Chemiluminescence from the Reaction of 9-Chlorocarbonyl-10-methylacridinium Chloride with Aqueous Hydrogen Peroxide[1]

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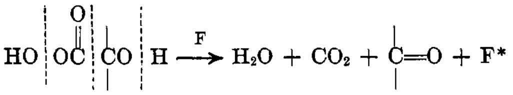
New chemiluminescent systems are described involving reactions of 9-chlorocarbonyl-10-methylacridinium chloride (V) or 9-chlorocarbonylacridine (XIII) with hydrogen peroxide. The quantum yield of the former was found to be about 1% under the conditions studied, making the reaction one of the most efficient known. Spectral comparisons suggest that the singlet excited state of the corresponding acridone is the emitting species. A mechanism is proposed involving concerted multiple-bond cleavage decomposition of a 9-carboperoxy-9-hydroxyacridan (VII) to the excited acridone. Related chemistry is described, including the anomalous hydrolysis of V to 10-methylacridone and carbon monoxide.

The results of an examination of acyl peroxide decomposition reactions for chemiluminescence have indicated that a concerted multiple-bond cleavage decomposition process is necessary for appreciable light emission.[2] Thus, in the general process



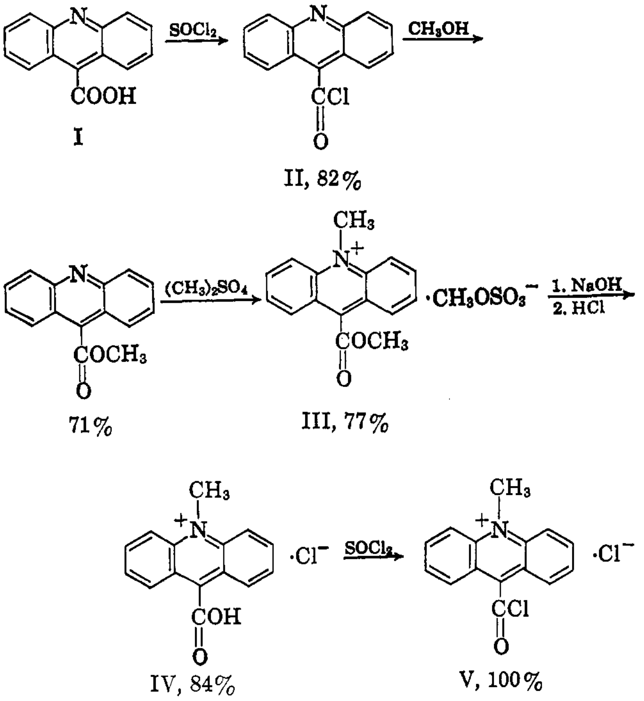
$$HO\,\vdots\,O\overset{O}{\overset{\|}{C}}\,\vdots\,\overset{|}{\underset{|}{C}}O\,\vdots\,H \xrightarrow{F} H_2O + CO_2 + \overset{|}{\underset{|}{C}}{=}O + F^*$$

where F is a fluorescent compound such as 9,10-diphenylanthracene (DPA), and F* is its light-emitting singlet excited state, it was postulated that concerted bond cleavage was necessary to provide for the simultaneous formation of several stable product molecules and the synchronous release of the 71 kcal. mole^{-1} of energy required to excite DPA. We are investigating the utility of the concerted multiple-bond cleavage concept as a criterion for the design of new chemiluminescent compounds, and we report below the discovery of new chemiluminescent reactions suggested to involve concerted multiple-bond cleavage decompositions of 9-carboperoxy-9-hydroxy-10-methylacridan and 9-carboperoxy-9-hydroxy-10H-acridan.

Results and Discussion

9-Chlorocarbonyl-10-methylacridinium chloride (V) was prepared by the route summarized in Chart I.

CHART I



Methylation of acid I to acridinium salt III was best accomplished by the three steps indicated. Direct methylation of I to III with dimethyl sulfate gave low yields. Hydrolysis of ester III to acid IV failed in several attempts under acidic conditions. Even solution of III in concentrated sulfuric acid, followed by water quenching, a standard technique for hydrolyzing hindered esters,[3] gave unchanged III. Evidently the intermediate acylium ion required for hydrolysis under such conditions is destabilized by the positive charge on nitrogen. Alkaline hydrolysis, however, proceeded easily to give the pseudo-base corresponding to IV, and IV was obtained on acidification. Evidently the steric deactivation toward hydrolysis expected for III itself[4] is relieved by formation of its pseudo-base. Reaction of acid IV with thionyl chloride gave V quantitatively.

Reaction of acid chloride V with 90% hydrogen peroxide or with anhydrous hydrogen peroxide in 1,2-dimethoxyethane or tetrahydrofuran gave little light emission or gas evolution. Dilution of such reaction mixtures with water, however, initiated a strong, long-lived chemiluminescent emission. Similar dilution with ethanol or tetrahydrofuran did not provide chemiluminescence. The reaction rate and chemiluminescent intensity were also found to be strongly pH dependent. Thus, reaction of V with hydrogen peroxide in an aqueous phthalate buffer at pH 6.7 provided a very strong but brief emission, reaction in a buffer at pH 4.0 provided a somewhat lower light intensity for periods exceeding 20 min., and in unbuffered solutions at an approximate pH of 2.5 relatively weak emission occurred over periods exceeding 1 hr. Moreover, the chemiluminescent emission from an aqueous reaction at pH 4 was essentially extinguished by the addition of dilute sulfuric acid, but chemi-

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[1] Presented in part at the Symposium on Chemiluminescence, U. S. Army Research Office, Durham, N. C., March 31 to April 2, 1965. It was learned at this meeting that F. McCapra and D. G. Richardson had independently observed chemiluminescence from V.

[2] M. M. Rauhut, D. Sheehan, R. A. Clarke, and A. M. Semsel, *Photochem. Photobiol.*, in press.

[3] L. P. Hammett and H. P. Treffers, *J. Am. Chem. Soc.*, **59**, 1708 (1937).

[4] See P. H. Gore, E. C. Vignes, and A. Feinstein, *Chem. Ind.* (London), 1514 (1958); M. Adam-Briers, P. J. C. Fierens, and R. H. Martin, *Helv. Chim. Acta*, **38**, 2021 (1955).

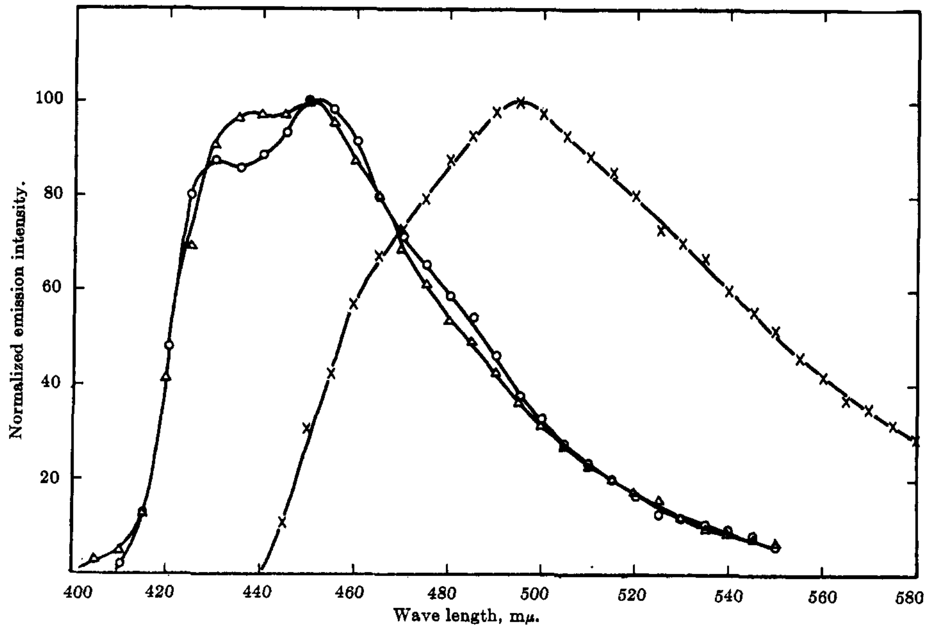


Figure 1.—Chemiluminescence and fluorescence spectra: ○, chemiluminescence emission from 1.0 × 10^{-4} M V and 1 M H_2O_2 in 55% t-butyl alcohol–water at a pH of about 3; △, fluoroescence emission from 1 × 10^{-4} M VIII in 55% t-butyl alcohol–water at pH 3; and ×, fluorescence emission from 1 × 10^{-3} M IV in water.

luminescence resumed on partial neutralization with dilute sodium hydroxide.

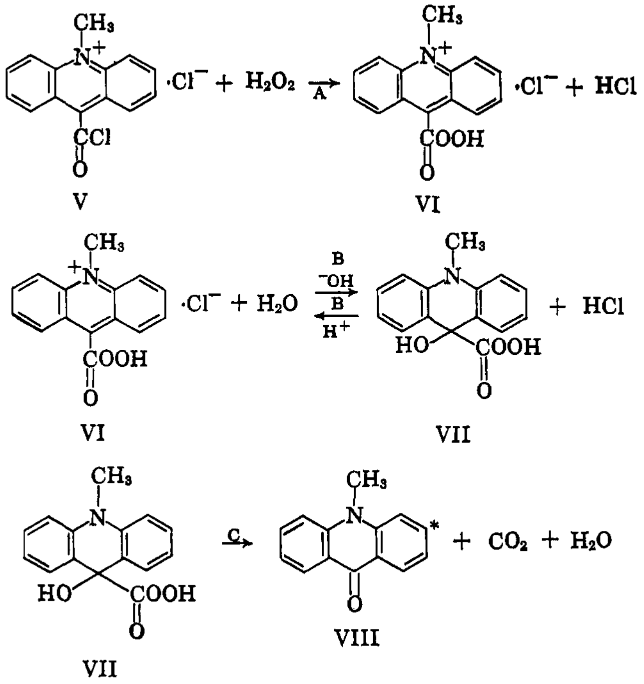
The principal product from the reaction was found to be 10-methylacridone, isolated in 52% yield from a typical chemiluminescent reaction. Smaller yields of hydrolysis product IV were also obtained. 10-Methylacridone also appears to be the emitting species in the reaction since the spectral distribution of chemiluminescence from V and hydrogen peroxide in aqueous t-butyl alcohol solution is closely comparable with the fluorescence spectral distribution of 10-methylacridone. The minor differences between the chemiluminescence spectrum and the fluorescence spectrum of VIII are within experimental error since reabsorption of chemiluminescence emission, where the light is emitted from the bulk of the solution, would not occur appreciably in the frontside fluorescence measurements, and reabsorption in chemiluminescence would account for the lower relative intensity at short wave length. We do not, however, regard comparisons of broad-banded fluorescence and chemiluminescence spectra as establishing conclusive identity of chemiluminescence emitters, since a number of different, related species can have very similar fluorescence spectra. It is clear, however, that the emitting species cannot be the acid IV, since the chemiluminescence spectrum in aqueous solution was almost identical with the spectrum pictured (Figure 1), and this is substantially different from the fluorescence spectrum of IV.

The chemiluminescent reaction does not specifically require hydrogen peroxide. Reactions of V with t-butyl hydroperoxide or peroxylauric acid were also strongly chemiluminescent, although an alkaline system was required and the reactions were short-lived.

The results are in accord with the mechanism diagrammed in Chart II.

Equilibrium B between acridinium salt VI and pseudo-base VII would be expected in aqueous solutions since pseudo-base formation is characteristic of acridinium salts.[5] In our experiments, equilibrium B is indicated by the specific requirement of water for chemiluminescence and by the increase in reaction rate with increasing pH. Moreover, formation of VIII seems best accounted for in terms of the decomposition of pseudo-base VII.

Chart II



The spectral evidence indicating that the excited state of VIII is the probable emitting species is in accord with the proposition that α-hydroxycarboperoxide VII decomposes by a concerted multiple-bond cleavage process, which accommodates the simultane-

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[5] R. M. Acheson and L. E. Orgel, "Acridines," Interscience Publishers, Inc., New York, N. Y., 1956, p. 239.

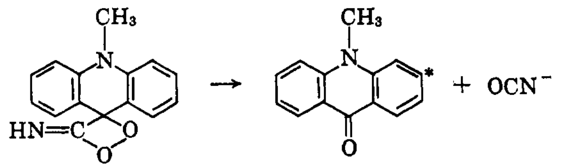
ous release of the 70 kcal. of energy required for the formation of excited singlet VIII.[2]

Reaction A is a relatively slow process in organic solvents as indicated by an experiment with 0.14 *M* V and 3.4 *M* hydrogen peroxide in anhydrous 1,2-dimethoxyethane, where the rate of disappearance of V was followed by infrared analysis. About 25 min. was required at 25° for the concentration of V to decrease by one-half, and about 60 min. to decrease to 5% of the starting concentration. Chemiluminescent reactions in aqueous solutions, however, were generally more rapid as indicated in Figure 2.

Peroxy acid VI appears to be fairly stable in acidic media, since solutions of V in 90% hydrogen peroxide retain the ability to generate light when added to water for periods of several days. A solution prepared from 0.0438 g. of V and 50 ml. of 90% hydrogen peroxide (3×10^{-3} *M* V) after standing at 25° for 3.5 hr. and diluted with water to 1×10^{-4} *M* retained 72% of the chemiluminescence efficiency of the freshly prepared solution.

Quantum Yield Determination.—The chemiluminescence quantum yield of a reaction of 2.00×10^{-4} *M* V with 0.86 *M* aqueous hydrogen peroxide was determined with a combination radiometer–fluorimeter designed and calibrated under the direction of Dr. R. C. Hirt.[6] The instrument provided for the determination of spectral distribution (Figure 1) as well as for the measurement of absolute intensity as a function of time (Figure 2). The quantum yield based on starting V was found to be 1.1%. This yield is approximately the same as the optimum reliable yields reported for 3-aminophthalhydrazide chemiluminescence under several reaction conditions.[7] Thus, V is one of the most efficient chemiluminescent materials now available. The variation of quantum yield with reaction conditions is currently under investigation and will be reported separately.

The chemiluminescence of V may be related to the recently reported chemiluminescent reaction between 9-cyano-10-methylacridinium nitrate and alkaline hydrogen peroxide, where it was suggested that cyclic peroxyimide IX was a key intermediate.[8] Additional work will be required, however, to verify this possibility.



IX

Hydrolysis of V.—Hydrolysis of V, like the reaction of V with hydrogen peroxide, occurs only slowly in acidic solutions. A solution of 2.8×10^{-2} *M* V in water after standing 1 hr. still generated strong emission on treatment with hydrogen peroxide. After 2 hr. the emission was weak. Moreover, hydrolysis does not proceed straightforwardly to acid IV, but instead gives a mixture of IV and 10-methylacridone (VIII) along with carbon monoxide. Carbon monoxide has been obtained in 34% yield along with a 57% yield of VIII.

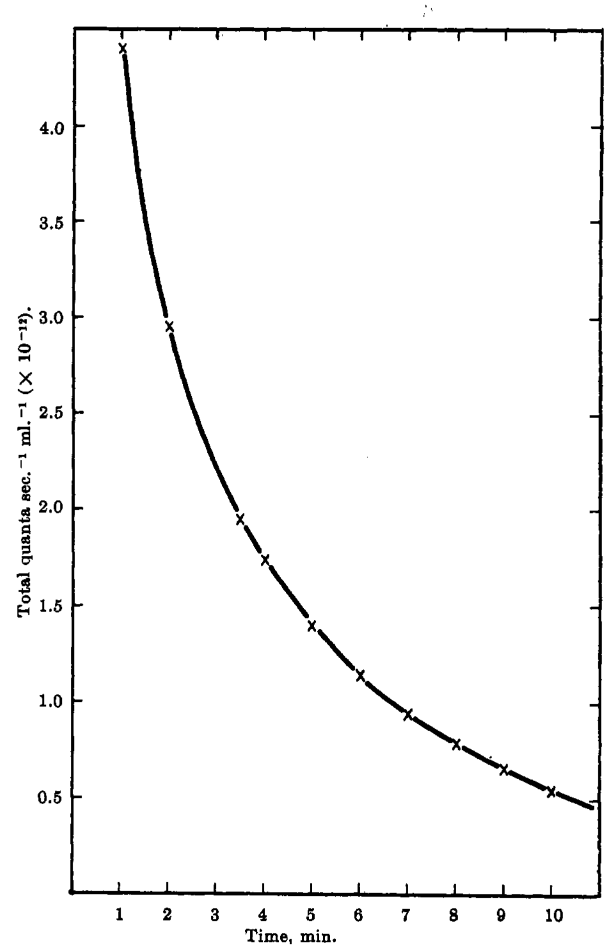
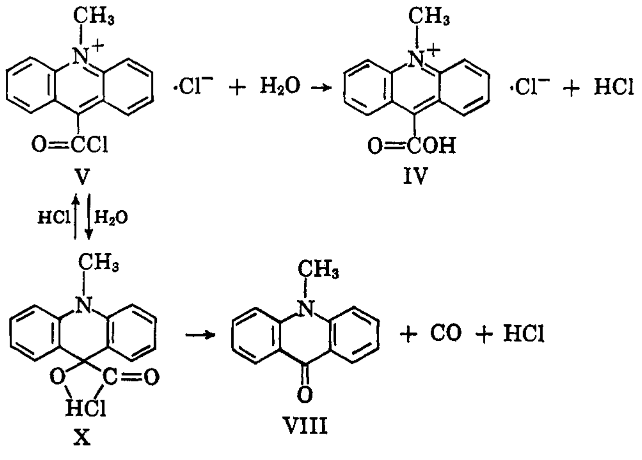


Figure 2.—Chemiluminescence emission from reaction of 2×10^{-4} *M* V with 1 *M* H_2O_2 as a function of time.

Since IV is stable (as the pseudo-base) in alkaline solution, as indicated by the conditions of its preparation, the formation of VIII and carbon monoxide from hydrolysis of V is best accounted for in terms of the intermediate pseudo-base X. Indeed, the formation of VIII from hydrolysis supports the assignment of pseudo-base VII as an intermediate in the chemiluminescent reaction of V with aqueous peroxide.



The unusual hydrolysis of V suggests that its reaction with hydrogen peroxide in the chemiluminescent reaction may give peroxy acid VI by an analogous mechanism (ee p. 3590, col. 1, top).

Reaction of 9-Carboxy-10-methylacridinium Chloride (IV) with Hydrogen Peroxide.—We examined the

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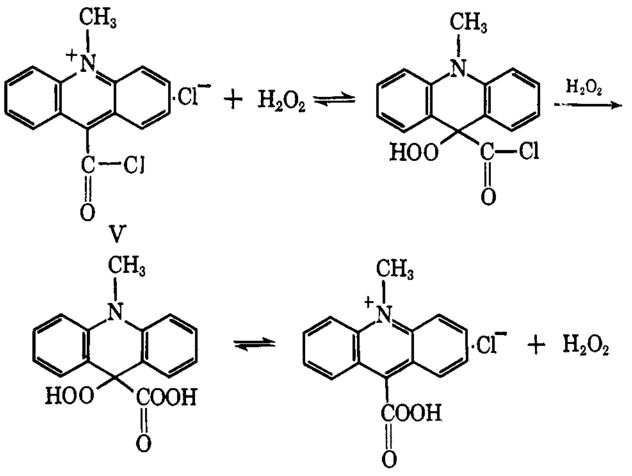
(6) B. G. Roberts and R. C. Hirt, *Photochem. Photobiol.*, in press.

(7) J. Lee and H. H. Seliger, *ibid.*, in press.

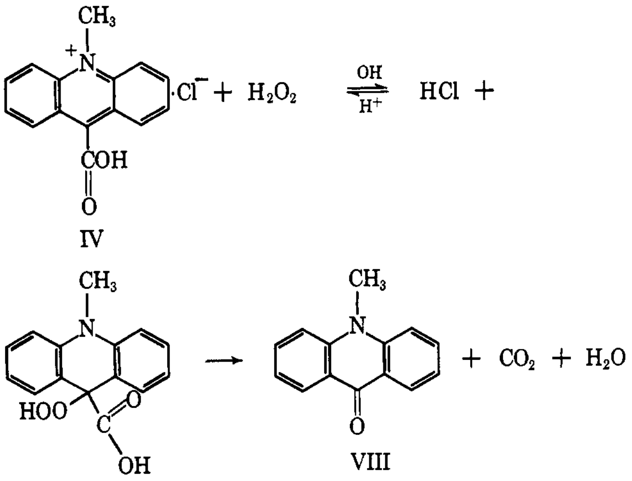
(8) F. McCapra and D. G. Richardson, *Tetrahedron Letters*, **No. 43**, 3167 (1964).



V

VI

possibility that reaction of IV with hydrogen peroxide might provide chemiluminescence according to the following process. The reaction in aqueous alkaline solution was indeed found to provide VIII very rapidly and essentially quantitatively. However, chemiluminescence was not detected. At a pH of 5.2 the reaction was substantially slower, presumably a consequence of the smaller equilibrium concentration of XI, but again VIII was obtained essentially quantitatively.

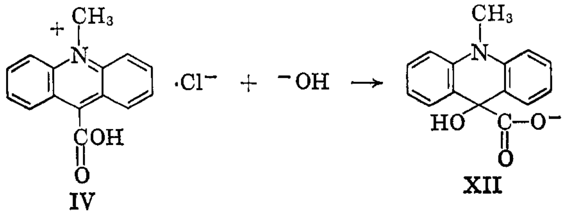


IV

XI

VIII

Reaction of IV with aqueous sodium hydroxide was accompanied by an immediate color change indicating the formation of pseudo-base XII.[5] Formation of XII in alkaline solution was further indicated by comparison of the ultraviolet spectrum of IV in water with the spectrum obtained in 0.1 *N* aqueous sodium hydroxide, where the disappearance of acridinium bands at 358, 399, and 419 mμ was noted along with other spectral changes (see Experimental Section). Addition of hydrogen peroxide to aqueous solutions of XII provided VIII only after standing at room temperature. Evidently conversion of hydroxyacridan XII to hydroperoxyacridan XI is slow under alkaline conditions where the equilibrium concentration of IV would be small.[5]

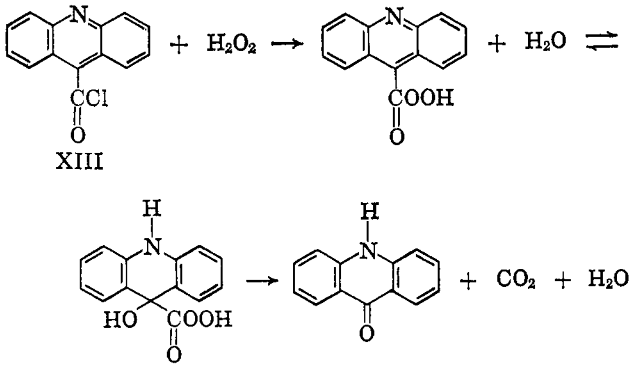


IV

XII

The failure of the decomposition of peroxyacridan XI to provide chemiluminescence is not subject to obvious interpretation. The energy released in the over-all process should be of comparable magnitude with the energy released in the chemiluminescent decomposition of VII and therefore sufficient to provide excited VIII. However, decomposition of XI is not necessarily concerted, and may proceed by a different mechanism. We are continuing to investigate this point.

Chemiluminescence from the Reaction of 9-Chlorocarbonylacridine Hydrochloride (XIII) with Hydrogen Peroxide.—Reaction of 9-chlorocarbonylacridine hydrochloride (XIII) with aqueous hydrogen peroxide in pyridine or water was accompanied by gas evolution and moderately strong blue light emission lasting on the order of 10 min. Both 9-carboxyacridine and acridone were isolated in small yields from the reaction mixture. The color of the chemiluminescent emission was that expected from acridone fluorescence. The observed chemiluminescence and formation of acridone suggest by analogy the formation of excited acridone from decomposition of hydrated 9-carboperoxyacridine.



XIII

Experimental Section[9]

9-Chlorocarbonylacridine Hydrochloride (II).—In a 100-ml. flask, 8 g. (30–33 mmoles) of the partially hydrated sodium salt of acridine-9-carboxylic acid[10] was added to 50 ml. of thionyl chloride, and the mixture heated on a steam bath for 4 hr. A clear brown solution was obtained which was added to excess hot methylene chloride, and the precipitated sodium chloride was filtered off. The filtrate was concentrated on a steam bath and heptane was added to precipitate the product. Filtration and drying afforded 7.45 g. of the yellow product, m.p. 218–218.5°. The yield, based on anhydrous starting material, was 82%.

Anal. Calcd. for $C_{14}H_9Cl_2NO$: C, 60.45; H, 3.26; Cl, 25.49; N, 5.03. Found: C, 59.77; H, 3.02; Cl, 25.42; N, 5.01.

9-Carbomethoxyacridine.—9-Chlorocarbonylacridine (30 g., 0.108 mole) was added slowly to 500 ml. of absolute methanol at room temperature. The resulting solution was neutralized with saturated aqueous sodium bicarbonate, then extracted with methylene dichloride. The methylene dichloride was removed at the aspirator, leaving a solid. The solid was recrystallized from heptane to provide 18.2 g. (71%) of the desired product, m.p. 127–128°.

Anal. Calcd. for $C_{15}H_{11}NO_2$: C, 75.95; H, 4.67; N, 5.90. Found: C, 75.50; H, 4.50; N, 6.22.

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[9] Melting points are uncorrected.

[10] K. Lehmstedt and F. Dostal, *Ber.*, **72**, 804 (1939).

9-Carbomethoxy-10-methylacridinium Methosulfate (III).—A mixture of 5.81 g. (0.025 mole) of 9-carbomethoxyacridine and 25 ml. of dimethyl sulfate was heated on the steam bath for 3 hr. The semisolid reaction mixture was triturated with toluene and the solid was collected on a filter. Recrystallization from ethanol–ether gave 6.88 g. (77%) of material, m.p. 229° dec. The infrared spectrum was in good agreement with that expected for the product.

Anal. Calcd. for $C_{17}H_{17}NO_6S$: C, 56.18; H, 4.77; N, 3.85; S, 8.82. Found: C, 56.35; H, 4.59; N, 3.99; S, 8.77.

9-Carboxy-10-methylacridinium Chloride (IV).—A solution of 21 g. (0.058 mole) of 9-carbomethoxy-10-methylacridinium methosulfate in 200 ml. water was added to 200 ml. of 10% sodium hydroxide. The deep purple solution was heated for 2 hr. on a steam bath, and the hot mixture was filtered. The dark amber filtrate was acidified with concentrated hydrochloric acid, yielding a dark green solution from which yellow needles precipitated. The solid was collected on a Büchner funnel, washed with isopropyl alcohol, and dried *in vacuo* to yield 13.3 g. (84%) of product, melting slowly above 205° dec. The infrared spectrum was in agreement with that expected for the product. Additional drying under reduced pressure at 100° over KOH for 72 hr. resulted in the loss of hydrochloric acid and the formation of the zwitterion, 10-methylacridinium-9-carboxylate, m.p. 231° dec., as indicated by infrared analysis.

Anal. Calcd. for $C_{15}H_{11}NO_2$: C, 75.93; H, 4.67; N, 5.90. Found: C, 76.28; H, 4.79; N, 6.14.

The ultraviolet spectrum of 9-carboxy-10-methylacridinium chloride in water and the spectrum of its pseudo-base (sodium 10-methyl-9-hydroxyacridan-9-carboxylate) in 0.10 *N* aqueous sodium hydroxide are summarized in Table I. The results reflect the expected loss of aromatic conjugation across the three-ring system, resulting from pseudo-base formation.[11]

TABLE I

9-Carboxy-10-methyl-acridinium chloride[a]		Pseudo-base[b]	
λ_{max}, mμ	Log ϵ	λ_{max}, mμ	Log ϵ
214	4.20	214	4.99
259	4.96	252	3.90
341	4.00	285	4.28
358	4.29	330 (s)	3.78
399	3.63		
419	3.66		

[a] 2.0×10^{-5} *M* in water. [b] 2.0×10^{-5} *M* in 0.10 *N* NaOH.

In an earlier hydrolysis attempt, a solution of 500 mg. of 9-carbomethoxy-10-methylacridinium chloride in 5 ml. of concentrated sulfuric acid was heated on the steam bath for 3 hr. The solution was poured over ice, then evaporated to dryness to obtain quantitative recovery of the starting ester. Attempted hydrolysis with refluxing concentrated hydrochloric acid overnight also gave only unchanged starting material.

9-Chlorocarbonyl-10-methylacridinium Chloride (V).—9-Carboxy-10-methylacridinium chloride, 5.0 g. (0.018 mole), was refluxed with 30 ml. of thionyl chloride for 6 hr. Boiling hexane was added to the refluxing solution until the solution became cloudy. The yellow product precipitated rapidly on cooling. The solid was dissolved in 30 ml. of boiling thionyl chloride and again boiling hexane was added to the cloud point to precipitate the product. Infrared spectral analysis indicated that the product at this stage was obtained as the SO_2Cl^- salt. The compound was heated for 24 hr. at 100° in the Abderhalden drying pistol over KOH to provide 5.3 g. (100%) of the chloride salt, m.p. 175–178° dec. (the melting point depends on the rate of heating).

Anal. Calcd. for $C_{15}H_{11}Cl_2NO$: C, 61.66; H, 3.80; Cl, 24.27; N, 4.80. Found: C, 62.14; H, 3.93; Cl, 24.08; N, 4.66.

Chemiluminescent Reactions of 9-Chlorocarbonyl-10-methylacridinium Chloride (V) with Hydrogen Peroxide. 1.—Acridinium chloride V (0.10 g.) was dissolved in 10 ml. of 90% aqueous hydrogen peroxide solution. A very weak chemiluminescent emission was observed. On dilution with water, the blue-green light emission increased in intensity with increasing dilution to a maximum at about 3 l. The lifetime of moderately strong emission under these conditions was about 20 to 30 min.

In a similar experiment using 0.0977 g. of V, 10 ml. of 30% hydrogen peroxide, and dilution to 500 ml. with water, 0.0369 g. (52%) of 10-methylacridone, m.p. 202° (lit.[12] m.p. 201–203°), identified by comparison of its infrared spectrum with that of an authentic sample, separated from the reaction mixture.

2.—Acridinium chloride V (0.05 g.) was dissolved in 5 ml. of 30% hydrogen peroxide causing a pH change from 2.5 to 1.5. Water was added to adjust the pH to 2.85. As the chemiluminescent reaction progressed the pH decreased gradually to 2.45 during 20 min. and then remained constant. Additional water was added to adjust the pH to 4.7. The intensity of emission increased substantially and then faded rapidly.

3.—A fresh solution of 0.05 g. of V in 5 ml. of 30% aqueous hydrogen peroxide was used in the following tests: (a) addition to excess 0.001 *N* hydrochloric acid provided a weak, long-lived light emission; (b) addition to excess phthalate buffer solutions at pH 4 gave a moderately bright, long-lived emission; and (c) addition to excess phthalate–phosphate buffer at pH 6.8 gave a strong brief light emission.

4.—A solution of 0.05 g. of V in 5 ml. of 30% hydrogen peroxide solution was diluted with about 100 ml. of water and treated with 25 ml. of 0.1 *N* hydrochloric acid. The emission was essentially extinguished. Addition of about 25 ml. of 0.1 *N* sodium hydroxide, then produced a bright flash of light.

Reactions of 9-Chlorocarbonyl-10-methylacridinium Chloride (V) with *t*-Butyl Hydroperoxide and with Perlauric Acid.—A solution of 0.05 g. of V in 5 ml. of *t*-butyl hydroperoxide did not yield any observable light when diluted with water. A brief light emission was produced when 1 ml. of 20% sodium hydroxide was added. Further dilution with water produced a weak, steady emission. The addition of a few drops of 50% sodium hydroxide to this solution provided bright, short-lived light.

The addition of about 0.05 g. of V to 10 ml. of a tetrahydrofuran solution of about 0.05 g. of perlauric acid did not produce an observable light emission. The dilution of the mixture with water provided a medium intensity emission.

Rate of Disappearance of 9-Chlorocarbonyl-10-methylacridinium Chloride (V) in Reaction with Hydrogen Peroxide.—Acridinium salt V (0.070 g., 0.25 mmole) was dissolved in a solution of 0.2 g. (0.059 mole) of 98% hydrogen peroxide in 1.26 g. of anhydrous 1,2-dimethoxyethane to give a 0.14 *M* solution. An aliquot was used to fill a 0.09-mm. infrared cell. A series of spectra were run in the region between 2000 and 1600 cm.$^{-1}$, using a reference cell containing the 1,2-dimethoxyethane peroxide solution. The results at 25° are summarized in Table II.

TABLE II

Time, min.	Absorbance			% acridinium acid chloride
	1780 cm.$^{-1}$ [a]	1700 cm.$^{-1}$ [a]	1720 cm.$^{-1}$ [b]	
0	Estd. 0.75	Estd. 0.65		100
5	0.67	0.57	0.06	89
10	0.61	0.51	0.09	82
15	0.57	0.47	0.15	76
25	0.34	0.28	~40	45–48
45	0.07	0.05	~60	9
60	0.04	0.03		5

[a] These bands were characteristic of 9-chlorocarbonyl-10-methylacridinium chloride. [b] This band appears to correspond to 9-carboxy-10-methylacridinium chloride formed by hydrolysis of V in a side reaction. The formation of water during the reaction interfered with measurements of the appearance of 10-methylacridone.

Hydrolysis of 9-Chlorocarbonyl-10-methylacridinium Chloride (V). 1.—Aliquots of a solution of 0.21 g. of V in 25 ml. of water were tested for chemiluminescent activity with hydrogen peroxide and water at intervals over a 3-hr. period. The light emission was still bright after 90 min. After 120 min. the emission was weaker. The chemiluminescent activity was completely gone after 180 min.

2.—The products of the hydrolysis of V were determined by dissolving 0.4953 g. of V in 100 ml. of water. After 12 hr. the

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[11] J. J. Dobbie and C. K. Tinker, *J. Chem. Soc.*, 269 (1905).

[12] I. Kröhnke and H. L. Honig, *Ber.*, **90**, 2215 (1957).

precipitate was collected, dried, and weighed to obtain 0.2058 g. (57%) of 10-methylacridone, m.p. 201–203°, lit.[12] m.p. 201–203°. The infrared spectrum was identical with that of an authentic sample.

The filtrate was evaporated to dryness, and the solid was dissolved in ethanol. Addition of ether caused the precipitation of 0.097 g. (20%) of 9-carboxy-10-methylacridinium chloride, identified by comparison of its infrared spectrum with that of an authentic sample.

3.—Evolved gas from the reaction of 22 mg. of V with excess water was swept by nitrogen into a 200-ml. syringe until the reaction was complete. The contents of the syringe were injected into a Liston Becker gas analyzer Model 15 for carbon monoxide analysis. The yield of carbon monoxide was 34.8 mole % of the V charged.

In a second experiment 35 mg. of V was treated with 1 *N* aqueous sodium hydroxide. The carbon monoxide yield was 32 mole %.

In both experiments, the CO yield is probably too low. It was observed that some of the evolved gas was trapped in a foam at the surface of the liquid phase.

Reactions of 9-Carboxy-10-methylacridinium Chloride (IV) with Hydrogen Peroxide.—A yellow solution of 10 mg. of IV in 1 ml. of 30% hydrogen peroxide was treated with 1 ml. of 5% sodium hydroxide. A yellow solid separated immediately, along with extensive gas evolution. Water was added to facilitate centrifuging the mixture. The solid was collected, washed with water, and dried. An infrared spectrum identified the product as 10-methylacridone.

In a second experiment the yellow solution of 10 mg. of IV in 1 ml. of water was treated with 1 ml. of 5% sodium hydroxide. After 10–20 sec. the solution became colorless. When there was no precipitate after 10 min., 2 ml. of 30% hydrogen peroxide were added to the solution. After 5 min. a precipitate began to form and gas evolution was observed.

In a third experiment, a solution of 0.27 g. (1 mmole) of 9-carboxy-10-methylacridinium chloride in 100 ml. of a pH 5.2 potassium acid phthalate buffer containing 10 wt. % hydrogen peroxide was prepared. After standing 2 to 3 min. a precipitate began to form. The reaction was allowed to continue for 2 hr. after which time the precipitate was collected and dried to obtain 0.19 g. (91%) of 10-methylacridone.

Light emission was not detected in these experiments.

Action of Aqueous Hydrogen Peroxide on 9-Chlorocarbonylacridine Hydrochloride (XIII) in Pyridine.—One gram of XIII was dissolved in 20 ml. of pyridine in a test tube. One-half of this solution was added to 10 ml. of a cold solution of 30% aqueous hydrogen peroxide. A steady light emission was observed changing from bright blue to blue-green with a lifetime of the order of 10 min., no solids separated and a mild gas evolution was observed. A similar result but more intense light emission was obtained using the remainder of the pyridine solution and reversing the order of addition.

The two reaction mixtures were combined, 100 ml. of water was added, and the solution was extracted twice with 100 ml. of ether. During the second extraction an orange-yellow solid precipitated from the aqueous phase and was separated. The solid was extracted with hot ethanol to leave a tan powder (300 mg., 37%) which exhibited an infrared spectrum superimposable with that of acridine-9-carboxylic acid. The ether extracts were dried over magnesium sulfate, filtered, and evaporated to afford a pale brown oil. Trituration of the oil with water gave a pale yellow solid. Filtration and recrystallization of the solid from aqueous ethanol yielded 150 mg. (21%) of acridone. The infrared spectrum of the product acridone was superimposable on that of a known sample.

A suspension of 2.8 g. of acridine chloride XIII in 50 ml. of methylene dichloride was treated at 0° with a cold solution of 1 ml. of 30% aqueous hydrogen peroxide and 1 ml. of pyridine in 10 ml. of water. A weak–medium emission developed slowly. The ice bath was then removed and emission continued for over 1 hr.

The addition of a few drops of 98% hydrogen peroxide to a suspension of acridinium chloride XIII in anhydrous ether did not produce observable light until pyridine or water was added. The water had a much greater effect on brightness.

Spectral and Quantum Yield Measurements.—The instrumentation, a combination radiometer–fluorimeter, incorporating a Bausch and Lomb grating monochromator and an Aminco amplifier with a 1P21 photomultiplier tube, and its absolute calibration has been described elsewhere.[6] The instrument provided for the absolute intensity determination of any 5-mμ wide wave length emission band as a function of wave length and as a function of time. For fluorescence spectra frontside excitation of the sample, contained in a 1.0-cm.-deep, 3.0-ml. cuvette, was provided by a General Electric F4T5/BLB fluorescent tube, which had been calibrated on an absolute basis.[6] Chemiluminescence spectra and quantum yields were determined in the same apparatus with the exciting lamp turned off.

The chemiluminescence quantum yield in einsteins per mole was calculated from the equation

$$\text{quantum yield} = \frac{\left(\int_0^{\infty} C_{\lambda'} I_{\lambda'} \mathrm{d}T\right)\left(\dfrac{\int_{\lambda^1}^{\lambda^2} C_{\lambda} I^{T}{}_{\lambda} \mathrm{d}\lambda}{C_{\lambda'} I^{T}{}_{\lambda'}}\right)}{(3.0 \times 10^{-3})[\mathrm{V}](6.02 \times 10^{23})}$$

The first product term in the numerator corresponds to the area of the corrected intensity *vs.* time decay plot at observed wave length λ′, where $I_{\lambda'}$ is the observed intensity and $C_{\lambda'}$ is the instrument calibration factor for λ′. This term was measured graphically. The second product term corresponds to the unit spectral area at time *T*. The spectral distribution and therefore the unit spectral area did not change appreciably during an experiment so that this was a constant factor for an experiment. This term can be calculated graphically from the area of a plot of $C_{\lambda} I^{T}{}_{\lambda}/C_{\lambda'} I^{T}{}_{\lambda'}$ *vs.* λ, but was actually determined with a Burroughs Datatron 205 computer, programmed with the calibration data.

Chemiluminescence Quantum Yield Measurements.—In a typical experiment, 0.00292 g. (1.00×10^{-5} moles) of V was dissolved in 50.0 ml. of 0.86 *M* hydrogen peroxide to provide a solution 2.00×10^{-4} *M* in V. Complete solution was obtained essentially instantaneously and, after shaking, a 3.0-ml. aliquot was transferred to the radiometer cuvette. The spectral distribution was measured at 3 and at 8 min. to obtain essentially identical spectra (Figure 1). The intensity as a function of time was measured at 450 mμ. A plot of quanta per second milliliter (total emission) *vs.* time is pictured in Figure 2. The calculated quantum yield was 1.1%.

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