Chemiluminescence Involving Acidic and Ambident Ion Light Emitters. The Chemiluminescence of the 9-Acridinepercarboxylate Anion

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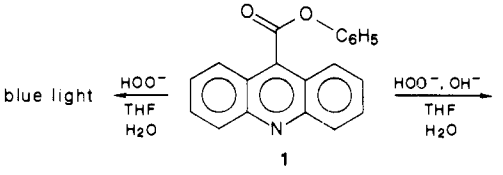
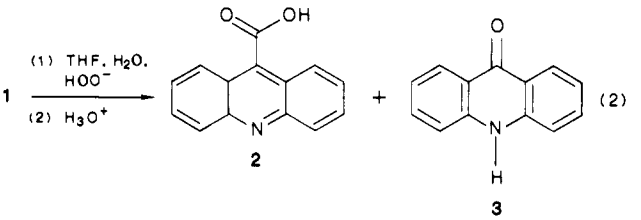
Abstract: The reaction of phenyl 9-acridinecarboxylate (**1**) with an excess of peroxide ion in THF/water (67/33 mol %) leads to the emission of either bright yellow-green light or bright blue light, depending on the reaction conditions. The blue emission is favored by high concentrations of hydrogen peroxide and water, for example. 9-Acridinepercarboxylic acid is a common intermediate in the reactions. The light emitter responsible for the blue chemiluminescence is acridone, whereas that responsible for the yellow-green chemiluminescence is the anion of acridone. The effects of base concentration and solvent composition on the relative proportions of these two emitters have produced evidence that, contrary to the expectation of simple theory, a dioxetanone is not an intermediate in the reaction. Other cases where chemiluminescence may involve percarboxylate and peroxide ions are discussed.

Chemi- and bioluminescent reactions that efficiently produce singlet states are generally considered to require dioxetane or dioxetanone intermediates as keys to the excitation process.[1,2] These reactions generate light emitters that are acids or ambident ions; most, however, are "silent" in the sense that only one of the potential light emitters is actually observed. The present work covers the chemiluminescent reaction of phenyl 9-acridinecarboxylate (**1**) with peroxide ion, a reaction in which light emission occurs from both acridone and its conjugate base. The reaction was discovered in unraveling the chemiluminescence that occurs when 9-acridinecarboxylic acid hydrazide is exposed to ambient light and then treated with water.[3] In the chemiluminescence of the phenyl ester, the effects of base concentration and solvent composition on the relative proportions of the acridone and acridone anion emitters have produced evidence that, contrary to the expectation of simple theory, a dioxetanone is not an intermediate in the reaction.

The Reaction. The reaction of phenyl 9-acridinecarboxylate (**1**) with an excess of peroxide ion in tetrahydrofuran (THF)/water (67/33 mol %) leads to the emission of either bright yellow-green light or bright blue light, depending on the reaction conditions (eq 1). The blue emission, e.g., is favored by high concentrations



of hydrogen peroxide and water. For the yellow-green emission, a rapid initial rise in intensity is observed followed by a pseudo-first-order decay (typical values for 0.1 mM **1**, 2.9 mM H_2O_2, and 7.4 mM OH^- are a rise time to maximum intensity of about 1 min and a decay rate constant of 3.8×10^{-3} s^{-1} at 27 °C). The products of the reaction are 9-acridinecarboxylic acid (**2**) and acridone (**3**) (eq 2). These compounds were isolated from the reaction product mixture and characterized; in 33 mol % H_2O, the yields are 41% **2** and 57% **3**. 9-Acridinecarboxylic acid under our reaction conditions is nonfluorescent and its formation, presumably via a simple hydrolysis reaction, is not relevant to the chemiluminescence. Chlorocarbonylacridine[4] is also chemiluminescent on reaction with hydroperoxide ion, and it also yields both blue and yellow-green light, but the quantum yield is lower than that for phenyl acridinecarboxylate, possibly because of the occurrence of side reactions such as an attack of the hydroperoxide ion directly on the chlorine atom.



Wavelengths and Light Emitters. The reaction of phenyl 9-acridinecarboxylate (**1**) with peroxide ion in aqueous tetrahydrofuran at pHs $> \sim 11$ yields yellow-green light, the spectrum of which shows vibrational fine structure (456, 481, 515 nm, Figure 1). The fluorescence of the product mixture, on the other hand, is blue in color under most conditions (up to pH ~ 12). The chemiluminescence of the phenyl ester at lower base concentrations or in the presence of excess hydrogen peroxide or water occurs in the blue region of the spectrum, again with vibrational fine structure (410, 430, 450 nm, Figure 1); under these conditions, the fluorescence is also blue and the chemiluminescence and fluorescence wavelength distributions are identical.

As outlined in the following section, the fluorescence of the anion of acridone is yellow-green in color; the fluorescence spectrum matches exactly the yellow-green chemiluminescence spectrum (Figure 1), and thus, acridone anion is the light emitter in the yellow-green chemiluminescence (eq 3). The blue chemiluminescence spectrum matches exactly the fluorescence spectrum of neutral acridone (Figure 1), and thus, acridone is the light emitter in the blue chemiluminescence (eq 3).

$$\text{Blue light} \leftarrow \mathbf{4} \xleftarrow{HOO^-} \mathbf{1} \xrightarrow[HO^-]{HOO^-} \mathbf{5} \rightarrow \text{yellow-green light} \quad (3)$$

(1) Reviews: White, E. H.; Roswell, D. F. *Acc. Chem. Res.* **1970**, *3*, 541. McCapra, F. *Prog. Org. Chem.* **1973**, *8*, 231. Gundermann, K. D. *Top. Curr. Chem.* **1974**, *46*, 61. Wilson, T. *Int. Rev. Sci.; Phys. Chem. Ser. Two.* **1976**, *9*, 265. Adam, W. *Adv. Heterocycl. Chem.* **1977**, *21*, 437. Schuster, G. B.; Schmidt, S. P. *Adv. Phys. Org. Chem.* **1982**, 187-238.

(2) (a) White, E. H.; Harding, M. J. C. *Photochem. Photobiol.* **1965**, *4*, 1129. (b) White, E. H.; Hopkins, T. A.; Seliger, H. H.; Rapaport, E. *J. Am. Chem. Soc.* **1969**, *91*, 2178. (c) Rapaport, E.; Cass, M. W.; White, E. H. *J. Am. Chem. Soc.* **1972**, *94*, 3153. (d) Rapaport, E.; Cass, M. W.; White, E. H. *J. Am. Chem. Soc.* **1972**, *94*, 3160. (e) Cass, M. W.; Rapaport, E.; White, E. H. *J. Am. Chem. Soc.* **1972**, *94*, 3168. (f) Wildes, P. D.; White, E. H. *J. Am. Chem. Soc.* **1973**, *95*, 2610. (g) White, E. H.; Miano, J. D.; Umbreit, M. *J. Am. Chem. Soc.* **1975**, *97*, 198. (h) White, E. H.; Steinmetz, M. G.; Miano, J. D.; Wildes, P. D.; Morland, R. *J. Am. Chem. Soc.* **1980**, *102*, 3199-3208.

(3) Taylor, S. P. B., The Johns Hopkins University, unpublished work.

(4) A blue chemiluminescence has been reported for the reaction of 9-chlorocarbonylacridine with hydrogen peroxide (Rauhut, M. M.; Sheehan, D.; Clarke, R. A.; Roberts, B. G.; Semsel, A. M. *J. Org. Chem.* **1965**, *30*, 3587-3592).

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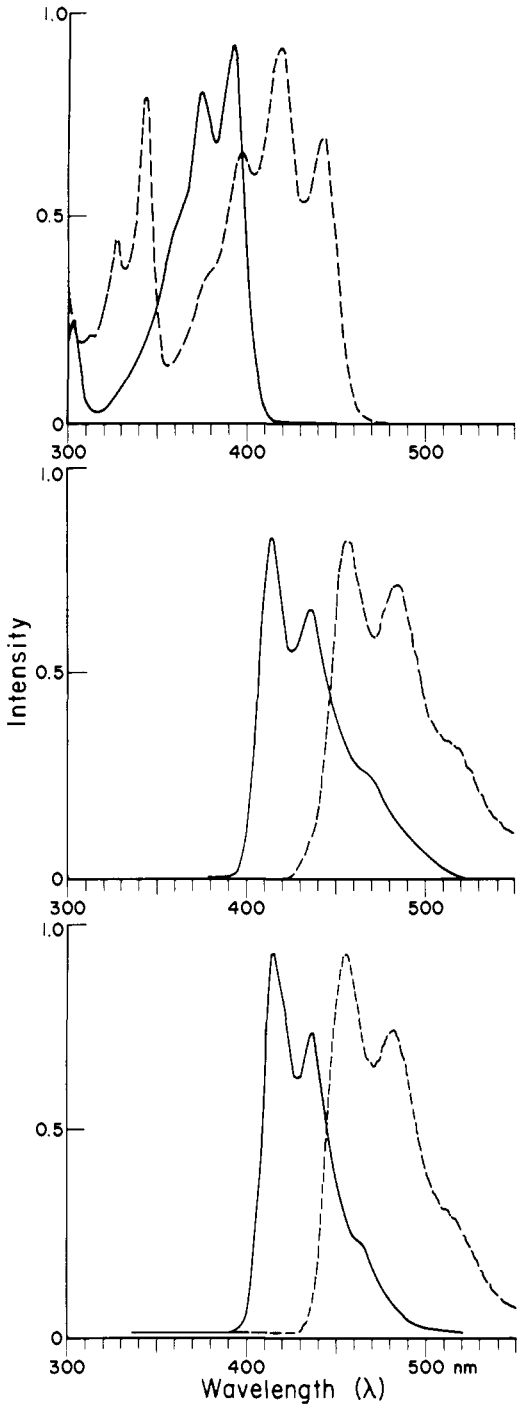
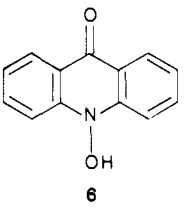


Figure 1. Spectra of acridine compounds (conditions listed in Figure 2). Upper set (each set was normalized): (—) absorption spectrum of acridone; (--) absorption spectrum of the anion of acridone. Middle set: (—) fluorescence emission spectrum of acridone (λ_{excit} 376 nm); (--) fluorescence emission spectrum of acridone anion (4×10^{-5} M, λ_{excit} 442 nm). Bottom set: (—) spectrum of the blue chemiluminescence (pH <11); (--) spectrum of the yellow-green chemiluminescence (pH >11).

N-Hydroxyacridone (**6**)[5] was also examined as a possible light emitter, but the fluorescence wavelengths proved to be appreciably longer than those of acridone (experimental section) and thus this compound is of no concern with respect to the chemiluminescence.

Acridone. Acridone (**3**) is a weak acid with a pK_A in water of 12.8 for the ground state and 9.9 for the first excited singlet state.[6] Acridone absorbs ultraviolet light with maxima at 292, 306, 376, and 394 nm; its anion (**7**) absorbs at 325, 344, 396, 420, and 442



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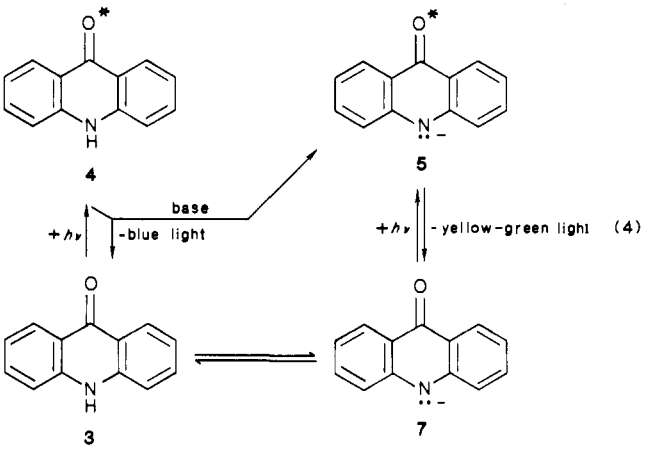
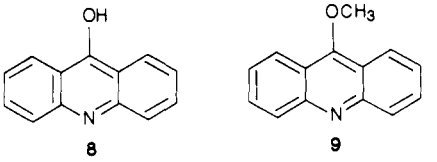
nm (Figure 1). The excitation spectra, as followed by the fluorescence emissions, are essentially the same as the absorption spectra. Irradiation of a mixture of acridone and its anion at 442 nm should excite solely the anion, whereas neutral acridone, largely, should be excited at 376 nm (eq 4).



In solutions of acridone in THF/H_2O (67/33 mol %) at [OH^-] and [H_2O_2] ~ 10^{-2} M, exciting specifically the anion present in the mixture at 442 nm leads to solely yellow-green fluorescence (Figure 2). Protonation to yield excited neutral acridone does not occur. Acridone anion is an ambident ion and protonation could occur on either nitrogen or oxygen. At least for ground-state molecules, the more electronegative atom of the free ion is the preferred site of protonation.[7] 9-Hydroxyacridine would be a stronger acid than acridone, however, and in the basic systems used, it would not accumulate to any degree. We have prepared 9-methoxyacridine (9) as a model of compound **8** and find it to be essentially nonfluorescent. It is of interest that in this series the only fluorescent structures are acridone and its anion, the spectra of which are different in wavelength but essentially identical in shape.



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When neutral acridone is specifically excited (376 or 398 nm) in a mixture with its anion, the blue fluorescence emission of acridone is observed accompanied by small amounts of the yellow-green fluorescence emission of the anion at low base concentrations and larger amounts in more basic media (Figure 2); thus, under the conditions used, partial ionization of excited acridone occurs. These observations show that the excited singlet states of acridone and its anion do not achieve equilibrium during their radiative lifetimes in our systems.

The major conclusion of this section is that under the conditions used for our experiments, excited acridone and its excited anion can be distinguished and also identified by means of their emission spectra.

Reaction Intermediates. The formation of 9-acridineper-carboxylic acid (**10**) as a crucial intermediate in the chemiluminescent reaction of the phenyl ester is reasonable in view of the

(5) Acheson, R. M.; Adcock, B.; Glover, G. M.; Sutton, L. E. *J. Chem. Soc.* **1960**, 3367.
(6) Schulman, S. G.; Sturgeon, R. J. *Anal. Chim. Acta* **1977**, *93*, 239–247.
(7) LeNoble, W. J. *Synthesis* **1970**, *2*, 1.

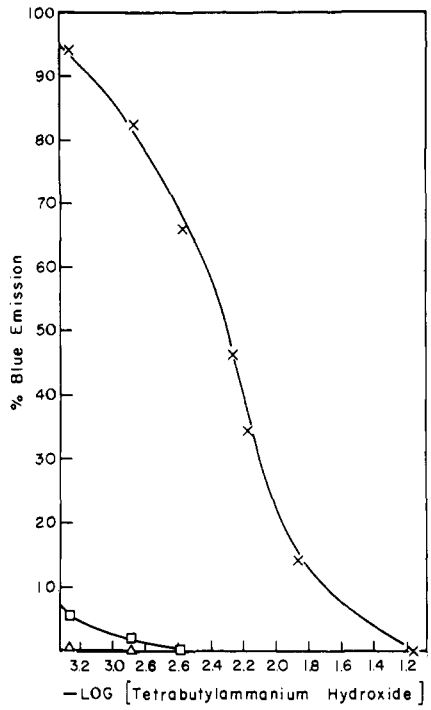
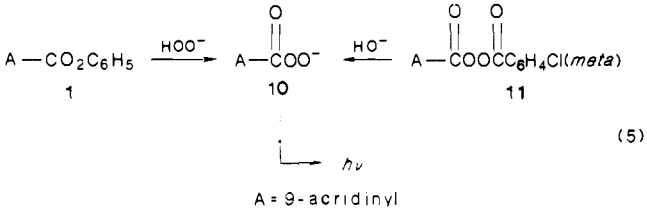


Figure 2. Amount of blue light obtained in the chemiluminescence of phenyl 9-acridinecarboxylate (**1**) and in related fluorescent emissions (in 33 mol % H_2O/67 mol % THF containing 0.03 M H_2O_2, 10^{-4} M substrate, and variable TBAH): (□) chemiluminescence; (×) fluorescence of acridone (λ_{excit} 398 nm); and (△) fluorescence of acridone anion (λ_{excit} 442 nm).

reagents used[8] and the structure of the products formed[1,2] (eq 2). Consistent with this view is the fact that the same chemiluminescence can be obtained from the reaction of base alone with 9-acridinoyl 3-chlorobenzoyl peroxide (**11**) (eq 5).



(5)

The time required to reach maximum intensity in the chemiluminescence of the phenyl ester is only slightly shorter than the half-life of the light decay; that is, the peracid anion (**10**) is formed at a rate similar to the rate of its destruction. We have been unable to isolate the peracid (conjugate acid of **10**) in a pure form, although it can be prepared in situ in several ways. Impure acridinepercarboxylic acid can be prepared as a glowing solid by treating the phenyl ester in THF with tetrabutylammonium hydroxide (TBAH) and an excess of 30% hydrogen peroxide (eq. 5) and, after a few seconds delay, pouring the solution over a mixture of granular dry ice and water.

Reaction Characteristics. Both the blue and yellow-green chemiluminescence emissions are about 1.5% efficient, a value coincidently similar to that observed for luminol[9] and for a chemiluminescent compound based on acridine.[10] The quantum yields and reaction rates were slightly depressed by oxygen and by sodium chloride. The presence of sodium chloride had no effect on the absorption spectrum of acridone anion, a small quenching effect on the fluorescence emissions, and no effect on the light distribution in a chemiluminescent system where both yellow-green and blue light were being emitted. The latter result is in sharp contrast to the effect of sodium ions on the emissive properties of 3-aminophthalate ion; the amount of ion pairing with sodium was found in that case to be different for the excited aminophthalate obtained by the absorption of light relative to that obtained via chemiluminescence.[11]

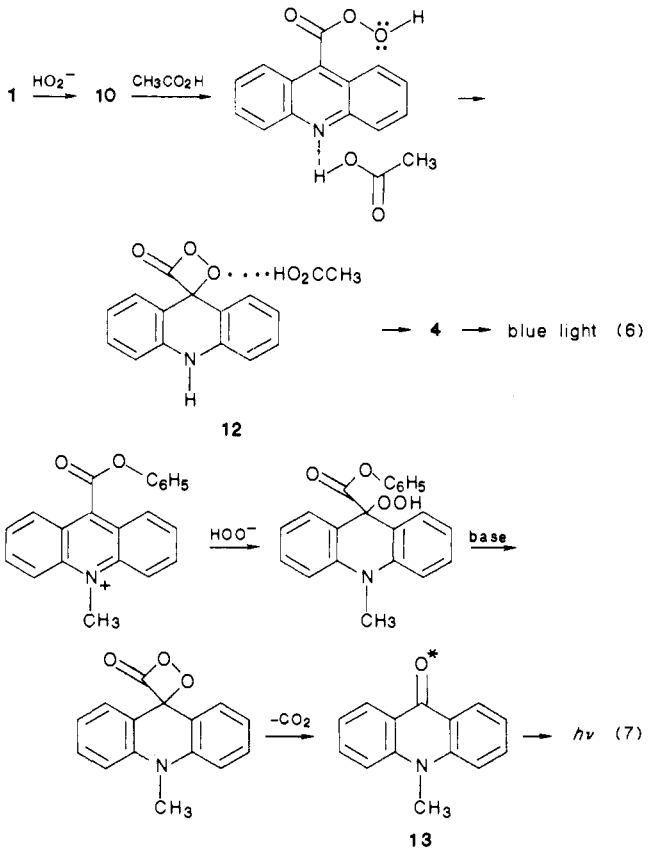
Yellow-Green Chemiluminescence. Over a considerable range of conditions (mol % H_2O <40; [OH] > 2.5×10^{-3} M), the chemiluminescence of the phenyl ester (**1**) is solely in the yellow-green region of the spectrum (Figures 1 and 2). The fluorescence emission of acridone anion is precisely the same (Figures 1 and 2). The fluorescence of neutral acridone measured under the same conditions is different; as shown in Figure 2 it is predominantly blue in color. Thus, the chemiluminescent reaction over the given range of concentrations in our protic media generates acridone anion (**5**) in the excited state, not excited neutral acridone (**4**). Two moles of base are consumed in the chemiluminescent reaction, and at low base concentrations a small amount of blue emission is seen (Figure 2).

In other cases of chemiluminescence where a neutral molecule is produced in the excited state or where the medium used does not allow proton transfer to occur, fluorescence of the reaction product mixture—over a range of excitation wavelengths—generally matches the chemiluminescence spectrum (except, of course, where subsequent chemical alteration of the product occurs).[1,2] In the present case (and presumably in other cases where the excited states are acids or bases) the correspondence does not necessarily hold. Although the chemiluminescence stems from the yellow-green emission of acridone anion, the resulting product mixture over a range of excitation wavelengths shows only the blue fluorescence of neutral acridone. This result follows from the difference of pK_A's for the ground and excited states of acridone and the nature of the chemical reaction leading to excitation. If base concentrations over $\sim 10^{-2}$ M and/or excitation wave lengths longer than 440 nm are used, however, the fluorescence emission of the product mixture shifts to the yellow-green range, matching the chemiluminescence emission.

Blue Chemiluminescence. Phenyl 9-acridinecarboxylate (**1**) and 9-chlorocarbonylacridine[4] produce a weak blue chemiluminescence on treatment with hydrogen peroxide. A much enhanced blue emission occurs if a buffered peroxide solution is used, or if reaction mixtures at a high pH are subsequently acidified. For example, if lower base concentrations are used ($<10^{-3}$ M; the reaction rate and chemiluminescence intensity decrease appreciably under these conditions) or if a water-rich medium is used (65 mol % H_2O) some blue chemiluminescence stemming from excited neutral acridone accompanies the yellow-green emission. At a pH of ~11 in 44 mol % H_2O–56 mol % THF (7 mM OH^-, 6 mM H_2O_2), equal amounts of blue and yellow-green light are observed.

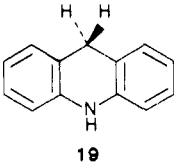
Exclusive blue emission is observed if high concentrations of hydrogen peroxide are used (>0.4 M). A particularly bright, blue chemiluminescence is observed if a solution of the phenyl ester and hydrogen peroxide is treated first with base and then with an excess of acetic acid. Under the latter conditions, the steady state amount of acridine-9-percarboxylate ion formed in the basic phase of the sequence is converted into acridinepercarboxylic acid in the acidic phase; subsequently, the step producing excited states may involve electrophilic catalysis of dioxetane formation and decomposition[12a] (eq 6). The process is related, in a sense, to that involved in the chemiluminescence of various *N*-alkylacridinium compounds (eq 7, e.g.).[12b]

(8) Brooks, B. T.; Brooks, W. B. *J. Am. Chem. Soc.* **1933**, *55*, 4310.
(9) Lee, J.; Seliger, H. H. *Photochem. Photobiol.* **1965**, *4*, 1015; **1972**, *15*, 227.
(10) The chemiluminescent reaction of 9-(chlorocarbonyl)-10-methylacridinium chloride with hydrogen peroxide has been reported to have a quantum yield of 1.1×10^{-2} (ref 4).
(11) White, E. H.; Roswell, D. F.; Wei, D. C.; Wildes, P. D. *J. Am. Chem. Soc.* **1972**, *94*, 6223. Wildes, P. D.; White, E. H. *J. Am. Chem. Soc.* **1973**, *95*, 2610.
(12) (a) Zaklika, K. A.; Burns, P. A.; Schaap, A. P. *J. Am. Chem. Soc.* **1978**, *100*, 318–320. Zaklika, K. A.; Kissel, T.; Thayer, A. L.; Burns, P. A.; Schaap, A. P. *Photochem. Photobiol.* **1979**, *30*, 35–44. (b) McCapra, F.; Richardson, D. G.; Chang, Y. C. *Photochem. Photobiol.* **1965**, *4*, 1111–1121.



The Absence of Dioxetanone Intermediates in the Yellow-Green Chemiluminescence. A major finding of the present study is that the excited state formed in the chemiluminescence of acridinepercarboxylate ion in protic media at millimolar base concentrations is that of the anion of acridone. The formation, exclusively, of excited acridone anion in protic media must find its explanation in the structure of reaction intermediates, in the structure of the transition state leading to the excited state, or in the acid–base equilibria of excited acridone and its anion. The process of interest is the step separating 9-acridinepercarboxylate ion from excited acridone anion, and a crucial aspect of this process is the extent of protonation of the nitrogen atom that occurs in protic media during the course of the reaction.

Scheme I outlines the extent of C–O bond formation in the ring closure of the percarboxylate ion and the attendant protonation of the nitrogen atom. Since the molecular ratio of water to ester **1** is $\sim 10^4$ (in the 33 mol % water–67 mol % THF medium) and since the ratio of the basicities of the acridine ring system[13] to THF[14] = $\sim 10^4$, ester **1**, percarboxylate **10**, and related species of equal or greater basicity would possess nitrogen atoms hydrogen bonded to hydrogen peroxide (e.g., **14**) or to water (in runs with $H_2O_2/OH^- < 1$). *N*-Protonated intermediates and excited acridone itself cannot be the precursors of excited acridone anion in the aqueous media used; excited acridone generated by light absorption in the fluorescence experiments (under the same reaction conditions as used in the chemiluminescence) was found to emit largely in the blue region of the spectrum as neutral acridone (Figure 2). Intermediate **17**, the N-protonated dioxetanone, would be relevant to the subject of this section if it were the precursor of dioxetanone anion **18**, a potential source of excited acridone anion **5**. However, **17** would be a very weak acid, the acidity of which would be determined largely by the diphenylamine moiety present in the molecule. The pK_A of diphenylamine is 24.95[15] and the strongest base present under the usual circumstances, where $H_2O_2/HO^- > 1$ (Figure 2), would be peroxide ion ($pK_A(H_2O_2 = 11.6)$;[16] considering the relative acidities ($\Delta pK_A = 12–13$), appreciable N–H ionization would not be expected to occur. Note that in the fluorescence measurements not even excited singlet acridone ($pK_A = 9.9$) ionizes appreciably in the reaction media used. The pK_A of acridane (**19**) would be more relevant, but it is unknown; however, it is estimated to be 24,[15] about one unit lower than that for diphenylamine, but high enough to suggest that the above conclusion should remain valid.



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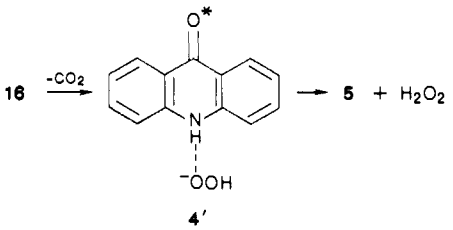
Proposing that dioxetanone anion **18** is the precursor of excited acridone anion (**5**) would be the most direct way to account for the observed yellow-green chemiluminescence. However, the addition of a weak base (the CO_3^- group of **10**, e.g.; $pK_A(ArylCO_3H) = \sim 8$)[17] to the acceptor system to form an intermediate with a strongly basic center ($pK_A \sim 24$) and a strained ring—in the absence of N-protonation—would be highly improbable on the basis of reaction characteristics of the Michael addition reaction.[18] The lifetime of a hypothetical dioxetanone anion such as **18** would be the time required to convert the hydrogen bond into a full N–H bond—essentially the time of one vibration. An electron transfer from nitrogen to the dioxetanone ring (next section) could, in principle, exceed in rate the proton transfer; but if so, the dioxetanone anion would again be bypassed as an intermediate in the usual sense of the word.

If full ring closure occurred to give a dioxetanone, **16**, e.g., the nitrogen atom would be hydrogen bonded initially to a hydroperoxide ion (or hydroxide ion). It is unlikely, however, that his hydroperoxide ion (or hydroxide ion) would maintain its special relationship to the nitrogen atom during the lifetime of intermediate **16** and the formation of **4′** (and thus of **5**) (eq 8). In view of the excess of hydrogen peroxide and/or water molecules in the medium, it is more reasonable that the hydroperoxide ion



(and HO^-) would be transferred to the various protic compounds in the medium (such reactions are diffusion controlled)[19] and thereby become indistinguishable from the excess of hydroperoxide and hydroxide ions normally present and which are also present, without a sizable effect, during the fluorescence measurements on neutral acridone (Figure 2).

A weak base could deprotonate a neutral dioxetanone (e.g., **17**), but only by a concerted E_2-type reaction, and this process would merely return the dioxetanone to **15**, the transition state for the ring closure reaction (Scheme I), or to **14**.

It thus appears that dioxetanone anion **18** is not formed as a reaction intermediate from percarboxylate ion **14** in the yellow-green chemiluminescence, and it is not formed by ionization of

(13) The pK_A of a protonated 9-acylacridine can be estimated to be ~ 2 based on the pK_A of acridine (5.6) and the effect of 4-cyano and acyl groups on the basicity of pyridine (Albert, A. *Heterocyclic Chemistry*, 2nd ed.; Oxford University Press: New York, 1968; Chapter 13).

(14) Arnett, E. M.; Wu, C. Y. *J. Am. Chem. Soc.* **1962**, *84*, 1684–1688. These authors report $pK_a = -2.08$. See also: Bonvicini, P.; Levi, A.; Lucchini, V.; Modena, G.; Scorrano, G. *J. Am. Chem. Soc.* **1973**, *95*, 5960–5964.

(15) Personal communication from Professor F. G. Bordwell, Northwestern University.

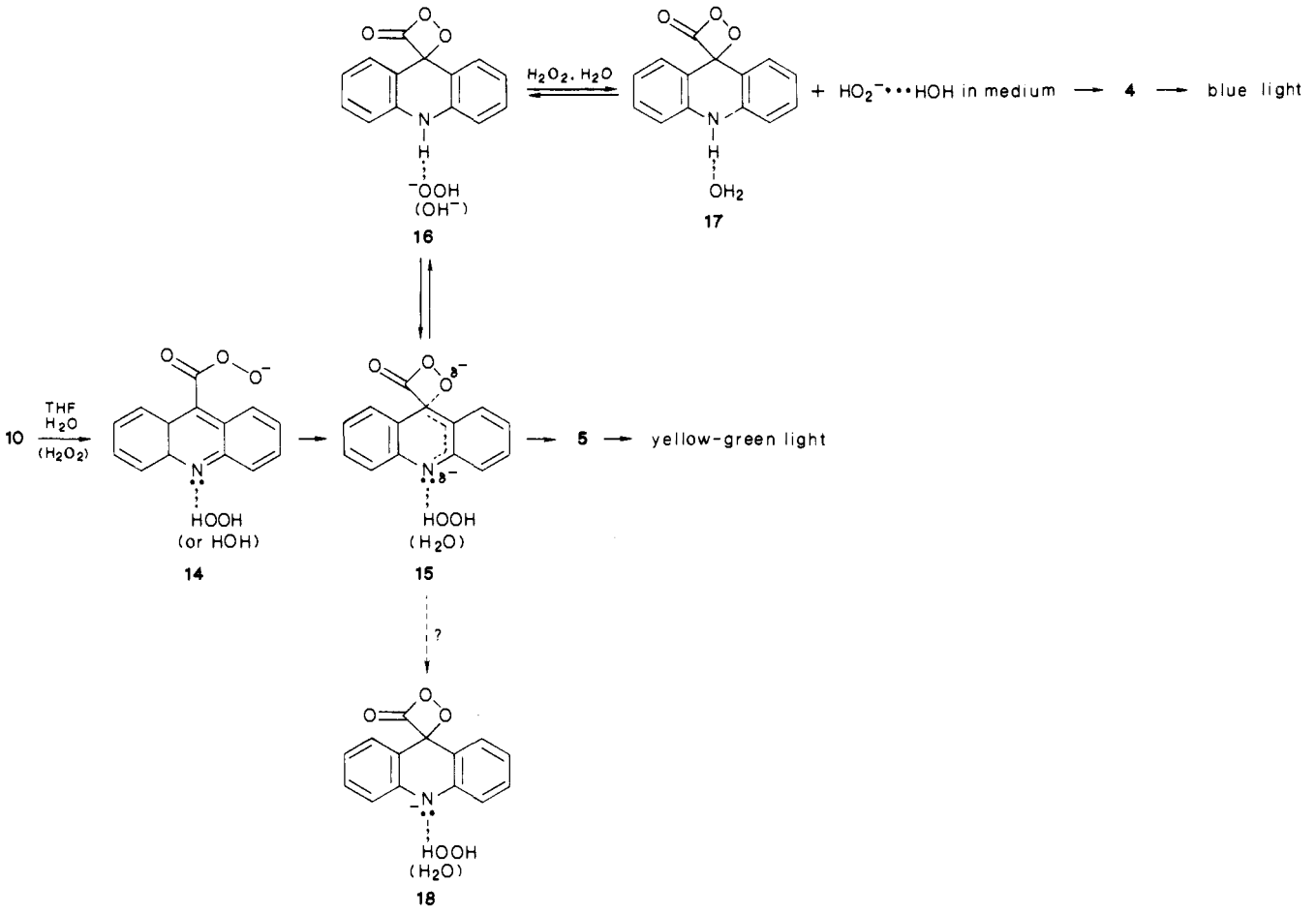
(16) Joyner, R. A. *Z. Anorg. Allg. Chem.* **1912**, *77*, 103.

(17) Swern, D. In *Organic Peroxides*; Swern, D., Ed.; Wiley-Interscience: New York, 1970; Vol. I, p 424.

(18) House, H. O. *Modern Synthetic Reactions*, 2nd ed.; W. A. Benjamin, Inc.: Menlo Park, CA, 1972; Chapter 9. Bergman, E. D.; Ginsburg, D.; Pappo, R. In *Organic Reactions*; R. Adams, Ed.; John Wiley and Sons, Inc.: New York, 1959; Vol. 10, Chapter 3.

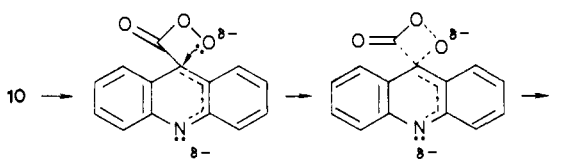
(19) Eigen, M. *Pure Appl. Chem.* **1963**, *6*, 97–115.

Scheme I



an N-protonated dioxetanone (**19** or **17**, e.g.). Yet the yellow-green chemiluminescence stems from acridone anion! The chemiluminescence has an apparent pK_A of ~11 with respect to the color of light emitted and not ~24 as would be expected if dioxetanone **16**, e.g., were an intermediate. The system behaves as if only a small negative charge develops on nitrogen during the course of the reaction.

Reaction Mechanisms. (A) Ionic Pathways. Estimates of the energy available from the conversion of acridine percarboxylate ion (**10**) into excited acridone anion (**5**) from bond energy values[20] indicate that the energy of C–O bond formation is needed to produce a species emitting at 456 nm; a minimum of ~63 kcal is required (chemical energy plus E_{act}). Yet full addition of the peroxide oxygen to the acridine ring to form dioxetanone anion **18** as an intermediate does not occur (previous section). The observation that only a small negative charge accumulates on the nitrogen atom would mean either that the transition state for acridone anion formation (from **14**) comes exceedingly early in the reaction (unlikely for the endergonic formation of excited states)[21] or that C–C and O–O bond breaking accompany the formation of the C–O bond (as in eq 9). The reaction, most probably, begins with a Michael-type addition of the peroxidic

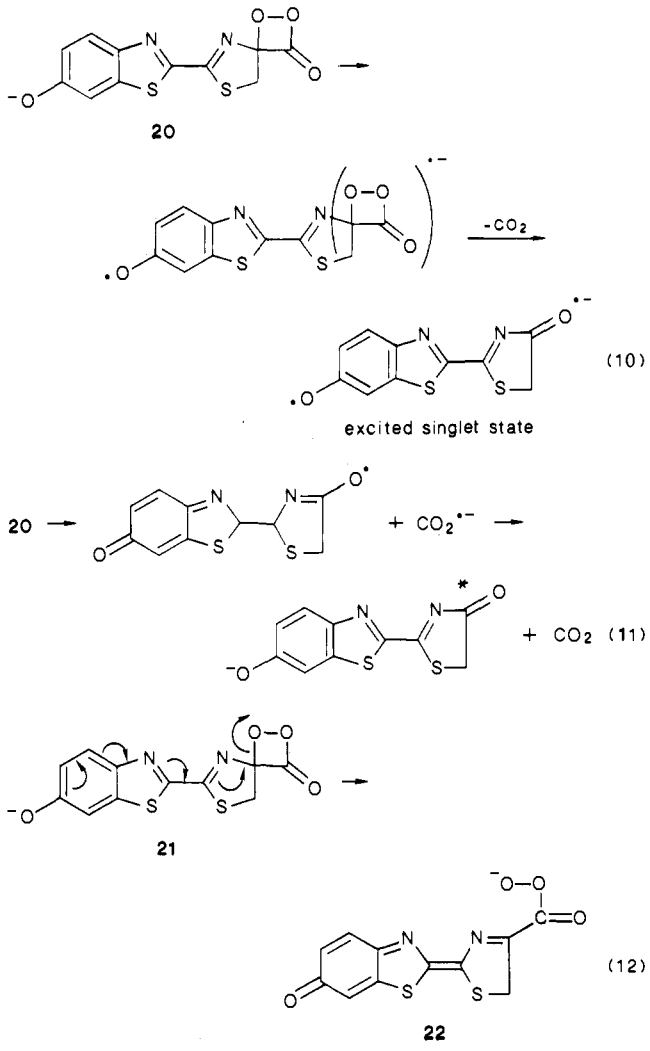


CO_2 + **5** (9)

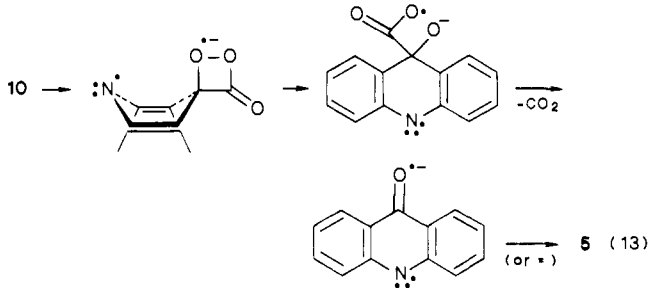
oxygen atom to the LUMO of the aromatic acceptor system.[22] If the normal Michael addition were to proceed to completion, the product would be dioxetanone **17**, which has been eliminated from consideration as an intermediate for the yellow-green light emission. Since the formation of dioxetanone anion **18** would involve the energy costly processes of forming a strained ring and converting a weak base into a strong one, we propose that in the early stages of the Michael addition, as the negative charge is fed into the LUMO of the aromatic system, a linked breaking of the C–CO and O–O bonds occurs. In this view, a transition state, but not an intermediate in the usual sense of the word, separates the starting percarboxylate ion (**14**) from the excited acridone anion **5**.

(B) Electron-Transfer Mechanisms. The requirement for a low charge on nitrogen can, in principle, be met by an intramolecular electron-transfer process. An intermolecular electron-transfer mechanism for the chemiluminescence attending fluorescer-induced decomposition of alkyl dioxetanones has been developed independently by two research groups.[23] An intramolecular version was subsequently advanced to explain the efficient generation of singlet excited states from putative dioxetanes and dioxetanones derived from highly conjugated molecules bearing electron-releasing groups, in particular —O^- and =N^-.[24,25] Two versions of the intramolecular excitation have been advanced with respect to firefly luciferin (eq 10[24] and 11[25]). It is not clear how these processes compete with those normally predictable in terms of resonance and electron delocalization[26] (eq 12). Nevertheless,

(20) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 2nd ed.; Harper and Row: New York, 1981; pp 146–147.
(21) Hammond, G. *J. Am. Chem. Soc.* **1955**, *77*, 334. LeNoble, W. J.; Miller, A. R.; Hamann, S. D. *J. Org. Chem.* **1977**, *42*, 338.
(22) Minato, T.; Fujimoto, H.; Fukui, K. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1621–1626.
(23) Schmidt, S. P.; Schuster, G. B. *J. Am. Chem. Soc.* **1978**, *100*, 1966. Adam, W.; Cueto, O.; Yany, F. *J. Am. Chem. Soc.* **1978**, *100*, 2587.
(24) Koo, J.-Y.; Schmidt, S. P.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 31.
(25) McCapra, F. *J. Chem. Soc., Chem. Commun.* **1977**, 946–948.
(26) See, however, ref 31 cited in ref 2h.

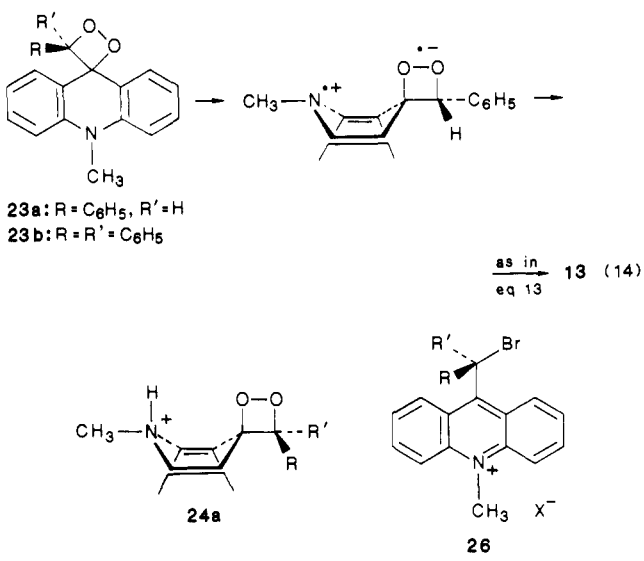


if the concept were to be applied to the chemiluminescence of the acridinepercarboxylate ion, one could propose that the negative charge developing on nitrogen—as a result of forming the new carbon–oxygen bond at C-9—is back-donated to the LUMO σ^* O–O of the developing dioxetane (eq 13). This mechanism is based on an electron-transfer mechanism advanced by Lee and

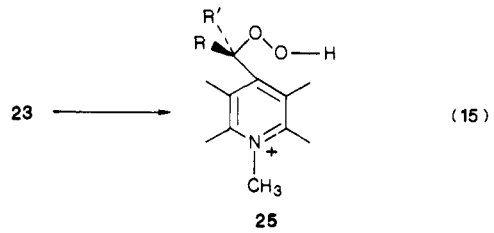


Singer[27] for acridine analogues with a preformed dioxetane ring (**23a,b**) (eq 14). The major experimental fact advanced in support of this mechanism was the observation that the chemiluminescence of **23a** could be quenched by acetic acid. The interpretation offered for this observation was that protonation of the nitrogen atom by the acetic acid (as in **24a**) would block the electron transfer. This explanation is not tenable since we have found that the chemiluminescence of analogue **23b** is *enhanced* by acetic acid (it is quenched by stronger acids such as formic and trifluoroacetic). It is unlikely that the energy of the LUMO σ^* O–O of the dioxetane would be changed much by the substitution of a phenyl group for the remaining hydrogen at the sp^3 carbon center.

(27) Lee, C.; Singer, L. A. *J. Am. Chem. Soc.* **1980**, *102*, 3823–3829.



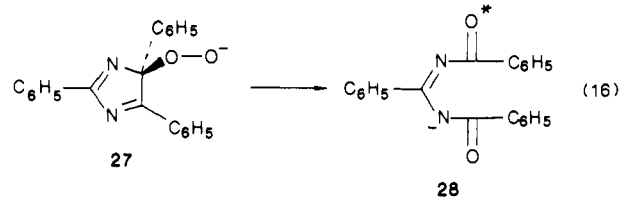
The strong acid quenching, which is partially reversible upon addition of base, probably stems from hydroperoxide formation (**25**, eq 15), where stronger acid catalysis is required for the more



highly substituted rings (Thorpe–Ingold Effect).[28] In this context it should be noted that treatment of **26** with basic hydrogen peroxide leads to strong light emission from *N*-methylacridone.[29] The weak acid effect noted above presumably stems from general acid catalysis facilitating the breaking of the O–C and/or the O–O bond of the dioxetane.[12a]

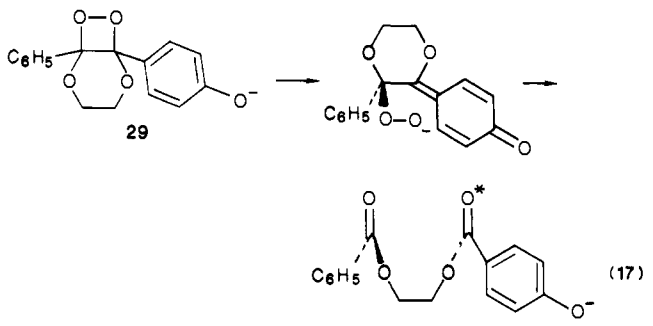
Intramolecular electron transfer to dioxetane rings has been invoked for a number of chemiluminescent reactions[30] in addition to those cited above; this mechanism may indeed prove to be the correct one, but definitive proof of its validity is needed.

Peroxide Reaction Intermediates. The chemiluminescence of lophine (and related imidazoles)[2a] (eq 16) appears to be closely related to that of acridinepercarboxylate ion (again, emission

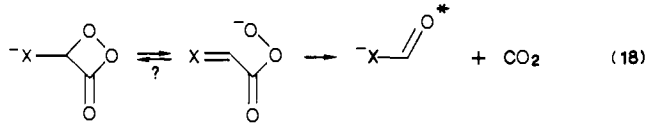


occurs from an anion). The efficient chemiluminescence of compound **29** reported by Schaap[30a] would also fit in this class if partial or complete ring opening were involved in the excitation step (eq 17). A possible fourth type involves the chemiluminescence of compound **23** (via **25**)[29] and the chemiluminescence of compound **26**[29] on reaction with hydroperoxide ion. Other examples may involve the chemi- and bioluminescence of firefly

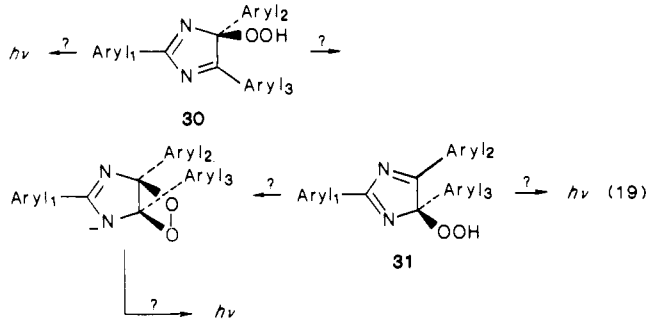
(28) Bruice, T. C.; Pandit, U. K. *J. Am. Chem. Soc.* **1960**, *82*, 5861.
(29) Unpublished work of H. W. Pinkert (J.H.U.).
(30) (a) Schaap, A. P.; Gagnon, S. D. *J. Am. Chem. Soc.* **1982**, *104*, 3504. (b) Reference 12a. (c) Handley, R. S.; Stern, A. J.; Schaap, A. P. *Tetrahedron Lett.* **1985**, *26*, 3183–3186. (d) Nakamura, H.; Goto, T. *Photochem. Photobiol.* **1979**, *30*, 27–33. (e) Wada, N.; Honda, M.; Suzuki, H. *J. Phys. Chem. Jpn.* **1985**, *54*, 4851–4860. In the context of electron-transfer chemiluminescence see also: Richardson, W. H.; Thomson, S. A. *J. Org. Chem.* **1982**, *47*, 4515–4520.



luciferin[2b,h] (via **22**) and cypridina luciferin.[31] In each case, a reaction center can be identified involving a percarboxylate or peroxide ion and a π-acceptor portion of a molecule which eventually can generate fluorescent products (eq 18). It has generally been assumed that the excitation pathway requires the dioxetane (or dioxetanone) structure of eq 18, and this appears to be correct for simple alkyl- and aryl-substituted dioxetanes, but the observations of anion emission in the chemiluminescence of acridinepercarboxylate and the imidazoles, in particular, suggest that the acyclic forms (or their transition states for excited state formation; as in eq 9, e.g.) may be the key species in cases where highly conjugated systems can be formed.

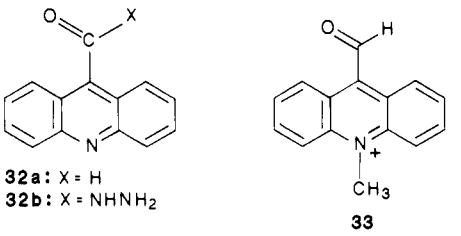


Isomeric imidazole hydroperoxides (intermediates in the "Lophine"-type chemiluminescence; eq 16) may lead to a resolution of the issue as to whether or not "open forms" of dioxetanes and dioxetanones (eq 18) can lead to excited states without passing through cyclic *intermediates*. If a dioxetane were an obligatory intermediate, the singlet/triplet ratios and probably the quantum yields for light production from isomers **30** and **31** would be the same (eq 19); if they proved to be different, reaction mechanisms involving allyl or acryloyl hydroperoxides (eq 18) would need to be considered.



In conclusion, both ionic (eq 9) and electron-transfer mechanisms (eq 13)[32] can be invoked for the conversion of acridinepercarboxylate ion into excited acridone anion; neither mechanism utilizes a dioxetanone as a discrete intermediate.

Related Systems. The same spectral distribution of light in the yellow-green region of the spectrum that was observed in the chemiluminescence of acridinepercarboxylate ion (**10**) also occurs in the chemiluminescent reactions of compounds **32a,b** and **33**[2c,d] with oxygen and a strong base. Intermediate **10** is presumed to



be the critical reaction intermediate in each case. The light emitter in each case is the singlet excited state of acridone anion and the transition state for production of the excited state is presumably the same in all cases. The chemiluminescence of **33** presumably involves first a demethylation reaction by the base to produce 9-acridinecarboxaldehyde (**32a**). In our earlier work we had attributed the light emissions to the corresponding carboxylic acid anions of **32a** and **33**.[2c,d] We have found, however, that the pure acid anions are essentially nonfluorescent. They are readily photooxidized in air and ambient light to produce acridone, however, and the presence of this compound in our samples of the acids led to the misassignments.

Experimental Section

Instrumentation. Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratory (Knoxville, TN). Proton magnetic resonance spectra were measured on a JEOL MH-100 or a Varian CFT-20 instrument, and values are reported relative to tetramethylsilane (Me_4Si). Photometric determinations were made by measuring the output of EMI 9558B or 1P21 photomultiplier-photometers exposed to the reacting solution. The values obtained were corrected for phototube spectral response. Quantum yields were measured relative to luminol.[9] Fluorescence and chemiluminescence spectra were determined on a Hitachi Perkin-Elmer Model MPF-2A spectrofluorimeter.

Materials. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride through a 2ft Vigreux column and stored in the dark under argon. Water was distilled and deionized. Tetrabutylammonium hydroxide (TBAH) was obtained as a 1.60 M aqueous solution from Sigma Chemical Co.

Acridone (Aldrich) was recrystallized twice from methanol: mp 358–361 °C dec (lit.[33] mp 354 °C); TLC on silica gel [methanol/chloroform, 5/95 (v/v)] gave one spot.

9-Cyanoacridine was synthesized in 65% yield from acridine by the method of Lehmstedt and Wirth,[34] mp 181–182 °C (lit.[34] mp 181 °C).

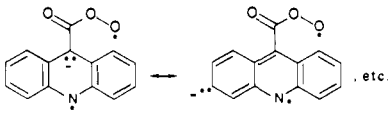
9-Acridinecarboxylic acid was prepared from 9-cyanoacridine by the method of Lehmstedt and Wirth.[34] The crude product obtained was treated with 1 M sodium hydroxide; the resulting mixture was filtered, and the filtrate was treated with 3 M sulfuric acid. The yellow precipitate was filtered, washed with copious amounts of water, and dried in vacuo to afford 94% of the carboxylic acid: mp 296.5–297 °C (lit.[34] mp 289–290 °C); IR (KBr) 3440, 3220, 2530, 1990, 1640, 1605, 1480, 1465, 1420 cm^{-1}.

9-Chlorocarbonylacridine Hydrochloride. Acridine-9-carboxylic acid (1.73 g, 0.01 mmol) was dissolved in thionyl chloride (25 mL) and the solution was refluxed for 6 h. The solution was concentrated in vacuo and hexane was added (20 mL) to precipitate the acid chloride (87% yield): mp 221–222 °C dec (lit.[4] mp 218 °C); IR (KBr) 2490, 2230, 1945, 1773, 1052, 870, 858, 765 cm^{-1}.

Phenyl 9-Acridinecarboxylate (1). 9-Chlorocarbonylacridine hydrochloride (1.5 g, 5.4 mmol) was added to a mixture of phenol (1.5 g, 15.9 mmol) and potassium *tert*-butoxide (2.5 g, 22.3 mmol) in THF (70 mL) under an argon atmosphere. The red mixture was stirred for 18 h in the dark, with the subsequent workup being performed under red light. Chloroform (150 mL) was added to the reaction mixture, which was then washed with 5% aqueous potassium bicarbonate solution and brine. The organic layer was dried over Na_2SO_4; it was then filtered and the solvent was removed on a rotary evaporator. The product, a yellow solid, was evacuated for 24 h (0.01 Torr) to remove excess phenol. The residue was passed through a short column of silica gel (with $CHCl_3$ as eluent) to remove traces of the parent acid. Removal of the solvent left a pale yellow solid, which was recrystallized from hexane/THF (1:1) four times to give very fine off-white needles (yield ca. 80%): mp 189–190 °C; IR

(31) McCapra, F.; Chang, Y. C. *Chem. Commun.* **1967**, 1011–1012.

(32) In view of the acceptor nature of acridine (with cyanide ion, e.g.)[34] and the extensive resonance delocalization in the radical anion i, electron transfer in a direction opposite to that illustrated in eq 13 and 14 should also be considered.



(33) Dictionary of Organic Compounds, 5th ed.; Chapman and Hall: New York, 1982; Vol. 1.

(34) Lehmstedt, K.; Wirth, E. *Chem. Ber.* **1928**, *61*, 2044.

(KBr) 3050, 1745, 1720 (sh), 1198, 1180, 1171, 980, 757, 737 cm^{-1}; NMR ($CDCl_3$) δ 7.3–8.35 (complex multiplet); UV (glyme) λ_{max} 252 nm (log ϵ 5.04), 350 (3.83), 362 (3.96), 382 (3.66); TLC on silica gel (toluene/ethyl acetate, 3:1) gave one spot with R_f 0.48. Anal. ($C_{20}H_{13}NO_2$) C, H, N.

9-Acridinoyl *m*-Chlorobenzoyl Peroxide (11). Following the method of Denney and Sherman,[35] and working under subdued lighting, a solution of 9-chlorocarbonylacridine hydrochloride (0.600 g, 2.16 mmol) and *m*-chloroperbenzoic acid[3] (0.372 g, 2.16 mmol) in 25 mol of methylene chloride (freshly distilled from sodium hydride) was cooled to −20 °C and to the stirred mixture was added dropwise at −20 °C a solution of pyridine (distilled from KOH) (0.341 g, 4.32 mmol) in 5 mL of methylene chloride. The temperature of the solution was maintained at −20 °C for 2.5 h while the solution was stirred; a yellow precipitate formed. Methylene chloride (25 mL) was added to the reaction mixture, which was then washed with 10% HCl (15 mL, 3×), whereby the yellow solid dissolved in the aqueous layer. The orange organic layer was washed with 10% bicarbonate solution (15 mL, 2×) and with brine (15 mL, 2×). The organic layer was dried over Na_2SO_4 and filtered and the solvent was removed on a rotary evaporator. The product, a yellow-orange oil, was evacuated for 8 h (0.10 Torr) to give an orange solid (0.380 g, 1.00 mmol, 47%), mp 55–60 °C. The solid was recrystallized from toluene–pentane (1:1) to give a bright yellow solid, mp 151–152 °C dec: TLC (toluene–ethyl acetate, 3:1, on silica gel) showed a single spot (R_f 0.22); NMR ($CDCl_3$) δ 8.40–7.75 (multiplet); IR ($CHCl_3$) 1795, 1730, 1695, 1650, 1350, 1200, 1025 cm^{-1}; UV (THF) λ_{max} 380 nm (log ϵ 3.47), 360 (3.73), 344 (3.63), 285 (3.58). This material decomposed during handling and it could not be prepared in a pure form.

***N*-Hydroxyacridone (6).** A solution of the title compound in aqueous 2 N sodium hydroxide was prepared by the method of Acheson et al.[5] Acidification with sulfuric acid yielded a crude product that was chromatographed on silica gel with chloroform as the eluant. An orange solid was obtained in ~4% yield: mp 254–6 °C dec (lit.[5] mp 256 °C dec); UV (THF) 308, 386, 405 nm [lit. UV (MeOH) 310, 390, 410 nm]; fluorescence (THF, λ_{exc} 394) 430, 445 nm; fluorescence (THF, TBAH at pH 13.5, λ_{exc} 386 or 404 nm) 420, 440, 465, 493, 530 (sh) nm.

9-Methoxyacridane (9). A slurry of 9-chloroacridine (5.0 g, 0.023 mol) in methanol (100 mL) was added to a stirred solution of sodium methoxide in methanol prepared by dissolving 0.7 g of freshly cut sodium in 25 mL of methanol under argon. The reaction mixture was refluxed under argon for 2 h and then cooled; a white solid precipitated. The mixture was poured into 200 mL of water, and the yellow precipitate formed was collected by filtration. Two recrystallizations from methanol/toluene (1:1 (v/v)) served to concentrate the desired material in the mother liquors which were concentrated and passed down a column of silica gel with chloroform as eluent. Collection of the desired fractions, followed by removal of the solvent, left a brown solid. Recrystallization from aqueous methanol (50:50 v/v) followed by sublimation (80 °C (0.1 mmHg)) gave a pale yellow powder (0.32g, 7%): mp 93.5–96 °C; IR (KBr) 1615, 1555, 1350, 1090, 967, 775, and 760 cm^{-1}; NMR ($CDCl_3$) δ 7.36–7.92 (complex multiplet, 8 H) 4.16 (s, 3 H); UV (THF): λ_{max} 340 nm (sh, log ϵ = 3.92), 349 (4.00), 356 (4.06), 365 (sh, 3.99), 383 (3.80). Anal. ($C_{14}H_{11}NO$) C, H, N.

Product Analysis. A solution of phenyl 9-acridinecarboxylate in THF (100 mL, 1×10^{-4} M) was treated with hydrogen peroxide in THF (10 mL, 10^{-1} M) followed by aqueous tetrabutylammonium hydroxide (10 mL, 10^{-3} M). The reaction solution contained 29 mol % H_2O–71 mol % THF; after mixing, a bright yellow-green chemiluminescence was observed for ~5 min. The solvent was removed by rotary evaporation and the residue was treated with 5% aqueous sodium bicarbonate solution (100 mL); the pH was adjusted to ~9. The aqueous phase was extracted with chloroform (3 × 30 mL), and the combined organic extracts were washed with water (70 mL) and made up to 100 mL with chloroform. The UV–vis spectrum of the solution was measured and the acridone content was determined from the intensity of its absorption at 392 nm (log ϵ 3.93). The aqueous washings were combined and made up to 200 mL and the UV–vis spectrum was recorded. 9-Acridinecarboxylic acid was determined from its absorption at 356 nm (log ϵ 3.97).

The spectra indicated that 55% acridone and 44% 9-acridinecarboxylic acid were formed. TLC (silica gel with toluene–ethyl acetate, 3:1, as eluant) of the product mixture indicated acridone (R_f 0.30) and 9-acridinecarboxylic acid (R_f 0.00) as the only products. The acridone bands was eluted and the spectral properties determined; the absorption and fluorescence spectra under neutral and basic conditions were identical with those of authentic acridone.

In a second run, 58% acridone and 44% acridine carboxylic acid were obtained.

Spectral Measurements. The reaction variables are listed in the figure captions. The percentage of blue light emission in the fluorescence of 1.19×10^{-4} M solutions of acridone excited at 398 nm (Figure 2) was calculated as follows:

$$\%\ \text{blue emission from excited acridone} = \{\text{area (blue emission)} \times (100)\}/\{\text{area (blue emission)} + \text{area (yellow-green emission from } \mathbf{4} \rightarrow \mathbf{5})\}$$

$$= \{\text{height}(410\ \text{nm}) \times (100)\}/\{\text{height (410 nm)} + \text{height (455 nm, from } \mathbf{4} \rightarrow \mathbf{5})(1.14)\}$$

True peak height at 455 nm = observed height at 455 nm − 0.3(height) observed at 410 nm (overlap from blue emission) − height at 455 nm due to excited anion (**7** → **5**).

peak height at 455 nm due to excited anion =

$$(\text{height at 455 for excitation of } 1.19 \times 10^{-4}\ \text{M acridone } \textit{anion} \text{ at } 398\ \text{nm})\left(\frac{\text{concentration of anion in solution}}{1.19 \times 10^{-4}}\right)$$

Concentration of anion in solution = absorbance (442 nm)/ϵ^{442}, where $\epsilon = 5.37 \times 10^3$.

Acknowledgment. Financial support was provided by Grant 36061 from the National Institute of General Medical Sciences of the U.S. Public Health Service and by the D. Mead Johnson Foundation.

(35) Denney, D.; Sherman, N. *J. Org. Chem.* **1965**, *30*, 3760.