Table I. Total Second Moments Obtained for ^{2}H Bchl (X% ^{25}Mg) at Two Temperatures

X% ^{25}Mg	300 K, G^2	77 K, G^2
10% ^{25}Mg	6.30 ± 0.23[a]	7.93 ± 0.13
92% ^{25}Mg	6.43 ± 0.08	9.05 ± 0.23

[a] Standard deviation evaluated from several determinations. For 300 K the difference in second moments is not statistically significant; however, the difference is always in the same direction, and we believe is indicative of a small coupling.

where G = gauss and **T** is the hyperfine coupling tensor assuming axial symmetry.

In comparing the second moments of the light-induced signal I in fully deuterated *R. rubrum* with those produced in fully deuterated *R. rubrum* enriched to 92% in ^{25}Mg there was no change within experimental error. In fact, we have calculated that this is the expected result for a monomeric hyperfine splitting of <0.3 G. Likewise, no change is expected in the low-temperature dimer second moments when we consider the observed monomer differences (Table I). This is yet another indication of the "special pair" nature of signal I in bacteria since this shows a reduction in the ^{25}Mg hyperfine coupling compared with the value determined for monomeric bacteriochlorophyll.

We can compare our determination of the ^{25}Mg isotropic hyperfine coupling in bacteriochlorophyll with values obtained for metal couplings in the related radicals of zinc *meso*-tetraphenylporphyrin ($ZnTPP^+$), ^{67}Zn coupling of 1.22 G,[12] and cobalt(III) tetraphenylporphyrin ($Co^{III}TPP^{2+}$), ^{59}Co coupling of 5.7 G,[13] and cobalt(III) octaethylporphyrin ($Co^{III}OEP^{2+}$), ^{59}Co coupling of 1.4 G.[13] The free ion isotropic coupling constant of ^{25}Mg is 692 MHz,[14] of $^{67}Zn^{II}$ is 1686 MHz,[15] and of $^{59}Co^{III}$ is 3666 MHz.[16] Thus the degree of metal s character is <1% in all three cases and may reflect a correlation of the ground-state wave functions for the three cations.[4,12] The relatively small coupling in $Co^{III}OEP^{2+}$ has been shown by Fajer to arise from a change in the ground-state wave functions relative to that of $Co^{III}TPP^{2+}$.[12]

It is significant that the result presented here provides a new probe of the nature of the primary reactants in bacterial photosynthesis. Although we cannot distinguish between signal I in the bacteria enriched in ^{25}Mg to that in bacteria containing the natural abundance of ^{25}Mg by conventional EPR techniques, we are using electron spin echo techniques to make this comparison.[17]

From MO calculations on the bacteriochlorophyll radicals, we expected[2] and have found[18] a considerably larger ^{25}Mg hyperfine coupling in the bacteriochlorophyll radical anion. We are using this to identify the first electron acceptor in bacterial photosynthesis.

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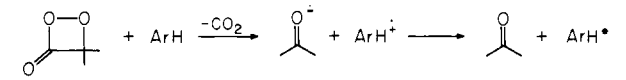
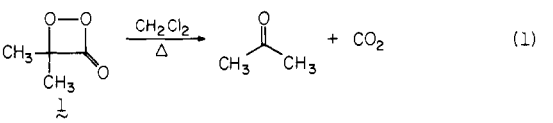
Dioxetanone Chemiluminescence by the Chemically Initiated Electron Exchange Pathway. Efficient Generation of Excited Singlet States[1]

Sir:

Many recent studies of chemiluminescence from organic molecules have centered on the reactions of the strained four-membered-ring peroxides known as dioxetanes.[2] These molecules have been identified or implicated in many of the most efficient chemi- and bioluminescent reactions. In particular, a carbonyl substituted dioxetane (dioxetanone) has been suggested as the key high energy molecule responsible for light production in the firefly.[3] In this paper we report our findings on the mechanism of light production from simply substituted dioxetanones. Our studies show that the most important light forming path for reaction of dioxetanone **1** is the bimolecular route which we have recently identified as chemically initiated electron-exchange luminescence (CIEEL).[4] Moreover, we have found that under conditions favoring CIEEL the fraction of reacting dioxetanone molecules that generate a photon of light approaches the most efficient bioluminescent reactions known. In addition, by analogy, it appears that the initiating reaction in firefly bioluminescence is an intramolecular electron transfer akin to the observed intermolecular reaction reported herein.[5]

Dimethyldioxetanone (**1**) was prepared and purified according to the procedure of Adam.[6] Thermolysis of **1** in





CH_2Cl_2 at 24.5 °C leads to the quantitative generation of acetone and to light emission.[7] The observed chemiluminescence under these conditions is a result of acetone emission. It has been reported previously that the addition of certain aromatic hydrocarbons to solutions of dioxetanone **1** results in the generation of hydrocarbon luminescence and markedly increases

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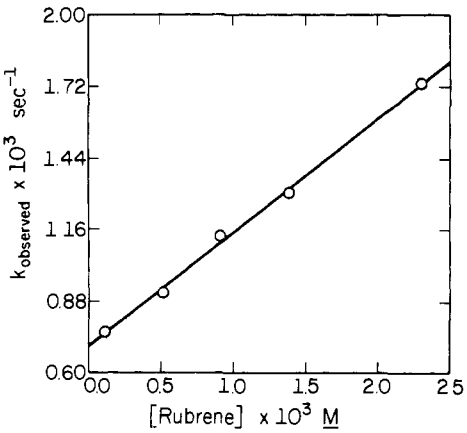


Figure 1. Observed dependence of rate of reaction for dioxetanone on the rubrene concentration. Note that the extrapolated zero rubrene concentration rate agrees, within experimental error, to the independently determined value.

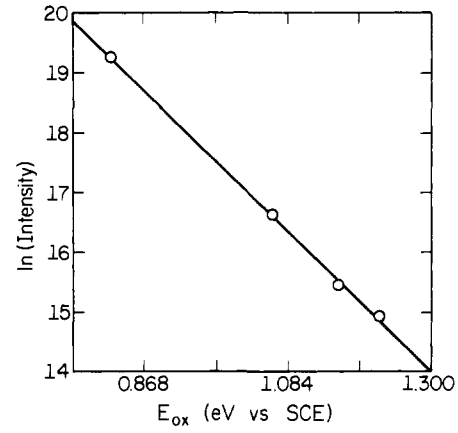


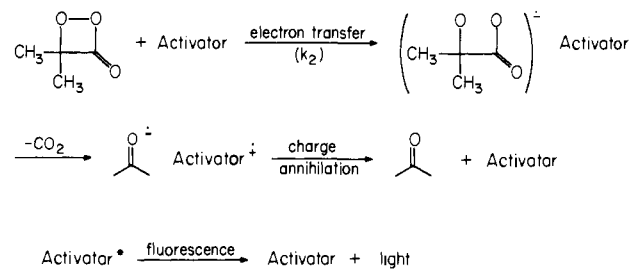
Figure 2. Dependence of the chemiluminescence intensity by the CIEEL path on oxidation potential (E_{ox}) of the activator. In order of increasing oxidation potential the points are rubrene, perylene, 9,10-diphenylethynylanthracene, and 9,10-diphenylanthracene.

the light yield.[8] We have also observed this effect and, significantly, have found that the aromatic hydrocarbon also increases the rate of reaction of the dioxetanone. The rate acceleration is directly proportional to the concentration of the hydrocarbon (catalytic chemiluminescence activator), as is shown for rubrene in Figure 1. Also, the observed rate acceleration is dependent on the structure of the activator. These findings are inconsistent with the previously proposed unimolecular pathway for excited state production. In the original mechanism, thermal reaction of the dioxetanone generated electronically excited acetone which, in a subsequent step, transferred energy to the hydrocarbon. If this scheme was solely responsible for generation of excited activator, the rate of reaction of the dioxetanone would be independent of the structure and concentration of the added hydrocarbon. This is contrary to our results.

To probe the nature of the interaction between dimethyldioxetanone (**1**) and the catalytic chemiluminescence activator, the efficiency of generation of light with various activators was examined. We observed that at identical hydrocarbon concentrations there is nearly a hundredfold range in the ability of the different activators to catalyze excited-state formation. The excited states detected under these conditions correspond to the fluorescing singlet of the activator in all cases.[9] These findings indicate that it is the bimolecular reaction between the dioxetanone and the activator that is responsible for the major fraction of the hydrocarbon chemiluminescence, since the singlet–singlet energy-transfer efficiency should be approximately independent of the identity of the hydrocarbon. Critically, the only predictor of activator efficiency is the one-electron oxidation potential of the hydrocarbon, as is shown in Figure 2. This result is required if electron transfer from the activator to the dioxetanone is the rate-determining step for formation of the activator excited singlet state. These findings are entirely consistent with excited-state production from dioxetanone **1** by the mechanism which we have recently identified as chemically initiated electron-exchange luminescence.[4]

Further evidence that the identity of the activator controls the light forming sequence is obtained from the study of the activitation energy (E_a) for the bimolecular reaction. Investigation of the temperature dependence of the chemiluminescence intensity with various activators revealed that E_a for this excitation path was proportional to the oxidation potential of the activator. For example, perylene activated chemiluminescence exhibits an E_a of *16.1 ± 0.6* kcal/mol while under identical conditions 9,10-diphenylethynylanthracene gives E_a = 18.0 ± 0.6 kcal/mol. Moreover, E_a for unimolecular formation of acetone singlet is 24.8 ± 0.4 kcal/mol. Thus, the aromatic hydrocarbons are truly catalytic activators.

Scheme I



The efficiency of production of excited singlet activator by the induced decomposition of **1** was investigated by measuring the absolute chemiluminescence yield.[10] When rubrene was employed as the activator in CH_2Cl_2 solution at 24.5 °C, it was found that 10 ± 5% of the dioxetanone molecules that proceed through the bimolecular path generate an excited rubrene singlet state. Even though this system is unoptimized, the remarkably high efficiency approaches that of the known bioluminescent reactions.[12]

The proposed mechanism for formation of the emitting singlet state of the chemiluminescence activator by the CIEEL path is shown in Scheme I. The first step is a one-electron transfer from the activator to the dioxetanone. The rate constant for this process, of course, depends upon the activation barrier for the reaction which is determined, in part, by the oxidation potential of the activator. Similiar electron-transfer reactions have been postulated, for example, to account for the catalytic induced decomposition of peroxides by transition metals and amines.[13] The activation energy for the electron transfer is also dependent upon the reduction potential of the peroxide. For dioxetanone **1** the bimolecular rate constant for the CIEEL path (k_2) in CH_2Cl_2 at 24.5 °C with rubrene is 0.44 M^{-1} s^{-1}. This compares to a value of 8 M^{-1} s^{-1} determined for diphenoyl peroxide under similar conditions.[4] Furthermore, we have not yet detected a CIEEL component to the chemiluminescence of simple alkyl substituted dioxetanes.[14] The difference in the catalytic rate constants for these peroxides is predicted by their reduction potentials.[15] The two electron-withdrawing carbonyl groups flanking the oxygen–oxygen bond of the diacyl peroxide results in more facile reduction and concomitantly a larger k_2 for diphenoyl peroxide than for the dioxetanone.

The next step along the chemiluminescence path is the rapid loss of CO_2 from the reduced dioxetanone. This generates the radical anion of acetone within the same solvent cage as the radical cation of the activator.[16] Subsequent charge annihi-

lation of the cage radical ions results in electronically excited-state generation.[17] One explanation for the observed 10% singlet yield is that the decarboxylation and charge annihilation are competitive with spin equilibration. Thus, the dioxetanones that react by the CIEEL path, but do not generate an excited singlet activator, may be generating the undetected triplet excited state of the hydrocarbon. The final step in the sequence is light emission from the excited activator, which we detect as chemiluminescence.

In competition with the CIEEL path, uncatalyzed unimolecular decomposition of the dioxetanone generates electronically excited acetone. The combination of these two excitation mechanisms accounts for all of the experimental observations on the chemiluminescence of dioxetanone **1**.

In summary, we have shown that an efficient CIEEL pathway is the major light generating process from dioxetanone **1** with any one of several easily oxidized activators. This is the third documented example of efficient chemiluminescence by this route.[4,18] We are continuing our investigation of the chemiluminescence of dioxetanones to further establish the details of the mechanism in this case. We are also investigating other chemiluminescent systems that appear to react by the CIEEL path.

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$$I_{chl} = k_2[\text{Act}][1]\phi_{\text{CIEEL}} + k_1[1]\phi_{ET}^{SS} \cdot \phi_S^S$$

where I_{chl} is the chemiluminescence intensity, ϕ_{CIEEL} is the efficiency of production of excited singlet activator by the induced decomposition, ϕ_{SS}^{ET} is the efficiency of energy transfer from acetone singlet to the activator, and ϕ_S^S is the efficiency of unimolecular acetone singlet generation. All solutions contained 20 μL of 5% aqueous Na_4EDTA to suppress metal catalyzed reactions.
(10) The yield of light was determined relative to tetramethyldioxetane (TMD) using 9-10-dibromoanthracene as the acceptor. The yield of acetone triplet was taken to be 30%,[11a] the triplet–singlet energy transfer efficiency 25%,[11] and the fluorescence quantum yield 10%.
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Molecular Recognition of DNA by Small Molecules. Synthesis of Bis(methidium)spermine, a DNA Polyintercalating Molecule

Sir:

The molecular recognition of DNA by small molecules is an important macromolecular receptor–drug interaction in the field of chemotherapy.[1] The formation of noncovalently bound nucleic acid–drug complexes produces profound pharmacological effects by interfering with biological processes in which nucleic acids participate.[1,2] The fact that DNA is a *defined macromolecular receptor* allows a rational approach to drug design and permits a unique opportunity for studying site-specific drug-binding processes. The therapeutic importance and the possibility for a detailed chemical understanding of the mechanism of action of these DNA–drug complexes provide sufficient stimulus to develop a rational methodology for optimizing the thermodynamics, kinetics, sequence specificity, structural specificity, and chemical specificity of these binding ligands.[3]

Some ligands that bind noncovalently to duplex DNA do so by a process called intercalation, the insertion of a flat molecule between the base pairs of a double helix.[4] Typical binding constants for these complexes are $K \sim 10^5$ M^{-1}.[5] We report here initial findings in our laboratories directed at increasing the binding affinity of drugs to nucleic acid by the synthesis of polyintercalating agents.[6] We describe the synthesis and study of a new molecule, bis(methidium)spermine (**1**, BMSp) and provide supporting evidence that (1) BMSp is a double intercalator, (2) has a binding site size of four base pairs, and (3) binds at least 10^4 times stronger to DNA than the simple monomer.

We chose to study the dimer of a well-characterized intercalating molecule the antitrypanosomal agent and nucleic acid probe, ethidium bromide **2** ($K \sim 10^5$ M^{-1}).[7] The synthetic strategy employed here preserves the major structural attributes of the ethidium monomer as an intercalator. The tetramine, spermine, was chosen to link the intercalators because of its known affinity for nucleic acid[8] and its length which allows a geometry sufficient to reach nonadjacent intercalation sites in accordance with the neighbor exclusion binding mode[9] (see Figure 1). For a bisintercalated species this polyamine connector should lie intimately in the groove of the DNA helix. Structural modifications of any linker with respect to charge, chirality, length, flexibility, and functionality are expected to play an important part in controlling the stability and nature of these polyintercalator–DNA complexes.

The synthetic sequence is outlined in Scheme I. Nitration of *o*-aminobiphenyl (**3**) (potassium nitrate/sulfuric acid),[10] condensation with *p*-cyanobenzoyl chloride, and cyclization (phosphorous oxychloride) yielded 6-(4-cyanophenyl)-3,8-dinitrophenanthridine. Successive methylation (dimethyl sulfate), hydrolysis, and reduction (reduced iron powder/HCl) afforded maroon crystals of 5-methyl-6-(4-carboxylphenyl)-3,8-diaminophenanthridinium chloride monochloride monohydrate (*p*-carboxylmethidium chloride, **4**) in an overall yield of 16%. The infrared and NMR spectra of compound **4** were

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