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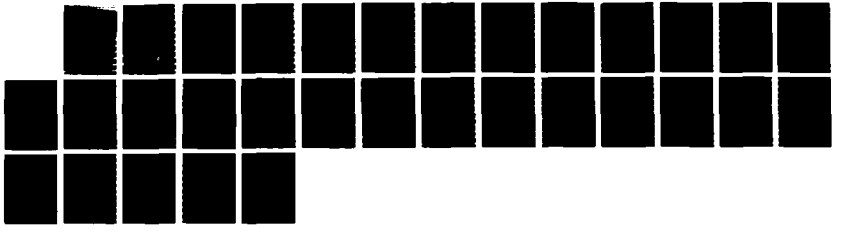

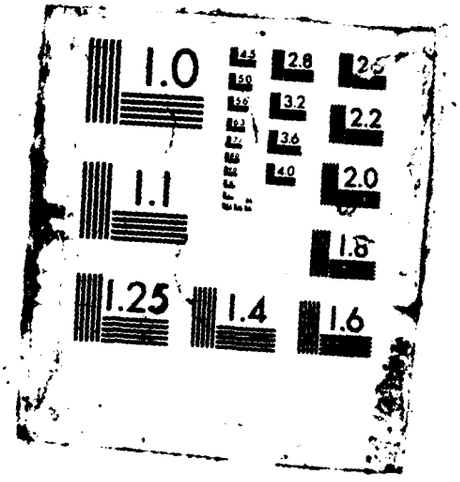
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CHEMILUMINESCENCE FROM AQUEOUS MICELLAR SYSTEMS

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ABSTRACT

Chemical and Enzymatic Triggering of 1,2-Dioxetanes. Our research group has recently discovered a series of dioxetanes which are thermally stable at ambient temperature but which can be "triggered" for efficient chemiluminescence on demand by the addition of the appropriate chemical catalyst or enzyme. The process involves the removal of a protecting group from a stable form of the dioxetane to generate an unstable aryloxide intermediate which decomposes spontaneously to yield the luminescence. The protected form of the dioxetane exhibits half-lives at 25 °C of several years while the aryloxide intermediate decomposes in seconds. Several methods for triggering the chemiluminescence have been developed including: (1) deprotonation of the hydroxy derivative; (2) removal of a silyloxy group with fluoride ion; and (3) enzymatic cleavage of an acetate-substituted dioxetane with aryl esterase. One particular example of chemical triggering generates the singlet excited product with an efficiency of 57%. Practical applications for these compounds include storable chemical light sources, liquid light standards for organic and aqueous systems, and new methods for immunoassay using the dioxetanes as luminescent substrates in enzyme-linked bioassays.

Chemiluminescence from Micellar Systems. We have found that cationic surfactants such as cetyltrimethylammonium bromide (CATB) can be used to significantly enhance rates of chemically triggered luminescence from appropriately substituted dioxetanes in aqueous solution. For example, CATB catalyzes the base-induced cleavage of an acetate-substituted dioxetane. Fluoride-triggering of silyloxy dioxetanes is also accelerated in the micelle. The electrostatic attraction of the cationic head group and the anionic reagent provides the observed micellar catalysis.

Effects of Heteroatom Substitutents on Dioxetanes. This study of the effects of heteroatom substitutents on the properties of dioxetanes resulted in the first report of activation parameters and rates of decomposition for unstable nitrogen- and sulfur-substituted dioxetanes. The results provide support for a mechanism for decomposition involving intramolecular electron transfer.

Hematoporphyrin-Chemiluminescence Cancer Therapy. Photoradiation therapy using a hematoporphyrin derivative (HPD) and visible light has shown considerable promise as an effective treatment for a wide variety of cancers. This procedure is being used clinically in many hospitals on an experimental basis. As part of our ONR-supported studies on chemiluminescence in aqueous systems, we conducted a preliminary investigation to determine if a chemiluminescent reaction could be used to activate HPD for tumor killing instead of an external light source. This new approach was targeted at improving the delivery of light to the HPD with an efficient, water-soluble chemiluminescent system that could be injected directly into the tumor. Very exciting results have been obtained in treating transplanted mammary tumors in mice and additional funding for this project is now provided by the American Cancer Society.

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FINAL TECHNICAL REPORT

CHEMILUMINESCENCE FROM AQUEOUS MICELLAR SYSTEMS

by

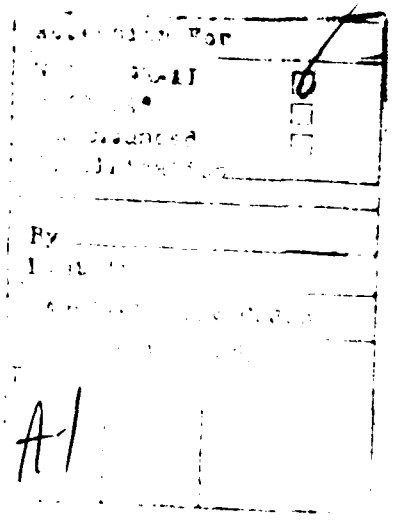
A. PAUL SCHAAP

Department of Chemistry

Wayne State University

Detroit, MI 48202

March 17, 1987



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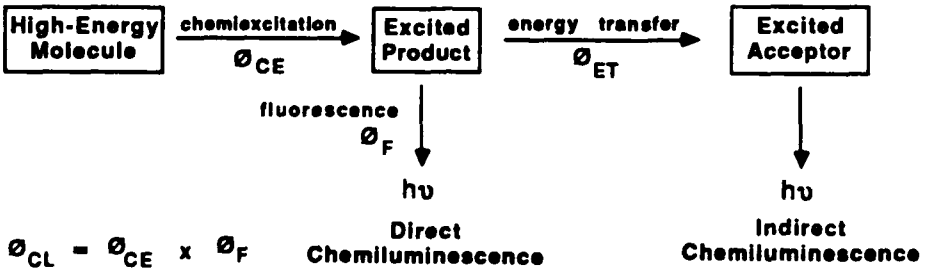
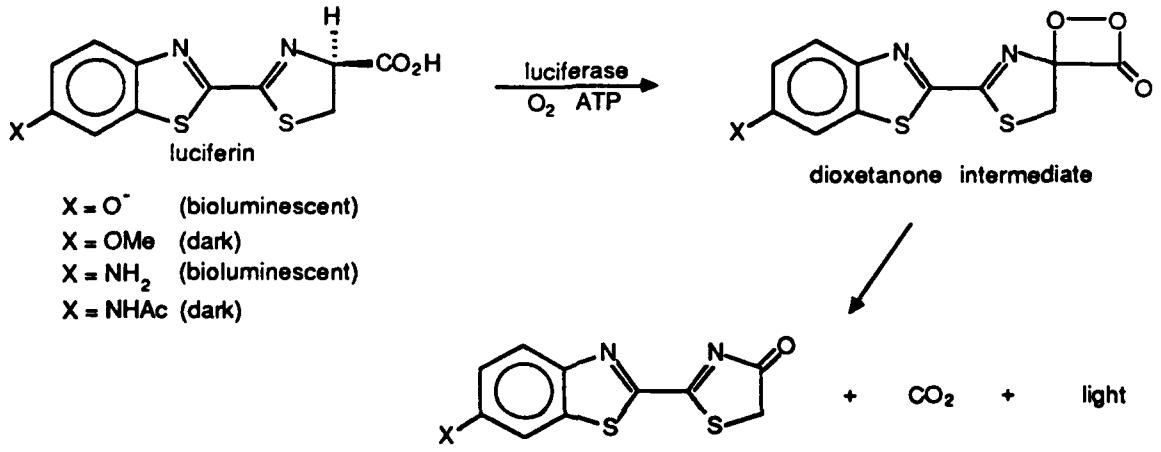
INTRODUCTION

Mechanisms of Chemiluminescence. Exothermic chemical reactions release energy during the course of the reaction. In virtually all cases, this energy is in the form of vibrational excitation or heat. However, a few chemical processes generate light or chemiluminescence instead of heat. The basic mechanism for light production involves thermal or catalyzed decomposition of a high energy material (frequently an organic peroxide) to produce the reaction product in a triplet or singlet electronic excited state. Fluorescence of the singlet species results in what has been termed direct chemiluminescence.[1] The chemiluminescence quantum yield is the product of the quantum yields for chemiexcitation and fluorescence. Energy transfer from the triplet or singlet product to a fluorescent acceptor can be utilized to give indirect chemiluminescence.



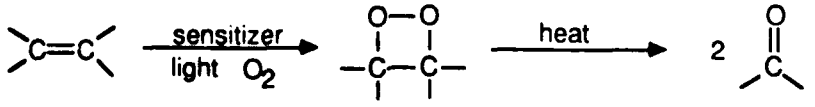
Bioluminescence. Light-producing reactions in living organisms give rise to bioluminescence. These biological processes are catalyzed by photoproteins or enzymes called luciferases which activate a substrate (luciferin) and molecular oxygen to produce an intermediate peroxide. The mechanism of the bioluminescence produced by the firefly (*Photinus pyrolis)* has been extensively studied.[2] The luciferin is converted in the presence of ATP and oxygen to a 4-membered ring peroxide (1,2-dioxetanone). This high energy intermediate decomposes to generate the observed yellow-green luminescence with an efficiency of 88%.[3] Although this species is apparently quite unstable and has not been isolated or observed spectroscopically, unambiguous evidence for its intermediacy in the reaction has been provided by oxygen-18 labeling experiments.[4] Other bioluminescent systems thought to involve dioxetanone intermediates are *Cypridina* and *Renilla*.[5]

Several years ago White and coworkers described the effects of various substituents on *in vitro* firefly bioluminescence.[6] A series of luciferins with different substituents X were prepared. White noted that both the synthetic luciferin with X = NH_2 and the natural luciferin (X = O^-) gave light while the reactions with the methyl ether and amide derivatives were dark. The mechanistic basis for these observations was not then understood. However, our group and others have since demonstrated similar substituent effects for model 1,2-dioxetanes (see below).



X = O^- (bioluminescent)
X = OMe (dark)
X = NH_2 (bioluminescent)
X = NHAc (dark)

Chemiluminescence from 1,2-Dioxetanes. In 1969 Kopecky and Mumford reported the first synthesis of an authentic dioxetane by the base-catalyzed cyclization of a β-bromohydroperoxide.[7] This method as been applied to the preparation of a variety of alkyl- and phenyl-substituted dioxetanes.[8] A second method for preparing dioxetanes was independently reported by Bartlett and Schaap[9] and Mazur and Foote.[10] Photooxygenation of alkenes bearing electron-donating groups in the presence of molecular oxygen and a photosensitizing dye produces dioxetanes in high yields. The mechanism of this reaction involves the photochemical generation of singlet oxygen which undergoes cycloaddition to the alkene to yield the dioxetane.[11] The use of a polymer-bound sensitizer which may be removed by simple filtration after the photooxygenation has made this method particularly convenient. [12]

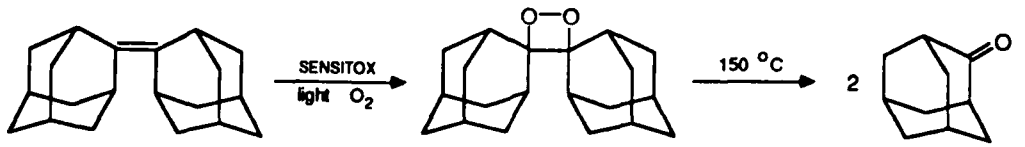


Several other synthetic procedures have been devised for the preparation of specific 1,2-dioxetanes. A recent review has outlined these various chemical, photochemical and electrochemical methods.[13] The scope of these methods is limited and none has received broad use. Dioxetanones or α-peroxylactones have been prepared by DCC-mediated cyclizations of α-hydroperoxy acids which in turn are derived from photooxygenation of ketene acetals.[14]

The properties of dioxetanes vary dramatically with the nature of substituents attached to the ring. Simple alkyl-substituted dioxetanes exhibit activation energies for decomposition of 22 - 25 kcal/mol and half-lives at ambient temperature of a few days.[1] Thermolysis of these peroxides yields predominantly triplet excited state species with ratios of triplet to singlet excited states in the range of 10-1000. Efficiencies for the formation of singlet excited states are typically much less than 1%. Several lines of evidence now support a decomposition mechanism involving rate-limiting homolytic scission of the O-O bond to give an intermediate biradical.[15] Richardson has shown that substitution of conjugating phenyl groups for alkyl groups does not destabilize the dioxetanes.[16] Further, rates of decomposition for these types of dioxetanes do not show any significant solvent[16] or secondary

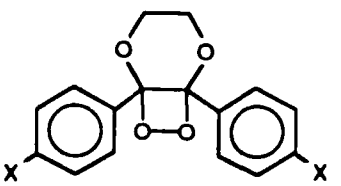
deuterium isotope effects.[17] Our group has provided additional evidence for the biradical mechanism through a study of substituent effects. Hammett plots of relative rates of decomposition for a series of dioxetanes derived from 2,3-diaryl-1,4-dioxenes gave small negative ρ values of -0.24 and -0.38 using σ^+ and σ substituent constants, respectively.[18] Richardson has also reported that 1-aryl-1-methyl dioxetanes yielded ρ values of -0.19 and -0.32.[19] These ρ values are smaller than expected for a concerted decomposition and are more consistent with a biradical mechanism.

Incorporation of sterically hindered alkyl groups such as adamantyl[20] or norbornyl[21] can provide remarkable stabilization for dioxetanes. For example, the dioxetane derived from adamantylidene-adamantane exhibits a melting point of 167 °C, an activation energy for decomposition of 37 kcal/mol, and a calculated half-life at 25 °C of over 20 years.[20b] This special stability is consistent with the biradical mechanism since stretching the O-O bond in the transition state leads to additional compression of the bulky alkyl groups.



adamantylideneadamantane

Dioxetanes bearing aromatic substituents with low oxidation potentials show quite different properties. They are much less stable, often decomposing with half-lives on the order of minutes at room temperature. Higher singlet chemiexcitation efficiencies are observed, in some cases exceeding 10%. Triplet/singlet ratios are much lower compared to the simple alkyl, alkoxy and phenyl-substituted dioxetanes. For example, we have shown that the *p*-dimethylamino-substituted dioxetane **1b** decomposes 382-times faster at 25 °C in toluene than the unsubstituted dioxetane **1a**. Further, the rate for **1b** increases 188-fold on going from hydrocarbon solvents to propylene carbonate with no appreciable change for **1a**. The activation energy for the dimethylamino-substituted dioxetane **1b** is also much lower (19.3 kcal/mol in toluene and 14.8 in propylene carbonate) than that exhibited by **1a** (24.8 kcal/mol with no solvent effect). Finally, **1b** shows brillant blue chemiluminescence at room temperature with a singlet chemiexcitation efficiency of 10% relative to the luminol standard while **1a** has a singlet chemiexcitation efficiency of 0.01%.[22] These results have been interpreted in terms of an alternate mechanism for decomposition involving intramolecular electron transfer from the aryl group to the peroxide σ* orbital as illustrated in structure **2**. McCapra,[23] Goto,[24] and Singer[25] have also reported other arylamino-substituted dioxetanes that exhibit low thermal stability and relatively efficient chemiluminescence.



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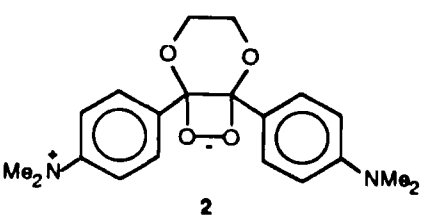
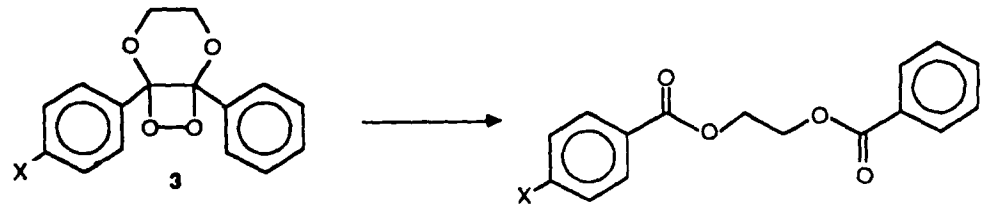
a, X = H (non-chemiluminescent)
b, X = NMe_2 (chemiluminescent)



2

Schuster[26] has shown that intermolecular electron-transfer reactions of dioxetanones and other types of peroxides can also afford excited states. Enhanced rates of decomposition in the presence of fluorescent aromatic hydrocarbons and amines have been explained in terms of this process. Evidence for this mechanism includes the observation of a correlation between the reaction rate constant and the oxidation potential of the fluorescer.

An additional example of the profound effect that particular substituents can have on the properties of dioxetanes is illustrated by a comparison of two other dioxetanes of the series from 2,3-diaryl-1,4-dioxene.[27] In 1982 we reported that the hydroxy-substituted dioxetane **3a** (X = OH) has a half-life at 25 °C of 57 hours and produces very low levels of luminescence upon heating at elevated temperatures. However, reaction of this dioxetane with a base at -30 °C affords a flash of blue light. Kinetic studies showed that the deprotonated dioxetane (X = O^-) decomposes 4.4×10^6 times faster at 25 °C than the protonated form (X = OH) with a half-life of 46 msec for **3b** calculated from the Arrhenius plot. The activation energy for decomposition of **3b** is much lower than that observed for **3a**, 13.4 vs. 24.4 kcal/mol, respectively. Further, the aryloxide-substituted dioxetane **3b** also exhibits a 100-fold higher singlet chemiexcitation efficiency. We have suggested that the contrasting properties of these two dioxetanes arise because they decompose by two different mechanisms. The more stable protonated dioxetane **3a** undergoes the "normal" cleavage by rate-limiting homolysis of the O-O bond while decomposition of **3b** is induced by intramolecular electron transfer from the easily oxidized phenoxide group.



3

a, X = OH (non-chemiluminescent)
b, X = O^- (chemiluminescent)

+ light

SUMMARY OF RESEARCH RESULTS

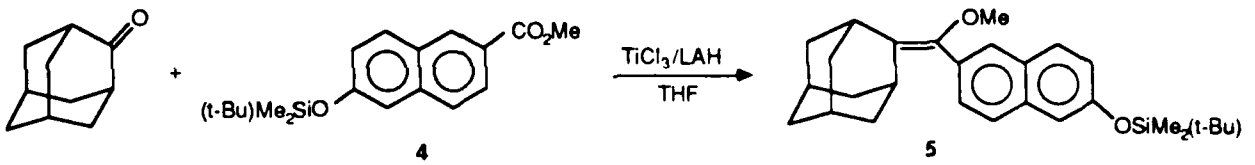
Chemiluminescence from Aqueous Micellar Systems

N00014-82-K-0696, 10/1/82 - 3/31/86

1. Chemical Triggering of 1,2-Dioxetanes

We have recently developed a series of dioxetanes which are thermally stable but can be triggered to generate efficient chemiluminescence on demand. To do this, we have brought together several key features from earlier types of dioxetanes: (1) the stabilizing influence of spiro-fused adamantyl groups has been utilized to provide dioxetanes that have long "shelf lives" at ambient temperature; (2) a fluorescent moiety has been incorporated in the structure so that direct chemiluminescence from the carbonyl cleavage product is obtained; and (3) the observation that the phenoxide-substituted dioxetane **3b** decomposes 4.4 x 10^6 times faster than the protonated form has also been exploited to give a method for triggering the luminescence.

We have synthesized the required alkenes by reaction of 2-adamantanone with aromatic methyl esters using titanium trichoride/LAH in THF. For example, reaction of methyl 6-*tert*-butyldimethylsilyloxy-2-naphthoate (**4**) with a 2-fold excess of 2-adamantanone gave the alkene in 83% yield based on starting ester. To the best of our knowledge, this is the first report of the condensation of ketones and esters to form vinyl ethers using the McMurry[28] procedure. This reaction is quite general and has been used by our group to couple a variety of esters and lactones to adamantanone.



4 5

Dioxetanes **6a**, **6b**, and **10** were prepared by photooxygenation of the vinyl ethers in CH_2Cl_2 using polymer-bound Rose Bengal (SENSITOX) and a 1000-W high-pressure sodium lamp. The dioxetanes are easily handled compounds that exhibit the desired thermal stability. Rate constants for the thermolysis were obtained at 80 to 120 ^oC from measurements of the decay of chemiluminescence intensity of 10^{-4} M solutions in *o*-xylene. Chemiluminescence spectra from thermolysis of all three dioxetanes exhibited maximum emission at 437 nm indicating that the luminescence under these conditions is derived from singlet excited adamantanone and not the phenyl or naphthyl esters. Rates showed variations of less than 3% and gave excellent Arrhenius plots ($r > 0.99$) with activation energies for **6a**, **6b**, and **10** of 29.7, 27.0, and 28.4 ($\pm$ 1) kcal/mol, respectively. Half-lives for these dioxetanes at 25 ^oC are estimated from the Arrhenius plots to be several years. Samples of the dioxetanes in *o*-xylene have remained on the laboratory bench for several months with no detectable decomposition.

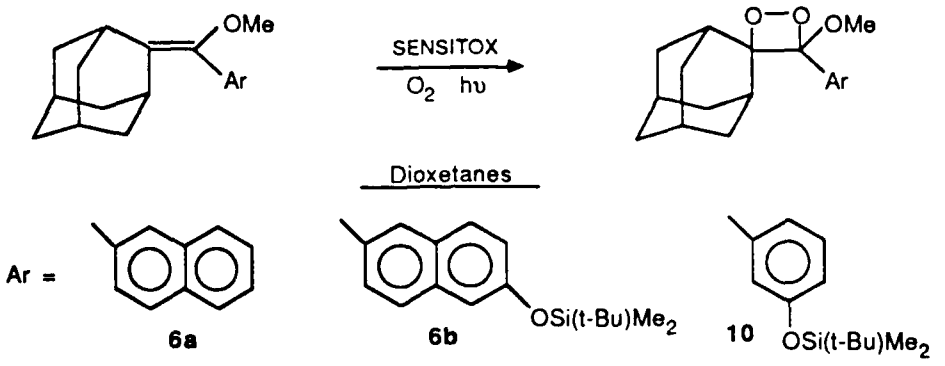
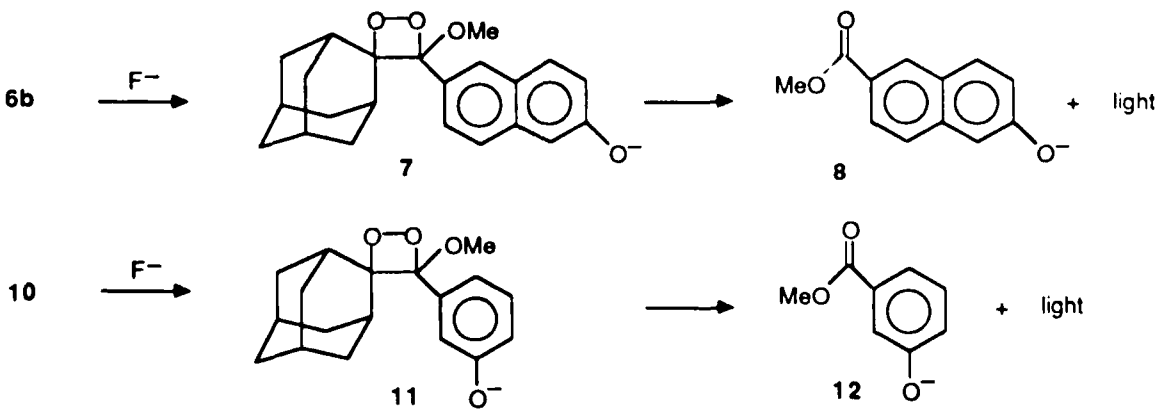


Table 1. Activation Parameters and Rates of Decomposition in o-Xylene.

dioxetane	E_a(kcal/mol)	log A	k(sec^{-1}) at 25 °C	half-life at 25 °C [a]
6a	29.7	13.2	3.17×10^{-9}	6.9 years
6b	27.0	11.7	8.72×10^{-9}	2.5 years
10	28.4	12.6	5.74×10^{-9}	3.8 years

(a) Calculated from the Arrhenius plots.

Deprotection of silyl ethers with fluoride is a widely used reaction in modern organic synthesis.[29] We have used this procedure as a convenient method for the generation of unstable, chemiluminescent aryloxide dioxetanes **7** and **11** from the thermally stable forms **6b** and **10**, respectively. In a typical experiment injecting an aliquot of an *o*-xylene solution of dioxetane **6b** into 3 mL of 0.001 M *n*-Bu_4NF in MeCN to give a final dioxetane concentration of 10^{-5} M produced a flash of blue chemiluminescence which decayed by a pseudo-first-order process with a half-life of a few seconds at room temperature. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm which was identical to the fluorescence of **8** and the fluorescence of the spent dioxetane solution under these conditions. No chemiluminescence derived from adamantanone fluorescence appears to be produced. Analysis of the crude product mixture resulting from treatment of a sample of **6b** with 1 equivalent of *n*-Bu_4NF by ^1H NMR and UV revealed only the expected cleavage products: adamantanone and the tetra-*n*-butylammonium salt of methyl 6-hydroxy-2-naphthoate in a 1:1 ratio.



Chemiluminescence quantum yields for the fluoride-triggered decomposition of dioxetane **6b** were measured relative to the luminol standard[30] using a photon-counting apparatus. Fluoride-triggered decomposition of 10^{-5} M solutions of **6b** in MeCN at 25 °C produced chemiluminescence with a quantum yield of 4×10^{-5} which was independent of fluoride concentration in the range 10^{-2} to 10^{-4} M. Correction for the fluorescence quantum yield of **8** in MeCN under identical conditions leads to a calculated chemiexcitation quantum yield of 1.1×10^{-4} or an efficiency for the formation of singlet excited **8** of 0.01% (Table 2).

Table 2. Fluoride-Induced Chemiluminescence from Dioxetanes 6b and 10.

dioxetane	solvent	Φ_{CL}^{a}	Φ_{F}^{b}	Φ_{CE}^{c}
6b	MeCN	4×10^{-5}	0.37	1.1×10^{-4}
10	MeCN	0.094	0.21	0.45
10	DMSO	0.25	0.44	0.57

(a). Chemiluminescence quantum yields. (b). Fluorescence quantum yields for cleavage products **8** and **12** relative to quinine sulfate with a value of 0.54. (c). Quantum yields for the formation of the singlet excited state of **8** and **12**.

In contrast to the low chemiluminescence quantum yield on triggering the decomposition of **6b** with fluoride, we find that similar treatment of **10** produces chemiluminescence with dramatically higher efficiency. Addition of excess *n*-Bu_4NF to 10^{-7} M solutions of dioxetane **10** in MeCN resulted in a rapid decomposition of **10** accompanied by bright blue chemiluminescence with a half-life of 5 sec at 25 °C. The pseudo-first-order rate constant was independent of fluoride concentration in the range 6.7×10^{-5} to 3.3×10^{-3} M. The spectrum of the resulting chemiluminescence exhibited a maximum at 470 nm in MeCN which matched exactly the fluorescence of **12** under these conditions (Figure 1). Similar experiments were carried out in DMSO. Quantum yields for the chemiluminescence of **10** with fluoride were determined in MeCN and DMSO relative to the luminol standard and found to be 0.094 and 0.25, respectively (Table 2). Correction for the fluorescence quantum yields of **12** in these solvents gives efficiencies for the formation of singlet excited **12** of 45 and 57%, the highest singlet chemiexcitation efficiencies yet reported for a dioxetane prepared in the laboratory.[31]

These results are explained by a mechanism initiated by cleavage of the Si-O bond by fluoride to generate the unstable dioxetanes **7** and **11**. The lack of any dependence of the rate of decay of the chemiluminescence on fluoride concentration suggests that the rate-limiting step under these conditions may be the cleavage of the aryloxide dioxetanes. The rapid decomposition of these intermediates is induced by an intramolecular elecron transfer from the electron-donating phenoxide substituent to the peroxide σ^* orbital. The reasons for the significant difference in the chemiexcitation efficiencies of **7** and **11** is not fully understood and is a subject for further investigation. It should be noted that we have prepared the corresponding *p-tert*-butyl-dimethylsilyloxyphenyl-substituted dioxetane and found the chemiexcitation efficiency to also be less than 0.01% in that case.

We plan to continue our investigations with these stable, efficient dioxetanes with a view towards the possible use of this system as a convenient liquid light standard. Stock solutions of **10** prepared in *o*-xylene exhibit high stability and can be stored for long periods. Typically, the calibration of a luminometer can be carried out by injecting 30 μL of a 10^{-3} M stock solution of **10** into 3 mL of a 0.01 M solution of *n*-Bu_4NF in dry DMSO. The luminescence is emitted over a 20 sec period with 1-2% reproduciblity for the total light emission.

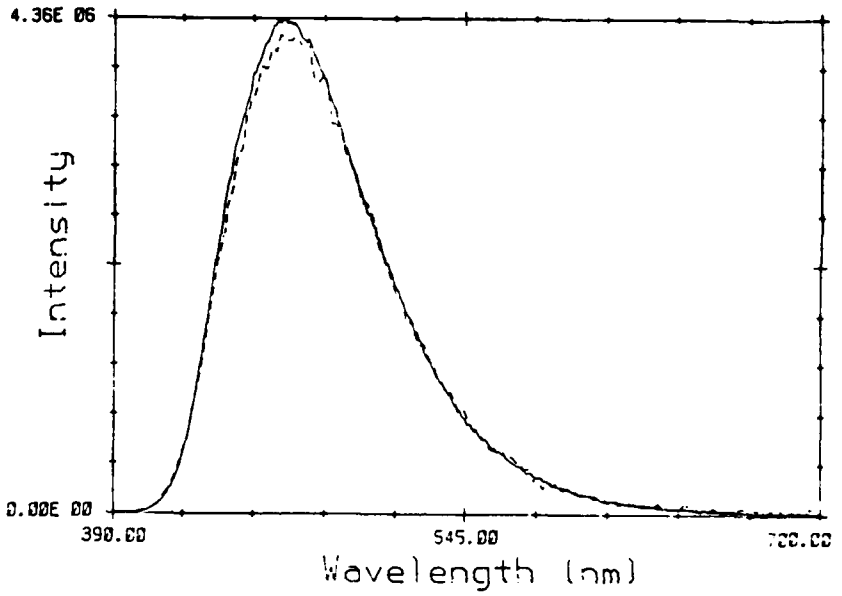
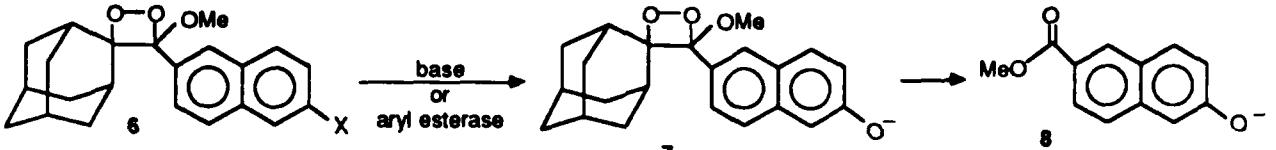


Figure 1. Chemiluminescence spectrum from fluoride triggering of dioxetane **10** in DMSO (----). Fluorescence spectrum of **12** under the same conditions (—).

2. Enzymatic Triggering of 1,2-Dioxetanes*

Biological assays involving enzymes utilize a wide variety of substrates which either form a color (chromogenic) or become fluorescent (fluorogenic) upon reaction with the enzyme.[32] As part of our investigation of triggering methods for dioxetanes, we have developed the first examples of chemiluminescent or lumigenic enzyme substrates.[33,34] Use of these peroxides in biological systems requires dioxetanes which are thermally stable at the temperature of the enzymatic reaction and do not undergo rapid spontaneous decomposition in the aqueous buffers. The spiro-fused adamantyl dioxetanes described in the previous section meet these requirements. We have, therefore, prepared a series of dioxetanes bearing functional groups which can be enzymatically modified to generate the aryloxide form. Decomposition of this unstable intermediate provides the luminescence. Dioxetanes have been synthesized which can be triggered by aryl esterase, alkaline phosphatase, and β-galactosidase. The last two examples are particularly significant because these enzymes are used extensively in enzyme-linked immunoassays.



The first case of enzyme triggering involved the acetate-substituted dioxetane **6d** and aryl esterase. Like the other naphthyl-substituted examples, this dioxetane is thermally very stable with a calculated half-life at 25 °C of 18.5 years (Table 3). In order to evaluate the stability of the deprotected form **7** of the dioxetane, chemical triggering experiments were conducted with the hydroxy derivative **6c** by treatment with excess potassium *tert*-butoxide in *o*-xylene. The luminescence was found to decay with a half-life of approximately 20 sec at 25 °C. Faster rates of decay were found with base-induced chemiluminescence from **6c** in methanol. Comparison of the chemiluminescence spectra with the fluorescence of anion **8** under the same conditions demonstrated that the luminescence is derived from chemiexcited **8**.

Table 3. Activation Parameters and Rates of Decomposition in o-Xylene.

dioxetane (X)	E_a(kcal/mol) [a]	log A	k(sec^{-1}) at 25 °C	half-life at 25 °C [b]
6a (H)	29.7	13.2	3.17×10^{-9}	6.9 years
6c (OH)	29.7	13.3	3.83×10^{-9}	5.1 years
6d (OAc)	32.5	14.9	1.19×10^{-9}	18.5 years

(a) Rates showed variations of less than 3% and gave Arrhenius plots with $r > 0.99$.
(b) Calculated from the Arrhenius plots.

*This project was supported in part by ONR and by a grant from Wayne State University.

Aryl esterase was used to catalyze the cleavage of the naphthyl acetate-substituted dioxetane 6d at ambient temperature in 0.05 M phosphate and 0.02 M Tris buffers. A 2 mM stock solution of 6d in 2-methoxyethanol was prepared. Aryl esterase (carboxylic ester hydrolase (E-3128)) from porcine liver was purchased from Sigma Chemical Co. as a suspension of 11 mg of protein per mL in 3.2 M $(NH_4)_2SO_4$ solution. When a 150 µL (0.3 µmol) aliquot of the stock solution of 6d was added to 3 mL of either buffer solution at 25 °C, no emission was detected. However, injection of 1 µL (0.26 units, final conc of protein = 3.6 µg/mL) of aryl esterase to the stirred solution generated chemiluminescence. The time required for one-half of the total light emission was found to be 7 min in both buffers. A similar time course for the consumption of 6d was found when the reaction was followed by UV spectroscopy. Additional experiments showed that total light emission is linearly dependent on dioxetane concentration over the range of 10^{-4} to 10^{-7} M (Figure 2). The rate of decay of the emission is a function of enzyme concentration while total light emission is independent of enzyme concentration (Figure 3).

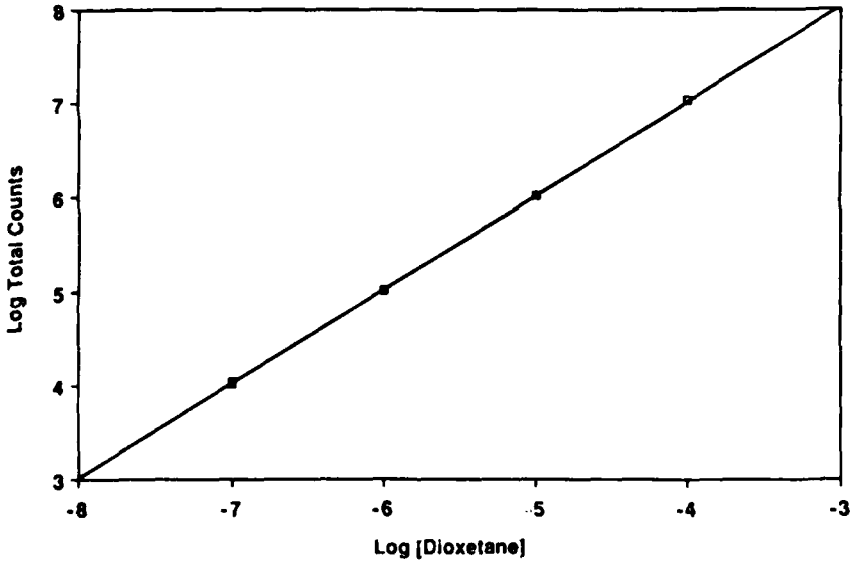


Figure 2. Plot of total light emission vs. dioxetane concentration for aryl esterase-catalyzed cleavage of 6d.

That this chemiluminescence is due only to an enzyme-catalyzed hydrolysis of acetate 6d is demonstrated by the following series of experiments: 1.) Addition of 5 µL of 3 M $(NH_4)_2SO_4$ to the dioxetane in the absence of enzyme produced no chemiluminescence. 2.) When distilled water was substituted for the Tris buffer in the presence of the enzyme, no chemiluminescence signal was observed; but on adding Tris buffer to this solution light emission was produced. 3.) In experiments where 150 µL of dioxetane stock in 3 mL of Tris buffer was triggered with 1 µL of enzyme at 25, 37 and 50 °C, the maximum light intensity (I_{max}) and the rate of decay both increased with increasing temperature. 4.) Denaturing the enzyme by heating 1 µL in 3 mL of Tris buffer to 90 °C and cooling to 25 °C resulted in no chemiluminescence when an aliquot of the dioxetane stock solution was subsequently added. Addition of untreated enzyme preparation to this solution again produced light.

5.) Addition of the known enzyme inhibitor, sodium dodecyl sulfate (SDS) at I_{max} caused an irreversible decrease in the intensity. The emission could be totally extinguished by addition of sufficient SDS. The decrease in light emission is not due to photophysical quenching of the excited state since thermal decomposition in the same solvent system with SDS at elevated temperatures results in readily detectable chemiluminescence. 6.) Sequential injection of ten identical aliquots of the dioxetane stock solution when light emission had stopped resulted in identical chemiluminescence curves, both in I_{max} and time for complete decay of the signal. 7.) Addition of esterase substrates (α-naphthyl and β-naphthyl acetates) also inhibited the chemiluminescence. These substrates were shown by UV spectroscopy to be hydrolyzed by the enzyme in seconds under the reaction conditions. A solution of 25 μL of the dioxetane stock in 3 mL of phosphate buffer maintained at 37 °C was treated with 5 μL of the enzyme to initiate the chemiluminescence. At I_{max} 10 μL of 0.011 M solutions of either α- or β-naphthyl acetate were added. A rapid decrease in light intensity was noted followed by restoration of the original intensity within 1 min.

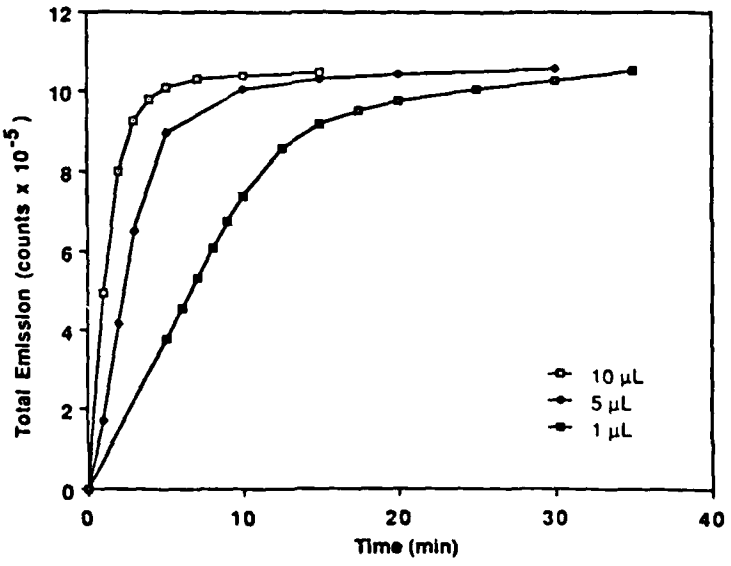


Figure 3. Plot of total chemiluminescence from esterase triggering of dioxetane 6d in Tris buffer with 1, 5, 10 μL of esterase suspension.

Many dioxetanes are known to be destroyed via non-luminescent pathways by amines[1a] and metal ions.[35] Therefore, a series of experiments was performed to assess the stability of 6d in the buffers over the time course of a typical run. A comparison was made between I_{max} with delays of 0 and 30 min before the enzyme was added. If the dioxetane was decomposing in the buffer, then I_{max} of the run where the dioxetane was exposed to the buffer for 30 min would be lower provided the enzyme is not saturated. Since constant light levels were not seen in any runs, it can be reasonably assumed that saturation kinetics did not apply here. In phosphate buffer at 25 °C, no decrease in I_{max} was observed after the 30 min delay with only a 12% decrease observed in Tris buffer.

The chemiluminescence spectrum for the enzyme-catalyzed decomposition of **6d** in Tris buffer at ambient temperature matches the fluorescence spectrum of methyl 6-hydroxy-2-naphthoate in the buffers and in strongly basic solution (Figure 4). The spectrum of the chemiluminescence from the spontaneous decomposition of the hydroxy-dioxetane **6c** under the same conditions was also identical. These findings demonstrate that rate-limiting enzymatic hydrolysis of the acetate group in dioxetane **6d** generates the unstable dioxetane **7** which subsequently yields singlet excited **8**.

The chemiluminescence quantum yield (Φ_{cl}) for the enzymatic triggering of **6d** is 1.6×10^{-6} in both buffers using luminol as a light standard. The fluorescence quantum yield for the naphthyl cleavage product is 0.65 and 0.55 in phosphate and Tris buffers so that the chemiexcitation efficiency for the formation of the singlet excited state of **8** is 2.4×10^{-4} % and 3.0×10^{-4} %, respectively. That the low value for Φ_{cl} is not due to quenching of **8** by the protein is shown by the fact that Φ_{cl} for the spontaneous chemiluminescence of **6c** is 1.4×10^{-6} in both buffers.

Although this particular system exhibits a relatively low chemiexcitation efficiency, these studies have demonstrated that thermally stable dioxetanes can be enzymatically triggered at biological temperatures. As demonstrated in the fluoride triggering work, the chemiexcitation efficiency of the dioxetanes can be dramatically altered by relatively simple changes in the structure of the aryloxide group. These results do, however, illustrate the potential sensitivity of this methodology for new types of biological assays involving appropriately substituted dioxetanes and enzyme triggering.

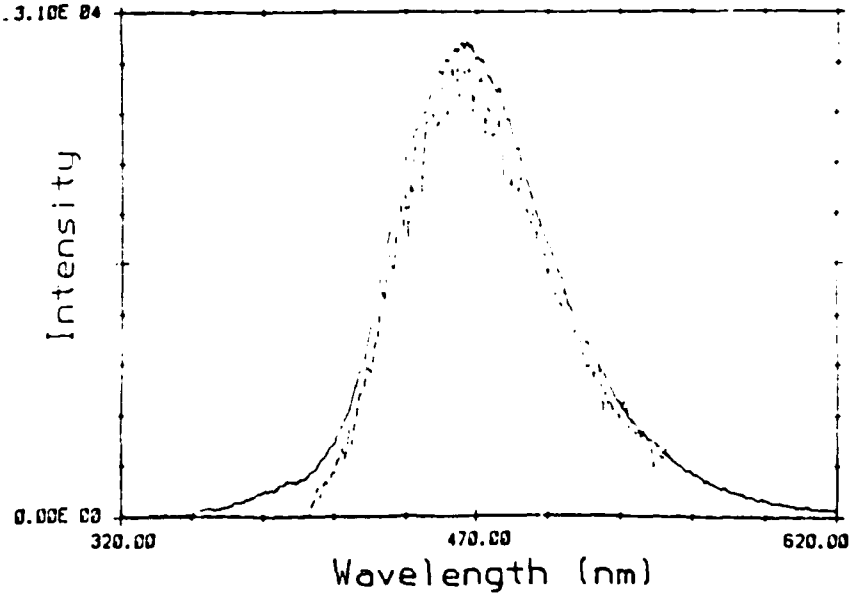
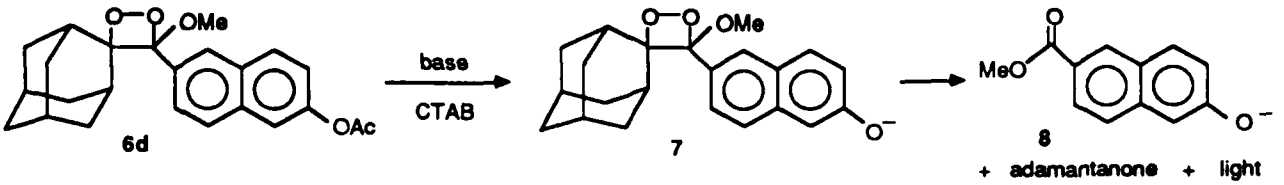


Figure 4. Chemiluminescence spectrum from esterase triggering of dioxetane **6d** in Tris buffer at ambient temperature (---). Fluorescence spectrum of **8** (—).

3. Chemiluminescence from Micellar Systems

Rates of various chemical reactions can be accelerated by micelles in aqueous solution.[36] Catalysis results from solubilization of the substrate in the micellar pseudophase and from electrostatic, hydrophobic, or polarity factors. As part of our ONR-supported research, we have conducted an investigation of the chemical triggering of thermally stable dioxetanes in micelles. This work represents the first report of the effects of the micellar environment on the chemiluminescent properties of isolable dioxetanes.[37] We have found that cationic surfactants such as cetyltrimethyl ammonium bromide (CTAB) can be used to significantly enhance rates of chemically triggered luminescence from appropriately substituted dioxetanes in aqueous solution. For example, CTAB catalyzes the base-induced chemiluminescent cleavage of the acetate-substituted dioxetane **6d**.



Dioxetane **6d** is solubilized in water with the surfactant and NaOH is added to initiate the luminescence. The electrostatic attraction of the cationic head group and the hydroxide anion provides the observed micellar catalysis. No rate enhancements are observed with anionic or neutral surfactants. A typical chemiluminescent decay curve is shown in Figure 5. The experiments were carried out with [6d] = 10^{-5} M, [CTAB] = 4.7 x 10^{-3} M, [OH^-] = 3 x 10^{-3} M at 25 °C. The half-life for dioxetane **6d** under these conditions is 0.05 min. The decay curve is first order with a correlation coefficient of >0.999. In the absence of surfactant, the half-life of the luminescence is > 30 min.

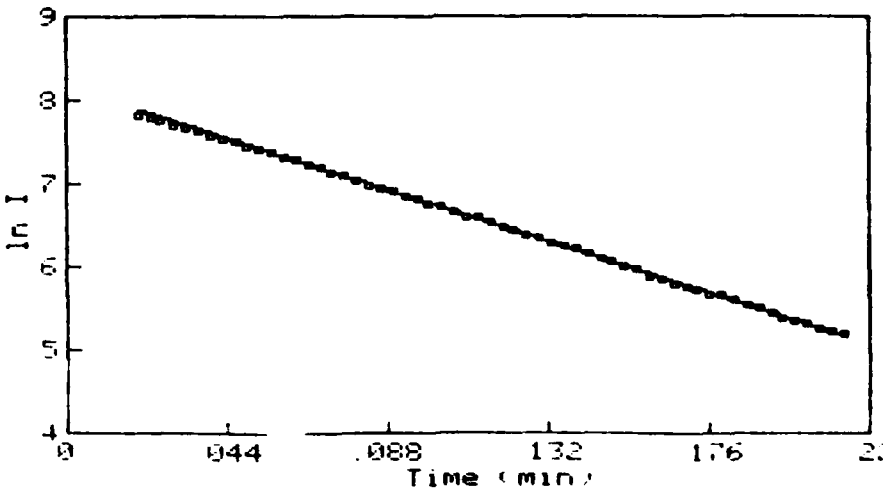


Figure 5. Plot of ln(light intensity) vs. time for the base-induced chemiluminescence of dioxetane 6d in CTAB micelles.

Rates showed the expected dependence on CTAB concentration indicating micellar catalysis above 5.8 x 10^{-4} M (Figure 6). In the range of 3.6 x 10^{-4} to 7.2 x 10^{-3} M, the rates were linearly dependent on hydroxide ion concentration (Figure 7). The chemiluminescence spectrum of the base-induced reaction of **6d** in CTAB is identical to the fluorescence of the cleavage product **8** under the same conditions. As expected from the earlier experiments with the various naphthyl-substituted dioxetanes **6**, the chemiluminescence efficiency for **6d** in CTAB is only 4.4 x10^{-4}%. However, recent experiments with more efficient dioxetanes related to **10** indicate that much higher efficiencies will be possible in aqueous micelles (see page 29). We have also conducted preliminary chemical triggering experiments with silyloxy-substituted dioxetanes using CTAB and fluoride ion in an aqueous system. We plan to continue these studies with the goal of developing efficient chemiluminescent systems for aqueous environments.

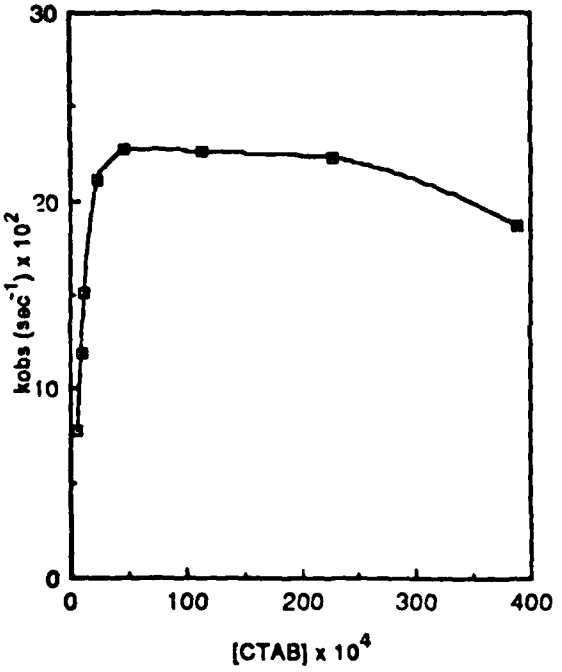


Figure 6. Rate of hydrolysis of 6d followed by chemiluminescence decay vs. concentration of CTAB.

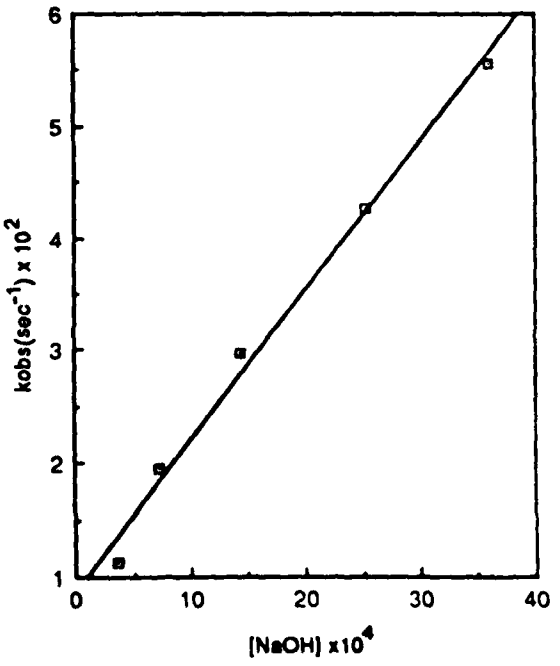
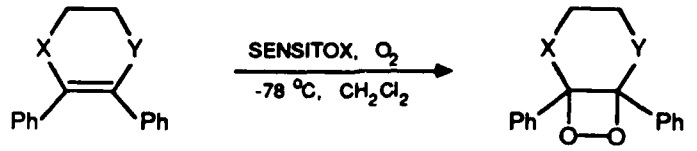


Figure 7. Rate of hydrolysis of 6d followed by chemiluminescence decay vs. concentration of base.

4. Effects of Heteroatom Substituents on 1,2-Dioxetanes

It has been recognized for some time that alkyl- or phenyl-substituted dioxetanes are relatively stable and decompose at elevated temperatures to give predominantly triplet excited states.[1] Alkoxy-substituted dioxetanes prepared by the photoxygenation of vinyl ethers exhibit properties similar to those of alkyl-substituted dioxetanes. For example, cis-3,4-diethoxy-1,2-dioxetane[38] and cis-3,4-diethyl-1,2-dioxetane[39] both have half-lives of several hours at 25 °C and thermolyze with Arrhenius activation energies of 24.4 and 24.5 kal/mol, respectively.

However, dioxetanes derived from enamines[40] and vinyl sulfides[41] were reported to be qualitatively much less stable, undergoing rapid decompostion (sometimes explosively) below °C. No mechanistic explanation for the striking instability of the nitrogen- and sulfur-substituted dioxetanes was provided by these investigators. Therefore, as part of our ONR-supported research, a systematic study of the effects of heteroatom-substitutents on the properties of dioxetanes was carried out. This investigation has resulted in the first report of activation parameters and rates of decomposition for nitrogen- and sulfur-substituted dioxetanes.



13 14

a, X = Y = O
b, X = Y = S
c, X = S; Y = O
d, X = Y = MeN
e, X = MeN; Y = O
f, X = PhN; Y = O

A series of structurally related alkenes **13a-f** was synthesized by acid-catalyzed condensation of benzoin with 1,2-ethanediol, 1,2-ethanedithiol, 2-hydroxyethanethiol, and N,N'-dimethyl-1,2-ethanediamine, 2-(N-methylamino)ethanol, and 2-anilinoethanol. Solutions of dioxetanes **14a-f** were prepared in CH_2Cl_2 by photooxgenation of the alkenes at -78 °C with polystyrene-immobilized Rose Bengal (SENSITOX) and a 400-W high pressure sodium lamp. The dioxetanes were characterized by their indirect chemiluminescence upon thermolysis in *o*-xylene in the presence of 9,10-dibromoanthracene (DBA), by ^1H NMR at low temperature and by identification of the cleavage products.

Rate constants for the decompostion of **14a-f** were determined from measurements of the decay of chemiluminescence intensity of 10^{-4} - 10^{-5} M solutions in *o*-xylene in the presence of 10^{-5} M DBA. The isothermal decompostions were monitored for at least three half-lives and were first order in all cases. Rates were found to be independent of DBA and dioxetane concentration. Activation parameters were calculated from Arrhenius plots.

In agreement with earlier qualitative observations we found that all of the dioxetanes with nitrogen- or sulfur-containing substituents **14b-f** are markedly less stable than the oxygen-substituted analog. The Arrhenius activation energy for decomposition of **14a** is 24.8 kcal/mol, consistent with

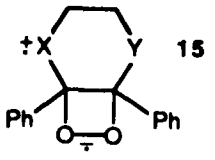
values observed for other oxygen-substituted dioxetanes. In contrast, dioxetanes **14b-f** exhibit much lower activation energies, ranging from 16.6 to 18.8 kcal/mol. An indication of the difference in stability of these two sets of dioxetanes is the temperature range over which decomposition rates could be conveniently measured (Table 4). From the Arrhenius plots it is calculated that the S- and N-substituted dioxetanes cleave between 10^4 and 5 x 10^5 times faster than **14a** at 25 °C.

Table 4. Activation Parameters and Rates for Heteroatom-Substituted Dioxetanes

Dioxetane (X,Y)	Temp. Range, °C	E_a, kcal/mol	Log A	k_{rel}, 25 °C
(O,O)	70.0 to 90.3	24.8	12.4	1 [a]
(S,S)	-11.5 to 20.5	18.8	13.6	3.96 x 10^5
(S,O)	-11.5 to 21 5	17.4	12.2	1.66 x 10^5
(MeN, MeN)	-0.7 to 29.6	16.6	11.1	5.12 x 10^5
(MeN, O)	-9.4 to 26.8	16.6	11.5	1.40 x 10^5
(PhN,O)	3.5 to 49.8	17.9	11.3	1.03 x 10^4

(a) Corresponds to a rate constant of 1.67 x 10^{-5} sec^{-1} in o-xylene at 25 °C.

As discussed previously, there is substantial evidence to indicate that "stable" dioxetanes such as **14a** decompose by a stepwise process involving homolysis of the peroxide bond to form a diradical with subsequent C-C bond cleavage. *Clearly*, an alternate mechanistic explanation is required to account for the distinct properties of nitrogen- and sulfur-substituted dioxetanes **14b-f**. By analogy to dioxetanes bearing heteroaromatic substituents, we have suggested that the mechanism for the deomposition of **14b-f** involves intramolecular electron-transfer from the heteroatom to the peroxide σ^* orbital (structure **15**). This mechanism requires that the stability of the dioxetane be related to the oxidation potential of the heteroatom substituents. Consistent with this suggestion are the results in Table 4 which show that dioxetanes bearing easily oxidized groups such as amines or sulfides (E_p^{ox}: Et_3N, + 0.96 V; Me_2S, + 0.88 V) are much less stable than a similar dioxetane with an alkoxy substitutent possessing a much higher oxidation potential (E^{ox} Et_2O, > +2.5 V). It should be noted that only one N- and S-group is sufficient to destabilize the dioxetane. This result also argues against a concerted mechanism involving both ring carbons in the transition state.



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In addition to the indirect "blue" chemiluminescence that can be observed visually from **14a-f** in the presence of DBA, we also found that injection of a cold solution of the mixed oxygen-sulfur dioxetane **14c** into *o*-xylene at ambient temperature results in an intense "yellow" chemiluminescence (λ_{max} = 553 nm). This direct emission is of particular interest because the diester cleavage product is

not detectably fluorescent. Figure 8 shows a chemiluminescence spectrum of **14c** in *o*-xylene obtained at -11°C with a Spex Fluorolog spectrofluorimeter. Correction was made for the decay of total light intensity during the scan by use of a second detector in a ratio mode. This luminescence is also observed in acetonitrile (λ_{max} = 533 nm) and in methylcyclohexane (λ_{max} = 561 nm). A plausible explanation for this unusual emission invlolves an intramolecular exciplex. Goto has reported chemiluminescene from an exciplex generated by the thermolysis of an indole-substituted dioxetane.[24] However, in that case emission from the fluorescent indole group as well as the exciplex was observed.

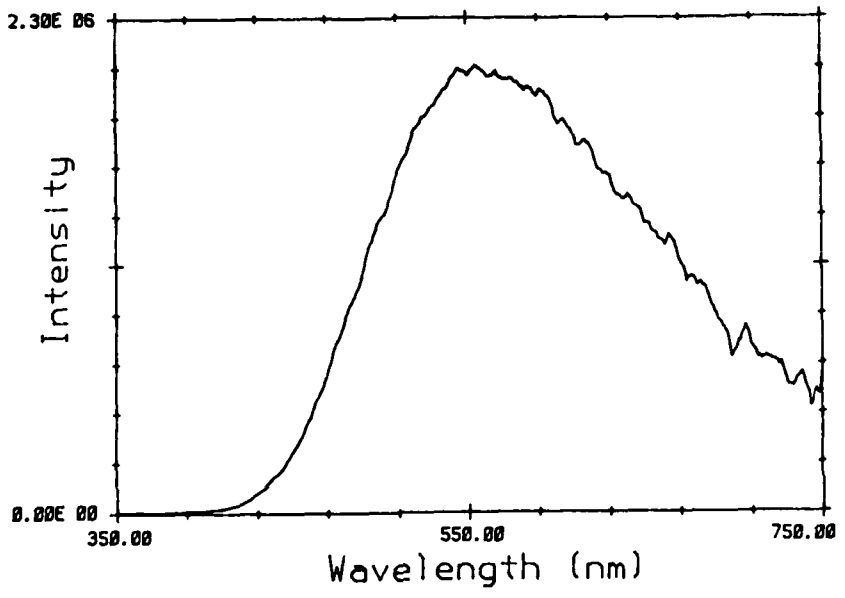


Figure 8. Chemiluminescence spectrum of dioxetane 14c in air-saturated o-xylene at -11 °C, corrected for the decay of total light intensity.

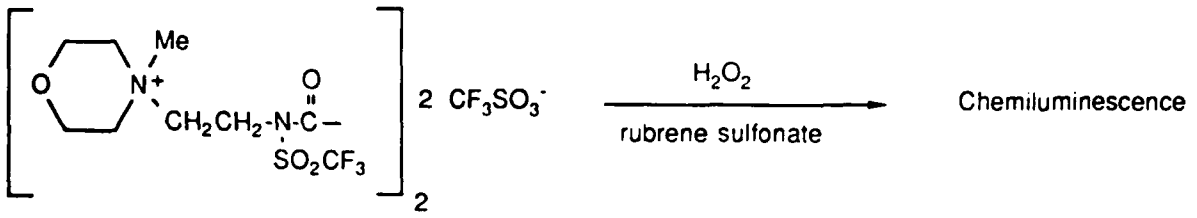
5. Hematoporphyrin-Chemiluminescence Cancer Therapy

Photoradiation therapy (PRT) has shown considerable promise as an effective treatment for a wide variety of cancers.[42] This new modality is based on the selective retention of hematoporphyrin derivative (HPD) in tumor tissue and on the photodynamic action produced by irradiation of HPD with visible light. The basic principles on which photoradiation therapy is based were developed over the last eighty years. In 1900 Raab discovered that certain fluorescent dyes could sensitize living organisms to visible light.[43] Some years later it was reported that various porphyrins and metalloporphyrins showed an affinity for malignant tissue.[44] Lipson and Baldes later described the synthesis of a derivative of hematoporphyrin that exhibited enhanced tumor localizing characteristics compared to hematoporphyrin itself.[45] This affinity of HPD for tumors has been utilized in the development of a sensitive method for the detection of malignant tissue. The active component of HPD has recently been isolated by Dougherty and found to be an ether-linked dimer of hematoporphyrin.[46] The mechanism for tumor localization has not yet been established.

The therapeutic potential of photoradiation therapy was first demonstrated in 1972 by Diamond who found that hematoporphyrin and light caused regression of transplanted tumors in rats.[47] Simultaneously, Dougherty observed that fluorescein and 488 nm light could be utilized to treat mouse mammary carcinoma. This group subsequently reported that cures could be effected in experimental tumor systems with HPD and red light.[48] Phototherapy of a human carcinoma with HPD was first described by Kelley and Snell for a patient with bladder carcinoma.[49] In pioneering studies by Dougherty and coworkers, PRT was successfully applied to the treatment of malignant tumors of the skin and subcutaneous tissue.[50] Dramatic therapeutic results have recently been reported for PRT with many types of solid tumors. Although the mechanism of tumor destruction by PRT has not yet been fully delineated, there is substantial evidence that singlet oxygen is involved as the active agent in the oxidation of biomolecules.

As part of our ONR-supported studies on chemiluminescence in aqueous systems, we conducted a preliminary investigation to determine if a chemiluminescent activator could be used to activate HPD for tumor killing instead of an external light source. This new approach was targeted at improving the delivery of light to the HPD by replacing the red laser with an efficient, water-soluble chemiluminescent system (CLS) that could be injected directly into the tumor. Very exciting results have been obtained in treating transplanted mammary tumors in C_3H/HeJ mice and additional funding for the project is now provided by the American Cancer Society.

As the chemical light source for this study, we used a system related to the peroxyoxalate chemiluminescence developed at American Cyanamid with ONR support. The luminescence is produced in aqueous solution by treatment of the substitutued oxamide **16** with 1% hydrogen peroxide in the presence of sulfonated rubrene as fluorescer.[51] The intense yellow-red light from this reaction lasts for 10-20 min.



An experiment is conducted as follows. A transplanted mammary adenocarcinoma from a female C_3H/HeJ mouse obtained from Henry Ford Hospital (Detroit) is excised after sacrifice of the animal. The tumor is carefully dissected and transplanted into the left axillary fold of C_3H/HeJ male and female mice. When the transplanted tumors become palpable, the animals are sensitized with hematoporphyrin derivative. Twenty-four hours after sensitization the animals are treated with the chemiluminescence system (CLS) injecting subcutaneously in the area of tumor localization. If the treatment is carried out in the dark, one can observe the tumor volume bathed in the luminescence of the reaction.

Typical results of an experiment are given in Figure 9. Curve B illustrates the growth of tumors for the untreated control animals. Curve A shows the dramatic effect of the hematoporphyrin-chemiluminescence cancer therapy in controlling the growth of the tumors. After 3 weeks the treated animals have an average tumor volume 10-fold smaller than the control animals. Of the 12 animals that were treated in this experiment, the tumors on 7 were completely eliminated. These animals have remained tumor free for over 3 months. These results under the American Cancer Society grant have lead the National Institutes of Health to issue an invitation for additional research on chemiluminescence-HPD cancer therapy.

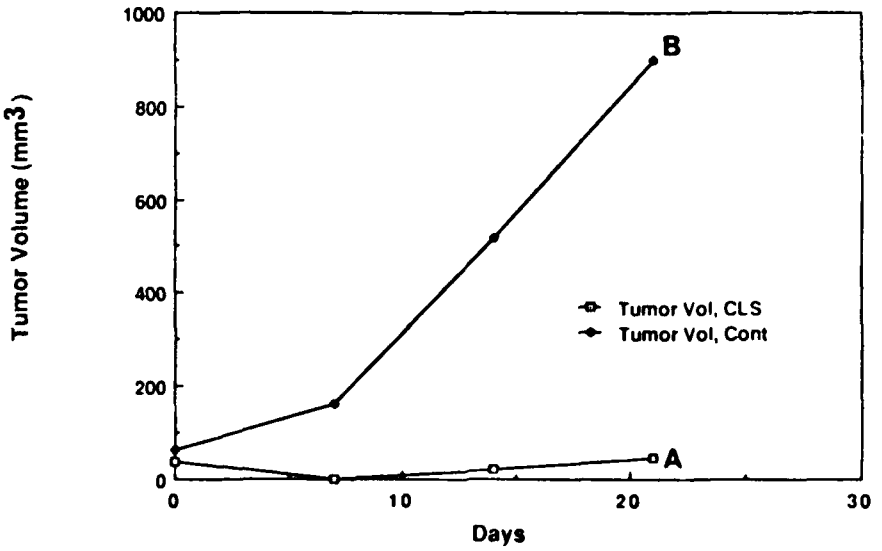


Figure 9. Plot of average tumor volume vs. days following treatment with HPD and the chemiluminescence system (curve A, 12 animals in the group); control group with no treatment (curve B, 10 animals).

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M. J. Phillip, J. D. McMahon, M. D. O'Hara, F. W. Hetzel, C. Amsterdamsky, and A. P. Schaap, Effects of Hematoporphyrin (HPD) and a Chemiluminescence System on the Growth of Transplanted Tumors in C3H/HeJ Mice, *Clayton Symposium on Porphyrin Localization and Treatment of Tumors,* Alan R. Liss, Inc., New York, 1985.

R. S. Handley, A. J. Stern, and A. P. Schaap, Effects of Heteroatom Substituents on the Properties of 1,2-Dioxetanes, *Tetrahedron Lett.,* 3183-3186 (1985).

A. P. Schaap, T. S. Chen, R. S. Handley, R. DeSilva, and B. P. Giri, Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 2: Fluoride-Induced Chemiluminescence from *Tert*- Butyldimethylsilyloxy-Substituted Dioxetanes, *Tetrahedron Lett.,* 935 - 938 (1987).

A. P. Schaap, R. S. Handley, and B. G. Giri, A Convenient Synthesis of Vinyl Ethers by the McMurry Coupling of Ketones and Esters, manuscript in preparation.

A. P. Schaap and H. Akhavan-Tafti, Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 4: Enhanced Chemiluminescence from Micellar Systems, manuscript in preparation.

TECHNICAL REPORTS

Technical Report 1. Effects of Hematoporphyrin (HPD) and a Chemiluminescence System on the Growth of Transplanted Tumors in C3H/HeJ Mice, M. J. Phillip, J. D. McMahon, M. D. O'Hara, F. W. Hetzel, C. Amsterdamsky, and A. P. Schaap, April 6, 1984.

Technical Report 2. Effects of Heteroatom Substituents on the Properties of 1,2-Dioxetanes, R. S. Handley, A. J. Stern, and A. P. Schaap, December 4, 1984.

Technical Report 3. Chemical and Enzymatic Triggering of 1,2-Dioxetanes. 2: Fluoride-Induced Chemiluminescence from *Tert*- Butyldimethylsilyloxy-Substituted Dioxetanes, A. P. Schaap, T. S. Chen, R. S. Handley, R. DeSilva, and B. P. Giri, March 16, 1987.

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REFERENCES AND NOTES

1. For reviews of 1,2-dioxetanes and chemiluminescence, see: (a) T. Wilson, *Int. Rev. Sci.: Phys. Chem., Ser. Two., 9*, 265 (1976). (b) P. D. Bartlett and M. Landis, In *Singlet Oxygen*, H. H. Wasserman and R. W. Murray, Eds., Academic Press, New York, 1979, Chapter 7. (c) G. B. Schuster and S. P. Schmidt, *Adv. Phys. Org. Chem. 18*, 187 (1982). (d) W. Adam, In *The Chemistry of Functional Groups, Peroxides*, John Wiley, New York, 1983, Chapter 24. (e) T. Wilson, In *Singlet Oxygen, Vol. II*, CRC Press, Boca Raton, 1985, Chapter 2.

2. For a review, see: W. D. McElroy and M. DeLuca,In *J. Bioluminescence in Action*, P. J. Herring, Ed., Academic Press, New York, 1978, p.109.

3. H. H. Seliger and W. D. McElroy, *Arch Biochem. Biophys., 88*, 136 (1960).

4. O. Shimomura and F. H. Johnson, *Photochem. Photobiol., 30*, 89 (1979).

5. O. Shimomura, In *Chemical and Biological Generation of Excited States*, Academic Press, New York, 1982, p. 249 and references therein.

6. (a) E. H. White, H. Worther, H. H. Seliger, and W. D. McElroy, *J. Am. Chem. Soc., 88*, 2015 (1966). (b) E. H. White and H. Worther, *J. Org. Chem., 31*, 1484 (1966).

7. K. R. Kopecky and C. Mumford, *Can. J. Chem., 47*, 709 (1969).

8. K. R. Kopecky, In *Chemical and Biological Generation of Excited States*, Academic Press, New York, 1982, p. 85.

9. P. D. Bartlett and A. P. Schaap, *J. Am. Chem. Soc., 92*, 3223 (1970).

10. S. Mazur and C. S. Foote, *J. Am. Chem. Soc., 92*, 3225 (1970).

11. For a review of this reaction, see: A. P. Schaap and K. A. Zaklika, In *Singlet Oxygen*, Academic Press, New York, 1979, Chapter 6.

12. (a) A. P. Schaap, A. L. Thayer, E. C. Blossey, and D. C. Neckers, *J. Am. Chem. Soc., 97*, 3741 (1975). (b) A. P. Schaap, A. L. Thayer, K. A. Zaklika, and P. C. Valenti, *Ibid., 101*, 4016 (1979).

13. W. Adam and G. Cilento, *Angew. Chem., Int. Ed. Engl., 22*, 529 (1983).

14. W. Adam and J. D. Liu, *J. Am. Chem. Soc., 94*, 2894 (1972).

15. (a) H. E. O'Neal and W. H. Richardson, *J. Am. Chem. Soc., 92*, 6553 (1970). (b) H. E. O'Neal and W. H. Richardson, *Ibid, 93*, 1828 (1971). (c) See also, E. J. H. Bechara and T. Wilson, *J. Org. Chem., 45*, 5261 (1980).

16. (a) W. H. Richardson, F. C. Montgomery, M. B. Yelvington, and H. E. O'Neal, *J. Am. Chem. Soc., 96*, 7525 (1974). W. H. Richardson, J. H. Anderegg, M. E. Price, W. A. Tapper, and H. E. O'Neal, *J. Org. Chem., 43*, 2236 (1978).

17. (a) J. Y. Koo and G. B. Schuster, *J. Am. Chem. Soc., 99*, 5403 (1977). (b) W. Adam and F. Yany, *Photochem. Photobiol., 31*, 267 (1979).

18. A. P. Schaap, S. D. Gagnon, and K. A. Zaklika, *Tetrahedron Lett.*, 2943 (1982).

19. W. H. Richardson and D. L. Stiggall-Estberg, *J. Am. Chem. Soc., 104*, 4173 (1982).

20. (a) J. H. Wieringa, J. Strating, H. Wynberg, and W. Adam, *Tetrahedron Lett.*, 169 (1972). (b) N. J. Turro, G. Schuster, H. -C. Steinmetzer, G. R. Faler, and A. P. Schaap, *J. Am. Chem. Soc., 97,* 7110 (1975). (c) F. McCapra and I. Beheshti, *J. Chem. Soc., Chem. Comm.*, 517 (1977). (d) W. Adam, L. A. A. Encarnacion, and K. Zinner, *Chem. Ber., 116,* 839 (1983). See also references 23 and 25.

21. (a) P. D. Barlett and M. S. Ho, *J. Am. Chem. Soc., 96,* 627 (1974). (b) P. Lechtken, *Chem. Ber., 109,* 2862 (1976).

22. (a) K. A. Zaklika, A. L. Thayer, and A. P. Schaap, *J. Am. Chem. Soc., 100,* 4916 (1978). (b) K. A. Zaklika, T. Kissel, A. L. Thayer, P. A. Burns, and A. P. Schaap, *Photochem. Photobiol., 30,* 35 (1979).

23. F. McCapra, I. Beheshti, A. Burford, R. A. Hann, K. A. Zaklika, *J. Chem. Soc., Chem. Comm., 944 (1977).*

24. H. Nakamura and T. Goto, *Photochem. Photobiol., 30,* 29 (1979).

25. C. Lee and L. A. Singer, *J. Am. Chem. Soc., 102,* 3832 (1980).

26. (a) G. B. Schuster, *Acc. Chem. Res., 12,* 366 (1979). (b) K. A. Horn and G. B. Schuster, *Tetrahedron, 38,* 1095 (1982) and references therein.

27. A. P. Schaap and S. D. Gagnon, *J. Am. Chem. Soc., 104,* 3504 (1982).

28. McMurry has mentioned that cyclic enol ethers can be obtained from this reaction but no examples have been published; see J. E. McMurry and D. D. Miller, *J. Am. Chem. Soc., 105,* 1660 (1983).

29. E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc., 94,* 6190 (1972). For additional references to the deprotection of alcohols with fluoride, see: T. W. Greene, In *Protective Groups in Organic Synthesis,* Wiley, New York, 1981.

30. (a) J. Lee and H. H. Seliger, *Photochem. Photobiol., 15,* 227 (1972). (b) P. R. Michael and L. R. Faulkner, *Anal. Chem., 48,* 1188 (1976).

31. For a listing of chemiexcitation efficiencies, see W. Adam, In *Chemical and Biological Generation of Excited States,* Academic Press, New York, 1982, Chapter 4. In comparing chemiluminescence efficiencies it is important to note the light standard used for calibration. Efficiencies relative to the radioactive standard should be divided by a factor of 2.6 for comparsion to chemiluminescence relative to luminol; consult reference 30b.

32. For a review, see: L. J. Kricka, *Ligand-Binder Assays: Labels and Analytical Strategies,* Marcel Dekker, Inc., New York, 1985.

33. (a) A. P. Schaap, R. S. Handley, and B. P. Giri, *Tetrahedron Lett.*, 935 (1987). (b) A. P. Schaap, M. D. Sandison, and R. S. Handley, *Tetrahedron Lett.,* in press.

34. Luminol derivatives and oxalate esters have been used with peroxidases or oxidases for chemiluminescent biological assays. See, for example, (a) H. R. Schroeder and F. M. Yeager, *Anal. Chem.., 50,* 1114 (1978). (b) H. Arakawa, M. Maeda, and A. Tsuji, *Anal. Biochem. , 79,* 248 (1979). (c) H. Arakawa, M. Maeda, and A. Tsuji, *Clin. Chem., 31,* 430 (1985). However, these systems differ from our case in that aryl esterase acts directly on the luminogenic substrate 6d.

35. (a) T. Wilson, M. E. Landis, A. L. Baumstark, and P. D. Bartlett, *J. Am. Chem. Soc., 95,* 4765 (1973); (b) P. D. Bartlett, A. L. Baumstark, and M. E. Landis, *Ibid., 96,* 5557 (1974).

36. (a) E. H. Cordes and R. B. Dunlap, *Acc. Chem. Res.*, *2*, 329 (1969). (b) J. H. Fendler and E. J. Fendler, *Catalysis in Micellar and Macromolecular Systems*, Academic Press, New York, 1975.

37. The effect of CTAB micelles on the chemiluminescent reaction of acridan esters in aqueous alkaline solution has been reported: F. McCapra, *Acc. Chem. Res.*, *9*, 201 (1976).

38. (a) T. Wilson and A. P. Schaap, *J. Am. Chem. Soc*, *93*, 4126 (1971); (b) T. Wilson, E. D. Golan, M. S. Harris, and A. L. Baumstark, *Ibid.*, *98*, 1086 (1976).

39. A. L. Baumstark and C. E. Wilson, *Tetrahedron Lett.*, 2569 (1979).

40. (a) C. S. Foote, A. A. Dzakpasu, and J. W.-P. Liu, *Tetrahedron Lett.*, 1247 (1975); (b) H. H. Wasserman and S. Terao, *Ibid.*, 1735 (1975); (c) F. McCapra, Y. C. Chang, and A. Burford, *J. Chem. Soc., Chem. Commun.*, 608 (1976); (d) I. Saito, S. Matsugo, and T. Matsuura, *J. Am. Chem. Soc., 101*, 4757 (1979); (e) W. Adam and L. A. A. Encarnacion, *Chem. Ber., 115*, 2592 (1982).

41. (a) W. Adam. L. A. Arias, and D. Scheutzow, *Tetrahedron Lett.*, 2835 (1982); (b) G. G. Geller, C. S. Foote, and D. B. Pechman, *Ibid.*, 673 (1983).

42. *Clayton Symposium on Porphyrin Localization and Treatment of Tumors*, Alan R. Liss, Inc., New York, 1985.

43. O. Rabb, *Z. Biol., 39*, 524 (1900).

44. (a) H. Auler, and G. Banzer, *Z. Krebsforsch, 53*, 65 (1942); (b) F. H. J. Figge, G. S. Weiland, and L. O. Manganiello, *Proc. Soc. Exp. Biol. Med., 68*, 640 (1948); (c) H. B. Gregorie Jr., E. U. Horger, J. Ward, J. Green, T. Richards, H. Robertson, and T. Stevenson, *Ann. Surg., 167*, 820 (1968); (d) J. Winkelman and D. S. Rasmussen-Taxdal, *Bull. Johns Hopkins Hosp., 107*, 228 (1969).

45. R. L. Lipson, E. J. Baldes, and A. M. Olsen, *J. Natl. Cancer Inst., 26*, 1 (1961).

46. T. J. Dougherty, Presented at the June 1983 meeting of the American Society for Photobiology, Madison, WI.

47. I. Diamond, S. Granelli, A. F. McDonagh, S. Nielsen, C. B. Wilson, and R. Jaenicke, *Lancet, 2*, 1175 (1972).

48. T. Dougherty, G. Grindey, R. Fiel, D. Weishaupt, and D. Boyle, *J. Natl. Cancer Inst., 55*, 115 (1975).

49. J. F. Kelly and M. E. Snell, *J. Urol., 115*, 150 (1976).

50. T. Dougherty, J. Kaufman, A. Goldfarb, K. Weisshaupt, D. Boyle, and A. Mittleman, *Cancer Res., 38*, 2628 (1978).

51. S. -S. Tseng, A. G. Mohan, L. G. Haines, L. S. Vizcarra, and M. M. Rauhut, *J. Org. Chem., 44*, 4113 (1979).

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