

THE CHEMILUMINESCENCE OF ACRIDINIUM SALTS

by

DONALD GEORGE RICHARDSON

B.Sc. (Honours), University of British Columbia, 1964

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

In the Department
of
Chemistry

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1969

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and Study.

I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemistry

The University of British Columbia
Vancouver 8, Canada

Date May 4, 1969

TO ANNA

Without whose help this thesis
would not have been a reality .

ABSTRACT

A series of 9-substituted methylacridinium salts have been devised, chemiluminescent in alkaline hydrogen peroxide solutions. Their relative light intensities correspond to the expected order of reactivity with the nucleophilic hydroperoxide anion, and the mechanistic evidence is consistent with the requirement for a four-membered cyclic peroxide intermediate as the direct precursor to chemiluminescence. In every case, the actual emitter is N-methylacridone, λ_{max} 442 m μ .

The brightest compounds are a series of substituted phenyl esters. They have large rate differences (with a Hammett dependence) but have equal quantum yields, equivalent to that of the closely-related "classical" light producer, lucigenin.

TABLE OF CONTENTS

	page
INTRODUCTION	1
EXPERIMENTAL	19
PART A: SYNTHESIS	20
PART B: PRODUCT STUDIES	34
PART C: CHEMILUMINESCENCE AND FLUORESCENCE SPECTRA	35
PART D: CHEMILUMINESCENCE	37
DISCUSSION	46
CONCLUSION	64
BIBLIOGRAPHY	65

LIST OF TABLES

	page
I CHEMILUMINESCENCE OF COMPOUNDS AT pH 12	40
II KINETICS OF THE NITROPHENYL ESTERS AT pH 8	42
III pH-DEPENDENT KINETICS OF 4-BROMO PHENYL ESTER <u>34d</u>	43
IV EFFECTS OF H ₂ O ₂ CONCENTRATION ON KINETICS OF ESTER <u>34c</u>	44
V EFFECT OF SUBSTRATE CONCENTRATION ON THE KINETICS OF <u>34c</u>	45

LIST OF FIGURES

	page
1. PRODUCT FORMATION IN CHEMILUMINESCENCE	7
2. SYNTHETIC ROUTES TO THE CHEMILUMINESCENT COMPOUNDS ...	19
3. CHEMILUMINESCENCE AND FLUORESCENCE SPECTRA FOR SELECTED ACRIDINIUM SALTS	36
4. LIGHT-MEASURING APPARATUS	37
5. A TYPICAL CHEMILUMINESCENCE DECAY CURVE	39
6. HAMMETT PLOT FOR THE SUBSTITUTED PHENYL ESTERS	41
7. LOG k VS. pH, 4-BROMO PHENYL ESTER	43

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Dr. Frank McCapra, whose abilities as a chemist should serve as an inspiration to anyone working under his direction.

INTRODUCTION

INTRODUCTION

Chemiluminescence is the process of light emission from molecules formed in an excited state by chemical reaction. Increasing awareness and interest among chemists is now becoming manifest by the increasing number of publications on the subject which have appeared in recent years. Several recent reviews¹⁻⁸ contain many of the current views on the subject, and the older reviews⁹⁻¹³ furnish material of historical interest.

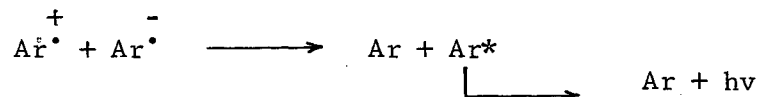
Many kinds of chemical reactions emit light. Examples are gas reactions¹⁴ and hydrocarbon oxidations^{3,7}, but often the quantum yields are so small, ie 10^{-8} to 10^{-15} , that emission cannot be detected by the naked eye.

The organic reactions in solution which emit visible light are much less common, and have aroused our interest. The quantum yields are still low, 10^{-1} to 10^{-5} , but nevertheless these reactions are efficient enough to provide a dramatic, visible demonstration of the energy released. Excepting perhaps bioluminescence (about which very little is known¹), it is possible to categorise most of these reactions into one of three general types: electron transfer reactions, excited oxygen formation, or peroxide decompositions.

Electron-Transfer Chemiluminescence

Chemiluminescence has been observed in the electrolysis of polycyclic aromatic hydrocarbons like anthracene and rubrene in the solvents DMF or acetonitrile when a supporting electrolyte is present.^{15,16} The emission occurs in the vicinity of the cathode, and its wavelength corresponds to that of the hydrocarbon fluorescence, indicating that the emitter is probably an excited singlet state of the hydrocarbon.

The proposed mechanism involves the formation of radical ions $Ar^{\cdot+}$ and $Ar^{\cdot-}$ at the anode and cathode respectively, the migration of the cation to the vicinity of the cathode, then a radical annihilation process involving an electron transfer:

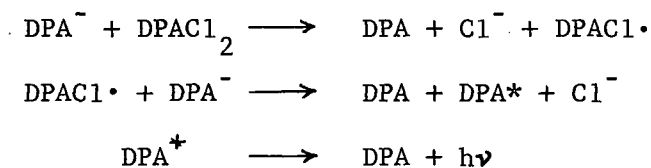


The cation could accept the electron into a high energy antibonding orbital, resulting in (most probably) the formation of an excited singlet.

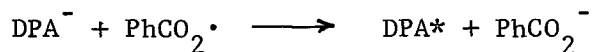
A more general type of chemiluminescent electron-transfer reaction among aromatic hydrocarbons has been described.¹⁷ This involves the addition of an electron to a radical cation or the removal of an electron from a radical anion yielding, in both cases, the parent hydrocarbon in an excited state.

In a typical example, the radical anion of 9,10-diphenylanthracene (DPA) is generated by treatment with sodium. When mixed with 9,10-dichloro-9,10-dihydro-9,10-diphenylanthracene (DPACl₂), probably reacting as $DPA^{\cdot-}$, a bright chemiluminescence is observed, and DPA is produced in very high yield. The light emitted is identical to the fluorescence of DPA.

A reasonable explanation for the formation of excited DPA (and hence the emission of light) is that DPACl₂ receives an electron from $DPA^{\cdot-}$ into its lowest antibonding orbital, resulting in excited DPACl₂⁻. This species could lose chloride ion to yield, directly, excited DPA with an antibonding electron which, when falling to a half-filled bonding orbital, ejects a photon.



Another chemiluminescent reaction, that of DPA^- and the oxidising agent benzoyl peroxide, is thought to involve the transfer of a bonding electron in DPA^- to a benzoate radical:



Excited Oxygen Formation

Because most of the organic chemiluminescent reactions involve either oxygen or hydrogen peroxide, speculation arose that the emission might be due to some species of excited oxygen.

Recent work by Ogryzlo *et al*¹⁸ has identified the principal emission bands of excited oxygen. The reaction $\text{Cl}_2 + (\text{H}_2\text{O}_2 + \text{NH}_3)$ emits a red glow in the region 550 to 1300 $\text{m}\mu$. The strongest bands (634 and 703 $\text{m}\mu$) are due to a complex of two excited O_2 molecules undergoing the transition $[\text{O}_2(^1\Delta_g)]_2 \longrightarrow [\text{O}_2(^3\Sigma_g^-)]_2$.

These results appear to exclude the participation of excited oxygen in the brighter organic chemiluminescent reactions, whose emissions are much more energetic, emitting light in the region 400 to 500 $\text{m}\mu$. A recent attempt¹⁹ to implicate excited oxygen in these more energetic emissions, by invoking a $[^1\Delta_g + ^1\Sigma_g^+]$ state, has been proven incorrect for at least two classes of compounds by work in our laboratories.

Organic Peroxide Decompositions

For all of the brightest organic chemiluminescent reactions in solution, the evidence so far obtained has either proven or, in some cases, strongly inferred that the light-producing step requires the decomposition of an organic peroxide to yield a molecule with an electronically excited carbonyl-containing chromophore. Before examining some of the best-known chemiluminescent reactions, some mention should be made of the more general aspects common to all.

That an excited carbonyl is responsible for light emission has been convincingly demonstrated in systems where the emission spectrum matches exactly with the fluorescence spectrum of a carbonyl product. The conditions under which these light-producing reactions occur are such that the singlet excited states⁴ are more likely than triplets⁴:

(1) The solutions are normally at room temperature, where vibrational and collisional deactivation of triplets is very pronounced.

(2) Oxygen, a proven triplet quencher, is often present.

As fluorescence is known⁴ to involve radiative transitions from an excited singlet to a ground state singlet, it is reasonable to say that the actual chemiluminescent emitter is the excited state of whichever product has a fluorescence spectrum identical to the light emission spectrum.

In cases where the reaction chemiluminescence and carbonyl product fluorescence do not match, identification of the emitter is more difficult. Colored byproducts can absorb some of the emitted light and hence shift the emission λ_{\max} ; alternatively, energy transfer from the excited product to a more highly fluorescent byproduct or impurity can result in a spurious emission spectrum. In these more difficult examples, high dilution (with less self-absorption) is helpful. This has been made feasible by the newer, more sensitive, spectrometers now available. The problem of interfering fluorescent compounds requires that a rigorous product study be made in order to identify the actual primary emitter.

The exact mechanism by which chemical energy results in an electronically excited species, capable of emitting light, is not known. However, work by our group^{20,21,22} and others^{23,24} has yielded a realistic working hypothesis which accounts for much of the behaviour observed for the brightest chemiluminescent reactions. This hypothesis has allowed us to predict

successfully the relative brightness of the new compounds synthesized during this investigation. The mechanism envisions the concerted multiple bond rearrangement or decomposition of an organic peroxide to yield a carbonyl chromophore in an electronically excited state.

There are some important requirements inherent in the proposed mechanism:

- (1) As the wavelength of the emitted light is usually in the region 400 to 500 μ , corresponding to ca. 70 kcal mole⁻¹ of energy, the reaction must be exothermic by at least this amount.²⁵ Peroxides, with their weak O-O bond (requiring only 33 kcal for cleavage) are especially suitable compounds.
- (2) The bond rearrangement process must be a very rapid single step, preferably concerted, because there is no mechanism available for energy accumulation and storage.
- (3) The geometry of the bond rearrangement must be favorable for efficient formation of the excited state. In this respect, the involvement of a transient four-membered cyclic peroxide transition state or intermediate is particularly attractive for several reasons:
 - (a) It is rigid and compact, less prone to vibrational deactivations;
 - (b) By O-O and C-C bond cleavage it yields directly two carbonyl groups, often within the same molecule; one of them could well have acquired the necessary 70 kcal of energy which is required (total energy released in the cleavage: ca. 108 kcal);

- (c) Its conformation resembles that of the resulting excited carbonyl group, tetrahedral²⁶. Moreover, it can be argued on orbital symmetry grounds that a concerted decomposition would yield one carbonyl in an antibonding excited state.²⁷
 - (d) A cyclic peroxide intermediate has been proposed in the non-chemiluminescent oxidations of α -diketones²⁸, nitriles²⁹, and enamines³⁰;
 - (e) Very recently, cyclic peroxides have been isolated and characterised; moreover, chemiluminescent decomposition is observed when heated in the presence of a fluorescent acceptor.³¹
- (4) For bright emission, the excited carbonyl chromophore should be in direct conjugation with a fluorescent π -system. Other things being equal, the brightness will be directly proportional to the fluorescence efficiency Φ , ie the fraction of energy which the excited singlet releases in a radiative decay to the ground state. In those cases where the carbonyl product is not fluorescent, the close proximity of a fluorescent molecule can result in energy transfer and subsequent emission by the latter molecule.

Although the exact mechanism of light production is still unknown, the following is a reasonable picture of the principles involved. The quantum yield Q is governed by at least three factors:

- (1) The fluorescence efficiency Φ for the emitter molecule,

- (2) The fraction of these molecules finding themselves in an excited state
- (3) The extent of "dark" side reactions giving non-fluorescent by-products.

Some of the "classical" chemiluminescent reactions give quantitative yields of a single product, the proven emitter, yet have a Q of 1% or less. Unless the fluorescence has been quenched by solvent or some other solute,³² then (2) must be of crucial importance in obtaining a bright emission.

Product formation can be envisioned as proceeding by either of the two routes:

- (1) A so-called "light" path leading directly to the excited product, and
- (2) a "dark" path resulting in ground-state product.

See Figure I.

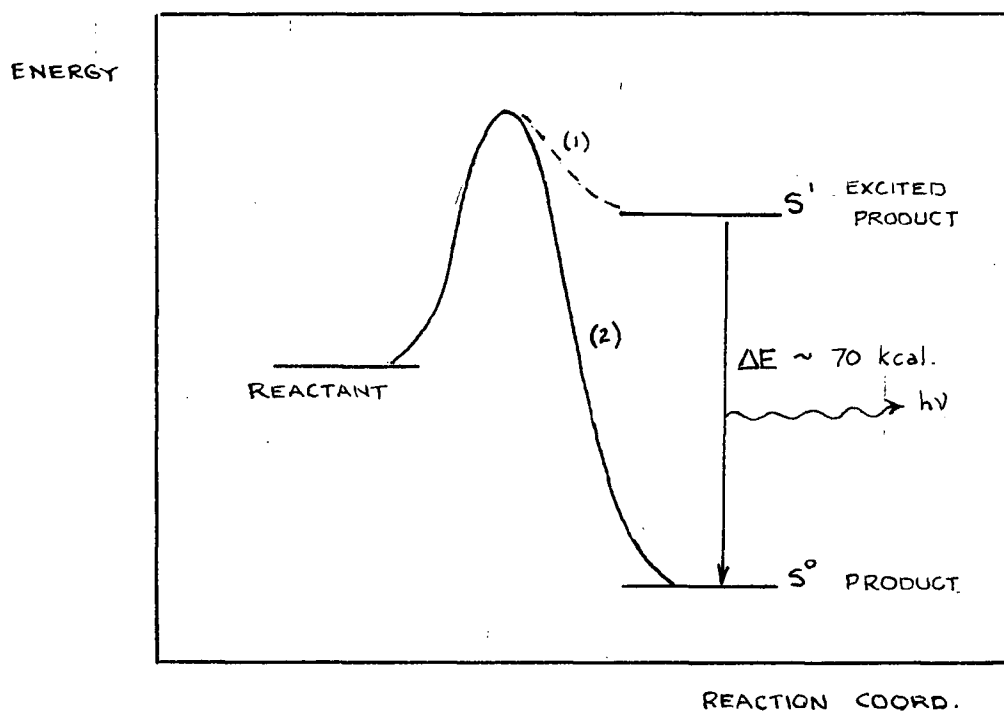


Figure I: Product Formation in Chemiluminescence

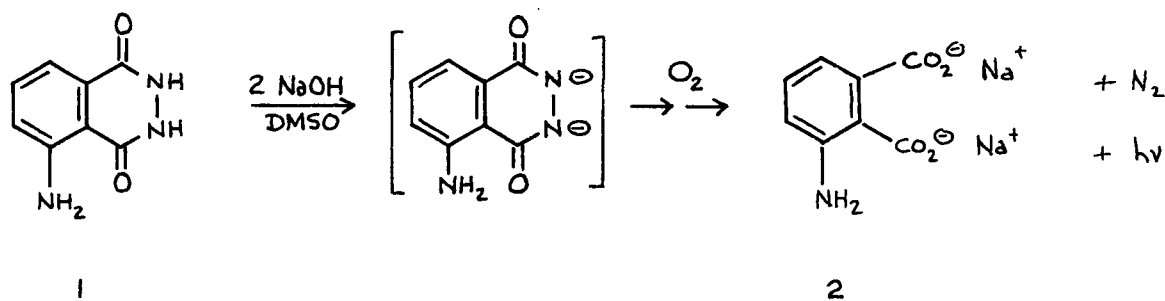
The differences, if any, in the early part of the two paths are unknown. The overall reaction mechanisms may differ, or, if the same mechanism is shared by both paths, then unknown geometric factors must play a part. We have obtained evidence that, in our series of compounds, both possibilities are contributing factors (see discussion).

Let us now apply these criteria to the brighter of the organic chemiluminescent reactions.

Luminol

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (1) is chemiluminescent when an alkaline solution containing oxygen or hydrogen peroxide is treated with an oxidizing agent such as potassium ferricyanide.^{5, 13}

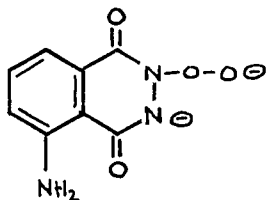
The chemical reaction and light emission step have been well characterized by White and co-workers,³³ who established the stoichiometry but not the mechanism for the reaction in dimethyl sulfoxide, where an oxidizing agent (other than oxygen) is not required.



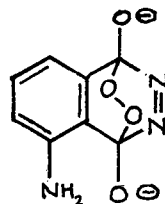
The aminophthalate anion 2 was unambiguously identified as the emitter by the congruence of its fluorescence spectrum with the emission spectrum ($\lambda_{\text{max}} 485\text{m}\mu$). Details of the mechanism are not known, but the oxidative addition of O_2 to the luminol dianion is thought to proceed through an adduct which, upon cleavage, could yield triplet ground-state nitrogen

and a vibrationally excited triplet aminophthalate molecule. Intersystem crossing to an isoenergetic level of the excited singlet state and subsequent emission of a photon completes the process.

Although no intermediates have been isolated, the nature of the adduct has been speculated upon⁵; 3 or 4 are possibilities.



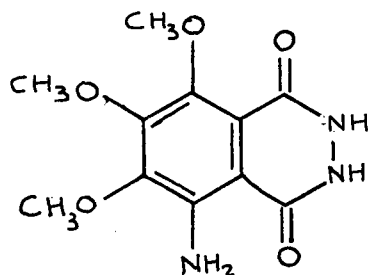
3



4

Note that both are peroxides, and that 4 resembles the four-membered cyclic peroxides described earlier.

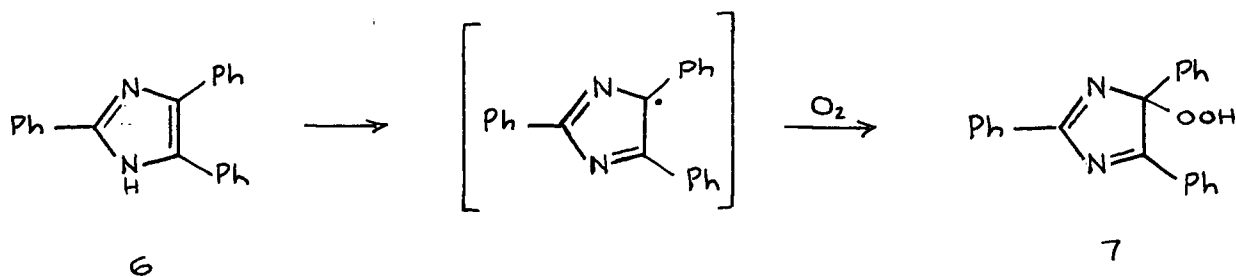
The quantum yield Q for luminol is about 5% in DMSO³³ and about 1% in aqueous systems.³⁴ Electron-releasing ring substituents enhance Q for the aminophthalate anion (10% with no substituents).^{35,36} This increases the quantum yield, so derivatives such as 5 are brighter than the parent luminol.



5

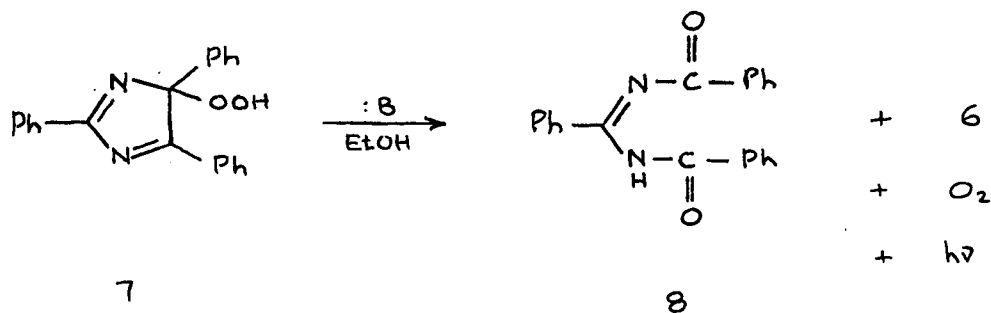
Lophine

Lophine (2,4,5-triphenylimidazole) (6) is chemiluminescent on reaction with oxygen in alkaline solutions, and the light is enhanced by oxidizing agents.³⁷ The suggestion³⁸ that a free radical may be involved has been substantiated³⁹; reaction of this radical with oxygen results in the corresponding hydroperoxide 7.^{40, 41}



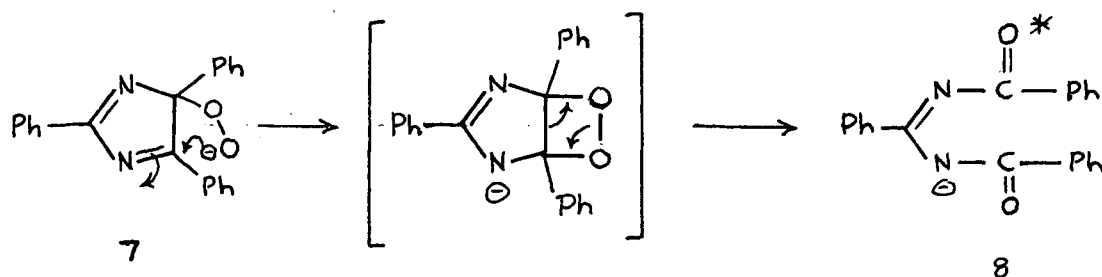
Lophine itself is weakly chemiluminescent in strongly alkaline solutions containing oxygen and, preferably, an oxidizing agent. Considerable degradation occurs, making identification of the emitter difficult.

The corresponding peroxide, however, is much more efficient, requiring only a mild base for bright emission. The products isolated from the peroxide decomposition are the dibenzoylbenzimidine 8 (70%) and lophine itself (20%).

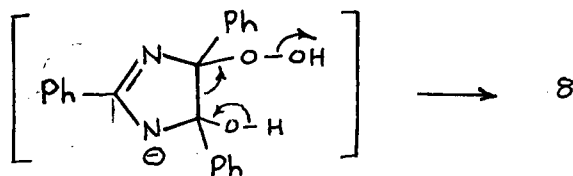


A series²⁴ of ring-substituted lophines, and their corresponding hydroperoxides, are also chemiluminescent. With at least two of these derivatives exact matching of the emission spectrum with the fluorescence spectrum of the amidine anion product has proven that the anion is the emitter. Moreover, the light enhancement by electron-releasing phenyl ring substituents again indicates that the fluorescence efficiency of the product is an important factor. A similar study⁴² of ring-substituted lophines has established a Hammett relationship for the light intensities, but the results are almost certainly due to differences in fluorescent efficiencies and not the oxidation rates.

The mechanism of lophine peroxide decompositions can be conveniently depicted as an internal rearrangement via the cyclic 4-membered peroxide, yielding directly the amidine anion in an excited singlet state, resulting in emission of light.



The observation²⁴ that chemiluminescence occurs in anhydrous solvents is evidence that an alternative mechanism by Rauhut *et al*²³ for the peroxide breakup does not operate here. This is an important

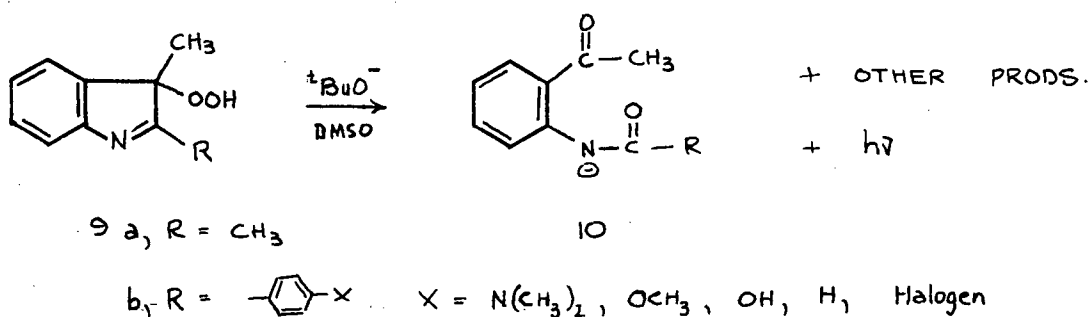


conclusion, and has direct bearing on our work. (see discussion.)

Indoles

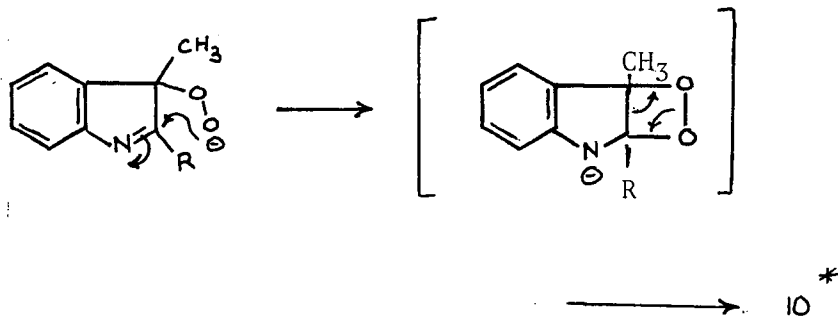
Interest in the chemiluminescence of substituted indoles is justified by the recent structural elucidation of the bioluminescent luciferin of the crustacean *Cypridina hilgendorffii*, in which an indole moiety is involved.⁴³

Work in these laboratories²² has resulted in the synthesis of a series of indolenyl 3-hydroperoxides 9, chemiluminescent in dimethyl sulfoxide/potassium t-butoxide.



The amides 10 have been isolated in high yield, and are the proven emitters by the fluorescence spectra of their anions, whose fluorescence efficiencies are directly proportional to the chemiluminescence intensities of the parent peroxides.

The preparation of an ¹⁸O-enriched peroxide and its subsequent rearrangement, without dilution of label, to the corresponding amide is offered as direct proof that the four-membered cyclic transition state is involved, analogous to that for lophine:

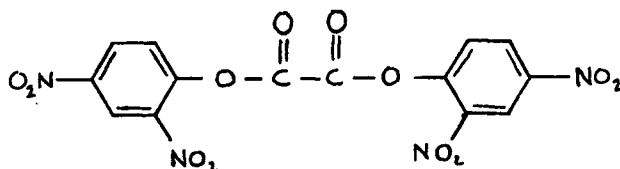


In the peroxides 9b, a Hammett relationship has been established for the light intensities, most probably due to the differing fluorescence efficiencies of the amides.

Secondary Emissions

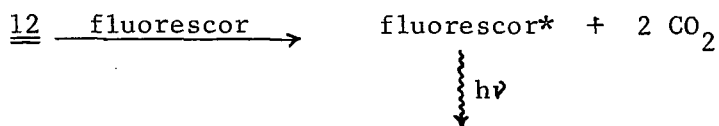
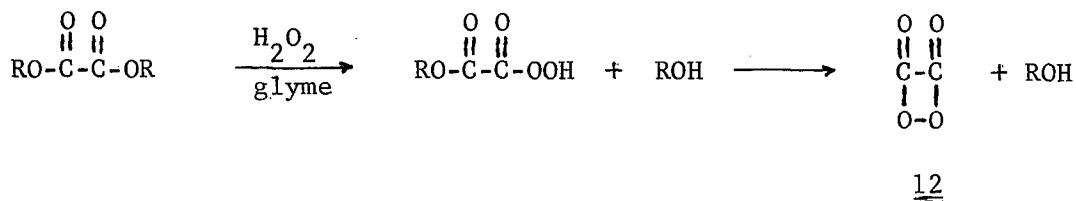
As previously discussed, bright chemiluminescence may occur if the newly formed excited carbonyl group is conjugated with a fluorescent π -system. In the cases where this product is not fluorescent, then energy transfer to a fluorescent molecule in situ can yield light.

Thus, the remarkable results of Rauhut and co-workers⁴⁴ have established a whole new family of chemiluminescent reactions with Q values as high as 23%. One class of reactants is of special interest. Selected aryl oxalate esters (eg, 11) are very efficient light producers when treated with hydrogen peroxide in the presence of a fluorescent acceptor like DPA or rubrene.



11

The reaction stoichiometry and emission spectra are consistent with the following proposed mechanism:



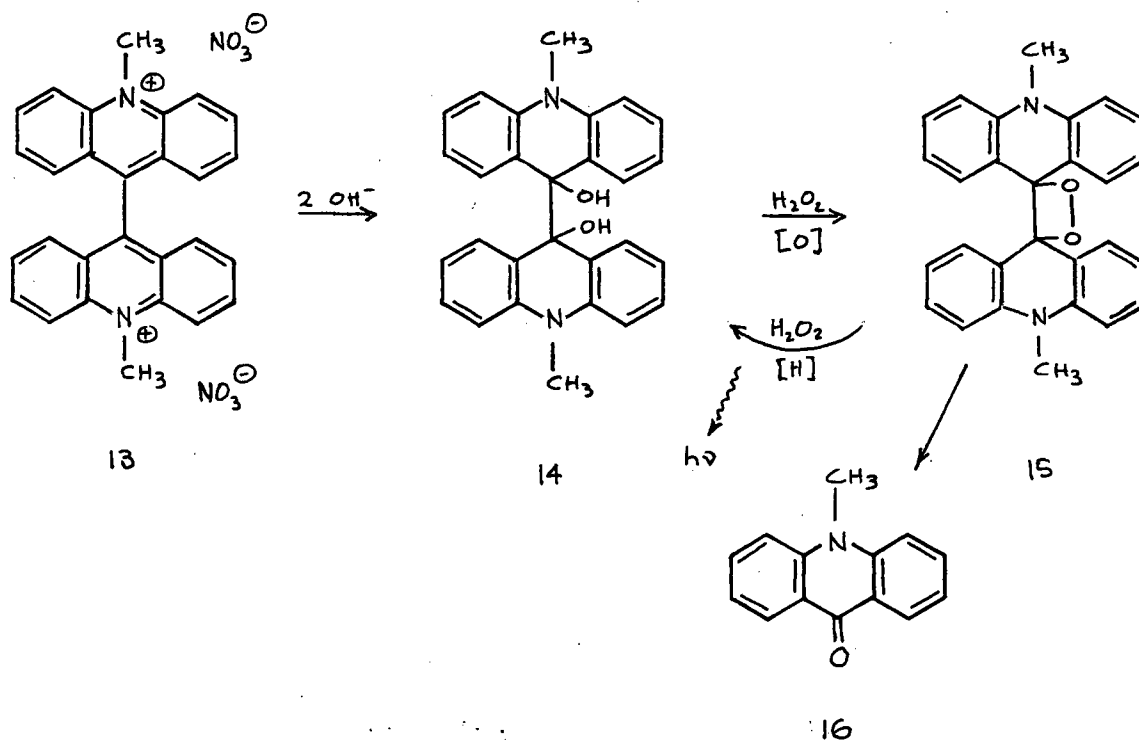
Moreover, there is evidence that cyclic peroxide 12, while not isolable, is a discrete intermediate.

Lucigenin

The investigation of the light emission by lucigenin (10, 10'-dimethyl 9,9'-biacridinium dinitrate) (13), have been, on the whole, rather unsatisfactory. The compound is chemiluminescent in aqueous alkaline solutions containing oxygen or, better yet, hydrogen peroxide. The blue-green emission is enhanced by the addition of alcohol, pyridine, ammonia, and (especially) osmium tetroxide;⁴⁵⁻⁴⁷ but the function of these "catalysts" has not been adequately explained to date.

Most investigators have sought to implicate various kinds of excited biacridinyl molecule as the emitter, on the grounds that lucigenin and its reduced forms are green-fluorescent, comparable to the usual emission spectrum of lucigenin (480-525 m μ).

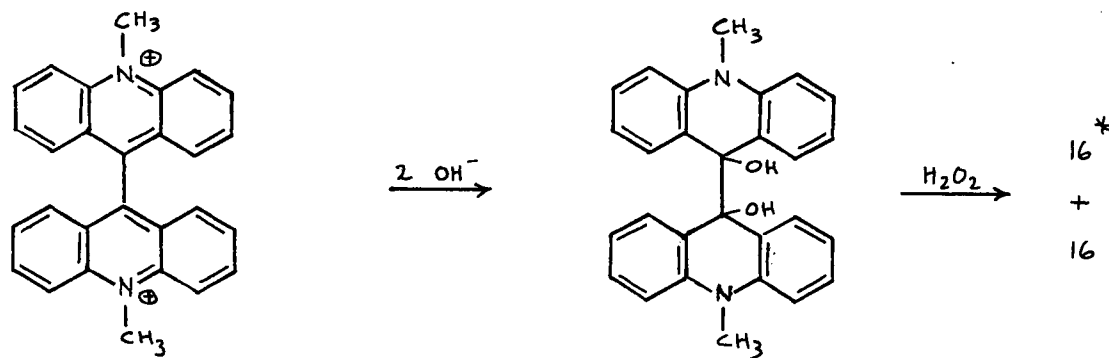
Gleu and Petsch,⁴⁷ the discoverers of this phenomenon, had postulated a mechanism involving the formation of a lucigenin dicarbinol pseudobase (14) which underwent oxidation by H₂O₂ to the cyclic peroxide (15) followed by reduction from a second H₂O₂ molecule, the actual light emission step:



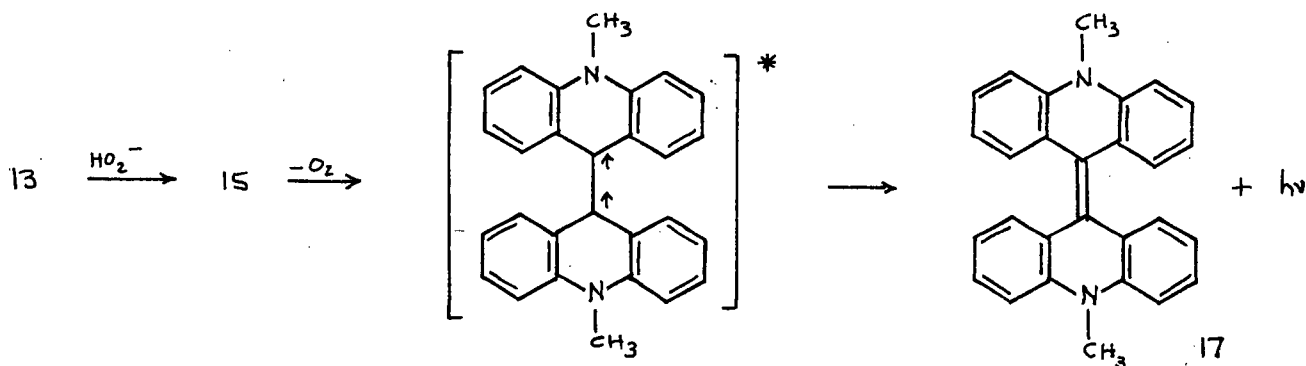
Decomposition of 15 to N-methylacridone 16, the only identifiable product, was thought to be a side reaction which terminated the emission by destroying the reactive peroxide substrate.

Tamamushi⁴⁸ envisioned the oxidation of lucigenin by molecular oxygen, resulting in a 10,10'-dimethyl-9,9' biacridinyl diradical which then formed the peroxide 15. Subsequent reduction by H_2O_2 supposedly resulted in excited pseudobase, the claimed emitter.

Kautsky and Kaiser³⁸ then re-examined the chemiluminescence spectrum of lucigenin and found that at lower concentrations and higher temperatures the emission was blue, not green. Moreover, the emission spectrum corresponded with the N-methylacridone fluorescence, thus indicating N-methylacridone was the primary emitter and that the lower-energy green chemiluminescence arose by energy transfer to a highly fluorescent species co-occurring. This species was assumed to be lucigenin itself. The overall reaction was proposed as:



In spite of these findings, later workers continued to devise mechanisms involving excited biacridinyl species in order to account for the green emission. Kurtz⁴⁹ proposed loss of O_2 by the cyclic peroxide 15 would yield an excited triplet of N,N-dimethylbiacridene 17.



The same process was suggested by Karyakin,⁵⁰ who claimed that phosphorescence of 17 was occurring (at room temperatures).

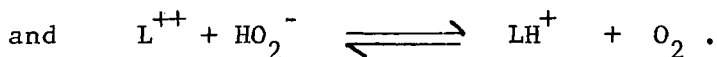
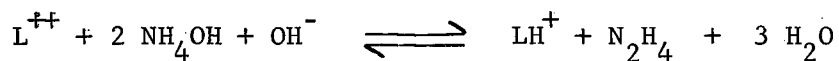
Kroh envisioned formation of the pseudobase 14 from the biacridene 17 or its oxide to be the light emission step.

Erdey^{52a} had originally supported the mechanism of Gleu, but more recently^{52b} reiterated the Kurtz proposal, in which the excited biacridene transferred energy to an added fluorescent acceptor molecule (eg, fluorescein).

Grigorovsky and Simenov⁵³ synthesized the biacridene 17 and its oxide, and found them to be spontaneously chemiluminescent in organic solvents without benefit of base or oxidizing agent. Brighter emission was obtained with added H_2O_2 , and the compounds remained unchanged without formation of N-methylacridone. Evidence was presented to indicate that these compounds were merely catalysts for the exergonic breakdown of hydrogen peroxide.

The claimed spontaneous chemiluminescence of 17 was also reported by Totter⁵⁴. He identified the primary emitter as N-methylacridone by performing the reaction in mixed solvents (aqueous pyridine or alcohol) at low lucigenin concentrations. The necessity for reduction of lucigenin when reacting with oxygen led him to propose an eleven step enzymatic

reduction process with complex rate equations, totally without experimental verification. In a subsequent consideration of the reaction without enzymatic reduction, Totter⁵⁵ proposed the reduction of lucigenin (L^{++}) by ammonia or hydrogen peroxide according to the reactions



With the exception of Kautsky and Kaiser, we consider all the investigations of lucigenin reported here as too contradictory to be reliable.

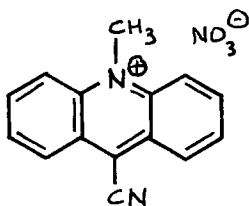
The lucigenin reaction is a complex one. The yields of the proven emitter, N-methylacridone, are only 50% at best. The alkaline peroxide solution is generally yellow and has a green fluorescence, indicating the possibility of a reduced biacridan species, arising perhaps by an unknown disproportionation process. Certainly we might expect the pseudobase 14 to be involved, since acridinium salts are known to react readily in this way.⁵⁶ Transformations thereafter have not been properly investigated to date.

We interpret the chemiluminescent portion of the lucigenin reaction as being a nucleophilic attack by hydrogen peroxide yielding, ultimately, the cyclic peroxide 15 which undergoes cleavage to give N-methylacridone 16 and light. The exact details of this reaction are not yet clear, but a partial picture now exists and will be discussed later. Certainly, much insight was gained by analogy with the acridinium salts devised and studied in this investigation.

Our initial approach to the lucigenin problem was as follows: if the chemiluminescence of biacridinium salts is accompanied by complicated side reactions that obscure the salient features of the light-producing steps,

then the synthesis of a suitable model compound, a 9-substituted N-methylacridinium salt (whose 9-substituent is prone to nucleophilic attack by hydroperoxide anion), might result in a chemiluminescent compound whose behavior under the same reaction conditions would be less complicated.

Synthesis of 9-cyano-10-methylacridinium nitrate 23 confirmed our predictions in every respect. The compound yielded light and, in virtually quantitative yield N-methylacridone, identified unambiguously as the emitter.



23

Encouraged by this early success,²⁰ we have now prepared a series of thirteen additional related compounds whose relative light intensities obey perfectly their predicted reactivities with hydrogen peroxide, providing strong evidence for the general reaction mechanism envisioned for them.

EXPERIMENTAL

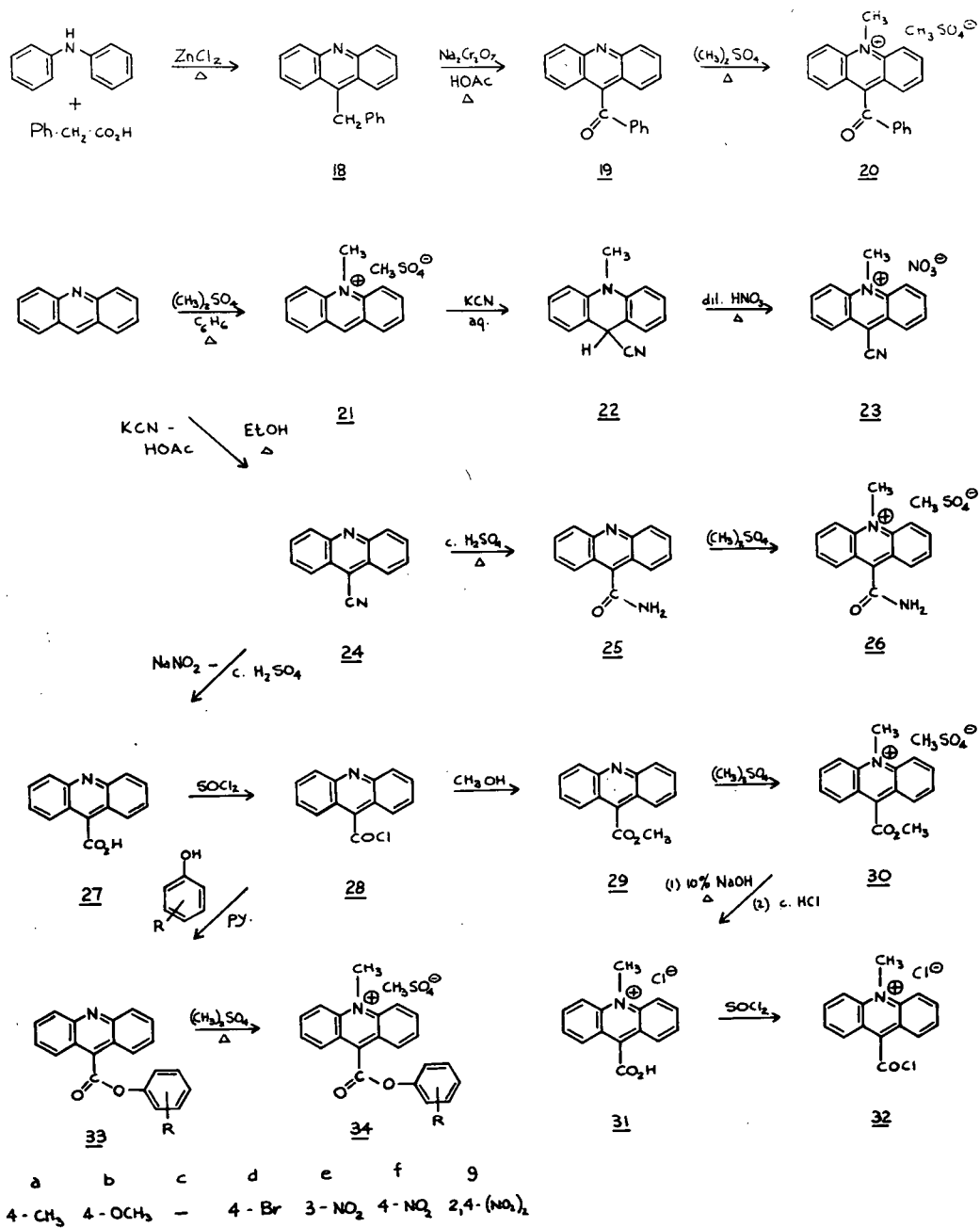


FIGURE 2: Synthetic routes to the chemiluminescent compounds.

PART A: SYNTHESIS

The synthetic routes to the chemiluminescent compounds are shown in Figure 2. For the most part, standard methods were employed, and the expected results were usually obtained. Occasionally, however, the reactions showed unusual features that merit special comment.

In the N-methylation of the acridine phenyl esters 33, the reactivity towards dimethyl sulfate decreased markedly when there was an electron-withdrawing ring substituent. This effect was not expected, as there is no direct conjugation between the functional groups involved. It would appear, therefore, that a large degree of inductive transmission through the ester oxygen exists.

Similarly, the acridine acid 27 was unreactive towards hot neat dimethyl sulfate. The reasons for this are even less clear, because the carboxylate group is essentially un-ionized in the acidic medium of dimethyl sulfate. Consequently, the desired acridinium acid 31 was obtained by alkaline hydrolysis of the corresponding methyl ester 30.⁵⁷

For some of the acridinium salts, melting points have not been precisely determined. There is evidence of slow decomposition upon heating, and the melting points varied according to how rapidly they were approached. A more satisfactory determination could probably be made using differential thermal analysis.

Unless otherwise noted, all infrared spectra were measured on a Perkin-Elmer 137 Infracord (nujol mulls), and ultraviolet spectra were recorded on a Beckman DK-2 spectrometer (95% ethanol, distilled). The melting points were measured on a Kofler hot stage, and are uncorrected.

9-Benzylacridine 18

The Bernthsen reaction⁵⁸ was utilized for the formation, in poor yield, of 9-benzylacridine. A mixture of 30 gm (0.22 moles) phenylacetic acid, 24 gm (0.14 moles) diphenylamine, and 40 gm (0.30 moles) freshly fused zinc chloride were heated at 200° for 15 hours with occasional stirring. The resulting black tar was poured into 300 ml of concentrated ammonia; a bright yellow solid and a black tar precipitated out. Careful decantation of the yellow compound, followed by suction filtration and washing with water, gave (after drying) 9.5 gm (25%) of crude product which, after repeated recrystallization from benzene, had mp 170-173° (lit.⁵⁹ mp 173°).

9-Benzoylacridine 19

A mixture of 5 gm (0.185 moles) 9-benzylacridine and 4 gm (0.135 moles) sodium dichromate in 100 ml glacial acetic acid was refluxed for 2.5 hours, and the resulting orange solution was diluted five-fold with water.

The insoluble reddish product was suction filtered and washed well with water, then air-dried. Upon sublimation (220°, 0.2 torr) a bright yellow solid was collected which was then recrystallized by dissolving up in chloroform and then adding ethanol. After boiling off most of the chloroform and cooling, a bright yellow mass of prisms was collected; 3.7 gm (70%), mp 214-216° (lit.⁶⁰ mp 214-216°).

10-Methyl-9-benzoylacridinium methosulfate 20

A mixture of 2 gm (0.007 moles) 9-benzoylacridine and 2 ml (2.7 gm, 0.0215 moles) methyl sulfate was heated on the steambath for 2 hours. The resulting semisolid was triturated several times with ether, then dissolved in methanol and precipitated out with ether.

Only a portion was water-soluble, so the product was washed with water and the aqueous washings were evaporated to dryness. Again, re-crystallized from methanol-ether, a bright yellow solid, 1.2 gm (40%), mp 293-295° was obtained.

anal. Calcd for $C_{22}H_{19}NO_5S$: C, 64.54; H, 4.68; N, 3.42%

found : C, 64.32; H, 4.27; N, 3.04%

ν_{\max} 1665 cm^{-1} (C=O)

λ_{\max} 242 m μ (sh) (ϵ 43,000), 248 (sh) (64,000), 253 (97,000),
350 (10,800), 365 (13,000)

N-methylacridinium methosulfate 21

A mixture of 25 gm acridine (K & K Chemicals), 5 ml of methyl sulfate, and 15 ml of benzene were warmed in a flask equipped with a reflux condenser. The exothermic reaction was occasionally moderated by brief cooling with an ice bath.

After 30 minutes, the bright yellow solid was triturated with ether and filtered. Washed well with ether and then with acetone, the bright yellow product, a strong lachrymator, was dried in vacuo to avoid air oxidation. The product, 14 gm (33%) was used without further purification. It blackens without melting above 300°.

9-cyano-10-methylacridan 22

To an aqueous solution of N-methylacridinium methosulfate was added, dropwise, a saturated solution of potassium cyanide until precipitation of the insoluble product was complete.

It was filtered by suction and washed well with water, dried in vacuo, then recrystallized from acetone; colorless prisms, mp 110-112^o, remelting at 160^o, was obtained (lit.⁶¹ mp, 143^o).

anal. Calcd for C₁₅H₁₂N₂ : C, 81.79; H, 5.49; N, 12.72%
found : C, 81.83; H, 5.29; N, 12.67%

ν_{\max} 2280 cm⁻¹ (CN)

λ_{\max} 278 m μ (ϵ 8,000)

9-cyano-10-methylacridinium nitrate 23

The acridan (10 gm) was dissolved in 50 ml of dilute nitric acid and warmed on the steambath for 1 hour. Upon cooling, large orange crystals deposited. Concentrating the mother liquors afforded a little more product, which was combined with the first crop and recrystallized from ethanol. A total of 9.5 gm (75%) of orange needles, mp 160-162^o (dec.) was isolated.

anal. Calcd for C₁₄H₈N₃O₃ : C, 64.04; H, 3.94; N, 14.94%
found : C, 64.27; H, 4.15; N, 14.81%

λ_{\max} 264 m μ (26,900), 368 (2,820), 386 (6,000)

9-cyanoacridine 24

To 45 gm (0.25 moles) acridine and 16 ml of glacial acetic acid in 200 ml of ethanol was added 25 gm (0.40 moles) potassium cyanide in 35 ml of water. The solution was heated to reflux with mechanical stirring; and, within a short time, the product began to crystallize out.

After 1 hour of refluxing, the reaction mixture was cooled to 0° and suction filtered. The yellow product was washed with a little ethanol, then with large amounts of water to remove traces of cyanide ion. The resulting yellow solid was allowed to air-dry for several days until there was no longer a green color visible when a sample was treated with conc. HCl.

Taken up in chloroform and treated several times with Norit, then recrystallized from a chloroform-ethanol solvent pair, 28 gm (52%) was isolated as fine, bright yellow needles, mp 182° (lit.⁶¹ mp 181°).

acridine-9-carboxylic acid 27

This compound⁵ was prepared by dissolving 12 gm (0.056 moles) 9-cyanoacridine in 100 ml of conc. sulfuric acid on the steambath for 2 hours, then cooling to 0° . To the bright red solution was cautiously added, in small portions, 40 gm (0.50 moles) of solid sodium nitrite. After the vigorous reaction had subsided, the reaction mixture was heated for another 2 hours, then diluted with water to precipitate out the product.

The finely divided, bright yellow solid was suction filtered and washed well with water, then was purified by repeatedly dissolving up in 10% alkali, suction filtering to remove any base-insoluble materials, and finally making strongly acidic with conc. HCl to precipitate the product.

The crude yield of product was 10.6 gm (75%), not recrystallized due to its insolubility in all the common organic solvents. The mp was above 300° (lit.⁶² mp, 300°).

Acridine-9-carbonamide 25

A small portion of the hot sulfuric acid solution of 9-cyanoacridine was diluted with water, precipitating out the intermediate amide. The pale yellow finely divided solid was recrystallized from acetic acid-water, with

mp 263-265° (lit.⁶³ mp 263-264°).

ν_{\max} 3400 cm^{-1} (NH); 1700 and 1660 cm^{-1} (CO).

10-methylacridinium-9-carbonamide methosulfate 26

0.5 gm (2.2 m moles) 25 was magnetically stirred with 1 ml (1.35 gm, .01 moles) dimethyl sulfate. After two hours only a small amount had gone into solution, so 5 ml dry methanol was added; within a few minutes the starting material was all in solution. Stirring was continued overnight, resulting in a bright yellow precipitate and a yellow solution. Adding ether threw out a little more solid, then the entire amount was collected by suction and washed well with ether.

The product (0.55 gm) was recrystallized from methanol: ether, depositing golden yellow prisms, mp 249-250°, homogenous on thin layer (silica: 50% ethyl acetate in chloroform). Recrystallized yield, 0.5 gm (70%).

anal. Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_5\text{S}$: C, 55.17 ; H, 4.63; N, 8.04%

found : C, 55.35 ; H, 4.80; N, 7.85%

ν_{\max} 3400 cm^{-1} (N-H), 1690 (C=O).

Phenyl ester syntheses.

The acridinium phenyl esters 34a to 34e were prepared by the following general route: acridine-9-carboxylic acid 27 was converted to its acid chloride 28 by stirring a suspension of 27 in neat, boiling, thionyl chloride (10-fold excess). After all the acid had gone into solution (generally within one hour), the excess SOCl_2 was carefully removed by vacuum distillation (80°, 100 mm). The solid residue was crushed and triturated several times with dry benzene to remove residual SOCl_2 , and the

resulting bright yellow solid was the acid chloride 28, sufficiently pure for the next step.

Esterification was accomplished by adding, to the acid chloride in anhydrous pyridine, an equivalent amount of the appropriate phenol dissolved in dry pyridine. After brief heating the reaction mixture became a homogenous solution which was cooled and then slowly diluted with 10% sodium hydroxide. The desired acridine phenyl ester 33 precipitated out, was collected on the Buchner, washed well with water, and air dried.

After recrystallization, the ester 33 was N-methylated by heating with an excess of neat dimethyl sulfate in the usual manner. After a suitable time the acridinium ester 34 was precipitated out with ether, filtered off, and purified.

9-carbo (4-methyl) phenoxyacridine 33a

The reaction between 2.5 gm (10^{-2} moles) of acid chloride 28 and 1.08 gm (10^{-2} moles) p-cresol yielded 1.65 gm (53%) of the crude 4-methyl phenyl ester. It was sublimed (200° , 0.1 torr) and crystallized from chloroform-methanol to give 1.5 gm of fluffy, off-white needles, mp $170-171^{\circ}$.

anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.83; N, 4.47%

found : C, 80.71; H, 5.09; N, 4.36%

ν_{\max} 1750 cm^{-1} (C=O)

9-carbo (4-methyl) phenoxy-10-methylacridinium methosulfate 34a

Methylation of 1.45 gm (4.65 m moles) 33a at 100° for 2 hours gave 1.55 gm (71%) of a bright yellow solid. It was recrystallized from methanol-ether to give yellow needles, mp $237-242^{\circ}$ with prior sintering, melting instantly if dropped on the hot stage at 200° .

anal. Calcd for $C_{23}H_{21}NO_6S$: C, 62.86; H, 4.82; N, 3.19%
 found : C, 63.02 ; H, 5.02; N, 3.29%

ν_{\max} 1750 cm^{-1} (C=O)

λ_{\max} 260 $m\mu$ (ϵ 91,500), 350 (11,300) 365 (12,700)

9-carbo (4-methyl) phenoxyacridine 33b

The reaction of 2.5 gm (10^{-2} moles) 28 and 1.24 gm (10^{-2} moles) hydroquinone monomethyl ether gave 1.85 gm (56%) of crude ester as a light brown solid. After sublimation (200° , 0.1 torr), the pure material (1.45 gm) was recrystallized from chloroform-methanol, giving colorless fluffy needles, mp $200-202^{\circ}$.

anal. Calcd for $C_{21}H_{15}NO_3$: C, 76.58; H, 4.59; N, 4.25%
 found : C, 76.85; H, 4.88; N, 4.16%

ν_{\max} 1755 cm^{-1} (C=O)

9-carbo (4-methoxy) phenoxy-10-methylacridinium methosulfate 34b

Methylation of 1.4 gm (4.25 m moles) 33b with 5 ml dimethyl sulfate at 100° for 2 hours gave 1.9 gm (98%) of a bright orange solid. It was recrystallized from methanol:ether to give bright orange tablets, mp $167-172^{\circ}$.

anal. Calcd for $C_{23}H_{21}NO_7S$: C, 60.66; H, 4.65; N, 3.08%
 found : C, 59.85; H, 5.05; N, 3.29%

ν_{\max} 1750 cm^{-1} (C=O)

λ_{\max} 242 $m\mu$ (ϵ 26,000), 248 (sh) (50,000), 253 (sh) (82,000),
 260 (105,000), 350 (9,100), 365 (11,400)

9-carboxyphenoxyacridine 33c

5.0 gm (.0225 moles of acid 27 was converted to the acid chloride 28, and to this was added 2.1 gm (.0225 moles) phenol in the usual manner. Routine workup gave 4.5 gm (67%) of fluffy, off-white needles from chloroform:methanol, mp 189-190°.

anal. Calcd for $C_{20}H_{13}NO_2$: C, 80.25; H, 4.38; N, 4.68%

found : C, 80.43; H, 4.48; N, 4.50%

ν_{\max} 1750 cm^{-1} (C=O)

9-carbophenoxy-10-methylacridinium methosulfate 34c

Heating 4.5 gm (.015 moles) 33c in 5 ml dimethyl sulfate at 100° for 2 hours resulted in 5.9 gm (92%) of a bright yellow solid. Crystallization from ethanol afforded bright yellow prisms, mp 228-230° after drying in vacuo.

anal. Calcd for $C_{22}H_{19}NO_6 S$: C, 62.12; H, 4.50; N, 3.29%

found : C, 62.00; H, 4.76; N, 3.21%

ν_{\max} 1752 cm^{-1} (C=O)

λ_{\max} 260 μ (ϵ 106,000), 350 (11,300), 365 (14,000).

9-carbo (4-bromo) phenoxyacridine 33d

The reaction between 2.5 gm (10^{-2} moles) of 28 and 1.73 gm (10^{-2} moles) p-bromophenol yielded 2.2 gm (58%) of crude product which, after sublimation (200°, 0.1 torr) and recrystallization from chloroform-methanol, afforded 2.0 gm of colorless needles, mp 190-191°.

anal. Calcd for $C_{20}H_{12}NO_2 Br$: C, 63.53; H, 3.47; N, 3.71%

found : C, 63.25; H, 3.48; N, 3.44%

9-carbo (4-bromo) phenoxy-10-methylacridinium methosulfate 34d

The methylation of 1.95 gm (5.15 m moles) of 33d in 5 ml neat dimethyl sulfate required 5 hours on the steambath before all had dissolved up. After precipitation with ether and recrystallization from methanol:ether, 2.0 gm (77%) of bright yellow needles, mp 261-263^o, were obtained.

anal. Calcd for C₂₂H₁₈NO₆ Br S : C, 52.39; H, 3.60; N, 2.78%
found : C, 52.89; H, 3.88; N, 2.85%

ν_{\max} 1755 cm⁻¹ (C=O)

λ_{\max} 262 m μ (ϵ 93,000), 350 (10,000), 365 (12,500)

9-carbo (3-nitro) phenoxyacridine 33e

The reaction of 0.6 gm (2.4 x 10⁻⁴ moles) acid chloride 28 and 0.27 gm (1.95 x 10⁻⁴ moles) 3-nitrophenol readily afforded the desired compound. It was recrystallized from chloroform:methanol to give 0.43 gm (64%) of pale yellow fluffy fibres, mp 170-172^o.

The sample prepared for analysis was, unfortunately, used up in analyses where the equipment was malfunctioning. But a molecular weight determination using the high-resolution MS-9 mass spectrometer indicated a parent peak of m/e 344.076658. C₂₀H₁₂N₂O₄ requires m/e 344.079700.

ν_{\max} 1750 (C=O)

9-carbo (3-nitro) phenoxy-10-methylacridinium methosulfate 34e

A mixture of 0.4 gm (1.16 m moles) 33e and 1.0 gm (180 m moles) dimethyl sulfate was heated at 150^o for 15 hours. Trituration with ether left a solid with a melting point range of 170-230^o, evidence of a mixture. Repeated fractional crystallization from ethanol:ether afforded 50 mg of a yellow solid

which was then recrystallized from minimum ethanol to give dark yellow prisms, mp 245-247^o with some prior sintering. The compound dropped on a hot stage at 210^o melts instantly.

The remaining material, a gelatinous mass, contained large amounts of the desired product, according to IR; but it was not practical to isolate.

anal. Calcd for C₂₂H₁₈N₂O₈S : C, 56.17; H, 3.86; N, 5.96%

found : C, 56.35; H, 4.17; N, 5.85%

ν_{\max} 1760 cm⁻¹ (C=O)

λ_{\max} 260 m μ (ϵ 108,000), 280 (sh) (27,500), 350 (11,000)

365 (11,000)

9-carbo (4-nitro) phenoxyacridine 33f

From 10 gm (.045 moles) of acid 27, the esterification with 6.2 gm (.045 moles) p-nitrophenol followed by recrystallization from chloroform: methanol yielded 8.9 gm (58%) of pale yellow, fibrous product, mp 188-190^o.

anal. Calcd for C₂₂H₁₂N₂O₄ : C, 69.76; H, 3.51; N, 8.14%

found : C, 69.84; H, 3.87; N, 8.25%

ν_{\max} 1750 cm⁻¹ (C=O)

9-carbo (4-nitro) phenoxy-10-methylacridinium methosulfate 34f

A mixture of 8.0 gm (.0233 moles) 33f and 5.0 gm (.040 moles) dimethyl sulfate in 150 ml dry benzene was mechanically stirred and refluxed for 20 hours. The resulting mixture was hot filtered; upon cooling, the filtrate deposited a large amount of starting material according to the infrared.

The bright yellow benzene-insoluble solid collected during the hot filtration was taken up in 200 ml methanol, and upon adding 50 ml water a little more starting material was thrown down and removed by filtration. The filtrate was evaporated to dryness, and the bright yellow residue was recrystallized from methanol to give 1.9 gm (17%) of prisms, mp 243-248°.

anal. Calcd for $C_{22}H_{18}N_2O_8S$: C, 56.17; H, 3.86; N, 5.96%

found: C, 56.49; H, 4.28; N, 5.64%

ν_{\max} 1750 cm^{-1} (C=O)

λ_{\max} 248 m μ (ϵ 58,500), 253 (97,000), 260 (126,000),
352 (12,000), 365 (13,500).

9-carbo (2,4-dinitro) phenoxyacridine 33g

The reaction of 3.2 gm (1.28×10^{-2} moles) acid chloride 28 and 3.0 gm (1.63×10^{-2} moles) 2,4-dinitrophenol gave 3.0 gm of a dark brown amorphous powder insoluble in all the common organic solvents. A sample was purified by sublimation (250°, 0.05 torr, 20 hours), and the pale yellow sublimate had mp 263-269°.

anal. Calcd for $C_{20}H_{11}N_3O_6$: C, 61.70; H, 2.85; N, 10.79%

found : C, 61.85; H, 3.22; N, 10.45%

ν_{\max} 1765 cm^{-1} (C=O)

9-carbo (2,4-dinitro) phenoxy-10-methylacridinium methosulfate 34g

0.35 gm of the sublimed 33g and 2 ml neat dimethyl sulfate were heated at 150° for 5 hours. The resulting mixture was triturated several times with ether to remove excess dimethyl sulfate. The ether-insoluble residue was leached with boiling methanol three times, and the combined methanol washings (bright yellow) were taken to dryness. The residue (0.25 gm) was recrystallized twice from ethanol to give golden yellow prisms, mp 174-176°.

anal. Calcd for $C_{22}H_{17}N_3O_{10}S$: C, 51.26; H, 3.32; N, 8.15%
 found : C, 51.39; H, 3.55; N, 8.20%

ν_{\max} 1765 cm^{-1} (C=O)

λ_{\max} 260 $\text{m}\mu$ (ϵ 90,000), 355, (9,600)

9-carbomethoxyacridine 29

This compound was made in the same manner as the phenyl esters, this time adding anhydrous methanol to the acid chloride 28 and working up in the usual manner. In a typical preparation, 14.0 gm (.063 moles) of acid 27 was converted to 28 then treated with 100 ml dry methanol. The crude ester 29 was recrystallized from methanol as pale yellow needles, mp 126-128° (lit.⁸⁰ mp 126.5-127.5°). The yield of pure material was 7.5 gm (50%).

ν_{\max} 1725 cm^{-1} (C=O)

9-carbomethoxy-10-methylacridinium methosulfate 30

5.8 gm (.0245 moles) 29 was heated in 25 ml neat dimethyl sulfate on the steambath for 3 hours. It was cooled and triturated with ether resulting in a bright yellow solid, which was recrystallized from methanol:ether to give 7.7 gm (86%) of bright yellow needles, mp 228-229°.

anal. Calcd for $C_{17}H_{17}NO_2S$: C, 56.18; H, 4.77; N, 3.85%
 found : C, 56.40; H, 4.65; N, 3.70%

ν_{\max} 1725 cm^{-1} (C=O)

λ_{\max} 260 $\text{m}\mu$ (ϵ 92,000), 350 (10,800), 365 (12,200)

9-carboxy-10-methylacridinium chloride 31

6.7 gm (.0185 moles) of 30 in 70 ml water was added to 70 ml 10% sodium hydroxide. The purple solution was heated on the steambath for 3 hours, then filtered hot. The filtrate was acidified with conc. HCl, giving a green solution and a mass of yellow-green needles, weighing 4.45 gm (88%) after drying in air. The product was recrystallized from methanol as yellow prisms, mp 242-244° (lit.⁵⁷ mp above 205° dec.)

$$\nu_{\max} 3400 \text{ cm}^{-1} \text{ (br)(O-H), } 1730 \text{ (C=O)}$$

9-chlorocarbonyl-10-methylacridinium chloride 32

A sample of 31, dried in vacuo over KOH, was suspended in neat boiling thionyl chloride (freshly distilled) for 15 minutes. By this time all the suspended material had gone into solution; removal of the excess thionyl chloride under reduced pressure left a bright yellow amorphous solid brilliantly chemiluminescent in solutions containing alkaline peroxide. The infrared (nujol) showed a small but significant peak for the O-H of the parent acid; but all attempts to recrystallize the compound resulted in extensive darkening. The mp was broad, starting above 210° with much decomposition (lit.⁵⁷ mp 175-178°)

$$\nu_{\max} 1790 \text{ cm}^{-1} \text{ (C=O)}$$

lucigenin bromide 13

8.0 gm of K&K lucigenin was dissolved up in 250 ml hot water and filtered hot. To the hot filtrate was added a hot solution of 80 gm KBr in water. Upon cooling to room temp a mass of golden yellow plates appeared. Two crops of product, 7.4 gm, mp above 300° were obtained.

PART B: PRODUCT STUDIES(a) 9-cyano-10-methylacridan (22)

0.60 gm (2.7×10^{-3} moles) of 22 were dissolved in 75 ml 95% ethanol. To this was added a solution of KOH (0.3 gm) in ethanol (10 ml). Upon bubbling oxygen through the solution, a steady blue glow was observed. After the light reaction was over, some pale golden needles had appeared; they were isolated by suction, filtration, washed well with water, and dried in vacuo. The filtrate was diluted with 30 ml water, concentrated under reduced pressure, and extracted with 4 x 10 ml chloroform. The combined chloroform extracts were dried over sodium sulfate and taken to dryness on the rotovap, giving a pale yellow solid. This, too, was dried in vacuo.

The two crops of solid (178 mg and 383 mg respectively) had identical infrared spectra, and melting points of 201-202° and 202-203° (lit⁶⁴ mp of N-methylacridone, 201-203°). The yield of N-methylacridone was 561 mg (95%).

(b) 9-cyano-10-methylacridinium nitrate (23)

In a similar manner, 0.5 gm of 23 in 50 ml 25% aqueous ethanol was treated dropwise with a solution of 0.5 ml 3% H₂O₂ in 5 ml 10% sodium hydroxide. After the bright, short-lived emission had ceased, the reaction solution was concentrated and worked up as before. The isolated N-methylacridone, mp 200-202°, weighed 480 mg (96%).

(c) 9-carbo (4-methyl) phenoxy-10-methylacridinium methosulfate (34a)

A solution of 50 mg 34a in 10 ml 50% aqueous ethanol was treated with alkaline H₂O₂ and, after chemiluminescence ceased, was concentrated under reduced pressure. The residue was leached with chloroform several times, then diluted with 10% HCl. The resulting acidic solution was extracted with several portions of chloroform.

Both chloroform extracts were examined by thin-layer (fluorescent silica, 50% ethyl acetate in chloroform). The former contained only N-methylacridone; the latter had mainly para-cresol with some N-methylacridone and also a small amount of yellow material at the origin. The relative amount of this yellow substance was increased when the workup involved concentrating the reaction solution at higher temperature.

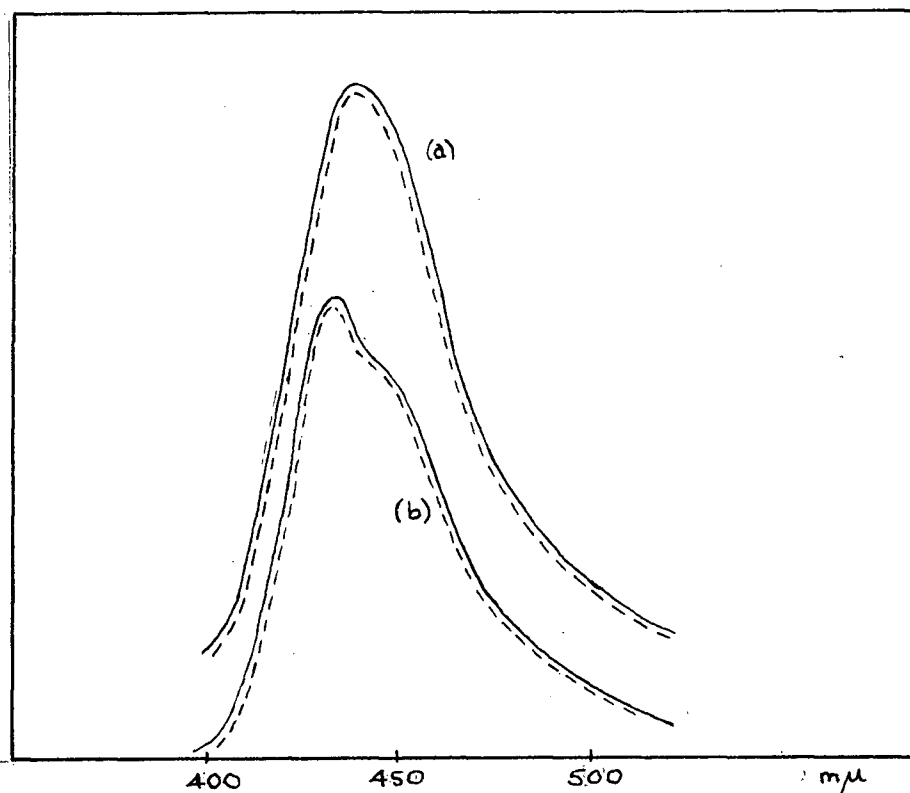
In the preceding experiments, no attempts were made to identify the other postulated products, cyanate ion (in (a) and (b)) and CO_2 (in (c)).

PART C: CHEMILUMINESCENCE AND FLUORESCENCE SPECTRA

The chemiluminescent emission spectrum of three bright compounds, 23, 34c and 32, were measured and compared with the fluorescence of N-methylacridone 16. These measurements were made with an Aminco-Bowman spectrofluorimeter and X-Y plotter. For the brightest compound; the acid chloride 32, it was necessary to use fast scan; the others, reacting more slowly, allowed use of the better resolution slow scan. In each case, congruence of the emission spectrum with the fluorescence of 16 identified it as the emitter.

(See Figure 3)

The fluorescence efficiency Φ of very pure N-methylacridone, 10^{-4}M in ethanol, was found by calibration against a known standard (10^{-4}M quinine sulfate in 1 $\text{N}_2\text{H}_2\text{SO}_4$)⁶⁶ using the method in Calvert and Pitts.⁶⁷ The value of 0.85 was unaffected by added water or alkali.



- (a): Emission of 32 vs. fluorescence spectrum of N-methylacridone 16 (both 10^{-3} M), fast scan. The curves have been normalized by adjusting the amplifier gain.
- (b) Emission of 34c or 23 (10^{-3} M), vs. fluorescence of 16; slow scan.

Figure 3: Chemiluminescence and fluorescence spectra for selected acridinium salts.

PART D: CHEMILUMINESCENCE

Solutions of reagents were prepared for the light emission studies. A $4.4 \times 10^{-2} \text{ M}$ solution of H_2O_2 was made up by diluting 0.1 ml 30% H_2O_2 to 25 ml with 50% aqueous ethanol. This concentration was verified by iodometric titration with 0.1 N sodium thiosulfate solution. The buffer solutions, in unit pH values from 7 to 12, were made up from stock solutions of 0.1 M HCl, NaOH, KH_2PO_4 , and "borax" according to published tables.⁶⁵ Their pH was checked against known standards (pH 7.0 and 10.0) on a Beckman pH meter and found to be accurate to within ± 0.05 pH. When 0.1 ml of any buffer solution was added to a 1 ml solution of 50% aqueous ethanol, the pH value did not change.

The compounds were examined for light emission in the following manner. A 50% aqueous ethanol solution containing substrates (10^{-4} M) and H_2O_2 ($4.4 \times 10^{-2} \text{ M}$) was prepared. To 1.0 ml of this solution in a 1 cm quartz cuvette was added 0.1 ml of pH 12 buffer, resulting in a flash of light. The light output was monitored with the apparatus in Figure 4.

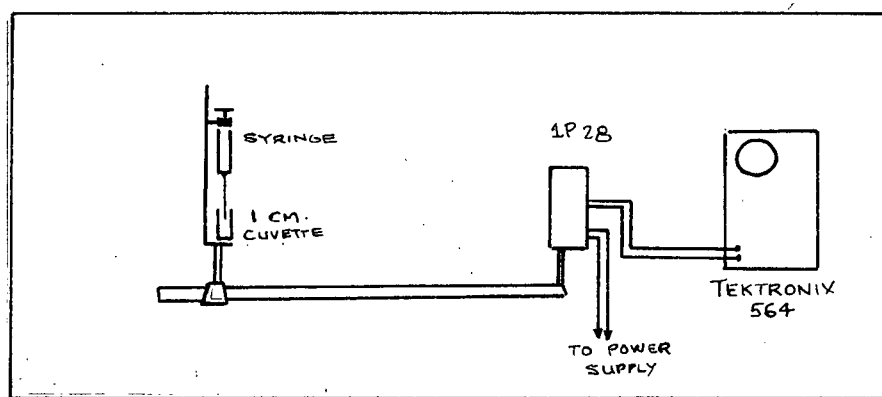


Figure 4: Light-Measuring Apparatus

The cuvette, its upper portion masked to overcome splash effects, was mounted in a clamp which could be moved along the optical bench, maintaining a fixed geometric relationship to the phototube while allowing

a variable path length. It was necessary to shroud the entire apparatus in black cloth and to provide a low level red illumination for the operator.

The path lengths chosen were 11 cm for the weaker compounds and 60 cm for the strongest. The intensities measured at the longer path were related to the others by using a moderately strong compound, the 4-methyl phenyl ester 34a, as a calibration standard at both path lengths; the attenuation was found to be nearly 100.

The most brilliant compounds overloaded the phototube even at the longer path length, so their light output was attenuated by placing, in the light path, a neutral density filter^{*} of O.D. 1.7 which afforded an attenuation factor of 30, determined by the use of a small incandescent bulb at low voltage. This filter⁶⁸ was known to transmit linearly over the entire visible spectrum, ensuring that its attenuation in the blue region was still 30.

The intensities were measured with a 1P28 phototube, whose output was fed directly to the input of a Tektronix 564 storage oscilloscope. the 'scope input impedance (1 Megohm) acted as a load for the phototube, and its voltage decrease as a function of time was imprinted on the face of the crt. Moreover, the instrument was set so that the initial light burst, resulting from the addition of base, triggered the vertical and horizontal scanning circuits.

The result was a record of the light intensity decay curve, accurate for reactions between 0.1 seconds and 2 minutes, over a wide range of intensities. For slower reactions, beyond the capabilities of the horizontal scan rate, the vertical (voltage) deflection was monitored over a time range measured by stopwatch.

* Kindly supplied by John McIntosh of this department.

The most rapid reactions (0.1 seconds and less) posed a special kind of problem, because the mixing time became comparable to the reaction lifetime. This caused, in general, a broadening of the decay curve and a much lower initial intensity than would be observed for perfect, instantaneous mixing. The mixing method found to be most satisfactory was the rapid, forceful injection of 0.1 ml of buffer from a 1 ml syringe equipped with a long 20-gauge needle whose tip was below the surface of the reaction solution. The addition of a magnetic stirring bar was not helpful; in fact, it only lengthened the mixing time.

The pH of the reaction solution was the same as that of the buffer which had been added; and, because the final base and peroxide concentrations (10^{-2} M and 4.4×10^{-2} M respectively) were in large excess with respect to the substrate (10^{-4} M), the reaction was expected to be pseudo first order in substrate.

This proved to be correct. The time-dependence of $\log I$ (intensity) was found by plotting I against time on semi-log paper. In all but the most rapid reactions, a linear relationship was obtained for at least three half-lives, indicating a true exponential decay and first order kinetics.⁶⁹

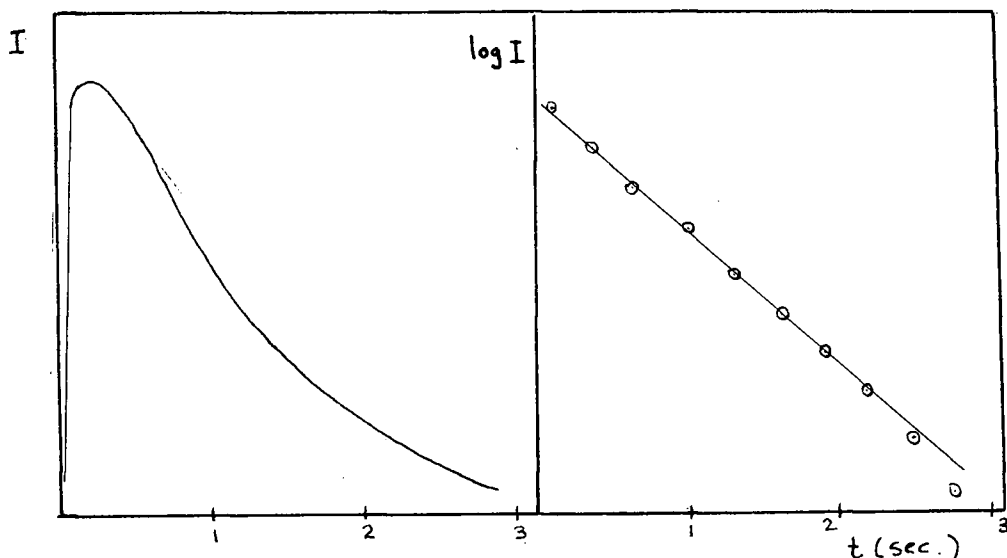


Figure 5: A typical chemiluminescence decay curve.

COMPOUND	No.	$t_{1/2}$ (sec)	k_1 (sec ⁻¹)	rel. rate	abs. I_{max} (V)	rel. I_{max}	rel. Q.Y	abs. Q.Y
Me ester	30	0.038	18.4	4,500	0.11 ^(a)	0.015	5.8×10^{-6}	10^{-8}
Benzoyl	20	2.1	0.33	80	0.15 ^a	0.020	2.9×10^{-4}	10^{-6}
Nitrile	23	30	0.023	5.6	0.5 ^a	0.066	1.25×10^{-2}	10^{-4}
Lucigenin bromide	13	169	0.004	1	7.6 ^a	1	1	10^{-2}
Phenyl esters	34							
4-OCH ₃	b	37	0.0187	4.6	35 ^b	4.6	0.98	10^{-2}
4-CH ₃	a	35.8	0.020	5.0	45 ^{a,b}	5.9	1.15	"
—	c	10.8	0.064	15.6	154 ^b	20.3	1.20	"
4-Br	d	0.42	1.635	400	4300 ^b	565	1.35	"
3-NO ₂	e	0.007	100	25,000	20,000 ^{c,d}	2650 ^{c,d}	-	"
4-NO ₂	f							

(a) phototube supply voltage 900V, path length 11cm

(b) phototube supply voltage 700V, path length 60cm

(c) not accurate because of mixing problems

(d) conditions as in (b), with neutral density filter added

Table I: Chemiluminescence of compounds at pH 12

[substrate]: 10^{-4} M

[H₂O₂] : 4.4×10^{-2} M in 50% aqueous ethanol

[OH⁻] : 10^{-2} M

In Table I the various compounds are ranked in order of observed quantum efficiency relative to lucigenin bromide. In all cases, the rate constants were obtained from the slopes of the log I-time plots, and the initial intensity (I_{\max}) values were found by extrapolation to zero time. In all but two cases, these corresponded closely to the observed I_{\max} . The initial intensities (measured in output voltage of the phototube) were then computed on an absolute basis, using the weakest compound as reference point and accounting for any subsequent attenuations. The relative quantum yields were obtained by graphing the absolute intensity decay curves and comparing the areas under the curves with that of lucigenin bromide. The absolute quantum yields are based on lucigenin.⁵⁴

The rate constants of the substituted phenyl esters 34 were compared to see if a Hammett relationship existed between them. Thus, in Figure 6 the substituent effect is shown. The σ -values are from Jaffee's review.⁷⁰

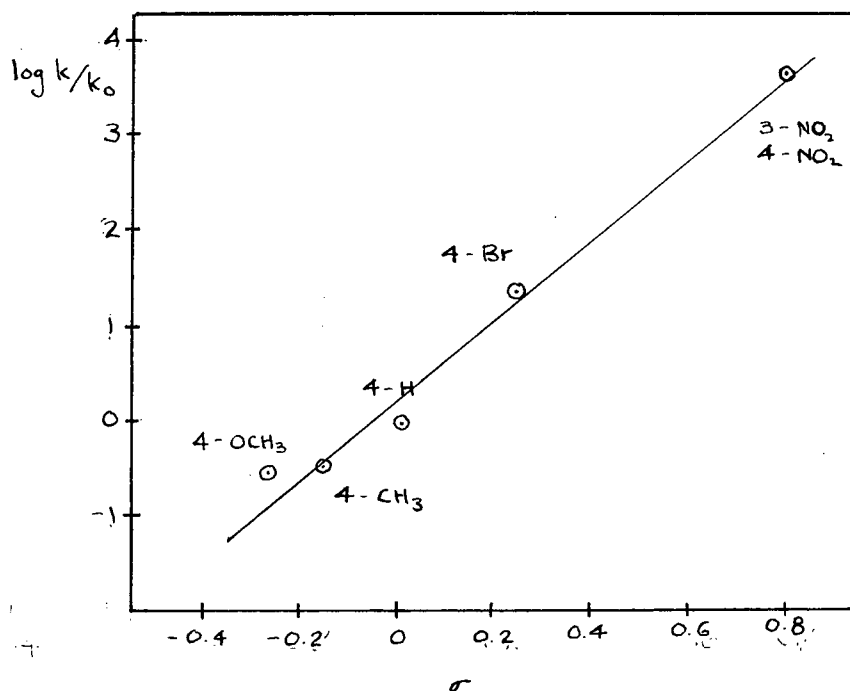


Figure 6: Hammett plot for the substituted phenyl esters.

The highly reactive nitrophenyl esters had the mixing problems described earlier; in fact, all three compounds had essentially identical reaction rates at pH 12. Re-testing of these esters at pH 8 disclosed that their rates, now slower by a factor of 20, did indeed show the difference predicted for them.

Phenyl ester	k_1 (sec ⁻¹)
3-NO ₂	4.1
4-NO ₂	5.5
2,4-(NO ₂) ₂	12.8

[substrate], 10⁻⁴M; [H₂O₂], 4.4 x 10⁻²M; [OH⁻], 10⁻⁶M

Table II: Kinetics of the nitrophenyl esters at pH 8.

The pH-dependent kinetics of one phenyl ester are shown in Table III and Figure 7. The concentrations of substrate and peroxide were as before, but the buffers added were of pH 7 to 12 inclusive, and double the quantity (0.2 ml) was added to ensure that accurate pH conditions were maintained. Again, the rate constants were determined from slopes of the log I plots, and relative quantum yields (in arbitrary units) were found by measuring the areas under the absolute intensity decay curves.

pH	k_1 (sec ⁻¹)	Quantum Yield (arbitrary units)
7	.00168	-
8	.023	98
9	.154	-
10	.71	100
11	.92	-
12	5.8	93

[substrate], 10^{-4} M, $[H_2O_2]$, 4.4×10^{-2} M, (1 ml 50% aq. etOH)

$[OH^-] = 10^{-7}$ to 10^{-2} M (0.2 ml)

Table III: pH-dependent kinetics of 4-bromo phenyl ester 34d

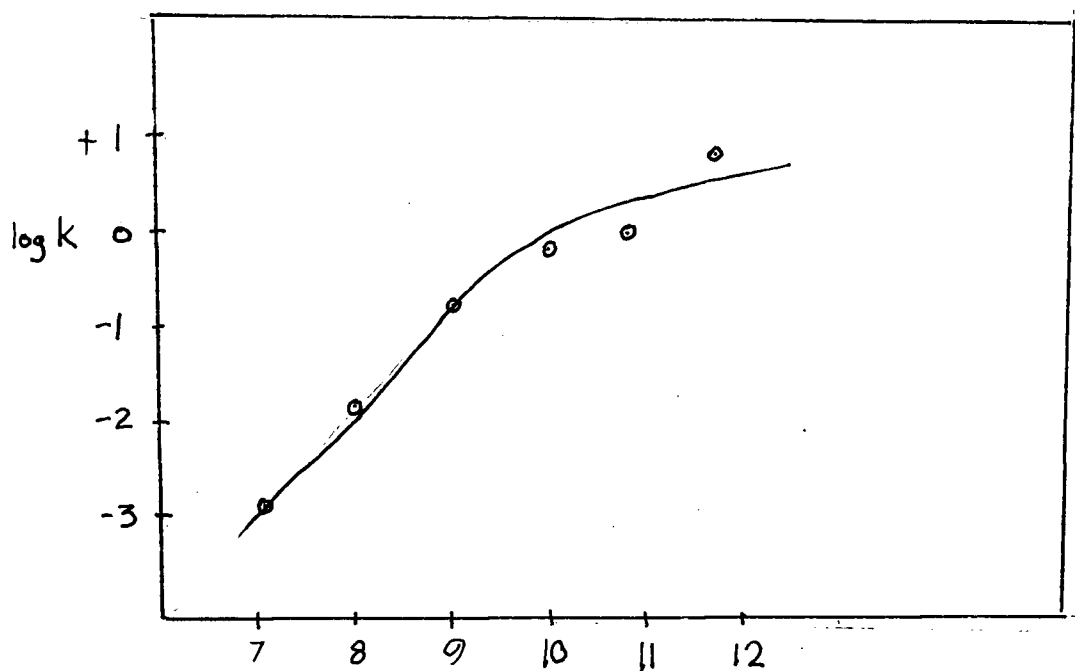


Figure 7: Log k vs. pH, 4-bromo phenyl ester

There is a specific requirement for hydrogen peroxide in the light-producing reaction, so the kinetics and quantum yield as a function of the H_2O_2 concentration were of interest. In Table IV, the unsubstituted phenyl ester 34c ($10^{-4}M$) with various H_2O_2 concentrations was treated with pH 12 buffer. In the reactions, the phenyl ester ($2 \times 10^{-4}M$) and an equal volume of H_2O_2 in aqueous ethanol ($4.4 \times 10^{-2}M$ etc.) were pre-mixed, then 0.1 ml of buffer was added.

H_2O_2 conc. (M)	k_1 (sec $^{-1}$)	Q Output (arbitrary units)
2.2×10^{-2}	0.21	100
$\times 10^{-3}$	0.49	105
$\times 10^{-4}$	0.66	61
$\times 10^{-5}$	0.62	13
$\times 10^{-6}$	0.21	2

[substrate], $10^{-4}M$; [H_2O_2], as above; [OH^-], $10^{-2}M$

Table IV: Effects of H_2O_2 concentration on kinetics of ester 34c

The effect of substrate concentration on the kinetics and quantum yield was examined (Table V) by varying the amount of phenyl ester 34c in $4 \times 10^{-2}M H_2O_2$ and treating with the pH 12 buffer.

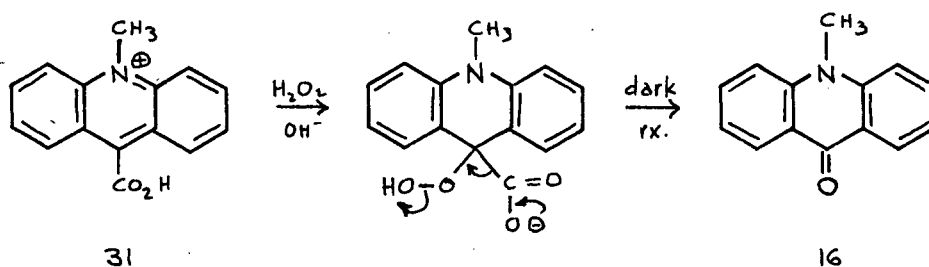
substrate	$k_1(\text{sec}^{-1})$	Rel. Q.Y.
10^{-4}	0.22	100
5×10^{-5}	0.39	32
2×10^{-5}	0.56	14
10^{-5}	0.66	5

[ester] - as above; $[\text{H}_2\text{O}_2] = 4 \times 10^{-2}\text{M}$; $[\text{OH}^-] = 10^{-2}\text{M}$

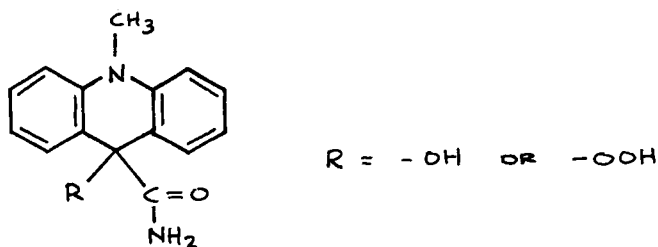
Table V: Effect of substrate concentration on the kinetics of 34c

It is now apparent that the compounds can be divided into three groups: those which emit no light at all, those which emit weakly, and the bright compounds whose quantum yield is about that of lucigenin.

The non-chemiluminescent compounds are the acridinium 9-acid 31 and the corresponding amide 26. The acid in an alkaline peroxide solution rapidly yields N-methylacridone without any light emission, probably by the following mechanism:



A solution of the yellow acridinium carbonamide 26 is rapidly decolorized by an alkaline peroxide solution, but there is no N-methylacridone formed, proven by the absence of its characteristic blue fluorescence. It is likely that the reactive 9-position has been attacked by base or (more likely) hydroperoxide anion, resulting in a metastable pseudobase which is not likely to react further.

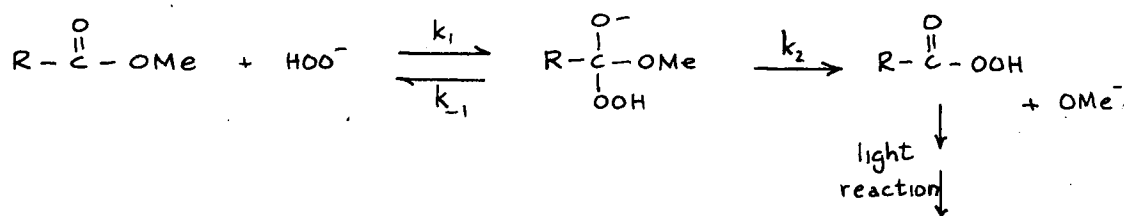


The most significant factor here is that neither the acid nor the amide is likely to have its carbonyl group attacked by hydroperoxide anion, and for this reason we did not expect light emission. Fortunately, none was observed.

DISCUSSION

The weakest emitters are the methyl ester 30, the benzoyl compound 20, and the nitrile 23, in order of increasing brightness. The first two are extremely weak, with quantum yields (using lucigenin as standard) of 10^{-8} and 10^{-6} respectively, and can be seen only in total darkness. (See Table I).

The methyl ester should be essentially unreactive to hydroperoxide anion, because the latter is a weaker base than methoxide anion. If (as we claim) the hydrolysis by hydroperoxide anion is necessary for light emission, the reaction



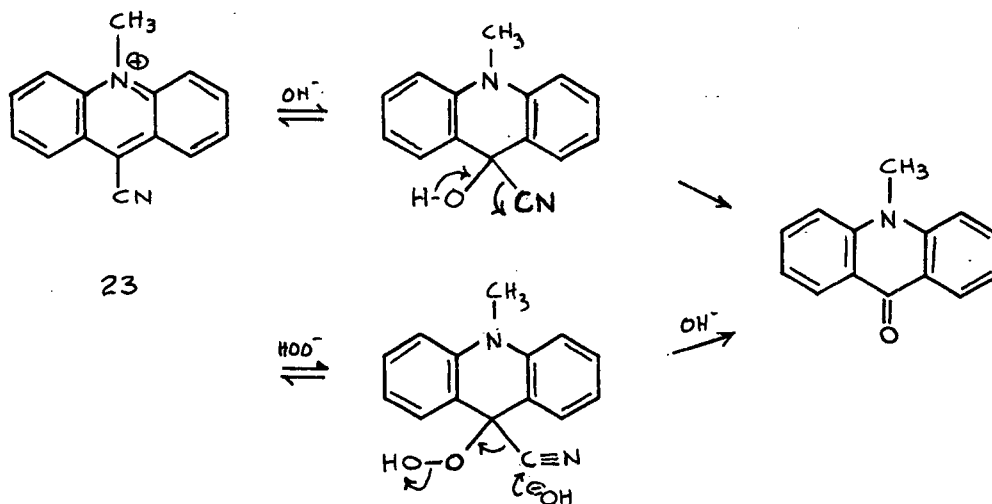
would have $k_{-1} \gg k_2$ for the methyl ester.⁷¹ Indeed, ethyl acetate is known to be unreactive to hydroperoxide anion.⁷² Moreover, the characteristic yellow color of the acridinium methyl ester is rapidly discharged, but there is no perceptible N-methyl acridone fluorescence. The behavior of the methyl ester may, therefore, be similar to that of the amide; and the very feeble light is an indication of the small degree to which the hydrogen peroxide hydrolysis occurs. On the other hand, one might expect the hydrolysis to be slow but continuous process, yielding light until the substrate had been completely destroyed by this or dark processes (eg, alkaline hydrolysis). The very brief flash observed suggests a trace impurity may be responsible, or that perhaps the substrate is transformed almost instantly into a substance incapable of chemiluminescence.

Similar behavior is shown by the benzoyl compound 20: its emission is 100-times stronger than that of the methyl ester, but is still very weak. Again, there is no perceptible N-methyl acridone formed, although rapid decolorization of the acridinium salt occurs. Thus, a metastable pseudo-base is highly probable, as in the previous cases. The light-producing reaction almost surely involves attack by hydroperoxide on the benzoyl carbonyl, but the subsequent mechanism differs from that of the methyl ester because there is no leaving group available. More will be said about this later. The benzoyl compound, with its electron-withdrawing aromatic ring, is expected to be more reactive to nucleophiles than is the methyl ester. This prediction is borne out, but the light-producing reaction is still a very unfavorable process.

The feeble light output of the methyl ester and benzoyl compound indicated that a detailed study of the reactions involved would not yield much useful information about the nature of efficient chemiluminescent processes, so at this point the investigation of these compounds was terminated.

The acridinium nitrile 23 is the most efficient of three weak emitters, with a quantum yield of ca. 10^{-4} . Its reactivity toward hydroperoxide anion is, therefore, at least 10,000 times that of the methyl ester, and 100 times that of the benzoyl compound.

In contrast with the weaker compounds, the nitrile reacts with alkaline H_2O_2 to give N-methylacridone in yields of >95%. Thus, the reaction is much cleaner than that of lucigenin (vide infra), but paradoxically has a much smaller quantum yield. We interpret this as an indication of side reactions producing N-methylacridone by dark pathways. Two possible routes are:



Evidence of these dark reactions is the observation that a solution of the nitrile and H_2O_2 in 50% aqueous ethanol gives considerable quantities of N-methylacridone (blue fluorescence) within 15 minutes, but no light is evolved. Subsequent addition of base gives light, but the intensity and duration is much reduced in comparison to that of a fresh solution. Similar behavior is observed if the nitrile is treated by the reagents in the reverse order. Moreover, the kinetics of the nitrile chemiluminescence are not cleanly first-order; non-linear plots are obtained, especially at high pH values. This indicates that the substrate is being partitioned into more than one reaction pathway.

Evidence will be presented later suggesting that the light-producing mechanism is different from those depicted above. The kinetics of the base-catalyzed reaction between hydrogen peroxide and aromatic nitriles were shown by Wiberg⁷³ to be first order in each of the three reactants involved, and that addition of hydroperoxide ion to the nitrile was the rate determining step. Further investigation of the reaction disclosed that hydroperoxide ion was 10^4 times more nucleophilic than hydroxide ion, the stronger base.⁷¹ These findings are consistent with our proposed mechanism.

A similar chemiluminescence is given by 9-cyano-10-methylacridan 22, which is oxidized to N-methylacridone when an alkaline ethanol solution is treated with molecular oxygen. The reaction does not appear to involve free radicals, because there is no enhancement with potassium ferricyanide, ferrous sulfate, or cupric bromide,⁷⁴ and no inhibition with arsenious oxide,⁷⁵ cyanide ion, or hydroquinone. The process could be envisioned as an oxidation to the acridinium nitrile 23 and hydroperoxide anion, which can then interact.

The standard of comparison was lucigenin bromide (N,N'-dimethylacridinium dibromide, 13), so chosen because it could be conveniently recrystallized and made much more pure than the commercially available lucigenin (the dinitrate). Besides this, the bromide was easily reduced to N,N'-dimethylbiacridene, 17, a compound of considerable interest.

Lucigenin bromide has a fairly strong, durable emission. Its half-life was nearly three minutes under the conditions selected, and its absolute quantum yield was assumed to be that of lucigenin itself: 10^{-2} , according to Totter.⁵⁴ The light was greenish instead of the blue color shown by all the other compounds in this series, and the resulting solution was yellow instead of water-white. The wavelength shift is a natural consequence of the self-absorption properties of colored solutions.

Thin-layer examination of the reaction mixture indicated the presence of several other compounds besides N-methylacridone; but the nature of these other products was not investigated.

Thus, lucigenin bromide gave 100 times more light than the nitrile 23, but the reaction was not as clean. This could be interpreted to mean that the energy transfer process resulting in excited N-methylacridone is much more efficient for lucigenin bromide than for the nitrile. Especially

significant is the observation that, according to our mechanism, the reaction of each molecule of lucigenin bromide with one of hydrogen peroxide yields two molecules of N-methylacridone, whereas the corresponding nitrile yields only one. Thus, the probability of forming excited N-methylacridone is greater with lucigenin bromide, although the extent of "dark" side reactions is unknown. An additional factor preventing efficient chemiluminescence of the nitrile is the large amount of N-methylacridone production by dark routes; similar processes could be involved with lucigenin bromide, but we do not know their extent.

The N-methylacridinium phenyl esters 34 are brilliant emitters under the standard conditions chosen. Their initial brightness relative to lucigenin bromide is anywhere from five to 4000 times as strong, but their total quantum yield (as measured by the total area under the emission curve) is, to all intents and purposes, the same as that of lucigenin bromide. Thus, only a kinetic factor is involved here, and initial brightness is gained at the expense of duration. We have already said that lucigenin bromide is expected to have a higher probability of excited N-methylacridone formation than will the acridinium nitrile 23. A similar comparison should be applied to the acridinium phenyl esters. This point will be amplified after the proposed chemiluminescent mechanism has been discussed.

The most significant property of the substituted phenyl esters is that their reaction rates obey a Hammett relationship (See Figure 6, Table I); and, moreover, this is the first such series in chemiluminescence that is dependent on rates only. Throughout the series we have the same emitter, N-methylacridone, whose fluorescence efficiency is constant. Thus, we have avoided the problem of substituent effects on the fluorescence efficiency of the emitter, a factor which for the lophine and indole peroxides was not determined. (See Introduction.)

At pH 12, the reaction lifetimes ranged from 4 minutes (the 4-methoxy phenyl ester) down to 0.05 seconds (the various nitrophenyl esters). For the fastest reactions, the mixing time was comparable to the total reaction time, and this resulted in a general broadening of the decay curve and deviations from true first-order kinetics. Indeed, the three nitrophenyl esters had essentially identical reaction rates at pH 12, in contradiction to the predicted reactivity differences. Re-measurement at pH 8 disclosed that these compounds, now reacting more slowly, did indeed follow the expected order of reactivity, with 3-nitro < 4-nitro < 2,4-dinitro. Thus, experimental difficulties with the fastest-reacting phenyl esters has made the Hammett plot at pH 12 somewhat less precise in the region of large σ , but the trend is clear.

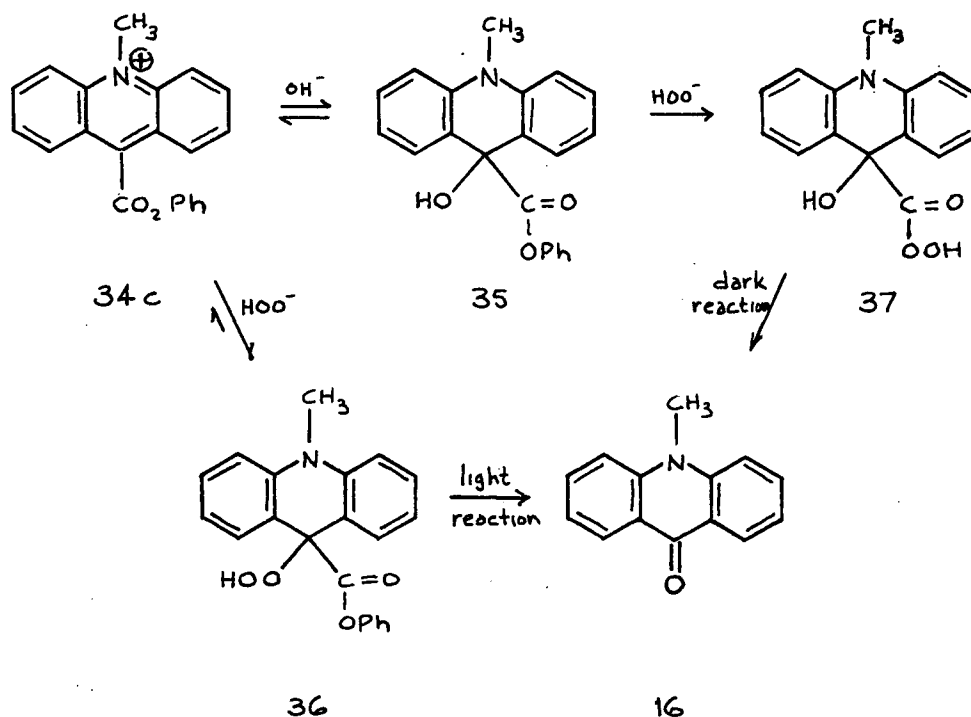
Unfortunately, the reaction conditions chosen for testing all the chemiluminescent compounds were ideal for the weaker ones but too vigorous for the bright phenyl esters. This prevents us from drawing more conclusions about the reactivity of those phenyl esters; for example, the 3-nitro compound might be expected to obey a linear relationship more closely than the 4-nitro isomer. We were not able to confirm this, however. The behavior of the 4-methoxy ester 34b is perplexing; it reacts more rapidly than one would expect. There is clearly no extra resonance-donating effect, however, because the nominal σ^+ value of -0.78 (from Brown⁷⁶) gives an even larger deviation. Perhaps the value for this particular functional group is more properly defined by the so-called " σ^n values" of van Bekkum et al.⁷⁷ We attempted to extend the Hammett plot by synthesis of a 4-N,N-dimethylamino phenyl ester, with σ -0.83; but difficulties encountered in the N-methyl-acridinium salt formation have prevented our including this compound in the report.

The ρ value was found to be 4.4; compare this with the ionization of phenols, whose ρ constant is 2.1.⁷⁰ This illustrates the extreme facility of the peroxide hydrolysis. We should point out that the phenyl esters were expected to react readily with hydroperoxide anion, precisely because their behavior with respect to nucleophilic attack is diametrically opposite to that of the methyl ester described previously. Here, the presence of a good leaving group (phenoxide ion, a weaker base than hydroperoxide ion) allows us to predict that ester hydrolysis by hydroperoxide would be considerably more facile. Indeed, the prediction has been completely substantiated.

Clearly then, the chemiluminescence of the acridinium phenyl esters 34 has, as its rate-determining step, the attack by hydrogen peroxide upon the ester carbonyl. The involvement of free hydroperoxide anion, however, has been ruled out by the following experiment: if (say) the phenyl ester 34c in aqueous ethanol is treated with a weak base, slow decolorization of the yellow solution occurs, resulting in the pseudobase 35 as proven by its ultraviolet spectrum.⁵⁷ (This is an equilibrium process; the acridinium form can be regenerated by adding mineral acid.) If hydrogen peroxide is now added to the pseudobase, there is a feeble but very long-lasting light emission.

In contrast, when a solution of the acridinium phenyl ester is treated in the reverse order (H_2O_2 , followed by base), instant decolorization and a bright, short-lived chemiluminescence is observed.

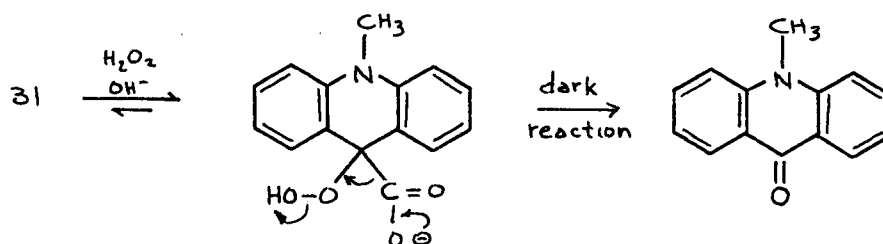
The interpretation is clear. In the former case, the 9-hydroxyl pseudobase 35 is capable of reacting with free hydroperoxide ion at the ester carbonyl, but this process does not result in rapid, efficient light production. In the second experiment, competition between hydroxide ion and the much more nucleophilic hydroperoxide ion is expected to produce the acridan peroxide 36 which can then yield bright light.



The feeble light resulting from the prior addition of base before H_2O_2 cannot be interpreted in terms of simple alkaline hydrolysis of the ester, because after standing for 15 minutes in a buffer at pH 12 the pseudobase 35 can be acidified generating an acridinium species which, upon adding alkaline H_2O_2 , gives (visually) as much light as a fresh solution. Thus, we see that the phenyl esters are quite resistant to base hydrolysis at room temperature. The light conceivably arises only after the hydroxyl of the pseudobase has undergone exchange with hydroperoxide anion, probably via the acridinium ester 34c; this is expected to be a very slow process due to the position of the equilibrium.

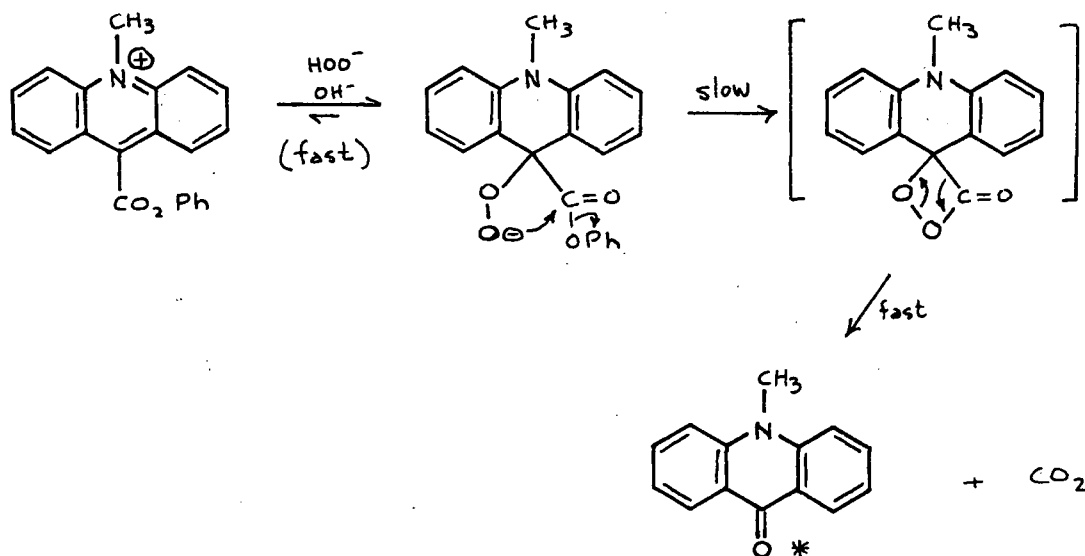
We do not believe that a decomposition of the peracid 37, while yielding N-methylacridone, is chemiluminescent. Indeed, the acridinium

acid 31 was shown to give N-methylacridone by a dark route:



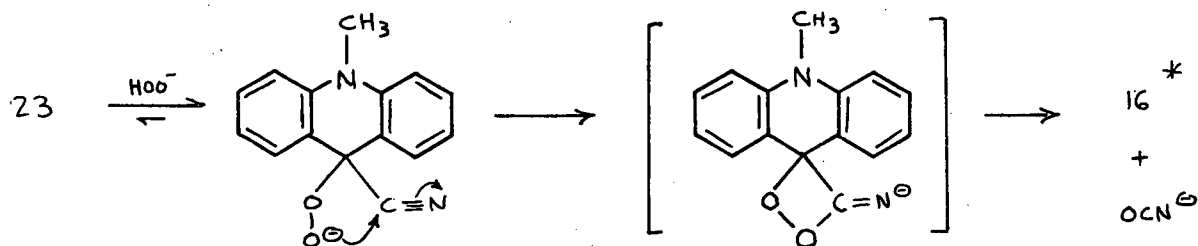
We admit the structures are not the same, but they do share one common feature: they both yield N-methylacridone by an acyclic process. Further evidence to disqualify the peracid is the analogous α -hydroxyl hydroperoxide of lophine, ruled out as a necessary intermediate. (See Introduction.) The reason for the absence of light is not readily apparent, but it could well involve an unfavorable transition state geometry preventing energy transfer to the newly-forming N-methylacridone carbonyl.

The observation that hydrogen peroxide is involved both at C-9 and at the ester carbonyl leads inevitably to our proposed chemiluminescence mechanism for the acridinium salts that was suggested in the Introduction; the necessity for the decomposition of a cyclic peroxide transition state or intermediate in the formation of excited N-methylacridone.



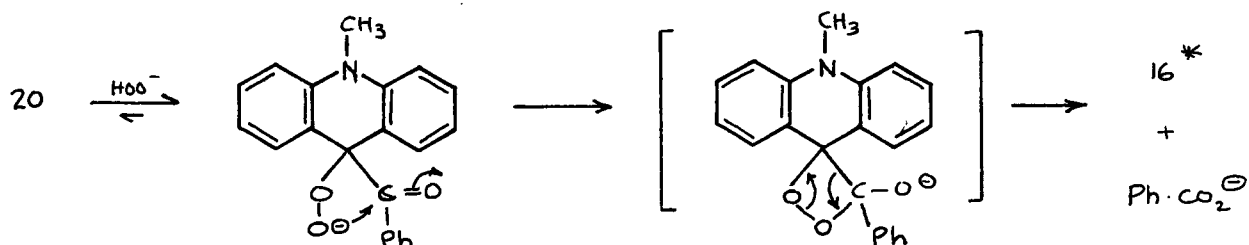
Moreover, this particular mechanism is the most reasonable one which reconciles all the kinetic data presently available.

The less efficient compounds are expected to have the same type chemiluminescence mechanism. The nitrile 23, for example, should have for its light-producing step the reaction



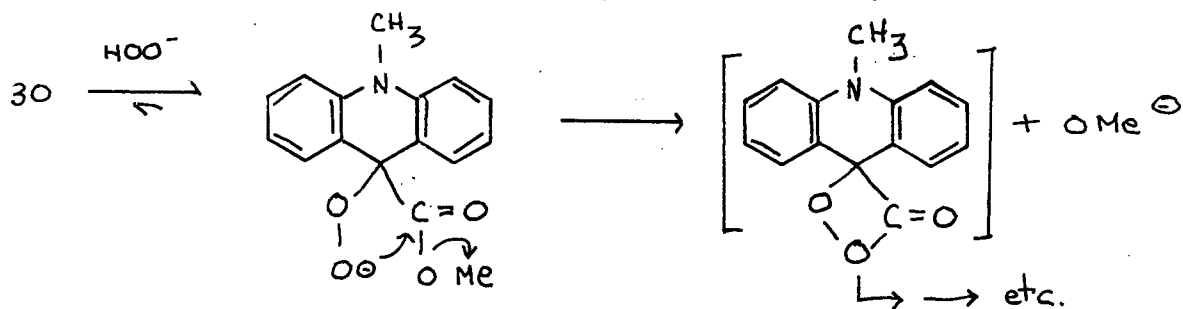
in addition to the dark reactions described previously. Here, the utilization of an electron sink has made possible the formation of a suitable transition state.

Like the nitrile, the benzoyl compound 20 cannot behave in the same manner as the phenyl esters because there is no leaving group. The alternative route, using an electron sink, is again available. Subsequent breakup of the 4-membered transition state yields benzoate ion.



An attempt was made using thin layer chromatography, to detect benzoic acid in the reaction mixture. We were unable to observe any, but this is hardly surprising in view of the observed quantum yield (10^{-6}).

The methyl ester has a leaving group, albeit a very poor one, on the carbonyl carbon. The feeble light production may therefore be an

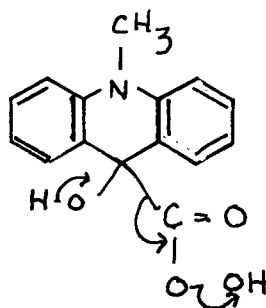


indication of the extent of this reaction, although there is not yet sufficient data available for definitive comment.

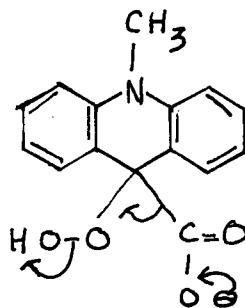
The relative light output of the phenyl esters 34 and of lucigenin bromide is roughly the same. This comparison can now be made in the same manner as for the nitrile 23.

As before, the probability of forming excited N-methylacridone (NMA) should be highest for lucigenin bromide, because (neglecting side reactions) it yields two molecules of product for every molecule of reactant. But, whereas the nitrile had only 1/100th the Q of lucigenin bromide, the phenyl esters have an equal amount. Also, significantly, the phenyl esters (like the nitrile) give NMA quantitatively.

The most reasonable explanation for the increased efficiency is that there are fewer "dark" pathways for NMA production. The two most obvious routes would be through the peracid 37 and the hydroperoxide carboxylate 38.



37



38

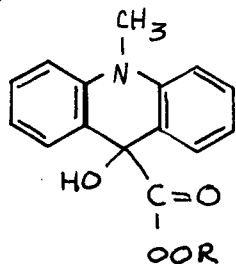
Neither path is expected to make a significant contribution. The formation of 37 is predicated on hydroxyl ion winning out over the much more nucleophilic hydroperoxide anion in competition for the reactive 9-position, followed by peroxide attack at the ester carbonyl. The second path would involve alkaline hydrolysis of 36 before internal attack, a much more favorable process, could occur. In both cases, hydroxyl ion would be functioning as a reactant instead of a catalyst. While such an effect might be more pronounced at pH values lower than the pK of hydrogen peroxide (11.5), we do not believe that under the conditions selected (pH 12, where the concentration of hydroperoxide anion is very high with respect to substrate) there is an effective competition by hydroxyl ion. In short, the light-producing reaction is likely to be much faster than either of the conceivable "dark" reactions. Why then is the quantum yield only 1% ?

There is no clear answer to this. If we can correctly assume that (for the phenyl esters at least) the majority of NMA formation goes via the cyclic peroxide, then the low quantum yield reflects the population of product molecules finding themselves in an electronically excited singlet state. Quenching effects can be ruled out, because the fluorescence efficiency Φ for NMA is 85% irrespective of solvent (ethanol, with or without water and base). Moreover, the high Φ value means that we cannot significantly enhance the quantum yield by making acridinium salts with an electron-releasing ring substituent, as with luminol. Certainly the quantum yield is decreased by having only one NMA molecule formed in the bond cleavage process. The other fragment, CO_2 , could well acquire the excess energy and lose it (most probably) by collisions with solvent. But after all the known variables are accounted for, we still can only speculate on the nature of the product excitation step, and how it can be optimized. Until more information is obtained the questions will remain unanswered.

The brightest compound in the whole series is the N-methylacridinium acid chloride 32. Unfortunately, it is so highly reactive that attempts to purify it were only partially successful. Moreover, when subjected to our standard conditions for light measurement, the compound began to hydrolyze and chemiluminesce too soon. The brightness was visibly greater than the phenyl esters, but of much shorter duration.

At this point, a paper appeared describing the independent discovery of this same compound.⁵⁷ Rauhut and co-workers at Cyanamid prepared 32 and studied its reactions. They found that, in a typical chemiluminescent reaction, 32 had a quantum yield of 1% (comparable to our phenyl esters) and gave NMA (52%) along with other products. These other products were thought to occur via aqueous or alkaline hydrolysis of 32, and turned out to be the acridinium acid 31, carbon dioxide, and carbon monoxide. As in our series, the emitter was NMA ($\lambda_{\text{max}} 440 \text{ m}\mu$).

A chemiluminescent mechanism was proposed, and involved the hydroxy-peracid 37a as a key intermediate. Evidence for 37a was



(a) R = H

(b) R = t-Bu

37

based on several factors:

(1) An infrared study of 32 in anhydrous 1,2-dimethoxyethane containing an equivalent amount of 98% hydrogen peroxide showed the acridinium acid chloride was fairly resistant to attack ($t_{\frac{1}{2}}$: 25 minutes);

(2) light emission was faster at higher pH, or with added water;

(3) weak chemiluminescence was observed when t-butyl hydroperoxide was substituted for hydrogen peroxide, suggesting 37b as the analogous intermediate.

Our work on 32 has been less extensive, but nevertheless we feel the conclusions drawn by the other group are valid only in part. Their mechanism is predicated on the attack by peroxide anion on the carbonyl, followed by hydroxy pseudobase formation and acyclic bond cleavage. Similar processes are possible in a series of acyl chlorides²³; and, while 32 may react in a similar fashion, the phenyl esters show properties incompatible with this scheme. Most significantly, 32 is weakly chemiluminescent with alkaline potassium t-butyl hydroperoxide, whereas the phenyl esters are much less so.

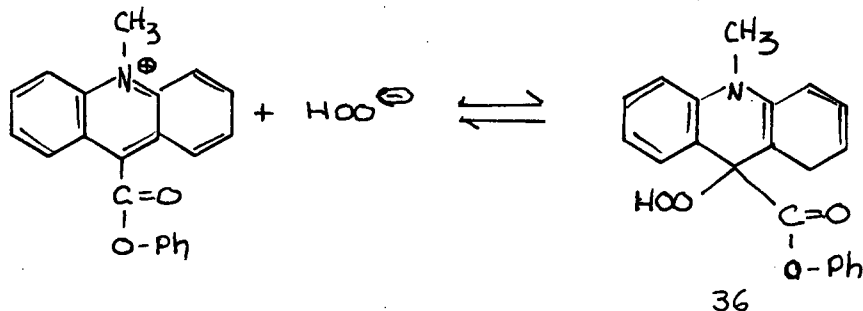
It could be argued that the lack of bright chemiluminescence using t-butyl hydroperoxide does not rule out peracid 37a as the key intermediate. That is, the low nucleophilic power⁷⁸ of t-butyl hydroperoxide might prevent its successful attack of the ester carbonyl; or, if the peracid did form, its facile decomposition might be retarded by the poor leaving ability of t-BuO⁻ anion (in 37a) as compared to OH⁻ ion (in 37b).

These objections can now be countered. The phenyl ester 34c has been treated with methyl hydroperoxide* in alkaline ethanol. This reagent, as nucleophilic as hydrogen peroxide,⁷⁹ yielded only a feeble light (due perhaps to traces of H₂O₂); and, moreover, NMA formed smoothly and rapidly.

* Kindly supplied by Prof. W. Jencks, Brandeis University.

The NMA formation can most reasonably be written as an acyclic decomposition, involving the expulsion of CH_3O^- anion (even less favored than $t\text{-BuO}^-$ expulsion). Most important, the impossibility of forming a cyclic peroxide intermediate seems to have prevented bright chemiluminescence.

In Table III, the effect of pH on the reaction rate and the light emission of phenyl ester 34d is shown. For three arbitrarily chosen pH values, the total amount of light is (within experimental error) the same. Thus, it would appear that the ratio of "light" to "dark" pathways is not influenced by the hydroxyl ion concentration. The quantum yield is so low, around 1%, that we do not wish to speculate on the exact nature of the various competing "dark" reactions occurring at different pH values. But the involvement of hydroperoxide anion in light production was shown in the logarithmic plot of the rate constants against the pH (Figure 7). The Brønsted slope β was 1.0 at low pH values, levelling off in the pH region 10 to 12, and consistent with the requirement for ionized hydrogen peroxide (pK 11.5) in chemiluminescence. Within this simple framework, however, there are more complex relationships. Evidence has already been presented for the 9-hydroperoxide 36 as the chemiluminescence precursor. Formation of this species would depend on the concentration of hydroperoxide anion and also on the equilibrium constant involved. High pH will drive the



equilibrium towards the right, and will also catalyze the further ionization of 36 because alkyl hydroperoxides are known to have pK values near that of H_2O_2 . That second ionization would be expected to result in internal attack of the ester group, giving the cyclic peroxide and chemiluminescence, and it

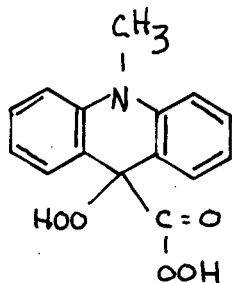
is reasonable to suppose that the second step is rate-determining. In our particular set of runs, the chemiluminescence lifetimes ranged from 20 minutes (pH 7) to 1 second (pH 12).

The effect of hydrogen peroxide concentration was of special interest in light of the claims made for an excited oxygen dimer as the energy source for chemiluminescence.¹⁹ For this to be valid, one would expect to find a square dependence on the peroxide concentration.

Our results (Table IV) do not substantiate these claims. In the range where hydrogen peroxide is in large excess over substrate, the amount of light is unchanged upon a 10-fold dilution of peroxide. This rules out the square dependence so necessary for the excited oxygen concept.

In the runs where the initial peroxide concentration is below that of the substrate (10^{-4} M), we see a reduction in light output, but the reduction is only about half as great as expected for each successive 10-fold dilution of peroxide. We have no explanation at the moment, although possibly the chemiluminescence quantum yield (ie, degree of excited product formation) might be increasing as the H_2O_2 concentration becomes low.

The variation in rate constants is also interesting. At high peroxide concentration the reaction has slowed down, and this could be caused by the attack on substrate by two molecules of peroxide, giving a relatively slow-reacting intermediate:



With lower peroxide concentration the rate is progressively faster, reaching a maximum where the two reactants are approximately equimolar. Below this point, the rates again decrease; this phenomenon is probably due to the low concentration of both reactants, at which point the magnitude of the "adduct" equilibrium constant becomes meaningful. Additional evidence here is that the two weakest peroxide concentrations give chemiluminescence curves having an induction period.

The effect of substrate concentration on the kinetics and light emission of the phenyl ester 34c has been studied (Table V). In the range of concentrations used, with peroxide and base in large excess, the reaction was expected to be pseudo first-order in substrate; the linear relationship of $\log I$ vs. time confirms this for all the runs in that series.

We notice a moderate rate increase with decreasing substrate concentration. This could be interpreted as an effect of side reactions becoming more prominent as the substrate is progressively diluted; but, within each run, the side reactions are not causing deviations from linear first-order kinetics. There is a parallel effect on the total light output; we see a larger loss in light emission than could be explained simply as the effect of dilution. Alternatively, the discrepancy in light output could be due to enhanced quantum yield (ie, efficiency of excited product formation) at higher substrate concentrations, due either to a more efficient energy transfer process or an absence of quenching effects. There is not sufficient data available to resolve these questions, however.

CONCLUSION

CONCLUSION

This investigation has yielded considerable information about the general mechanism proposed for efficient chemiluminescent processes. That is, the involvement of a 4-membered cyclic peroxide transition state or discrete intermediate seems necessary, and for bright light production, a high reactivity with hydroperoxide anion appears to be necessary. But there are many aspects of the reactions which we do not yet fully understand. For example, it was found that water, in amounts far greater than kinetically significant, was necessary for bright rapid light emission. This may be a solvation phenomena, but further evidence is lacking. The brightest compounds, the acridinium phenyl esters, were weakly chemiluminescent in the absence of hydrogen peroxide; trace amounts of H_2O_2 may have been responsible, but again, further work is needed to clarify this matter. The weakest compounds in the series show anomalous properties and the light that they emit may not indeed be a result of hydrolysis by hydrogen peroxide. In this respect, a detailed product study appears in order.

The kinetic studies provided some evidence to support our general hypothesis, but because the light-producing part of the reaction is so small, and the possibility of complex side reactions so high, that care must be exercised in interpreting the results. Principally, this has been a preliminary investigation designed to explore the feasibility of more elaborate experimentation, and this is now in progress.

BIBLIOGRAPHY

1. F. McCapra, Quart. Revs. 1966, 485
2. K.D. Gundermann, Angew. Chem. Int. Ed. 4, 566 (1965)
3. R.F. Vassil'ev et al, Russian Chem. Revs. 1965 , 599
4. E.J. Bowen, Chemistry in Britain, Vol. 2 No. 6, June 1966, p. 249
5. E.H. White in "Light and Life", (ed. McElroy and Glass), Johns Hopkins Press, Baltimore, 1961, p. 183
6. T. Quickendon, J. New Zealand Inst. Chem. 28, 10 (1964)
7. Symposium on Chemiluminescence, Photochem. Photobiol. 4 No. 6 December (1965)
8. E.J. Bowen, Pure Appl. Chem. 473 (1964)
9. E.N. Harvey, "A History of Luminescence", Amer. Phil. Soc. (1957)
10. R.S. Anderson, Ann. N.Y. Acad. Sci. 49, 337 (1948)
11. H. Stork, Chem. Ztg. 85, 467 (1961)
12. A. Bernanose, Bull. Soc. Chim. Fr. 1950, 567; 1951, 329; 1952, 39D.
13. W. Vaughan, Chem. Revs. 43, 447 (1948)
14. B.A. Thrush, Chemistry in Britain, Vol. 2 No. 7, July 1966, p. 207
15. R.E. Visco and E.A. Chandross, J. Amer. Chem. Soc. 86 5350 (1964)
16. D.M. Hercules, Science 145 , 808 (1964)
17. E.A. Chandross and F.L. Sontag, J. Amer. Chem. Soc. 88, 1089 (1966)
18. J.S. Arnold, R.J. Browne, and E.A. Ogryzlo, Photochem. Photobiol. 4 963 (1965)
19. A.U. Khan and M. Kasha, J. Amer. Chem. Soc. 88, 1574 (1966)
20. F. McCapra and D.G. Richardson, Tetr. Letters 1964, 3167
21. F. McCapra, D.G. Richardson, and Y.C. Chang, Photochem. Photobiol. 4, 1111 (1965)
22. F. McCapra and Y.C. Chang, Chem. Comm. 1966, 522
23. M.M. Rauhut, D. Sheehan, R.A. Clarke, and A.M. Semsel, Photochem. Photobiol. 4, 1097 (1965)

24. E.H. White and M. Harding, *ibid.* 4 1129 (1965)
25. H. Eyring, F.H. Johnson, and M.J. Polissar, "The Kinetic Basis of Mol. Biology", Wiley, New York (1954)
26. S.F. Mason, *Quart. Revs.* 15, 306 (1961)
27. F. McCapra, *Chem. Comm.* 1968, 155
28. C.A. Bunton in "Peroxide Reaction Mechanisms" (Ed. Edwards), Interscience, New York 1962, pp. 16-18
29. H.G. Aurich, *Tetr. Letters* 1964, 657
30. J.E. Huber, *Tetr. Letters* 29, 3271 (1968)
31. K. Kpecky and C. Mumford, *Can. J. Chem.* 47, 709 (1969)
32. An excellent discussion of fluorescence is found in "Light: Physical and Biological Action", ed. Siliger and McElroy, Academic Press, New York, 1965, Chapter 2.
33. E.H. White, O. Zafirionou, H. Kägi, and J. Hill, *J. Amer. Chem. Soc.* 86, 941 (1964)
34. J. Lee and H.H. Seliger, *Photochem. Photobiol.* 4, 1015 (1965)
35. K.D.H. Drew and F.H. Pearman, *J. Chem. Soc.* 506 (1937)
36. A Spirit - Van Der Burg, *Rec. Trav. Chim.* 69, 1536 (1950)
37. B. Radziszewski, *Ber.* 10; 70, 321 (1877)
38. H. Kautsky and K.H. Kaiser, *Naturwiss.* 31, 505 (1943)
39. T. Hayashi and K. Maeda, *Bull. Chem. Soc. Japan*, 35, 2057 (1962); 36, 1052 (1963)
40. D.M. White and J. Sonnenberg, *J. Amer. Chem. Soc.* 86, 5685 (1964)
41. E.H. White and M. Harding, *ibid.* 86, 5686 (1964)
42. G.E. Philbrook, M.A. Maxwell, R.E. Taylor, and J.R. Totter, *Photochem. Photobiol.* 4, 1175 (1965)
43. Y. Kishi, T. Goto, Y. Hirata, O. Shimomura, and F.H. Johnson, *Tetr. Letters* 1966, 3427
44. M.M. Rauhut et al, *J. Amer. Chem. Soc.* 89, 6515 (1967) and references cited therein
45. K. Weber, *Z. Phys. Chem.* 50, 100 (1941)

46. O. Schales, Ber. 72, 1155 (1939)
47. K. Gleu and W. Petsch, Angew. Chem. 48, 57 (1935)
48. B. Tamamushi and H. Akiyama, Trans. Faraday Soc. 35, 491 (1939)
49. R.B. Kurtz, Trans. N.Y. Acad Sci. 16, 399 (1954)
50. A.V. Karyakin, Optika i Spektrosk. I, 123 (1959)
51. J. Kroh, Acta Chimica 5, 115 (1960)
52. (a) L. Erdey, Acta Chim. Acad. Sci. Hung. 3, 81, 105 (1953);
(b) 39, 295 (1963)
53. A.M. Grigorovsky and A.M. Simenov, J. Gen. Chem. USSR 21, 653 (1951)
54. J.R. Totter, Photochem. Photobiol. 3, 231 (1964)
55. J.R. Totter and G.E. Philbrook, *ibid.* 5 177 (1966)
56. R.M. Acheson, "The Acridines", the Chemistry of Heterocyclic Compounds (ed. Weissberger), Interscience, New York, 1956
57. M.M. Rauhut, D. Sheehan, R.A. Clarke, B.G. Roberts, and A.M. Semsel, J. Org. Chem. 30, 3587 (1965)
58. R. Bernthsen, Ann. 224, 1 (1884)
59. L. Decker and C. Hoch, Ber. 37, 1564 (1904)
60. K. Lehmstedt and F. Dostal, Ber. 72, 804 (1939)
61. C. Kaufmann and A. Albertini, Ber. 42, 1999 (1909)
62. K. Lehmstedt and E. Wirth, Ber. 61, 2044 (1928)
63. G.I. Braz and S.A. Gore, J. Gen. Chem. USSR 23, 909 (1953)
64. I. Kröhnke and H. Honig, Ber. 90, 2215 (1957)
65. Handbook of Chemistry and Physics, 42nd edition, The Chemical Rubber Publishing Co., Cleveland, Ohio, 1960, p. 1718
66. W.H. Meluish, J. Phys. Chem. 65, 229 (1961)
67. J. Calvert and J. Pitts, "Photochemistry", Wiley, New York 1966, p. 799
68. M. Banning, J. Opt. Soc. America 37, 686 (1947)
69. A. Frost and R. Pearson, "Kinetics and Mechanism, " Wiley, New York, 1953

70. H. Jaffe, Chem. Revs. 53, 191 (1953)
71. K. Wiberg, J. Amer. Chem. Soc. 77, 2519 (1955)
72. R.A. Joyner, Z. Anorg. Chem. 77, 103 (1912)
73. K. Wiberg, J. Amer. Chem. Soc. 75, 3961 (1953)
74. M.S. Karasch and G. Sosnovsky, Tetr. 3, 97 (1958)
75. G.A. Russell, J. Amer. Chem. Soc. 76, 1595 (1954)
76. H.C. Brown and Y. Okamoto, J. Amer. Chem. Soc. 80, 4979 (1958)
77. H. van Bekkum et al, Rec. Trav. Chim. 78, 815 (1959)
78. G. Baddeley and R.M. Topping, Chem. and Ind. 1693 (1958)
79. W.P. Jencks and J. Carriuolo, J. Amer. Chem. Soc. 82, 1778 (1960)
80. H. Gensen and F. Rethwisch, J. Amer. Chem. Soc. 50, 1144 (1928)