Chemiluminescent Reaction of 9-Carboxy-*N*-methylacridinium Chloride with Potassium Peroxydisulfate

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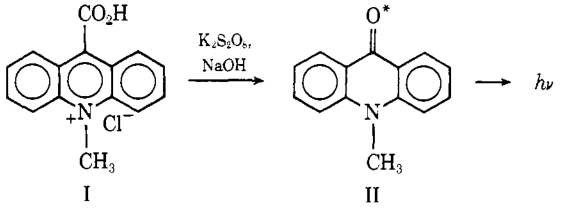
Abstract: The reaction of 9-carboxy-*N*-methylacridinium chloride (I) with potassium persulfate in aqueous base yields a strong blue chemiluminescence. *N*-Methylacridone is the light emitter. The reaction was found to be first order in I and first order in persulfate. Both the quantum yield and reaction rate increase with increasing hydroxide ion concentration. Allyl alcohol, an efficient sulfate radical-ion trap, causes a decrease in the rate of chemiluminescence decay although it causes an increase in the chemiluminescence quantum yield. At high base concentrations the quantum yield approaches 0.02. The mechanism proposed involves a nucleophilic displacement on persulfate by an intermediate alcoholate anion (IV) followed by dioxetanone formation, and subsequent dissociation to excited *N*-methylacridone. A chemiluminescent reaction of I with potassium peroxymonosulfate is also reported.

Derivatives of acridine have proved useful in a number of studies that have contributed to our present knowledge of chemiluminescence.[1] The chemiluminescence of derivatives of *N*-methylacridinium salts containing "activated" carbonyl groups such as acid chlorides[1b,c] or esters[1c] have been reported. Previous investigators have looked for, but not observed chemiluminescence from the free acid, 9-carboxy-*N*-methylacridinium chloride (CMC, I).[1b,c] We now report a chemiluminescent reaction of this compound (eq 1).



(1)

Results

The Reaction. The reaction of compound I with persulfate in aqueous base produces an intense blue light. At high base concentrations (8 to 10 *M* sodium or potassium hydroxide) the light is clearly visible in a brightly lit room. At initial CMC (I) concentrations above 10^{-3} *M* the main product of the reaction, *N*-methylacridone (II), separates immediately from solution as a flocculent precipitate. Pure *N*-methylacridone was isolated from such a reaction mixture in a 54% yield. Spectral analysis of more dilute reactions (10^{-4} *M* I) indicated over 75% formation of *N*-methylacridone. Further, *N*-methylacridone is the only product detectable by tlc of the chloroform extracts of the reaction mixture. In a control experiment, *N*-methylacridone was found to be nonchemiluminescent under the reaction conditions.

Hydroxide Adduct (III) as an Intermediate in the Chemiluminescence of I. The hydroxide adduct of CMC (III)[1b] is an example of the pseudobases formed from acridinium salts.[2,3] The equilibrium (eq 2) strongly favors the pseudobase form, which is stable in base; acidification regenerates I quantitatively. The participation of pseudobase III in the chemiluminescence of I was initially suggested by the observation of an induction period on addition of I to persulfate in aqueous base. During this period, which is base concentration dependent, the chemiluminescence intensity rises slowly to a maximum. The base dependence was readily determined since the addition of hydroxide to CMC (I) can be followed by the disappearance of the absorption of I at 355 nm (the longest wavelength absorption maximum of pseudobase III is at 285 nm). In this way the total times necessary for essentially complete conversion of I to III were determined for several base concentrations (Table I). The formation of pseudobase III with excess base is pseudo first order, and the reaction is first order in base.

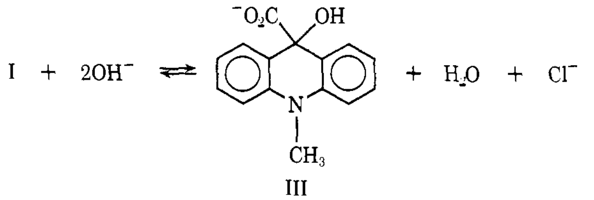


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Table I. Hydroxide Addition to CMC *vs.* Time of Maximum Chemiluminescence Intensity

NaOH concn, *M*	Time of complete pseudobase formation,[a,b] sec	Time of max chemiluminescence intensity,[c] sec
0.10	135–40	100–105
0.50	33–36	30–34
1.0	16–18	16–18
2.0	8–9	8

[a] 1.0×10^{-4} *M* CMC at 23°. [b] The point of completion was taken to be the point at which the OD of I at 355 nm dropped to 1% of the initial value. [c] 10^{-2} *M* $K_2S_2O_8$ and 10^{-4} *M* CMC at 23°.

The time required to form the pseudobase correlates well with the time to reach the maximum in chemilum-

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(1) (a) K. Gleu and W. Petsch, *Angew. Chem.*, 48, 57 (1935); (b) M. M. Rauhut, D. Sheehan, R. A. Clarke, B. G. Roberts, and A. M. Semsel, *J. Org. Chem.*, 30, 3587 (1965); (c) F. McCapra, D. G. Richardson, and Y. C. Chang, *Photochem. Photobiol.*, 4, 1111 (1965); (d) J. R. Totter, *ibid.*, 3, 231 (1964); (e) E. G. Janzen, J. B. Pickett, J. W. Happ, and W. DeAngelis, *J. Org. Chem.*, 35, 88 (1970).

(2) R. M. Acheson, "Acridines," Interscience, New York, N. Y., 1956, p 239.

(3) M. Decker, *J. Prakt. Chem.*, 45, 161 (1892).

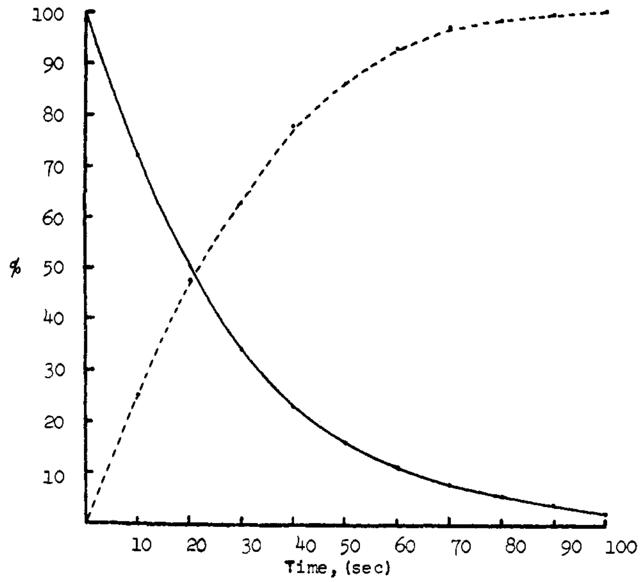

Figure 1. Decrease in concentration of CMC compared to the increase in chemiluminescence emission intensity: (- - - -) per cent of maximum emission intensity (1.0×10^{-4} M I, 1.0×10^{-2} M $K_2S_2O_8$, 0.10 N NaOH, 23°); (——) per cent OD at 355 nm, relative to initial OD of I (0.10 N NaOH and 1.0×10^{-4} M initial concentration of I; $T = 23°$).

inescence intensity (Table I). A similar correlation can be seen in a comparison of plots of (a) chemiluminescence intensity, and (b) pseudobase formation in 2 N base as a function of time (Figure 1). The chemiluminescence curve reaches a maximum slightly before the pseudobase formation peaks, because only in the chemiluminescence experiment is the concentration of I + III decreasing (through reaction with persulfate). If persulfate is added to a solution of preformed pseudobase III, the rise to maximum chemiluminescence intensity is instantaneous for all base concentrations in Table I. This result and the results summarized in Table I and Figure 1 show that pseudobase III is an intermediate in the chemiluminescence of I.

The Emitting Species. The chemiluminescence emission spectrum of the reaction of CMC with potassium persulfate in aqueous base (λ_{max} 432, 450 nm; fwhm[4] 53 nm) showed a good correspondence (Figure 2) with the fluorescence spectrum of *N*-methylacridone (II) under comparable conditions (λ_{max} 431, 450 nm; fwhm 53 nm), demonstrating that the first singlet state of *N*-methylacridone is formed in the reaction and that it is the emitting species.

Rates of Reaction. Most of the rate experiments were carried out under pseudo-first-order conditions with an excess of persulfate and aqueous base. Under these conditions the first-order rate equation (a) can be used in the form of eq (b) provided that the intensity

$$\ln (x)^0/(x) = kT \qquad \text{(a)}$$

$$\ln I^0/I = kT \qquad \text{(b)}$$

of chemiluminescence (I) is directly proportional to the concentration of an intermediate (x) derived from CMC.[5]

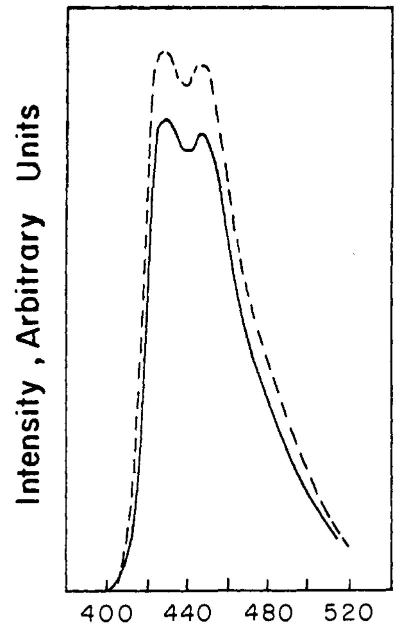

Figure 2. (- - - -) Fluorescence emission spectrum of *N*-methylacridone (II) (10^{-5} M in 10% aqueous ethanol). (——) Chemiluminescence emission spectrum of 9-carboxy-10-methylacridinium chloride (I) (10^{-4} M I, 10^{-2} M $K_2S_2O_8$, 2 N NaOH).

It was found that the chemiluminescence quantum yield remained essentially constant at initial CMC concentrations between 10^{-4} and 10^{-5} M. However, the quantum yield decreased above this range (Table II), presumably because of concentration quenching of the excited state of II. Therefore all kinetic runs were carried out with the initial CMC concentration of 10^{-4} M.

Table II. Dependence of the Chemiluminescence Quantum Yields on CMC Concentration

CMC concn, $M \times 10^4$	Rel quantum yield,[a] arbitrary units	CMC concn, $M \times 10^4$	Rel quantum yield,[a] arbitrary units
0.10	51	1.00[b]	44
0.20	46	3.00	26
0.50	45	5.00[c]	

[a] 10^{-2} M $K_2S_2O_8$ and 10 M NaOH. [b] Pseudo-first-order intensity decay constant 2.0×10^{-3} sec^{-1} (10^{-2} M $K_2S_2O_8$, 2 N NaOH, 23.0°). [c] Pseudo-first-order intensity decay constant 2.2×10^{-3} sec^{-1} (conditions as in footnote *b*).

Plots of ln I^0/I *vs.* time were linear for at least three half-lives for aerated runs carried out under pseudo-first-order conditions. In the rate experiments, persulfate was added to preformed solutions of pseudobase III; thus maximum chemiluminescence intensity was reached within 1 sec. An intensity value within a few seconds after this maximum was taken as I^0. The decay of emission intensity is first order (thus first order in CMC) and the rate constant is essentially independent of the initial concentration of CMC (Table II).

The rate of reaction was also determined by following the increase in the concentration of *N*-methylacridone (II) through measurement of its absorption at 393 nm. The first-order rate constant determined by this method

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[4] Fwhm is the full band width between half-maximum intensity points of the spectrum.

[5] A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," Wiley, New York, N. Y., 1953, p 28.

was 0.8×10^{-3} sec^{-1}, as compared with 1.1×10^{-3} sec^{-1}, the rate constant determined by chemiluminescence intensity decay measurements (both reactions run in 5% aqueous dimethoxyethane). Attempts to measure the rate of reaction in aqueous solution by following the disappearance of CMC were unsuccessful because of the increase in overlap of the absorption bands with the longer wavelength absorption of *N*-methylacridone as the reaction progresses. However, the initial (first 120 sec) rate found in this way in water, 1.6×10^{-3} sec^{-1}, was comparable to that obtained from light intensity decay measurements (2.2×10^{-3} sec^{-1}).

The effect of persulfate concentration on reaction rate is summarized in Table III. Second-order intensity decay constants were calculated by dividing the pseudo-first-order decay constants (obtained from log *I vs.* time plots) by persulfate concentration. The results indicate a first-order dependence of rate on persulfate.

Table III. Persulfate Dependence of Reaction Rate and Quantum Yield[a]

$K_2S_2O_8$, $M \times 10^2$	Second-order[b] intensity decay constant, M^{-1} sec^{-1} $\times$ 10	Uncorrected[c] quantum yield, arbitrary units	*N*-Methylacridone[d] fluorescence intensity, arbitrary units	Corrected quantum[e] yield, arbitrary units
0.5	2.0, 1.8	100	100	35
0.8	1.9			
1.0	2.0, 1.9, 1.8	88	85	37
2.0	1.8			
3.0	1.9	65	45	51
5.0	1.9, 1.7	52	30	62
10.0		31	11	100

[a] 1.0×10^{-4} *M* CMC in 2.0 *N* NaOH. [b] Defined as k_{obsvd}/M $K_2S_2O_8$; $T = 23.0 \pm 0.1°$. [c] $T = 23.3 \pm 0.2°$. [d] 1.0×10^{-5} *M* *N*-methylacridone in 9% aqueous ethanol. [e] From (uncorrected quantum yield/fluorescence quantum yield of *N*-methylacridone at each molarity of $K_2S_2O_8$) (35).

The concentration of persulfate in 6 *M* sodium hydroxide was followed by iodometric titration; no decrease in active oxygen content occurred over a 45-min period. It seems unlikely that this result stemmed from hydrolysis to give products at the same oxidation level, such as peroxymonosulfate ($SO_5{}^{2-}$) or hydrogen peroxide, since peroxydisulfate is known to decompose to these products only under highly acidic conditions.[6] It was therefore concluded that peroxydisulfate does not decompose in concentrated hydroxide solution at a rate comparable with the rate of the chemiluminescent reaction of CMC. This view is in agreement with the report that under the conditions of the Elbs reaction (1 to 2 *N* hydroxide), no decomposition of persulfate by hydroxide was observed.[7]

There is a positive salt effect on the rate of reaction (summarized in Table IV). The data in Tables IV and V show that at constant ionic strength the rate of reaction decreased slightly on going from 0.10 to 2.0 *M* sodium hydroxide, then increased on going above 2 *M* sodium hydroxide. The rate of a reaction containing 2.0 *N* sodium hydroxide and 1.0 *N* potassium chloride is slower than the rate of a reaction containing 3.0 *N* sodium hydroxide, and the rate of a reaction containing 2.0 *M* sodium hydroxide and 2.0 *M* sodium nitrate is slower than a reaction containing 4.0 *M* sodium hydroxide. At concentrations above 2 *N* sodium hydroxide it is impossible to determine the contribution of the salt effect to the reaction rate due to problems of solubility of the salts used. Also, application of the Debye–Hückel equation of solutions of high ionic strength has no theoretical justification.[8] For these reasons, the kinetic order with respect to base (Table V) was not determined.

Table IV. Ionic Strength Dependence of Reaction Rate and Quantum Yield[a]

Concentration, *M*: NaOH	Concentration, *M*: Electrolyte	Pseudo-first-order intensity decay constants, sec^{-1} $\times$ 10^3	Relative quantum yield, arbitrary units	μ
0.10			1.1	0.1
0.10	2.0 NaCl		1.0	2.1
2.0		1.9	36.0	2
2.0	1.0 NaCl	2.6		3
2.0	2.0 NaCl	3.2	39.8	4
2.0	3.0 NaCl	4.5		5
2.0	2.0 $NaNO_3$	2.3		4
2.0	4.0 $NaNO_3$	2.5		6
2.0	1.0 KCl	2.1		3

[a] 10^{-4} *M* CMC and 10^{-2} *M* $K_2S_2O_8$.

Table V. Dependence of Reaction Rate and Quantum Yield[a] on Sodium Hydroxide Concentration

NaOH concn, *M*	Pseudo-first-order intensity decay constant, sec^{-1} $\times$ 10^3	Absolute quantum yield,[b] einstein mol^{-1} $\times$ 10^2
0.10	1.4	0.01
0.10[c]	5.6	
0.50	1.5	0.04
0.50[c]	3.2	
1.0	1.3	
2.0	1.9	0.36 ± 0.02[h]
4.0	2.6	0.68[e]
4.0		0.59[f]
7.0	8.2	
8.0	12.7	1.01
10.0	21.0	1.07 ± 0.02[h]
12.0	29.4	1.01
12.0[d]		2.04 ± 0.2[i]
10.0		1.12[g]

[a] Conditions: 10^{-4} *M* CMC, 10^{-2} *M* $K_2S_2O_8$; $T = 23.0°$ for rate runs only. [b] Uncorrected for $K_2S_2O_8$ quenching of fluorescence of II. [c] NaCl added; (NaCl) + (NaOH) = 2.0 *M*. [d] 12.0 *M* KOH used; CMC = 2.0×10^{-6} *M*. [e] $T = 22.8°$. [f] $T = 42°$; all other runs near 23°. [g] 1.0×10^{-2} $(NH_4)_2S_2O_8$ used. [h] Average of three experiments. [i] Average of two experiments. A value of 3.0 has been obtained by a different operator.

Quantum Yields for Aerated Runs. The dependence of the chemiluminescence quantum yield on potassium persulfate concentration is summarized in Table III. The apparent quantum yield decreases with increasing persulfate concentration. However, when a correction is made for the quenching of *N*-methylacridone fluorescence by persulfate, an increase in the chemilumines-

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(6) I. M. Kolthoff and I. K. Miller, *J. Amer. Chem. Soc.*, 73, 3055 (1951).

(7) E. J. Behrman, *ibid.*, 85, 3478 (1963).

(8) Reference 5, p 139.

cence quantum yield is observed. Potassium persulfate and ammonium persulfate give almost identical quantum yield results (Table V).

The effect of hydroxide ion concentration on the quantum yield of chemiluminescence is presented in Table V. The use of potassium hydroxide rather than sodium hydroxide resulted in higher quantum yields; however, because of difficulties in maintaining known concentrations of potassium hydroxide in stock solutions (due to the solubility of potassium carbonate in potassium hydroxide solutions), sodium hydroxide was used in most of the experiments. As shown in Table V, under conditions of high potassium hydroxide and low CMC concentrations, the quantum yield, uncorrected for the quenching of the fluorescence of II by persulfate, approaches 0.02, making this one of the more efficient chemiluminescent reactions known.[1] The quantum yield increases 20-fold between 1 and 10 M sodium hydroxide. The fluorescence efficiency of 10-methylacridone is independent of sodium hydroxide concentration, and the chemiluminescence quantum yield is almost unaffected by changes in the ionic strength of the reaction medium (Table VI). The quantum yield of chemiluminescence was slightly less at 42 than at 22.8° (Table V).

Table VI. Effect of Allyl Alcohol and Oxygen on the Reaction Rate and Quantum Yield[a]

Conditions	First-order chemiluminescence intensity decay constant, $\sec^{-1} \times 10^3$	Relative quantum yield, arbitrary units[b]
(1) No allyl alcohol, aerated by stirring	2.0	1.0
(2) 10^{-3} M allyl alcohol (aerated)	1.6	
(3) 10^{-2} M allyl alcohol (aerated)	1.4	1.4[c]
(4) Oxygen bubbling	1.5	1.1
(5) 10^{-2} M allyl alcohol and oxygen bubbling	1.2	1.4
(6) Nitrogen bubbling[d]	4	0.6
(7) 10^{-2} M allyl alcohol[d] and nitrogen bubbling	3[f]	0.9
(8) Oxygen bubbling[d,e]		3.3
(9) No additions[d,e] (aerated)		3.8

[a] Initial concentrations (except runs 8 and 9): 1.0×10^{-4} M I, 1.0×10^{-2} M $K_2S_2O_8$, 2 N NaOH, plus added radical inhibitor. [b] Determined from relative areas under intensity decay curves. [c] Determined relative to run 1 by direct measurement with a capacitor, as described in Experimental Section. [d] Values from a single experiment. [e] 1.0×10^{-4} M I, 1.0×10^{-2} M $K_2S_2O_8$, 10 N NaOH. [f] Calculated from first half-life.

Effect of Allyl Alcohol and Oxygen on the Reaction Rates and Quantum Yields. Many persulfate oxidations are essentially first order in persulfate alone.[9] Such reactions have been interpreted in terms of a free-radical chain process involving the sulfate radical ion ($SO_4\cdot^-$) as the chain carrier.[10] Allyl alcohol is known to strongly inhibit sulfate radical-ion oxidations by trapping this species. Dissolved oxygen is also known to be a radical trap in $SO_4\cdot^-$ reactions.[11]

It is evident from Table VI that both allyl alcohol and dissolved oxygen cause a decrease in the decay rate relative to a reaction exposed to the atmosphere but containing no added inhibitor (Table VI). Also, the presence of either allyl alcohol or oxygen leads to an increase in the quantum yield (Table VI). In contrast, when a reaction was purged of oxygen by continuous flushing with nitrogen, a rate increase and quantum yield decrease were observed. The addition of allyl alcohol to a nitrogen flushed reaction restored the quantum yield to 85% of the value observed in an air saturated solution.

These results show that the chemiluminescent reaction is a nonradical path which competes with one or more dark radical reactions. The observed decrease in decay rate with increasing inhibitor concentration is due to the fact that both light (nonradical) and dark (radical) reaction paths contribute to the overall decay rate. Run 5, saturated with oxygen and containing 10^{-2} M allyl alcohol, is probably almost free of radical reactions since the effect of the two radical inhibitors is not much different than the effect of either alone (Table VI). Thus, the rate 1.2×10^{-3} sec^{-1} probably most accurately represents the contribution of the chemiluminescent reaction to the decay rate.

Discussion

The mechanism for the chemiluminescent reaction of CMC (I) with persulfate in aqueous base must account for the following observations: (a) the rate of reaction of hydroxide with I to form pseudobase III correlates with an increase in the chemiluminescence intensity (Table I). (b) The reaction rate is decreased by the presence of allyl alcohol and dissolved oxygen. The quantum yield is increased by the presence of these reagents (Table VI). (c) The reaction is first order in both CMC and persulfate (Tables II and III). (d) The reaction rate increases substantially with increasing hydroxide ion concentration above 2 M sodium hydroxide (Table V). (e) The reaction rate shows a positive salt effect (Table IV). (f) The quantum yield, corrected for persulfate quenching of N-methylacridone fluorescence, increases with increasing persulfate concentration (Table III). (g) The quantum yield increases substantially with hydroxide ion concentrations up to a limiting value (Table V) and is relatively independent of ionic strength (Table IV).

A mechanism which conforms to the experimental observations is given in Chart I. Step 1, the reaction of hydroxide with I to give III, is supported by the correlation between pseudobase formation and chemiluminescence intensity (Table I and Figure 1). Step 2 represents the ionization of compound III to give alcoholate IV. Step 4 is bimolecular displacement by IV on the peroxy bond of persulfate ion; such reactions are well documented.[12,13]

The kinetic results, showing a first-order dependence on both CMC and persulfate, are the same as those observed for the Elbs persulfate oxidation of phenols, in which the rate-determining step is an ionic displacement on the persulfate peroxy bond by phenolate

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(9) (a) D. A. House, *Chem. Rev.*, **62**, 185 (1962); (b) W. K. Wilmark and A. Haim in "Peroxide Reaction Mechanisms," J. O. Edwards, Ed., Wiley, New York, N. Y., 1962, p 175.

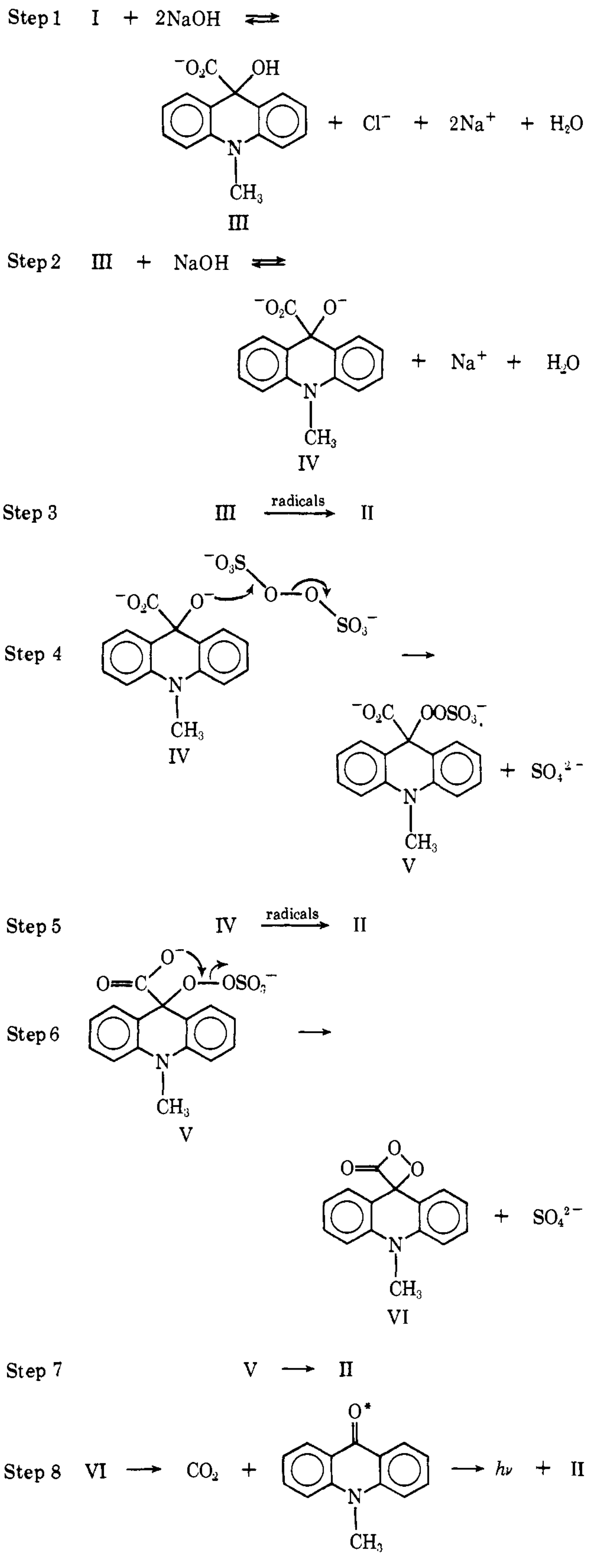
(10) (a) I. M. Kolthoff, E. J. Meehan, and E. M. Carr, *J. Amer. Chem. Soc.*, **75**, 1439 (1953); (b) K. B. Wiberg, *ibid.*, **81**, 252 (1959).

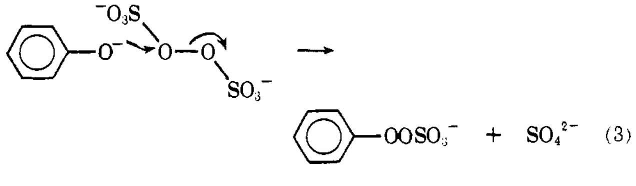
(11) D. L. Ball, M. M. Crutchfield, and J. O. Edward, *J. Org. Chem.*, **25**, 1599 (1960).

(12) J. O. Edwards in ref 9b, p 67.

(13) R. Curci and J. O. Edwards in "Organic Peroxides," D. Swern, Ed., Vol. I, Wiley-Interscience, New York, N. Y., 1970, p 199.

Chart I





anion (eq 3);[7] thus step 4 is rate determining. As in the Elbs reaction,[7] there is a positive salt effect on the rate of the reaction of CMC with persulfate.

The reaction of alcoholate III with persulfate is probably related also to the proposed rate-determining two-electron oxidation by persulfate of 3-aminophthalhydrazide in the chemiluminescent reaction of that compound.[14] The base dependence of the rate of reaction of CMC with persulfate is similar to the base dependence reported for the Elbs reaction,[7] in which a sharp increase in rate is observed as hydroxide concentration is increased into the range corresponding to the pK_a of the phenol. The pK_a of pseudobase III is not known; however, an estimate can be made by comparison with mandelic acid monoanion ($C_6H_5CHOHCO_2^-$) in which the pK_a of the alcohol function is 15.1.[15] As Table V shows, the sharp increase in rate of the chemiluminescence reaction occurs between 1 and 10 N sodium hydroxide. It thus appears that alcoholate IV reacts with persulfate in a manner similar to the reaction of phenolate anions in the Elbs reaction[7] (eq 3). A heterolysis of persulfate to give sulfur tetroxide and sulfate has been shown by Kolthoff and Miller[6] to be a significant reaction only in strongly acidic solution. These investigators found that the heterolysis of persulfate was undetectable in 0.1 M sodium hydroxide; thus, prior ionic cleavage of persulfate need not be considered in the present case. A homolysis of persulfate[7,9] in the chemiluminescent reaction of I is precluded by the first-order dependency of the rate on both I and persulfate. The observation of a salt effect in the chemiluminescent reaction is also not in accord with initial persulfate homolysis, since persulfate homolysis is independent of ionic strength at high pH.[9a]

Dark radical paths must contribute to the chemiluminescence emission decay rate, as shown by the decrease in rate caused by allyl alcohol and by oxygen. Such radical paths must be dark or inefficient since these same radical inhibitors cause an increase in the quantum yield. The radical contribution (indicated by the data of Table VI) is apparently not sufficiently large or different in kinetic order to alter the kinetics of chemiluminescence intensity decay from the pseudo-first-order dependence. Obligatory participation of sulfate radicals after the rate-determining step of chemiluminescence may be ruled out by the increase in quantum yield caused by the addition of allyl alcohol or dissolved oxygen to the reaction. Oxidations of carboxylate ions involving $SO_4\cdot^-$ are known, but they proceed at a measurable rate only at elevated temperatures and with silver(I) catalysis.[16]

Continuous oxygen bubbling through a reaction mixture 10 N in sodium hydroxide did not increase the quantum yield of light emission relative to a run saturated with air (runs 8 and 9, Table VI). The radical component is thus not competitive with the fast, ionic chemiluminescent reaction in 10 N sodium hydroxide solution. At lower base concentrations, resulting in lower concentrations of IV, the radical component apparently becomes dominant, as shown by the sharp drop in light emission (Table V).

(14) M. M. Rauhut, A. M. Semsel, and B. G. Roberts, *J. Org. Chem.*, **31**, 2431 (1966).

(15) M. T. Beck and M. Halmes, *Nature (London)*, **186**, 388 (1960).

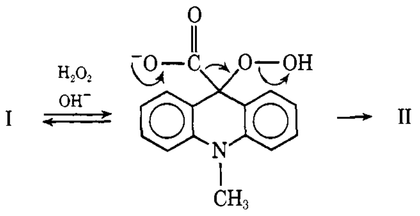
(16) J. A. Anderson and J. K. Kochi, *J. Amer. Chem. Soc.*, **92**, 1651 (1970).

Step 3 is included to account for the increase in quantum yield with base concentration and also the increase of the reaction rate with base (over 2 *N* base). Species III, which is a diphenylamine, would be expected to be readily oxidized by persulfate and by radicals.[17] Step 5 accounts for the increase in quantum yield with an increase in persulfate concentration (Table III).

Step 6, the nucleophilic displacement of sulfate by carboxylate to give dioxetanone VI, has precedent in the reaction of acetate ion with the oxygen of peroxy bonds.[12] Consideration of a space-filling model of V shows that the intramolecular displacement of sulfate (step 6) is feasible in terms of steric requirements.

It is generally accepted that the thermal decomposition of the dioxetane ring results in the formation of a carbonyl fragment in the singlet excited state.[18-21] One might also consider a rapid multiple bond cleavage of V to give excited II, a process which has been proposed to explain chemiluminescence in a related system;[1b] we feel, however, that steps 6 and 8 have more theoretical appeal[18] and experimental support.[18-21]

Although there is no evidence for step 7, it is included since it is unlikely that the intermediate V decomposes exclusively *via* the dioxetanone VI. A possible analogy for the dark decomposition of V is the rapid nonchemiluminescent reaction[1b,c] of I with alkaline hydrogen peroxide (eq 4) to yield *N*-methylacridone, presumably *via* adduct VII. This adduct could decompose *via* a multiple bond cleavage as shown.[1b] On addition of I to alkaline hydrogen peroxide (2 *M* sodium hydroxide), the reaction was complete in about 10 sec as determined by the rapid increase in the *N*-methylacridone absorption at 393 nm.



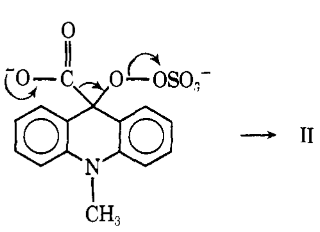
VII

(4)

Reaction with Peroxymonosulfate. It might be expected, *a priori*, that intermediate V could be formed by the addition of dipotassium peroxymonosulfate (K_2SO_5) to I. However, the reaction of peroxymonosulfate with I within the pH range from 9 to 12 gave II *via* a dark reaction. At pH 7.5 there was no reaction, indicating the dianion (SO_5^{2-}) may be required for the dark pathway.[22]

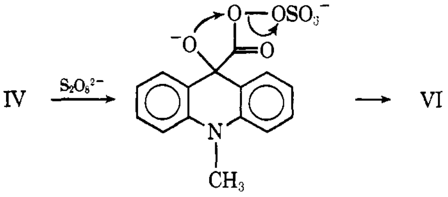
Peroxymonosulfate does undergo a chemiluminescent reaction with I at hydroxide concentrations above 1 *N;* a blue light is emitted similar to that observed in the reaction of I with perdisulfate. The rate of chemiluminescence intensity decay in the reaction of CMC with peroxymonosulfate did not follow strict pseudo-first-order kinetics, in that the decay rate increased by approximately 20% over the first two half-lives of the reaction. The rate measurements for reactions of peroxymonosulfate with CMC in base were not as reproducible as were those for the peroxydisulfate reactions. The quantum yield of the chemiluminescent peroxymonosulfate reaction does show the same type of base dependence as that observed in the case of peroxydisulfate. In 4, 6, and 8 *N* potassium hydroxide, the ratios of the quantum yields of the peroxydisulfate reactions to those of the peroxymonosulfate reactions were 15, 10, and 3, respectively. Thus it appears that at low base concentrations, peroxymonosulfate effectively destroys IV, etc., in dark reactions.

Alternative Mechanisms. The dark reaction of peroxymonosulfate with CMC at pH 9-12 suggests a possible, but less likely, alternative to the mechanism shown in Chart I. It is possible that intermediate V may be formed in the reaction of peroxymonosulfate with CMC and that V decomposes only *via* a dark reaction (as in eq 5, *e.g.*). If so, the light-producing path in the peroxydisulfate reaction might arise from the nucleophilic attack of the carboxylate group of IV on peroxydisulfate (eq 6) to give intermediate VIII; intermediate VIII could then give dioxetanone VI by an intramolecular displacement of sulfate by the alcoholate anion. Dioxetane VI would then yield excited *N*-methylacridone, as in the mechanism outlined in Chart I. The rates of known nucleophilic displacements on peroxydisulfate by carboxylate anions are much slower than the chemiluminescent reaction of I with peroxydisulfate.[12] However, the nucleophilicity of the carboxylate group of IV may be enhanced by the α-alcoholate group.



V

(5)



VIII

(6)

One further mechanism, which conforms to much of the evidence presented above, involves the homolytic cleavage of the peroxy bond in intermediate V to give IX; decarboxylation would then yield the ketyl of 10-methylacridone (X). Chemiluminescence has been observed in the oxidation of X to II,[23] and in our hands X gave an easily visible chemiluminescence on reaction with persulfate. The observation that radical scavengers such as allyl alcohol and oxygen *increase* the quantum yield of the chemiluminescence argues against the ketyl pathway, however.

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[17] L. Horner and E. Schwenk, *Angew. Chem.*, **61**, 441 (1949); *Justus Liebigs Ann. Chem.*, **566**, 69 (1950).

[18] D. R. Kearns, *J. Amer. Chem. Soc.*, **91**, 6554 (1969).

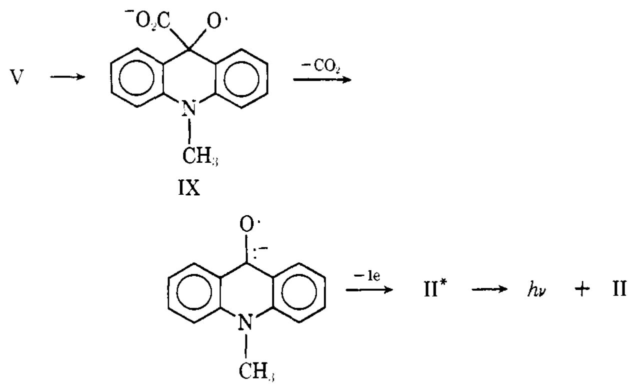
[19] E. H. White and M. J. C. Harding, *ibid.*, **86**, 5686 (1964); E. H. White, J. Wiecko, and D. R. Roswell, *ibid.*, **91**, 5194 (1969).

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

[21] F. McCapra, *Chem. Commun.*, 155 (1968).

[22] The pK_a of the peroxy hydrogen of peroxymonosulfate is 9.4: D. L. Ball and J. O. Edwards, *J. Amer. Chem. Soc.*, **78**, 1125 (1956).

[23] E. A. Chandross and F. I. Sonntag, *ibid.*, **88**, 1089 (1966).



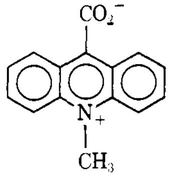
IX

X

Experimental Section

Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were determined on a Perkin-Elmer Model 337 infrared spectrophotometer, and were calibrated against known absorption bands of polystyrene. Ultraviolet spectra were determined on a Cary Model 14 spectrophotometer.

Materials. Baker reagent grade potassium persulfate and Merck reagent ammonium persulfate were used without further purification. The 0.20 *M* potassium persulfate stock solutions used in all runs were always prepared on the day of the experiment. Potassium peroxymonosulfate was obtained from E. I. DuPont de Nemours and Co. as a mixture of potassium bisulfate, potassium sulfate, and 43% potassium peroxymonosulfate (determined by iodometric titration). Baker reagent grade 3% aqueous hydrogen peroxide was used without further purification. Sodium hydroxide stock solutions were stored in plastic bottles with Ascarite-filled side arms, and portions were withdrawn by syringe through rubber septums and injected directly into reaction solutions. Solutions stored in this way never precipitated any sodium carbonate (insoluble in solutions of 1 *N* sodium hydroxide or above) even after weeks of storage. Periodic checks by titration with standard acid showed the hydroxide concentration to remain unchanged for weeks.



XI

9-Carboxy-10-methylacridinium Chloride (I). Acid I was prepared and purified by the method of Rauhut;[1b] ir λ_{max} (KBr) 3370, 1707, 1601, 1440, 1186, 1030, 870, 753 cm^{-1}; uv (ethanol) 440 nm (sh) (log ε3.47), 417 (3.66), 398 (3.62), 355 (4.17), 257 (4.93). This sample was heated at 100° *in vacuo* over KOH for 3 weeks, resulting in the loss of hydrochloric acid and the formation of the zwitterion XI, 10-methyl-9-acridiniumcarboxylate,[1b] mp 229° dec (lit.[1b] 231° dec); ir λ_{max} (KBr) 1606, 1545, 1312, 1266, 1190, 1030, 872, 790, 756 cm^{-1}.

Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.93; H, 4.67. Found: C, 75.83; H, 4.61.

Chemiluminescent Reaction of CMC (I) with Potassium Persulfate in Aqueous Base. Acid I (274 mg, 1 mmol), dissolved in approximately 10 ml of water, was added to 100 ml of 1 *N* potassium hydroxide, containing potassium persulfate (2.70 g, 10 mmol). The resulting intense blue chemiluminescence decayed slowly and was noticeably dimmer after 5 min. After the reaction was stirred for 1 hr, addition of a few crystals of persulfate in darkness brought no further emission. The solution and the flocculent precipitate which had formed were washed into a separatory funnel and extracted three times with 150-ml portions of chloroform. The combined extracts were dried with sodium sulfate and evaporated, giving large clusters of light brown needles of crude II (129 mg, 60%); the only component detectable by tlc (silica gel in chloroform) was identical in R_f and fluorescence color with *N*-methylacridone. The crude product was crystallized from ethanol–water to give 114 mg (54%) of *N*-methylacridone, mp 194–196° (lit. 201–203°).[24] The infrared spectrum of this sample was identical with that of an authentic sample of II.

The aqueous reaction solution after chloroform extraction was acidified with aqueous hydrochloric acid. The basic aqueous solution showed two broad absorptions, one at 250 nm and the other in the region of 400 nm, which did not change on acidification. The solution was taken to dryness and extracted for approximately 10 hr with ethanol in a Soxhlet extractor. The resulting solution (100 ml) had an absorption at 392 nm, OD 0.49. Dilution by approximately 10-fold revealed an absorption at 252 nm, 11.8 times as intense as that at 392 nm. This pattern is characteristic of acridine compounds, which absorb in the regions of 250 nm (ε ~10^5) and 350 to 400 nm (ε ~10^4).[25] Assuming that ε is 10^4 for the 392-nm peak, the absorption at 392 nm corresponds to 5×10^{-3} mmol of an acridine compound, or 0.5% yield from I.

Chemiluminescent Reaction of CMC with Potassium Peroxymonosulfate in Aqueous Base. A solution of approximately 1 mg of I in 2 ml of water was added to a stirred solution (5 ml) of 5 *N* sodium hydroxide and approximately 10^{-1} *M* potassium peroxymonosulfate. A blue light was emitted which was easily visible in a dimly lit room, and which decayed to a fraction of its original intensity within several minutes. The ultraviolet spectrum of the spent reaction mixture was identical with the spectrum of *N*-methylacridone.

Experiments with the Pseudobase III. The reaction of I with hydroxide to give III[1b] can be easily observed visually. Acid I gives a green solution which rapidly becomes colorless on addition of a pellet of sodium hydroxide. Similarly, the intense green fluorescence of I disappears on addition of hydroxide. Two experiments of a more quantitative nature were carried out.

(a) **Rate of Reaction of CMC with Hydroxide.** The cell compartment of a Cary 14 spectrophotometer, thermostatically kept at 23°, was fitted with a cover which contained a hole plugged with a rubber serum stopper. The arrangement was such that a long syringe needle could be stuck into the cuvette while the instrument was recording. With the instrument recording at 355 nm (an absorbtion maximum of I, log ε 4.17) and the chart moving at a known speed, 0.16 ml of 10^{-3} *M* I was injected into a thermostated solution of sodium hydroxide (initial concentration 0.50×10^{-4} *M* I, 0.10 *N* sodium hydroxide). Approximately 0.1 cc of air was injected with I to ensure mixing. The volume of the injection was corrected for the volume of the long needle (0.07 ml). Within 150 sec the recorder needle had come to rest at a constant value. The data obtained in this way are presented in Figure 1. The decrease in OD at 355 nm is first order, and the first-order rate constant is 3.7×10^{-2} sec^{-1}. Similar experiments were carried out at sodium hydroxide concentrations of 0.50, 1.0, 2.0, and 8 *N*. The reaction is first order in hydroxide (Table I). After reaction of base with I was complete, I was regenerated by acidification; after dilution to a standard volume the OD at 355 nm was recorded and in all cases showed complete regeneration of I from III. For runs in 0.5, 2.0, and 8.0 *N* sodium hydroxide, the final optical densities, measured on diluted aliquots, were 0.61, 0.59, and 0.61, respectively (theory for quantitative recovery = 0.59).

(b) **Stability of Pseudobase III in Base.** The optical density at 393 nm was measured every 5 min for two solutions 10^{-4} *M* in I, one in 0.10 *N* sodium hydoxide and the other in 2 *N* sodium hydroxide (absorption of II, log ε 3.94). After 263 min the OD at 393 nm had increased from zero to 0.023 in the 2.0 *N* hydroxide solution and from zero to 0.047 in the 0.10 *N* hydroxide solution. A 10^{-4} *M* solution of II would have an OD of 0.860.

Fluorescence and Chemiluminescence Emission Spectra. Fluorescence and chemiluminescence emission spectra were determined on a Hitachi Perkin-Elmer Model MPF-2A spectrophotofluorimeter and are reported uncorrected. Chemiluminescent spectra were obtained by measuring emission from solutions with time zero concentrations of 10^{-4} *M* CMC and 10^{-2} *M* persulfate in 2 *N* sodium hydroxide, running the spectrophotofluorimeter with the source off. The chemiluminescence emission spectrum was recorded several times during the course of the reaction and was found to remain constant in spectral distribution throughout the reaction. Fluorescence emission spectra (excitation at 271 nm) were recorded using solutions of 10^{-5} *M* II in 10% aqueous ethanol.

Rate of Reaction Based on the Appearance of *N*-Methylacridone. In a cuvette of 10-mm path length the following reagents were mixed: 1.19 ml of 5.41 *N* sodium hydroxide, 0.32 ml of 10.3 *M* I,

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[24] I. Krönke and H. L. Honig, *Chem. Ber.*, 90, 2215 (1957).

[25] A. Albert, "The Acridines," Edward Arnold, London, 1951.

0.16 ml of 1,2-dimethoxyethane (glyme), and 1.53 ml of water. The cuvette was placed in the cell compartment of a Cary 14 spectrophotometer thermostated at 23°. After the temperature of the solution had reached 23°, 0.16 ml of 0.20 M potassium persulfate was added by syringe, the reaction was given a quick stir, the cell compartment was closed, and the increase in absorption of II at 393 nm *vs.* time was recorded. The initial concentrations were 2 N sodium hydroxide, 10^{-4} M I, 10^{-2} M potassium persulfate, and 5% glyme. Preliminary experiments had shown that under the reaction conditions more than 95% of the absorption at 393 nm is due to *N*-methylacridone (II). The highest absorption maximum of III is at 285 nm (log ϵ 4.28). The change in OD at 393 nm with time was used to calculate the first-order rate constant $K = 0.8 \times 10^{-3}$ sec^{-1}. The equation used was $\ln [1/(1 - \mathrm{OD}/0.85)] = kt$, which was derived from $\ln a/(a - x) = kt$, where a = initial concentration of I (10^{-4} M) and x = concentration of II = OD/e = (OD/0.85) × 10^{-4}. The rate constant for an identical reaction, calculated from chemiluminescence intensity decay data, was 1.1×10^{-3} sec^{-1}. Duplicate runs gave rate constants identical with the ones above.

Rate of Reaction Based on the Disappearance of CMC. To 47.5 ml of a solution containing the appropriate proportions of sodium hydroxide and potassium persulfate and preadjusted to 23° was added 2.5 ml of 10^{-3} M CMC. The initial concentrations were thus 5×10^{-4} M CMC, 10^{-2} M potassium persulfate, and 2 N sodium hydroxide. At 1-min intervals, aliquots of 1 ml were withdrawn, quenched in aqueous hydrochloric acid, and brought to 10-ml volume by addition of water. The solutions also contained 5% glyme to keep *N*-methylacridone in solution. The OD at 355 nm, an absorption maximum of CMC (I), was measured for each aliquot. The data were plotted as log OD *vs.* time, giving, after the first 120 sec, a curve falling below the line drawn through the first two points. The pseudo-first-order rate constant calculated for the first 120 sec of reaction was 1.6×10^{-3} sec^{-1}. The rate constant from chemiluminescence intensity decay data from an identical reaction was 2.2×10^{-3} sec^{-1}.

Reaction of Hydrogen Peroxide with CMC in Aqueous Base. The following reagents were mixed in a cuvette: 1.19 ml of 5.41 N sodium hydroxide, 0.16 ml of 0.2 M hydrogen peroxide, and 1.53 ml of water. The cuvette was placed in a spectrophotometer cell compartment and the base line was set at zero. To this solution was added 0.32 ml of 1.0×10^{-3} M I, giving time zero concentrations of 2 N sodium hydroxide, 10^{-2} M hydrogen peroxide, and 10^{-4} M I. The solution was given a quick stir, the cell compartment was closed, and recording of the OD at 393 nm (an absorption band of *N*-methylacridone) was begun 8 sec after the addition of I. The OD at 393 nm was 0.59 at t = 8 sec and remained at this value, indicating 70% formation of II within 8 sec. The only product detected by silica gel tlc (chloroform) of such reaction mixtures was *N*-methylacridone (II).

Intensity Decay Kinetics and Total Light Emission Studies. Chemical-screening experiments were performed in a dark room after dark adaption of the eye, and witnessed by a second observer. Chemiluminescence intensity decay rates and relative efficiencies were determined using an RCA 1P21 phototube, biased by a Fluke Model 4128 dc power supply. The output of the phototube was amplified with a unit designed and built by Mr. John Veise (Department of Biology, The Johns Hopkins University).[26] The amplifier signal[27] was recorded on a Techni-rite chart recorder. Absolute quantum yields were determined by direct comparison with luminol using the method of Lee and Seliger.[28] A glass vial (25 mm × 75 mm) in a totally light-tight enclosure immersed in a constant-temperature bath formed the reaction cell, and the detector was placed about 12 cm above the reaction surface. In all intensity decay measurements, the appropriate volumes of stock solutions of CMC and hydroxide were mixed and brought to the desired temperature within the sample compartment. To initiate the reaction, 0.25 ml (except in experiments where persulfate was varied) of 0.20 M persulfate solution was injected through a rubber septum into the stirred sample solution to give a total volume of 5.0 ml, assuming no deviation from ideal total volume of mixing. The same procedure was used in quantum yield determinations, except that the order of addition of the persulfate and CMC solutions was reversed. Reactions were initiated by addition of 0.50 ml of a solution of CMC. Kinetic runs and quantum yield determinations with peroxymonosulfate were carried out in the same way as with peroxydisulfate. In all experiments involving allyl alcohol, the alcohol was added before the start of the chemiluminescent reaction.

Reactions in which there was continuous nitrogen or oxygen bubbling through the reaction solution were measured with the same apparatus and techniques used for other intensity decay and quantum yield measurements. To bring the desired gas into the sample compartment a long syringe needle was wired onto the gas line. Then, after adjustment of the gas flow rate, the needle was inserted through a rubber septum and into the sample solution. In runs where temperature control of the gas was desired, the gas was passed through approximately 10 ft of Tygon tubing immersed in the same constant-temperature bath used to control the reaction temperature.

Reaction of *N*-Methylacridone Ketyl with Potassium Persulfate. *N*-Methylacridone (0.42 g, 2 mmol) was added to dry dimethoxyethane (50 ml) in which sodium (0.046, 2 mg-atoms) had been dispersed. The mixture was stirred for 24 hr at room temperature under a nitrogen atmosphere. Portions (5 ml) of the dark green solution were added to solutions of potassium persulfate (0.047 g, 0.2 mmol) in water (10 ml) or in 10% aqueous sodium hydroxide solution (10 ml). Bright blue chemiluminescence was observed in both cases. Injection of the dark green ketyl solution into aqueous sodium hydroxide or into water did not produce any observable light emission.

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(26) For a more detailed description of this instrument, see D. R. Roswell, Doctoral Thesis, The Johns Hopkins University, Baltimore, Md., 1968.

(27) The amplified signal could be measured as potential *vs.* time, giving a plot of chemiluminescence intensity *vs.* time; the signal could also be collected on a capacitor, the charge of which affords a measure of the total number of photons produced by the reaction. All quantum yields except those in Table VI were determined in the latter way.

(28) The quantum yield of luminol is 0.0125: J. Lee, A. S. Wesley, J. F. Ferguson, III, and H. H. Seliger in "Bioluminescence in Progress," F. H. Johnson and Y. Haneda, Ed., Princeton University Press, Princeton, N. J., 1966, p 35.