solid line. Excitation (366 nm) was supplied for the fluorescence spectrum of **6** by a Model 098 Perkin-Elmer prism monochrometer using a Leitz 200-W Hg light source isolated by two Corning 7-60 filters. Both CL and fluorescence emission spectra were determined on a 1.0-m F/3 Fastie-Ebert grating spectrometer.

during photosensitization. The longest period of photosensitization in these reactions was 6 min. The slope of the log–log plot in Figure 2 is 0.99, indicating that the reaction is first order in concentration of $^1O_2$. The limit of sensitivity for the detection of $^1O_2$ by 50 nmol of 7,8-Diol was approximately 12 nmol of $^1O_2$ formed in a 15-s irradiation. The limit of sensitivity was defined as 7,8-Diol CL_0 equal to the blank CL of a rose bengal and 0.1 M SDS solution following a 15-s irradiation.

CL Emission Spectra from 7,8-Diol and *c*- and *t*-MVP. Figure 3 shows the $^1O_2$-initiated CL emission spectra of *c*-MVP and *t*-MVP. These CL spectra were identical with the fluorescence of the product pyrene-1-carboxaldehyde (solid line); the symbols (○) represent the $^1O_2$ spectrum of 7,8-Diol published previously.[11] These emission spectra demonstrate that the shapes of the two CL emissions were similar with a full width at half-maximum (fwhm) of 3170 cm^{-1}; ν_{max} for both the *c*- and *t*-MVP CL emissions is 2.15×10^4 cm^{-1}. The CL emission spectrum from 7,8-Diol has a fwhm of 3.19×10^3 cm^{-1} and a ν_{max} of 2.08×10^4 cm^{-1}, a shift of 670 cm^{-1} or 1.9 kcal mol^{-1}.

Table II. Relative CL Yields from 7,8-Diol and *t*-MVP in Different Solvents[a]

solvent	rel CL yield[b] 7,8-Diol	*t*-MVP
0.1 M SDS	1.0	1.0
methanol	0.25	0.25
ethanol	0.11	0.033
actonitrile		0.0056
chloroform	0.080	0.0044
chlorobenzene	0.075	
diethyl ether	0.000 11	0.000 21
dimethyl sulfoxide	9.1×10^{-5}	2.6×10^{-7}

[a] Concentrations (μM) of 7,8-Diol and *t*-MVP were varied in 1 mL of methanol containing 10 nmol of rose bengal. These solutions were irradiated for 1 min, and aliquots were placed in a vial with the solvent of interest. The concentration of the solvent of interest was always greate than 95%. Solvents used for these studies were anhydrous except for aqueous 0.1 M SDS. CL yield is defined as the nmber of photons emitted divided by the number of initial substrate molecules. [b] Relative to the CL yield in 0.1 M SDS.

Table III. Solvent Dependence of ϕ_{CL}(dioxetane) and ϕ_{EX} for the CL from *t*-MVP Dioxetanes and ϕ_{Fluor} for Pyrene-1-carboxaldehyde[a]

solvent[b]	ϕ_{CL}(dioxetane)[c]	ϕ_{Fluor}	ϕ_{EX}
0.1 M SDS	$2.7 \pm 0.5 \times 10^{-2}$	0.454	0.061
methanol	$6.8 \pm 1.3 \times 10^{-3}$	0.12	0.057
ethanol	$8.9 \pm 1.5 \times 10^{-4}$	0.052	0.017
acetonitrile	$1.5 \pm 0.2 \times 10^{-4}$	0.044	0.0034
chloroform	$1.2 \pm 0.2 \times 10^{-4}$	0.021	0.0057
diethyl ether	$5.7 \pm 0.5 \times 10^{-6}$	0.0036	0.0016
Me_2SO	$7.2 \pm 1.2 \times 10^{-9}$	0.041	1.8×10

[a] In all measurements of ϕ_{CL}(dioxetane) for *t*-MVP, 1 mL of methanol containing 10 nmol of rose bengal and 9.5 nmol of *t*-MVP were irradiated for 2 min, and aliquots of the irradiated solution were added to the solvent of interest. Parallel samples were analyzed by HPLC to quantitate the amount of *t*-MVP reacted in the methanol with $^1O_2$ and the amount of **6** produced. Fluorescence quantum yields (ϕ_{Fluor}) for pyrene-1-carboxaldehyde were measured relative to quinine sulfate in a SLM 8000 photon counting spectrofluorometer using the same solvent compositions as those used for ϕ_{CL}(dioxetane). ϕ_{EX} is derived from eq 3 utilizing values of α,ϕ_{CL}(dioxetane,) and ϕ_{Fluor} obtained by direct measurements. [b] Solvent concentration is 95%, 5% methanol from photosensitization reaction. [c] S.D. (n = 4)

CL Yields from Dioxetanes of 7,8-Diol and *t*-MVP in Different Solvents. The CL yields of *t*-MVP and 7,8-Diol in different solvents normalized to the yields in 0.1 M SDS (Table II) indicate that polar solvents such as 0.1 M SDS, ethanol, and methanol produce the highest yields. The chemical yield of **6** in each solvent was the same, indicating that the measured CL was dependent only on ϕ_{EX} and ϕ_{FL} over a range of 10^6 in relative CL yield.

Table III shows the solvent dependence of ϕ_{CL} from *t*-MVP dioxetanes, the fluorescence quantum yields, ϕ_{Fluor}, of pyrene-1-carboxaldehyde (**6**), and the singlet excitation yields, ϕ_{EX}, calculated from eq 4, assuming that ϕ_{Fluor} equals ϕ_{FL}. The solvents with the highest ϕ_{CL}, ϕ_{Fluor}, and ϕ_{EX} are the polar solvents 0.1 M SDS, methanol, and ethanol. Since α was held constant by carrying out all photosensitizations in a single solvent, methanol, the product of $\phi_{EX}\phi_{FL}$ in eq 3 accounted for the observed range in ϕ_{CL} in Table III and therefore for the range in the relative CL yields in Table II.

The addition of rose bengal to preformed dioxetanes *t*-MVP (**8**) increased the observed first-order CL decay rate (k), analogous to the effect of methylene blue on 7,8-Diol CL reported previously.[11] This was proposed to be the result of a dark *inter*molecular electron-exchange mechanism.

CL Yields of 7,8-Diol and *t*-MVP in H_2O/Methanol Mixtures. The $^1O_2$-induced CL of 7,8-Diol in water/methanol mixtures (Figure 4) has a maximum at 50% water/methanol. Increasing the water concentration lowered the lifetime of the CL intermediate formed from 7,8-Diol. In similar experiments with *t*-MVP in Figure 4, a maximum in ϕ_{CL} of *t*-MVP was also observed in

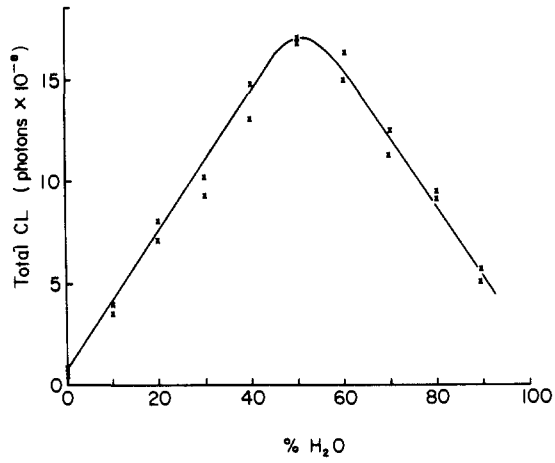

Figure 4. CL yields of 7,8-Diol in water/methanol mixtures. Samples containing 0.9 nmol of 7,8-Diol and 10 nmol of rose bengal in 1 mL of methanol were irradiated for 1 min with a tungsten lamp (1000 W) and subsequently transferred to 19 mL of solvent and placed in the photometer. Percent H_2O (v/v) refers to the final 20-mL water/methanol mixture.

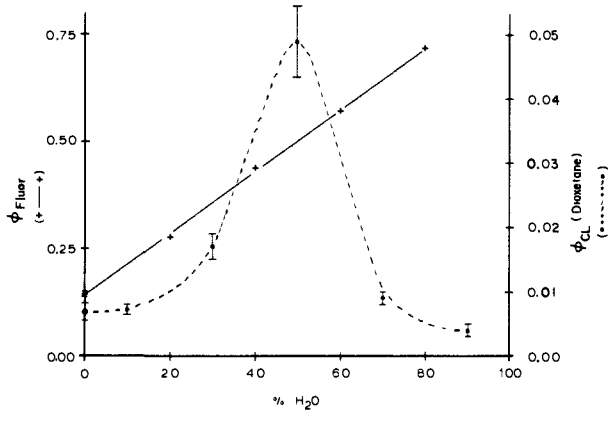

Figure 5. Pyrene-1-carboxaldehyde ϕ_{Fluor} and *t*-MVP ϕ_{CL}(dioxetane) as functions of water concentration in water/methanol (v/v) mixtures. Conditions for *t*-MVP ϕ_{CL}(dioxetane) (●) and pyrene-1-carboxaldehyde ϕ_{Fluor} (+) were identical with those in Table III. Error bars indicate the standard deviation of replicate measurements (n = 4).

50% water/methanol mixtures. The lifetime of the *t*-MVP dioxetane also decreased with increasing water concentrations. The total CL from 7,8-Diol increased by a factor of 20 between 100% methanol and 50% water/methanol, compared with a factor of 6 for *t*-MVP.

ϕ_{Fluor} and Fluorescence Emission Spectra of Pyrene-1-carboxaldehyde in Water/Methanol Mixtures. ϕ_{Fluor} for pyrene-1-carboxaldehyde was directly proportional to the concentration of water, increasing from 0.14 in methanol to 0.73 in 80% water/methanol mixtures (Figure 5). This increase in ϕ_{Fluor} was accompanied by a red shift in the fluorescence emission maximum from 455 nm in pure methanol to 475 nm in 80% water/methanol. Figure 6 shows wavenumber plots of the fluorescence emission spectra of pyrene-1-carboxaldehyde in various water/methanol mixtures. The fwhm for each of the water/methanol mixtures was identical, 3170 ± 30 cm^{-1}, indicating similar aldehyde singlet excited states. The observed spectral shifts and the increased ϕ_{Fluor} are attributed to stronger hydrogen bonds forming between the carbonyl group of **6** and water than existed between the carbonyl group of **6** and methanol.[29-31] Similar red shifts and enhanced

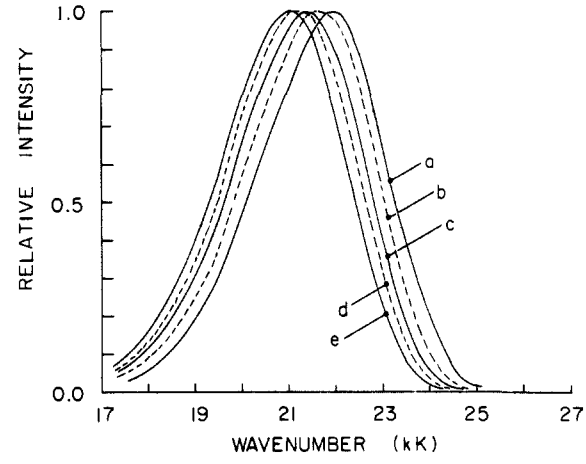

Figure 6. Fluorescence emission spectra (relative quanta per wavenumber interval) of pyrene-1-carboxaldehyde in water/methanol mixtures (v/v): a, methanol; b, 20% H_2O; c, 40% H_2O; d, 60% H_2O; e, 80% H_2O. Pyrene-1-carboxaldehyde concentrations were approximately 1 μM. Spectral measurements were made with a SLM 8000 photon counting spectrofluorometer with 347-nm excitation.

fluorescence quantum yields for pyrene-1-carboxaldehyde were also observed for the polar solvents listed in Table III.

Activation Parameters of 7,8-Diol and *t*-MVP Dioxetane Decomposition. E_A's, determined for the dioxetane formed from 7,8-Diol in the solvents 0.1 M SDS and methanol, were 15.3 and 15.6 kcal mol^{-1}, respectively. The corresponding values for log A were 8.76 and 8.63. Activation parameters for *t*-MVP dioxetane decomposition in methanol were E_A = 10.9 kcal mol^{-1} and log A = 5.73.

IV. Discussion

There are two substituent effects to be considered in pyrenyldioxetane CL: (a) the effect of electron-donating substituents on the vinyl bond increasing the likelihood of a $^1O_2$ reaction and (b) the effect of easily oxidizable units such as pyrene which will decompose the dioxetane with high singlet excited state yields by intramolecular chemically initiated electron-exchange luminescence (CIEEL).[32-35] The latter was evidenced by the low (~14 kcal/mol) activation energies for both *t*-MVP and 7,8-Diol dioxetane decompositions.

Aromatic aldehydes exhibit red-shifted fluorescence and increased fluorescence quantum yield in polar, hydrogen-bonding solvents. This fluorescence enhancement is due to the lowering of the $\pi \rightarrow \pi^*$ state relative to the $n \rightarrow \pi^*$ state by hydrogen bonding.[30] The fluorescence quantum yields of pyrene-1-carboxaldehyde in Table III for 95% solvent/5% methanol would be much lower in pure nonpolar solvents. The small amount of methanol in these solvent mixtures has a strong fluorescence activation effect similar to that described by Cherkasov[31] for 2-acetylanthracene. The methanol was necessary to keep the lifetime of $^1O_2$ and the quantum yield for $^1O_2$ production of dioxetane constant for the rose bengal photosensitization reaction.

Model studies on the $^1O_2$ reaction with (methoxyvinyl)-naphthalene produced results similar to those of Matsumoto and Kuroda.[36] The primary product was an endoperoxide and the minor product was naphthalene-1-carboxaldehyde. The $^1O_2$ production of naphthalene-1-carboxaldehyde and the observed $^1O_2$-initiated CL from (methoxyvinyl)naphthalene are interpreted

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as further substantiation of the dioxetane CL mechanism for *t*-MVP and 7,8-Diol.

The only experiment in which the CL yield of 7,8-Diol did not parallel that of *t*-MVP was in the low-temperature photosensitization reactions. At room temperature, CL from 7,8-Diol was directly proportional to the amount of $^1O_2$ produced during the irradiation period. At −78 °C, CL from 7,8-Diol shows rapid saturation with the time of irradiation. This inability to accumulate a chemiluminescent intermediate from 7,8-Diol at −78 °C during long intervals of irradiation could be due to (a) secondary photochemical reactions of $^1O_2$ with 7,8-Diol competing with the formation of a 9,10-dioxetane from 7,8-Diol or (b) low-temperature inhibition of dioxetane formation, possibly by steric hinderance. The absence of accumulation, upon low-temperature irradiation, of a chemiluminescent intermediate from *trans*-(methoxyvinyl)-2-methylpyrene implies that ortho-substituted vinylpyrenes may be better models to explain the anomalous behavior of 7,8-Diol at low temperature.

The absence of a product of $^1O_2$-induced CL of 7,8-Diol, isolable by HPLC and exhibiting a fluorescence identical with the CL, implies that the dialdehyde product is either unstable or reactive. However, the pyrene-1-carboxaldehyde product of *t*-MVP dioxetane decomposition is isolable and stable, and its fluorescence emission spectrum is identical with the *t*-MVP dioxetane emission.

Singlet oxygen induced CL from *t*-MVP has striking similarities with the CL from 7,8-Diol. These encompass (a) solvent effects on CL, (b) lifetimes of CL, (c) CL emission spectra, (d) activation energies, and (e) microsomal enzymatic CL. The differences in ϕ_{CL} can be attributable to the fact that *t*-MVP is much more reactive with $^1O_2$ and that an ene reaction is possible with 7,8-Diol but is not possible with *t*-MVP. The evidence we have presented demonstrates the dioxetane mechanism for the CL of *t*-MVP by isolation of pyrene-1-carboxaldehyde, the expected dioxetane decomposition product whose fluorescence spectrum was identical with the observed *t*-MVP CL dioxetane emission spectra and by the parallel effects of solvent on *t*-MVP dioxetane CL and the fluorescence quantum yields of pyrene-1-carboxaldehyde. Except for the isolation of the proposed dialdehyde product of 7,8-Diol CL, the CL lifetimes, solvent effects, activation energies, and spectra of 7,8-Diol dioxetane parallel that of *t*-MVP.

The factor ϕ_{FL} for a product excited state is probably lower than the photophysical fluorescence quantum yield of **6** (ϕ_{Fluor}). Excited states produced by photon absorption are governed by photoselection rules and initially have the conformation of the ground-state molecule in its ground-state solvent cage. Chemically produced excited-state products are not governed in their formation by photoselection rules, and their initial conformations relative to their solvent cages may be quite different. Therefore quenching of CL excited states may be significantly different from the quenching of photoexcited states. Lee and Seliger[20] measured ϕ_{CL}(luminol) = 0.0125, whereas photoexcited ϕ_{Fluor} of the aminophthalic acid product was 0.3. Whether this large difference is attributable to a difference between chemiexcited ϕ_{FL} and photoexcited ϕ_{Fluor} or to an effect on ϕ_{EX} cannot be determined. The result is that the components of the product $\phi_{EX}\phi_{FL}$ cannot readily be evaluated from substitution of independently measured values of ϕ_{Fluor} measured by photoexcitation. Therefore the derived values of ϕ_{EX} in Table III are minimum values.

From Table III for *t*-MVP, ϕ_{CL}(dioxetane) $\cong$ 0.03 which is more than a factor of 2 higher than for luminol. However $^1O_2$ reactions with *t*-MVP (Scheme III) result in both dioxetane (10% yield) and apparently endoperoxide (87% yield) with an overall ϕ_{CL}(*t*-MVP) of 0.003. This high ϕ_{CL} and the high specificity of this CL for $^1O_2$ make *t*-MVP an excellent probe for $^1O_2$,[37] since photon detection in the CL assay has a greater advantage in sensitivity than the use of non-CL chemical traps for $^1O_2$, similar to fluorescence assay techniques compared with a spectrophotometric assay, i.e., a factor of 10^5–10^6. It should be possible to detect $^1O_2$ or $^1O_2$ equivalents in cells using trace amounts of *t*-MVP or other second generation CL probes, where the presence of the probe may not interfere with normal physiological processes.

Registry No. 1, 61443-57-0; **2**, 77267-10-8; **4** (R_1 = OMe; R_2 = H), 93265-41-9; **4** (R_1 = H; R_2 = OMe), 102699-15-0; **4** (R_1,R_2 = 1,3-dithian-2-yl), 102699-16-1; **4** (R_1 = OMe; R_2 = H) (2-methoxy), 102699-17-2; **4** (R_1 = Cl; R_2 = H), 102699-18-3; **4** (R_1 = Bu-*t*; R_2 = H), 102699-19-4; **4** (R_1 = R_2 = H), 17088-21-0; **4** (R_1 = H; R_2 = OMe) (2-methoxy), 102699-20-7; **6**, 3029-19-4; O_2, 7782-44-7; 2-(trimethylsilyl)-1,3-dithiane, 13411-42-2; (methoxymethyl)triphenylphosphonium chloride, 4009-98-7; neopentyltriphenylphosphonium iodide, 3740-00-9; 2-methylpyrene-1-carboxaldehyde, 102699-21-8; *N,N,N'*-trimethylethylenediamine, 142-25-6; naphthalene-1-carboxaldehyde, 66-77-3; (methoxyvinyl)naphthalene, 102699-14-9.

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Permanganate Ion Oxidations. 17. Kinetics and Mechanism of the Oxidation of (*E*)-3-(2-Thienyl)-2-propenoates and (*E*)-3-(3-Thienyl)-2-propenoates in Phosphate-Buffered Solutions[1]

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Abstract: The kinetics and mechanism of the permanganate ion oxidation of (*E*)-3-(2-thienyl)-2-propenoates and (*E*)-3-(3-thienyl)-2-propenoates have been studied at 418, 526, 584, and 660 nm in phosphate-buffered solutions (pH 6.83 ± 0.03). The reaction is first order in permanganate ion and first order in substrate. A rate-determining step leading to the formation of a metallacyclooxetane or a cyclic manganate(V) diester is supported by low enthalpies of activation, large negative entropies of activation, small substituent effects, steric effects, and an inverse secondary deuterium kinetic isotope effect. The ambiphilic nature of permanganate ion is considered.

Recently, there has been considerable discussion concerning the oxidation state of the manganese species observed during the permanganate ion oxidation of carbon–carbon double bonds.[1,3-29] Although it was thought that the observed manganese species was

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