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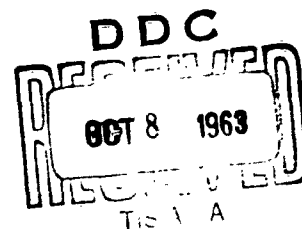
TECHNICAL REPORT NO. 1

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AND
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ARPA ORDER NO. 299, AMEND. 3
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CHEMILUMINESCENT MATERIALS

AMERICAN CYANAMID COMPANY
CENTRAL RESEARCH DIVISION
STAMFORD, CONNECTICUT

JUNE 1 - AUGUST 31, 1963



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SUMMARY

Work during this period has been divided between (1) assembly and calibration of radiometric apparatus, (2) synthesis designed to test the validity of several hypotheses, and (3) exploration of the behavior of a recently reported new chemiluminescent system.

Apparatus intended for quantitative measurement of chemiluminescence as a function of time, temperature and wavelength has been assembled and calibration is virtually complete. Optional front side illumination has been included in the design to enable investigation of fluorescence and energy transfer phenomena at high concentrations.

None of the six synthetic approaches initiated during the period have yet been completed and it appears likely that one or two will be abandoned. Three syntheses are under investigation involving preparation of energy rich precursors which should collapse on heating or reaction to give the known fluorescing agent N-methylacridone, or a derivative. These reactions resemble processes which may occur in the known lucigenin chemiluminescence. Routes to a cyclic diacyl peroxide which can collapse to a fluorescent cyclic anhydride on reaction with a phosphine are described. Preparation of a key intermediate for the synthesis of a highly fluorescent or, alternatively, a polymeric derivative of luminol (aminophthalhydrazide) is described;

however, the desired products have not yet been obtained.

A literature report that oxalyl chloride and hydrogen peroxide liberate light only in the presence of an energy acceptor has been confirmed and amplified. Our results require non-radiative energy transfer, but do not yet enable choice of a mechanism. The system is important since it demonstrates light emission proceeding from structurally simple reactants and indicates that many well known reactions might be chemiluminescent if proper methods for transferring energy could be established.

INTRODUCTION

The research program to be described in this and ensuing reports is concerned with all elements of the process by which chemical energy is converted into visible light. Its over-all objective is to seek an understanding of chemiluminescence, using the information obtained to design new systems of greater brightness or efficiency than those presently available.

In the broadest sense chemiluminescence can be divided into two successive processes--the first involving one or more chemical steps by which molecules in an electronic ground state react to form a product molecule in an electronic excited state and the second involving the physical process by which the excited state energy is converted into light of a suitable wavelength. The over-all yield of a chemiluminescent reaction is, then, the product of the yields of the chemical process and the physical process and an improvement in either will result in increased over-all efficiency.

To the scientist, the most mechanistically significant measure of the over-all efficiency of chemiluminescence would be quantum yield--that is, the number of quanta of light produced in a given system divided by the number of molecules reacting. Therefore, quantum yield will frequently be discussed in these reports. However, it should be remembered that visual output or luminosity is the practical measure of chemiluminescence and involves primarily quanta emitted close to 555 m μ , the region of maximum eye sensitivity. Thus visual efficiency requires

a weighting of quanta at different wavelengths in proportion to the relative sensitivity of the eye at those wavelengths. This emphasizes the importance to this program of methods for controlling not only the quantum yield but also the spectral distribution of chemiluminescent systems.

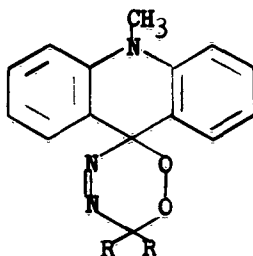
This report is divided into two parts--chemical and physical. The chemical section describes work intended to establish the effect of chemical structures (or conditions) on chemiluminescence while the physical section concerns problems of measurement and factors affecting the emission of excited state energy or its transfer to an energy acceptor. Various energy transfer mechanisms are well known in studies of fluorescence and their importance in the quenching or enhancement of chemiluminescent emission will be discussed when the principles are pertinent to the work being reported.

SECTION 1 - CHEMISTRY

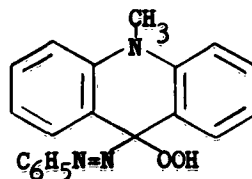
New Chemiluminescent Reactions

Two basic and obvious requirements for a chemiluminescent reaction are (1) that the reaction liberate sufficient energy to generate quanta in at least the visual portion of the spectrum (72 kCal/mole to 41 kCal/mole) and (2) that a compound be available to convert this energy to a radiative electronic excited state. A third requirement is that an efficient pathway be available for conversion or transfer of the available chemical energy to the molecular electronic energy required for emission. While it is a straightforward matter to design energetic reactions providing products known to be fluorescent (capable of emission), it is clear from the literature that most such reactions are not substantially chemiluminescent, and that therefore the third requirement is not commonly met.

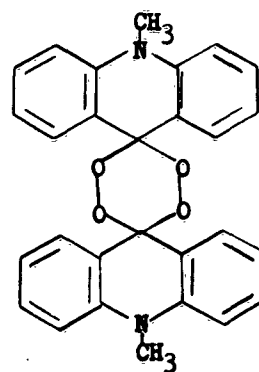
To provide information upon which to base structural criteria for the third requirement, we are attempting to prepare the compounds shown below.



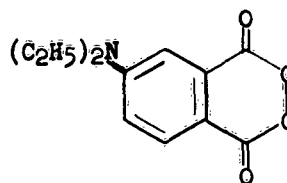
I



II

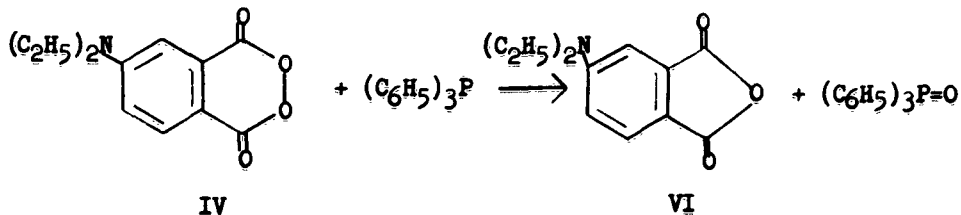
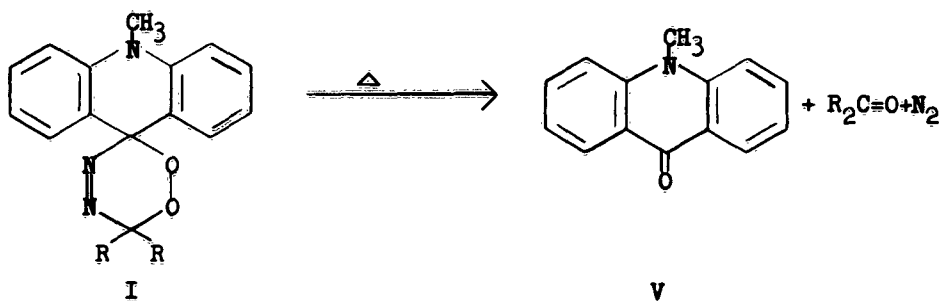


III



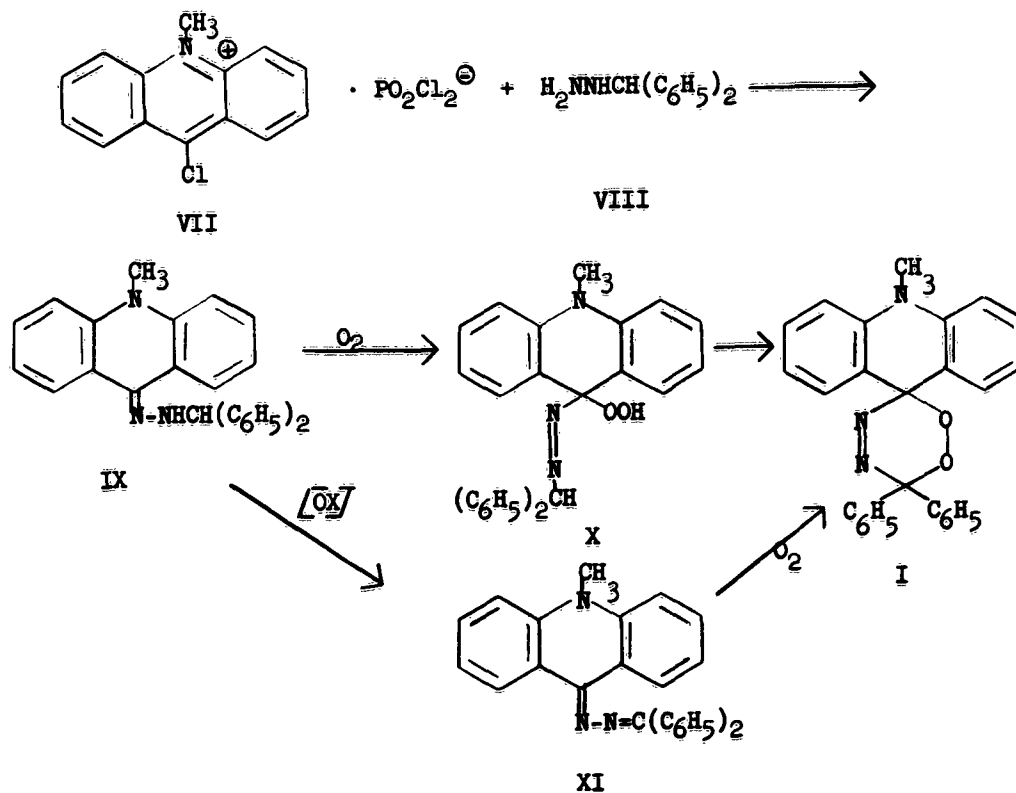
IV

These compounds, as a consequence of their peroxidic structures, should meet requirement (1) and by suitable choice of reaction conditions can be expected to meet requirement (2). Thus thermal decompositions of I, II, and III should produce the highly fluorescent N-methylacridone, V while reaction of IV with triphenylphosphine should generate 4-diethylaminophthalic anhydride, VI¹. Although the latter has not been reported previously, its fluorescence can be predicted confidently by analogy with the known and substantially fluorescent² 3-aminophthalic anhydride.



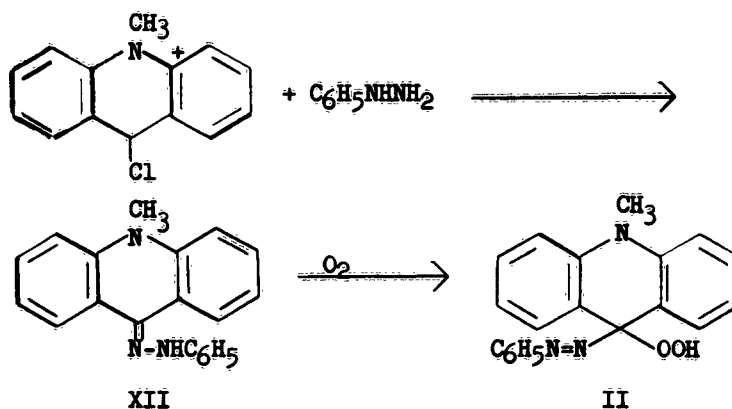
Since these test reactions meet requirements (1) and (2), their ability to accommodate requirement (3) can be determined by examining them for chemiluminescent behavior. Moreover, the results will have direct mechanistic significance bearing on the third requirement, since the chemical mechanisms of the four reactions will differ. Synthesis of Compounds I through IV is currently underway, and the synthetic aspects of this effort are described in the following paragraphs.

Synthesis of I ($R=C_6H_5$) has been attempted by the route diagramed below.

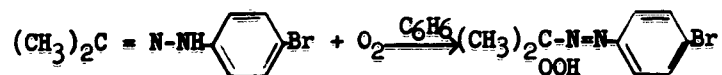


Reaction of 9-chloro-10-methylacridinium dichlorophosphate VII with benzhydrylhydrazine, VIII, would be expected to produce the benzhydrylhydrazone, IX³. Autooxidation of IX might be expected to provide the desired azoperoxide I directly by way of the intermediate azohydroperoxide X. Alternatively, oxidation of IX could give the azine XI, which might then be autooxidized to I. When the reaction was carried out, azine XI was obtained directly, presumably by in situ oxidation of IX. Attempts to convert XI to I will be made in the near future.

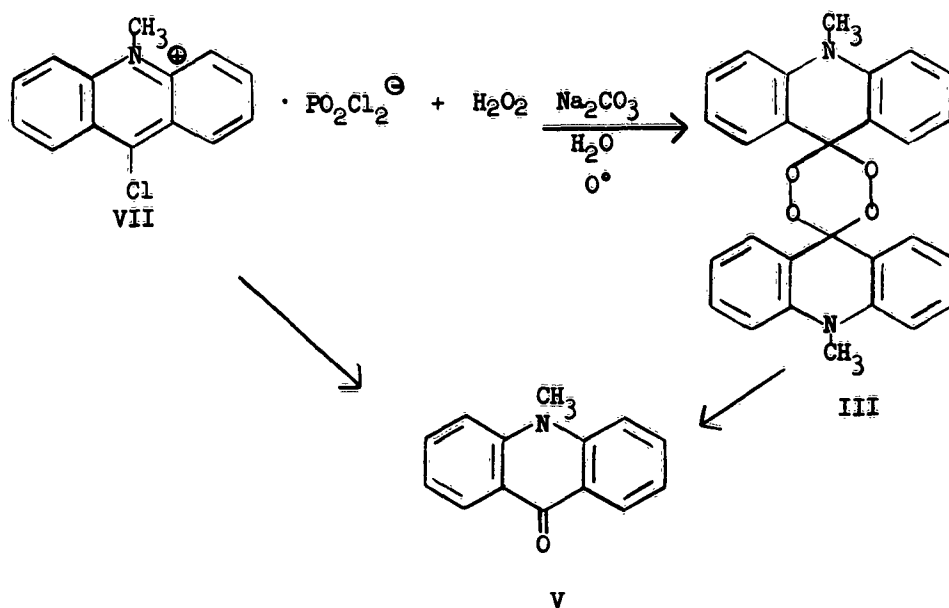
The preparation of II will be carried out by a similar route.



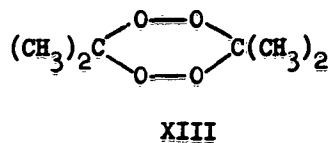
Autooxidation of the phenylhydrazone XII is expected to be straightforward by analogy with the known autooxidation reaction shown below⁴.



Two attempts to synthesize III by the following reaction have given only 10-methylacridone, V.

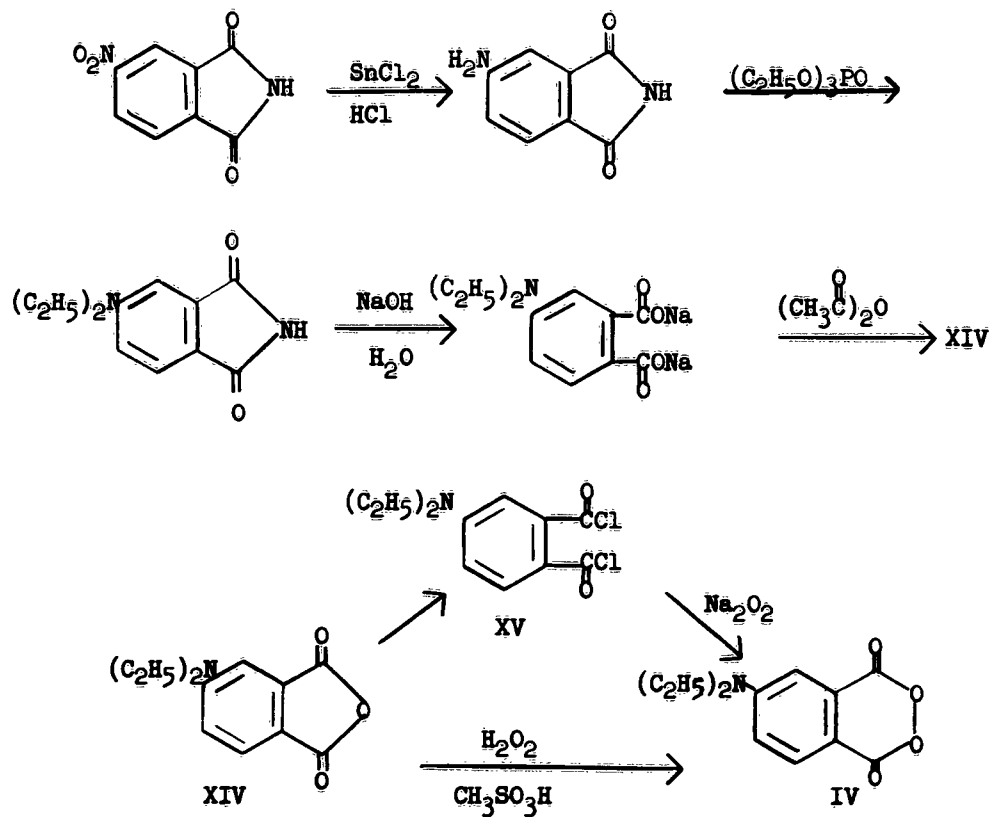


While III would be expected to give the acridone on decomposition, we have no compelling reason to suspect it as an intermediate in the over-all reaction. Moreover, a peroxide of structure III might be expected to have reasonable stability by analogy with the appreciably stable "diacetone peroxide," XIII⁵.



The over-all reaction as carried out was not chemiluminescent. An attempt will be made to prepare III by an alternate route.

The preparation of IV has been attempted by the following route.



Reduction of 4-nitrophthalimide under conditions described in the literature⁶ gave 4-aminophthalimide in 100% yield. Attempted alkylation of the amine with dimethylsulfate under literature conditions⁷ gave meager amounts of the 4-diethyl derivative, but alkylation with triethylphosphate⁸ gave 4-diethylaminophthalimide in 34% yield. Hydrolysis

of the imide followed by reaction with acetic anhydride gave the desired 4-diethylaminophthalic anhydride XIV, which was found to have the expected strong fluorescence.

A number of attempts to convert the anhydride XIV to 4-diethylaminophthaloyl peroxide IV, however, have failed. Since phthaloyl chloride is known to give phthaloyl peroxide on treatment with sodium peroxide⁹, we first attempted to prepare 4-diethylaminophthaloyl chloride. Reactions of anhydride XIV with thionyl chloride-zinc¹⁰, benzotrichloride-zinc¹⁰, or phosphorus pentachloride¹¹, however, gave only unchanged anhydride and tars.

An attempt to convert the anhydride directly to peroxide IV by reaction with hydrogen peroxide in methane sulfonic acid was also unsuccessful¹².

Polymeric Phthalhydrazides

Concentration quenching, well known in fluorescence spectroscopy¹³, is characterized by the loss of emission efficiency with increasing concentration of luminant above about 10^{-3} molar in solution. Since the emission process corresponding to the radiative decay of an excited molecule, is the same in chemiluminescence as in fluorescence, concentration quenching would also be expected in chemiluminescence. Loss of chemiluminescent efficiency with increasing concentration has been well established experimentally, particularly in the phthalhydrazide chemiluminescent system. In chemiluminescence, moreover, the concentration problem is further worsened by the

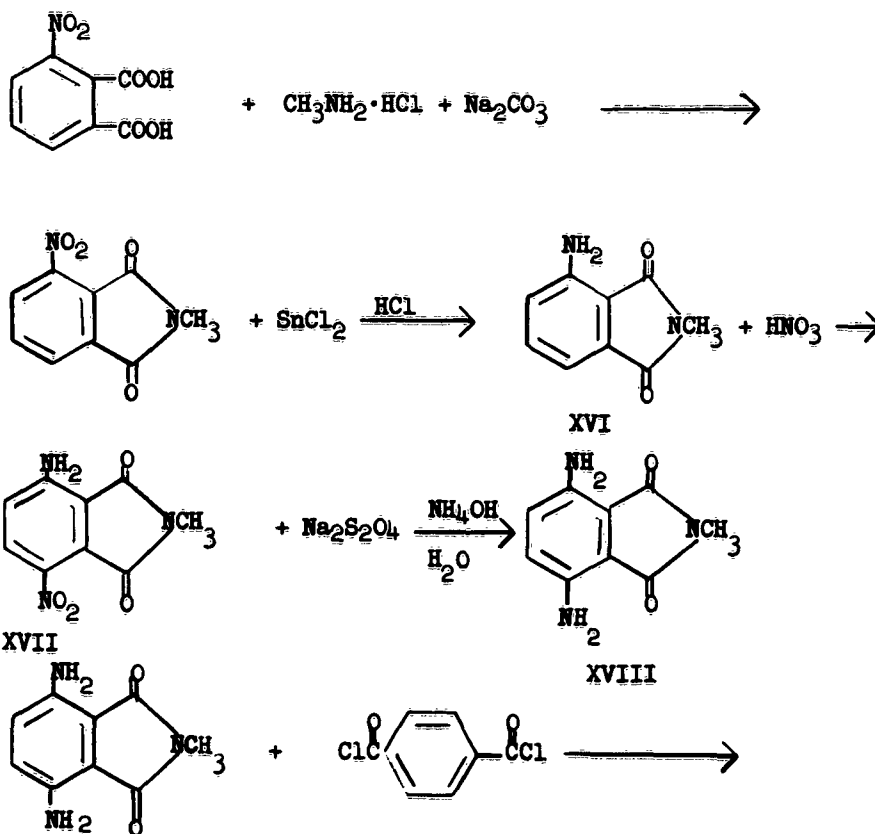
probability that key radical intermediates required for the chemiluminescent process will be destroyed by coupling or disproportionation at higher concentrations. It can be calculated that chemiluminescent intensities suitable for broad use will require luminant concentrations on the order of one-tenth molar. Since efficiency loss begins at about one-thousandth molar, it is clear that concentration quenching, both spectroscopic and chemical, represents a serious problem in the development of practical chemiluminescence.

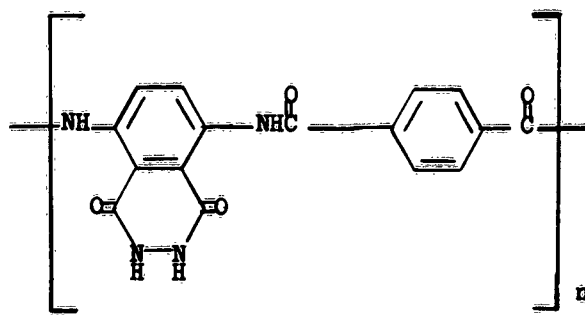
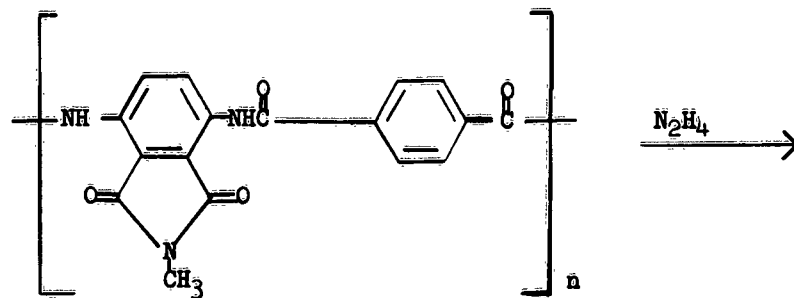
Both quenching processes require close approach of intermediates derived from the luminant. Thus a chemiluminescent system that decreases the probability of such collision should permit higher efficiency at high concentration. In fluorescence this can frequently be achieved by increasing the viscosity of the solvent or by lowering the temperature. Early attempts in this laboratory to increase chemiluminescent efficiency by increasing viscosity did not give encouraging results. However, in at least three chemiluminescent systems, efficiency does increase with decreasing temperature.

A third method of limiting collision at high concentration might be to tie the luminescent structure into a relatively immobile polymer backbone. In a polymeric system, where molecular motion is damped it can be shown that the time required for an initial collision between active sites is considerably lengthened. Since concentration quenching occurs generally at every encounter, and since the lifetime of excited species are short (10^{-8} seconds), even a small decrease in

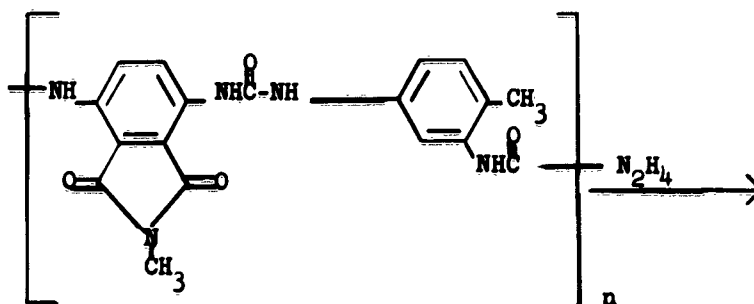
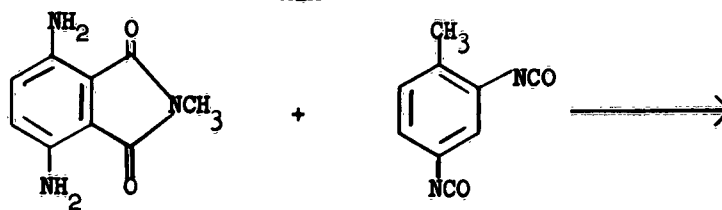
the collision frequency can be expected to increase emission efficiency. Polymers containing ionized groups should be particularly effective since charge repulsion would reduce coiling and intramolecular approach. An optimum result might be expected in a cross-linked polymer where mobility is severely restricted.

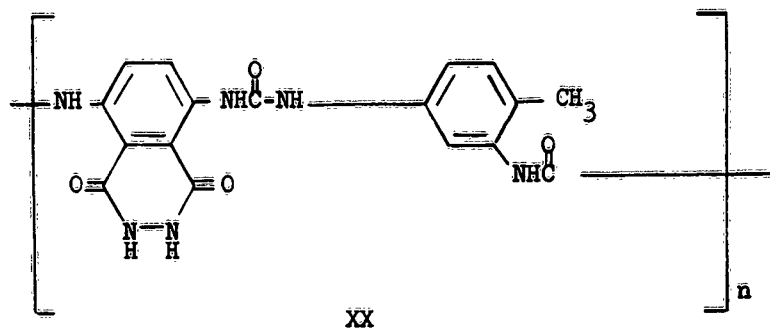
To test this general approach the polyamide XIX and the polymer XX are being prepared by the following routes.





XIX



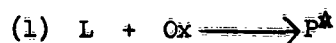


Fusion of 3-nitrophthalic acid with methylamine hydrochloride and sodium carbonate gave 3-nitro-N-methylphthalimide in 91% yield. Reduction with tin II chloride and hydrochloric acid gave 3-amino-N-methylphthalimide, XVI in 82% yield.

Several early attempts to nitrate the acetyl derivative of XVI by the "traditional" method of nitrating aryl amines gave poor yields. It was subsequently found, however, that direct nitration of the free amine gave the desired 3-amino-6-nitro-N-methylphthalimide XVII in 90% yield. Reduction of XVII to the diamine XVIII proceeded in only 20% yield, although additional work is expected to improve this. Conversion of the diamine to the desired polymers will begin in the near future.

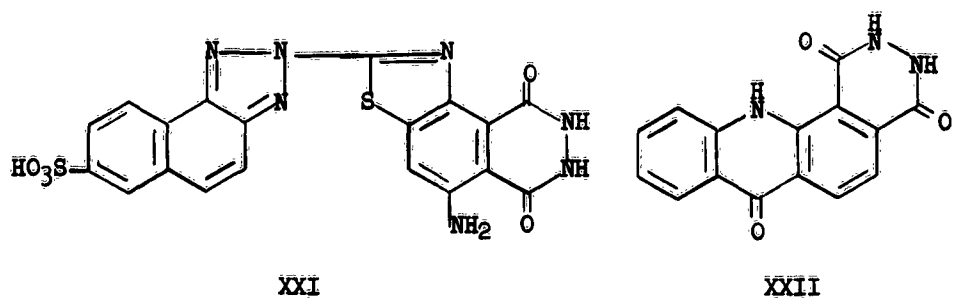
Highly Fluorescent Phthalhydrazide Derivatives

The generalized mechanism of chemiluminescence requires the formation of an excited state by chemical reaction followed by the radiative decay of that excited state as diagramed below.

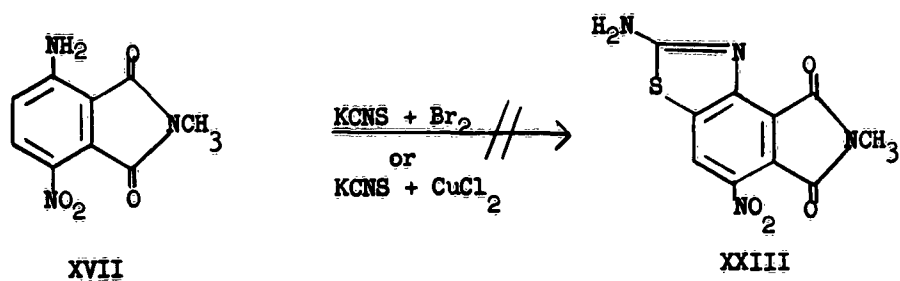


Equation (1) represents a chemical reaction, actually a series of steps, between a "luminant", L, and an oxidant, Ox. A product, P^* , of this reaction appears in an excited state which then radiates as in equation (2). As stated in the introduction, the efficiency of the over-all process will be the product of the efficiency of steps (1) and (2). Since step (2) is identical to the radiative process in fluorescence, the over-all efficiency will depend on the fluorescence efficiency of the emitting species, P. In phthalhydrazide chemiluminescence, as in most chemiluminescent systems, the identity of the emitter has not been established. However, a high fluorescence efficiency of the emitter might be assured by incorporating the chemiluminescent phthalhydrazide structure into a highly fluorescent backbone.

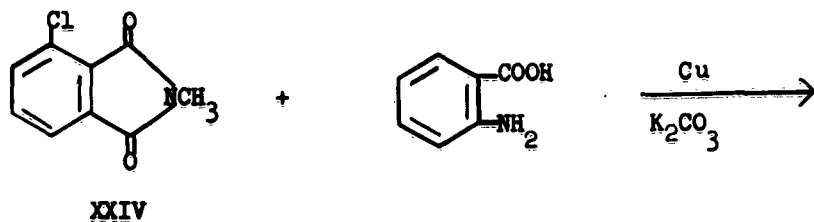
Attempts to synthesize the benzothiazole XXI and the acridone XXII, designed to test this possibility, have thus far been unsuccessful.

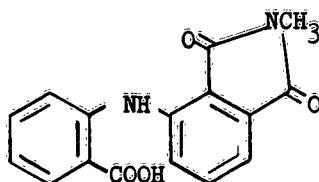


In the former case, application of two standard thiazole syntheses¹⁴ to 3-amino-6-nitro-N-methylphthalimide, XVII, failed to give the required intermediate thiazole XXIII.



In the latter case reaction of 3-chloro-N-methylphthalimide XXIV with anthranilic acid in the Ullmann reaction¹⁵ failed to give the required intermediate diphenylamine XXV.





XXV

Failure of XVII to react with thiocyanogen ($\text{KCNS} + \text{Br}_2$) may be attributed to the low electron density of the aromatic ring resulting from the electronegative nitro and cyclic imide substituents, although p-nitroaniline undergoes reaction successfully¹⁴. The failure of the Ullmann reaction, however, is less easily accounted for since the chlorine is activated toward nucleophilic substitution by the imide ring¹⁶.

A further attempt will be made to prepare XXII by another route.

Experimental (a) (Chemistry Section)

Materials - 9-Chloro-10-methylacridinium dichlorophosphate (VII) was prepared from 10-methylacridone (VIII) and phosphorus oxychloride¹⁷. Benzhydrylhydrazine was prepared from benzophenone and hydrazine followed by reduction with lithium aluminum hydride¹⁸. 4-Aminophthalimide was prepared by tin II chloride-hydrochloric acid reduction of the corresponding nitrophthalimide⁶. Other starting materials were obtained from commercial sources.

10-Methylacridon-9-ylidiphenylmethylazine (XI) - A solution of 5.4 g. (0.028 mole) of benzhydrylhydrazine in 50 ml. of dry dimethylformamide was added slowly at room temperature to a stirred solution of 9.5 g. (0.028 mole) of 9-chloro-10-methylacridinium dichlorophosphate in 250 ml. of dry dimethylformamide. After stirring 2 hours at room temperature, the mixture was made alkaline by the addition of 200 ml. of 5% sodium bicarbonate. A tarry orange precipitate was obtained. The tarry solid was dissolved in acetone, treated with activated charcoal, and the acetone was evaporated under vacuum. The residual red oil crystallized upon treatment with hexane. Recrystallization from hexane gave 2.5 g. (23.3%) of material, m.p. 125-6°.

NMR and infrared spectral analyses were in agreement with the azine structure.

Anal. Calcd. for $C_{27}H_{21}N_3$: C, 83.69; H, 5.47; N, 10.84.

Found: C, 83.42; H, 5.39; N, 10.69.

Attempted Synthesis of 10,10"-Dimethylspiro[acridan-9-3'-s-tetroxane-6',9"-acridan](III) - To 1.0 g. (0.005 mole) of 10-methyl, 9-acridanone was added 10 ml. of phosphorus oxychloride and the resulting mixture was refluxed for three hours. On cooling a bright yellow solid was obtained and was collected and washed well with ether. The solid (9-chloro-10-methylacridinium dichlorophosphate) was dissolved in 250 ml. of ice water and treated in the dark with 100 ml. of a cold (0°) solution of 10% hydrogen peroxide in 5% aqueous sodium bicarbonate. The reaction mixture was stirred at 0° for 20 hours. No luminescence was observed. A brown solid separated and was collected and washed well with water to obtain 0.9 g. (90%) of 10-methyl, 9-acridanone, m.p. 197-199° (lit.¹⁹ m.p. 201°) further characterized by comparison of its infrared spectrum with that of an authentic sample.

[(a): Melting points and boiling points are uncorrected.]

4-Diethylaminophthalimide - A mixture of 5.0 g. (0.03 mole) of 4-aminophthalimide and 20.0 g. of triethylphosphate was refluxed (210° to 230°) for 5 hours. The mixture was cooled to 50° and poured over excess crushed ice. The resulting solution was neutralized to pH 7 with solid sodium carbonate, and the solution was extracted with three 50 ml. portions of chloroform. The extracts were combined, dried over calcium sulfate and evaporated under reduced pressure to about 30 ml. The solution was chromatographed on a 33 by 3 cm. neutral alumina column to obtain 3.2 g. (32%) of yellow solid, m.p. 175-6° (lit.⁷ m.p. 177°).

An earlier attempt to carry out the alkylation with dimethyl-sulfate⁷ was unsuccessful.

Disodium 4-Diethylaminophthalate - A mixture of 1.6 g. (0.007 mole) of 4-diethylaminophthalimide and 25 ml. of 50% aqueous sodium hydroxide was boiled for 30 minutes. The disodium salt (1.3 g. 63%) separated on cooling and was recrystallized from a mixture of water, ethanol, and acetone to obtain an analytical sample, m.p. 250°.

Anal. Calcd. for $C_{12}H_{13}O_4NNa_2$: C, 51.25; H, 4.66; N, 4.98.

Found: C, 50.76; H, 4.85; N, 5.17.

4-Diethylaminophthalic Anhydride (XIV) - A mixture of 3.0 g. (0.011 mole) of disodium 4-diethylaminophthalate and 50 ml. of acetic anhydride was heated on a steam bath until evaporation to dryness was complete. The residue was extracted with cold chloroform. The extract was concentrated and treated with petroleum ether to obtain 1.0 g. (43%) of material melting at 94-98°. Recrystallization from a benzene petroleum ether mixture gave material melting at 102-3°.

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39.

Found: C, 65.51; H, 5.73; N, 6.55.

Attempted Preparation of 4-Diethylaminophthaloyl Chloride (XV) - Attempts to react 4-diethylaminophthalic anhydride with thionyl chloride¹⁰, benzotrichloride¹⁰, or phosphorus pentachloride¹¹ under conditions successful for the conversion of phthalic anhydride to phthaloyl chloride^{10,11} gave only mixture of unchanged anhydride and tars.

Attempted Preparation of 4-Diethylaminophthaloyl Peroxide (IV) - A solution of 1.05 g. (0.005 mole) of 4-diethylaminophthalic anhydride in 15 ml. of methane sulfonic acid was treated with 0.7 g. (0.019 mole) of 90% hydrogen peroxide and allowed to stand at 35° for four hours.

The resulting solution was poured over 50 g. of ice, and the clear solution was neutralized to pH 7 with aqueous sodium carbonate solution. Since precipitation did not occur, the neutral solution was extracted with three 100 ml. portions of chloroform. The extract was in turn extracted with 5% aqueous sodium bicarbonate. Evaporation of the chloroform gave only a trace of tar. The bicarbonate extract was neutralized with acetic acid and extracted with chloroform. The chloroform extract on evaporation gave 0.15 g. of 4-diethylaminophthalic acid, m.p. 149°, characterized by comparison with an authentic sample.

3-Nitro-N-methylphthalimide - 3-Nitrophthalic acid (50.0 g., 0.24 mole) was mixed thoroughly with 34.0 g. (0.50 mole) of methylamine hydrochloride and 26.5 g. (0.25 mole) of sodium carbonate. The reaction mixture was heated at 160° for 3 hours with occasional stirring and then cooled. The solid was washed well with 250 ml. of water and the yellow product was collected on a filter and dried to obtain 45.0 g. (91.0%) of material, m.p. 110-112° (lit.²⁰ 112-113°).

Anal. Calcd. for $C_9H_6N_2O_4$: C, 52.43; H, 2.93; N, 13.59.

Found: C, 52.13; H, 2.86; N, 13.35.

3-Amino-N-methylphthalimide (XVI) - 3-Nitro-N-methylphthalimide (45.0 g., 0.213 mole) was added with stirring to a solution of 250 g. of tin II chloride dihydrate in 900 ml. of conc. hydrochloric acid and 300 ml. water. The solution was magnetically stirred and maintained at 38-43° for 1 hour, at which time the hydrochloride of the product began to separate. The product was collected when the reaction mixture returned to room temperature, then washed with 100 ml. water to liberate the bright yellow free base, which was collected and dried to give 30.7 g. (82%) of material, m.p. 198-200° (lit.²¹ m.p. 199°).

3-Acetamido-6-nitro-N-methylphthalimide - 3-Amino-N-methylphthalimide (22.0 g., 0.125 mole) was converted to the 3-acetamido derivative by refluxing for 5 minutes with 250 ml. of acetic anhydride. The mixture was cooled to room temperature and 87.5 ml. of 90% HNO_3 was added dropwise with stirring to the solution, keeping the temperature between 35-40°. The bright orange solution was stirred an additional two hours after the addition and then poured over 2 kg. of crushed ice. An orange, tarry semi-solid separated from solution and was collected on a filter. The product was recrystallized from a 10% solution of acetone in 2B alcohol to give 2.6 g. (7.9%) of material, m.p. 174-176°.

Anal. Calcd. for $C_{11}H_9N_3O_5$: C, 50.19; H, 3.45; N, 15.96.

Found: C, 49.9; H, 3.24; N, 15.98.

Several other attempts to carry out the nitration using varying reaction times and temperatures either failed or gave poor yields. A sulfuric acid-nitric acid nitration at 0° was unsuccessful.

3-Amino-6-nitro-N-methylphthalimide (XVII) - To 300 ml. of conc. sulfuric acid cooled to 0° was added 40.0 g. (0.22 mole) of 3-amino-N-methylphthalimide. A solution of 16 ml. of 70% nitric acid, 4 ml. water and 80 ml. conc. sulfuric acid was added dropwise with stirring during one hour at 0° to 5°. The reaction mixture was stirred 6 hours at 0-5°, then poured into 5 l. of an ice-water mixture. The orange precipitate was collected on a filter, washed well with water, and dried to obtain 44.0 g. (90.3%) of product, m.p. 195-200°. Recrystallization from alcohol gave material, m.p. 215-217°.

Anal. Calcd. for $C_9H_7N_3O_4$: C, 48.87; H, 3.19; N, 19.00.

Found: C, 49.41; H, 2.90; N, 18.90.

3,6-Diamino-N-methylphthalimide (XVIII) - A. To a solution of 25 ml. conc. ammonium hydroxide in 75 ml. water was added 5.0 g. (0.023 mole) of 3-amino-6-nitro-N-methylphthalimide. Sodium hydro-sulfite (12.0 g., 0.069 mole) was added to the mixture in three portions with stirring. The reaction mixture was boiled 5 minutes, filtered, and heated on a steam bath for 30 minutes. The product separated on cooling and was recrystallized from water to obtain 0.8 g. (18.2%), m.p. 240-45° (dec.).

Anal. Calcd. for $C_9H_9N_3O_2$: C, 56.53; H, 4.74; N, 21.97.

Found: C, 56.25; H, 4.34; N, 22.19.

B. To a solution of 6.2 g. (0.12 mole) of ammonium chloride in 20 ml. water and 80 ml. 2B alcohol was added 5.0 g. (0.023 mole) of 3-amino-6-nitro-N-methylphthalimide. Powdered iron (6.4 g., 0.115 mole) was added, and the mixture was refluxed 2 hours. The solvents were evaporated on the steam bath, and the residue extracted with several portions of chloroform totalling 800 ml. The chloroform was evaporated to obtain 0.9 g. (20.5%) of product, m.p. 242-46°.

C. An attempt to reduce 3-amino-6-nitro-N-methylphthalimide with tin II chloride and hydrochloric acid failed.

Attempted Preparation of 2-Amino-N-methyl-6-nitro-4,5-Benzothiazoledicarboximide (XXI) - A. To a solution of 200 ml. glacial acetic acid and 10 ml. methyl alcohol were added 9.0 g. (0.04 mole) of 3-amino-6-nitro-N-methylphthalimide and 15.6 g. (0.16 mole) potassium thiocyanate. The solution was cooled to 15°, and 13.3 g. (0.08 mole) of bromine in 50 ml. glacial acetic acid was added dropwise with rapid stirring over a one period. The reaction was stirred for 45 minutes, and then heated to 70°. Water (50 ml.) was added, and the mixture was heated to 100°. The mixture was allowed to stand at 100° for 15 minutes, then filtered while hot to recover 5.5 g. of the starting material, m.p. 185-194°. The filtrate was made basic by addition of ammonium hydroxide, and the precipitate was collected. Infrared analysis indicated that the product was not the desired benzothiazole; the material was not further identified.

B. 3-Amino-6-nitro-N-methylphthalimide (5.0 g., 0.022 mole) was mixed thoroughly with 19.0 g. (0.14 mole) of anhydrous copper II chloride and 17.0 g. (0.17 mole) of potassium thiocyanate. Glacial acetic acid (8 ml.) was added and the mixture was heated 1 hour at 40° and then 4 hours at 100°.

The reaction mixture was treated with 500 ml. water and heated to boiling. The insoluble material was collected on a filter, washed well with water, and dried. Only starting material was obtained.

Attempted Ullmann Condensation of Anthranilic Acid with 3-Chlorophthalimide - To 550 ml. n-amyl alcohol were added 43.9 g. (0.32 mole) of anthranilic acid and 182.3 g. (1.32 mole) of potassium carbonate. The mixture was boiled with stirring for 1 hour, during which time ten per cent of the alcohol was boiled off to remove water of neutralization.

To the stirred mixture was added 45.4 g. (0.25 mole) of 3-chlorophthalimide and 0.9 g. of active copper catalyst²². The mixture was refluxed for 30 hours, and then steam distilled. The deep purple non volatile residue remaining was acidified with hydrochloric acid, and a dark-colored tar separated. Reprecipitation from sodium bicarbonate solution gave only unchanged anthranilic acid (35.0 g., 79%).

SECTION 2 - PHYSICS

I Energy Transfer Studies

Chemiluminescence from Oxalyl Chloride and Hydrogen Peroxide -

Weak chemiluminescence from hydrogen peroxide oxidations has been frequently observed²³. Very recently, however, bright chemiluminescence has been reported from the reaction of hydrogen peroxide with oxalyl chloride in the presence of a fluorescent material such as anthracene or N-methylacridone²⁴.



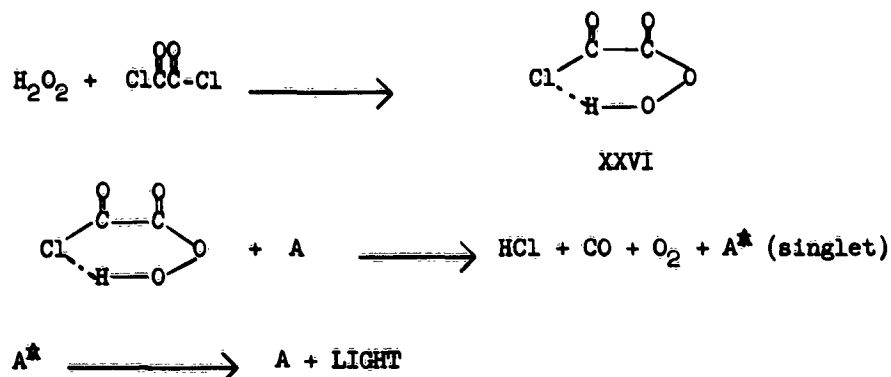
This reaction is of considerable interest because of its simplicity. Moreover, because little light is produced in the absence of a fluorescent material, the reaction appears to be a clear-cut case of energy transfer²⁵ in a chemiluminescent system. In view of these important factors we have studied certain points bearing on the energy transfer process. Our results to date are summarized below:

- (1) While the reaction at room temperature is no more than a bright flash, at -78° in ether solution the intensity is substantially stronger, and the lifetime increases to about ten minutes.
- (2) The original report²⁴ that the vapors from the reaction give rise to chemiluminescence in contact with a fluorescent substance is unequivocally substantiated by experiments at -78° , where the reaction is less violent.
- (3) The intensity is increased when anthracene is replaced by the more strongly fluorescent 9,10-diphenylanthracene. This observation is in agreement with the prediction that chemiluminescence

efficiency can be increased by improving the fluorescence yield of the emitter.

(4) There is negligible emission, even in the ultraviolet, in the absence of a fluorescent material. This was established by searching for and failing to find to find ultraviolet emission with a photomultiplier tube detector and by failing to induce 9,10-diphenylanthracene fluorescence through a quartz separator. This fact rules out the possibility of an emission-reabsorption energy transfer process and indicates (by elimination) a non radiative process of energy transfer. While non radiative energy transfer is well known in fluorescence spectroscopy²⁵, this system appears to be the first clear-cut case of its application to chemiluminescence.

(5) A well known form of non-radiative energy transfer is the resonance process²⁵. As it is customarily viewed, this process requires close overlap between an emission band defining the electronic energy of the donor and the absorption band of the acceptor. If such a process were involved in oxalylchloride-hydrogen peroxide chemiluminescence, only acceptors having at least one closely matched absorption band could be effective. To examine this point eleven potential acceptors having varying absorption bands were tested for their ability to generate light. All of these chemiluminescent compounds were at least partly active, and the simple resonance transfer process must be ruled out. The most likely remaining mechanism is a variation of a suggestion by the discoverer²⁴.



In this mechanism a species with substantial chemical energy such as XXVI interacts with the ground state of the acceptor, A, in such a way that the bulk of the available chemical energy converts A to an excited singlet state. In such a process A might act as a catalyst providing a new path for removal of the liberated energy. Such a process would be less bound by the rigorous quantum restriction of ordinary energy transfer since the decomposition products could carry away varying amounts of energy. This mechanism is still of doubtful validity and substantial work yet needs to be done. It is very unlikely, for example, that a species such as XXVI would be sufficiently volatile at -78° to provide the observed vapor phase chemiluminescence. Moreover, the vapor phase experiment indicates that the energetic intermediate has a substantial lifetime. Stability would not be expected in a species such as XXIV.

Experiments with Oxalyl Chloride and Hydrogen Peroxide -

A stock solution of one molar oxalyl chloride in diethylether was used in the following experiments:

(1) Reaction at Room Temperature - Two mg. of 9,10-diphenylanthracene was combined with 5 ml. of the stock solution and treated in the dark with 2 drops of 30% aqueous hydrogen peroxide. A violent reaction occurred during which a bright but brief blue chemiluminescence was observed. No luminescence was observed in the absence of the anthracene.

(2) Effect of Cooling - Experiment (1) was repeated at a temperature of -78° (dry ice-acetone bath). The reaction was mild and the intense chemiluminescence had a lifetime of five to ten minutes.

(3) Search for Ultraviolet Emission - Experiment (1) was repeated but in the absence of any fluorescent acceptor and in a quartz cell at -78° . No emission of light was detected when the cell was placed against a 1P28 ultraviolet-sensitive photomultiplier tube. In a similar experiment the U.V. cell was immersed in a dilute solution of 9,10-diphenylanthracene in ether. No fluorescence of this external solution was detected. When a piece of filter paper which had been soaked in 9,10-diphenyl anthracene solution and dried was held against the cell in another experiment, no induced fluorescence of 9,10-diphenylanthracene was observed, but vapors escaping from the cell produced fluorescence on the paper. The fluorescence of 9,10-diphenylanthracene was observed when the filter paper was irradiated with a lamp. That

the fluorescence observed near the opening of the U.V. cell was truly from vapors was ascertained by holding the paper 2 to 3 inches from the opening and observing the weak fluorescence of the paper.

(4) Effect of Fluorescent Materials - A 5 ml. aliquot of 1 molar ethereal oxalyl chloride was added to 10 ml. of diethyl ether containing 2-3 mg. of a fluorescent material. The solution was then cooled in a dry ice-acetone bath and 2 drops of 30% hydrogen peroxide added. Results are summarized below:

<u>Fluorescent Compound</u>	<u>U.V. Absorption Bands of Fluorescent Compound (mμ.)</u>	<u>Emitted Light</u>	
		<u>Color</u>	<u>Intensity</u>
rubrene	303, 465, 495, 530	yellow	strong
quinine sulfate			weak
phenazine	250, 350, 360, 390	yellow	medium
trans-stilbene	228.5, 295.5, 307.5	violet	medium
pentaphenyl phosphole		green	weak
2-phenylbenzo- thiazole	226, 248, 256, 297		weak
1,3-diphenyliso- benzofuran		green	medium
<u>7H</u> -Benz(de)- anthracene-7-one	230, 253, 283, 306, 397	yellow	medium
10-methyl, 9- acridanone	256, 295, 306, 387, 404	blue	medium
9,10-diphenyl anthracene	258.4, 337.0, 353.0, 371.5, 392.0	blue	strong
anthracene	230, 250, 310, 325, 340, 355, 380	blue	medium

II. Absorption and Fluorescence Spectra of Selected Compounds:

Energy transfer studies require that a large number of fluorescent materials of varying absorption and emission characteristics be available. In addition to those known in the chemical literature, a number of fluorescent materials have been prepared by the company for use as solids but never characterized in solution. Of particular interest were materials likely to emit in the region 450-600 mμ. Company files were surveyed and the most interesting materials obtained for further study.

The absorption spectra of the compounds in solution were obtained using a Cary Model 14 Recording Spectrophotometer. The wavelength positions of the absorption and fluorescence maxima, and the values of the specific absorptivity are summarized in Table 1. The fluorescence spectra were obtained using a Beckman KD-2A Ratio Recording Spectrophotometer. No fluorescence intensities are given, for the instrument used was not calibrated for absolute intensity measurements.

Table 1

Wavelength Absorption Maxima and Intensities and
Wavelength Emission Maxima for Several Fluorescent Compounds

<u>Compound</u>	<u>Solvent</u>	<u>Absorption Maximum λ in mμ</u>	<u>Specific Absorptivity at λ max.</u>	<u>Absorption Maximum with NaOH added λ in mμ</u>	<u>Emission Maximum λ in mμ</u>
Calcofluor Yellow H.E.B.	Methanol	440	52.0	-	535
Calcofluor 6G.	Methanol	435	41.8	440	535
Calcofluor Yellow H.E.B. Super Conc.	Methanol	440	49.3	-	535
Calcofluor: Yellow 7G.	Methanol	445	52.7	-	538
Calcocid Uranine B. 4315	H ₂ O	490	83.5	490	532
Calcofluor White 5B. conc.	Methanol	332 and 372	44.5 and 17.0	332 and 372	470

III. Radiometric Equipment

Qualitative data alone provides an inadequate basis for the mechanistic analysis of chemiluminescent systems. Suitable equipment is required which will permit intensity, spectral, lifetime, and absolute quantum yield determinations. Moreover, the equipment should also provide for fluorescence spectral and quantum yield measurements to allow comparison with corresponding chemiluminescent systems. In addition, a suitable instrument should provide for luminosity measurements of chemiluminescent light, since even for practical purposes, the eye is a poor judge of visual intensity.

Thus optical studies on systems which are chemiluminescent or fluorescent require measurements to be made under well-defined and reproducible conditions, with suitable calibrations in both radiometric and luminous units. Quantum yields or efficiencies for both chemiluminescence and fluorescence should be obtained from data obtained under identical conditions. Measurements on fluorescence are needed both from external optical excitation and also from internal excitation due to absorption of chemiluminescent radiation or from radiationless transfer.

Many of the difficulties encountered in the measurement of intensity of fluorescence arise from the mode of excitation and the absorption of the exciting light. Most conventional fluorimeters and spectrofluorophotometers use 90° excitation and are limited thereby in the range of concentrations that may be reliably determined. However, since these instruments are largely used in the field of trace analysis,

deviations at relatively high concentrations are not important. Excitation from an angle as near as possible to that of the emission to be measured is desirable to permit studies at higher concentrations and to allow the separation of the effects of excitation absorption and of concentration quenching. The spectroradiometer-fluorimeter described in the experimental section was designed to have near-zero angle excitation for fluorescence measurement, and to be capable of measuring chemiluminescence, delayed fluorescence, phosphorescence, transmission, and also to be used as a spectroradiometer. Construction of the equipment and three of the four steps involving calibration have now been completed.

Equipment Construction:

Previous experience with the construction, calibration, and use of a solar spectroradiometer²⁶ led to the selection of a small Bausch and Lomb grating monochromator (model 33-86-25), which covers the range from 3500 to 8000 Å with a reciprocal linear dispersion of 64 Å/mm. A 1P22 photomultiplier tube was used in the housing supplied with an Aminco photomultiplier power supply and amplifier. The housing also held a shutter and space for a quartz actinometer cell. This housing was attached to the monochromator behind the exit slit. The apparatus is diagrammed in Figure 1.

The housing furnished for the tungsten light source was used as the base for the excitation sources. The tungsten lamp and transformer were removed and replaced with two General Electric type F4T5/BLB

SPECTRORADIOMETER - FLUORIMETER

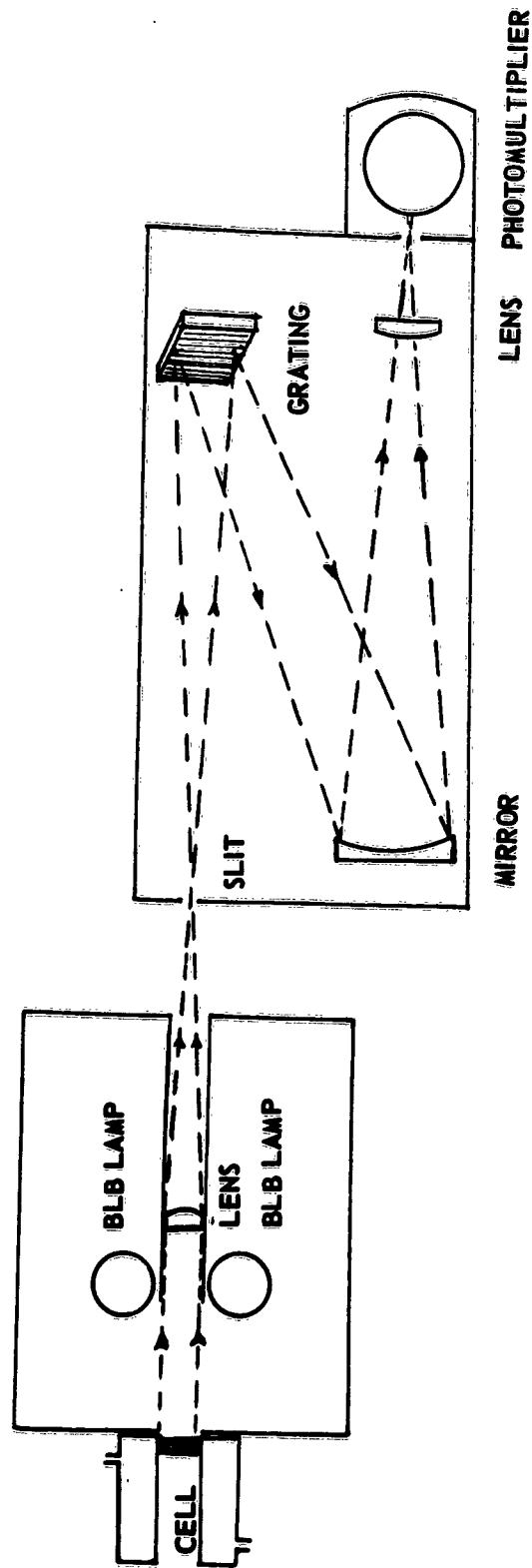


FIGURE 1

fluorescent tubes, mounted vertically on either side of a brass tube serving as a light shield and lens holder. A 12 cm. focal length glass lens was placed at its own focal distance from the slit to receive parallel light and focus it on the entrance slit of the monochromator. A hole was cut in the back wall of the housing to permit the exciting light to strike the sample, which is housed in a jacket through which temperature-controlled water is circulated.

The instrument may be used in five ways: (1) as a fluorimeter, with the exciting tubes irradiating the sample, (2) as a "chemiluminometer," with exciting lamps off and a chemiluminescent sample, (3) as a combination of (1) and (2) for measuring a combination of chemiluminescent and fluorescent systems, (4) for transmission measurement, with an external light source and a cell-in, cell-out technique, and (5) as a spectroradiometer with an external source of light and suitable optics.

Provision was also made to mount a micrometer Baly cell²⁷ in place of the temperature-controlled cell holder. This cell is variable from 0.01 to 5.00 mm., and will be used in studying the effect of light path (cell length) on chemiluminescent and fluorescent systems. The regular cell holder can accommodate cells from 5 to 50 mm. in length.

Equipment Calibration:

The wavelength dial of the monochromator was calibrated with an Osram mercury lamp, and found to be accurate to well within the error

in reading the dial markings. Fixed slits of 1.34 mm. for the entrance and 0.75 mm. for the exit were selected. (The image of the entrance slit is reduced by 56%.)

The wavelength response of the 1P22 photomultiplier tube was calibrated by the technique of ferri-oxalate actinometer previously described^{26,28}, together with a National Bureau of Standards lamp having a color temperature of 2864° K. The use of a light source of known spectral energy distribution gave a relative correction curve. Actinometer tests (with the actinometer mounted just before the photomultiplier and behind the exit slit) at two wavelength settings permitted the scale of the meter to be calibrated in terms of microwatts per cm^2 per 50 Å falling on the photomultiplier. The calibration curve is shown in Figure 2.

To check the spectral distribution of the exciting lamps, a mirror was placed in the sample position to reflect the light from one of the lamps into the monochromator. The corrected spectral distribution curve agreed well with that of a quite similar 15-watt lamp²⁶, and is shown in Figure 3.

The excitation input energy to the fluorescent cell was measured by substituting the actinometer solution in the 1-cm cell and exposing for a known length of time. The integrated flux amounted to 3 milliwatts/ cm^2 . This value will be used in calculating fluorescent quantum yields (modified if necessary by the transmission curve of the sample being measured).

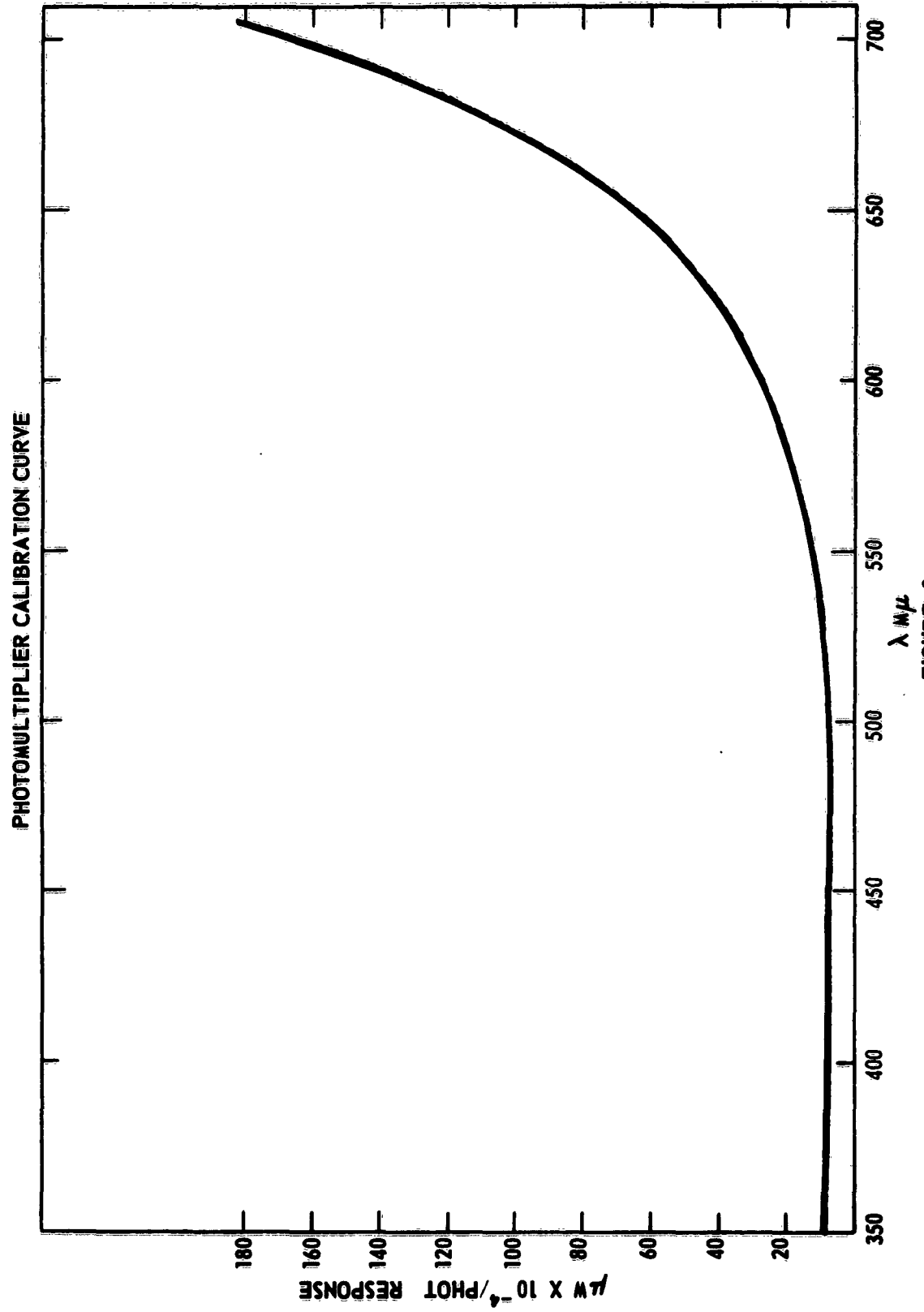


FIGURE 2

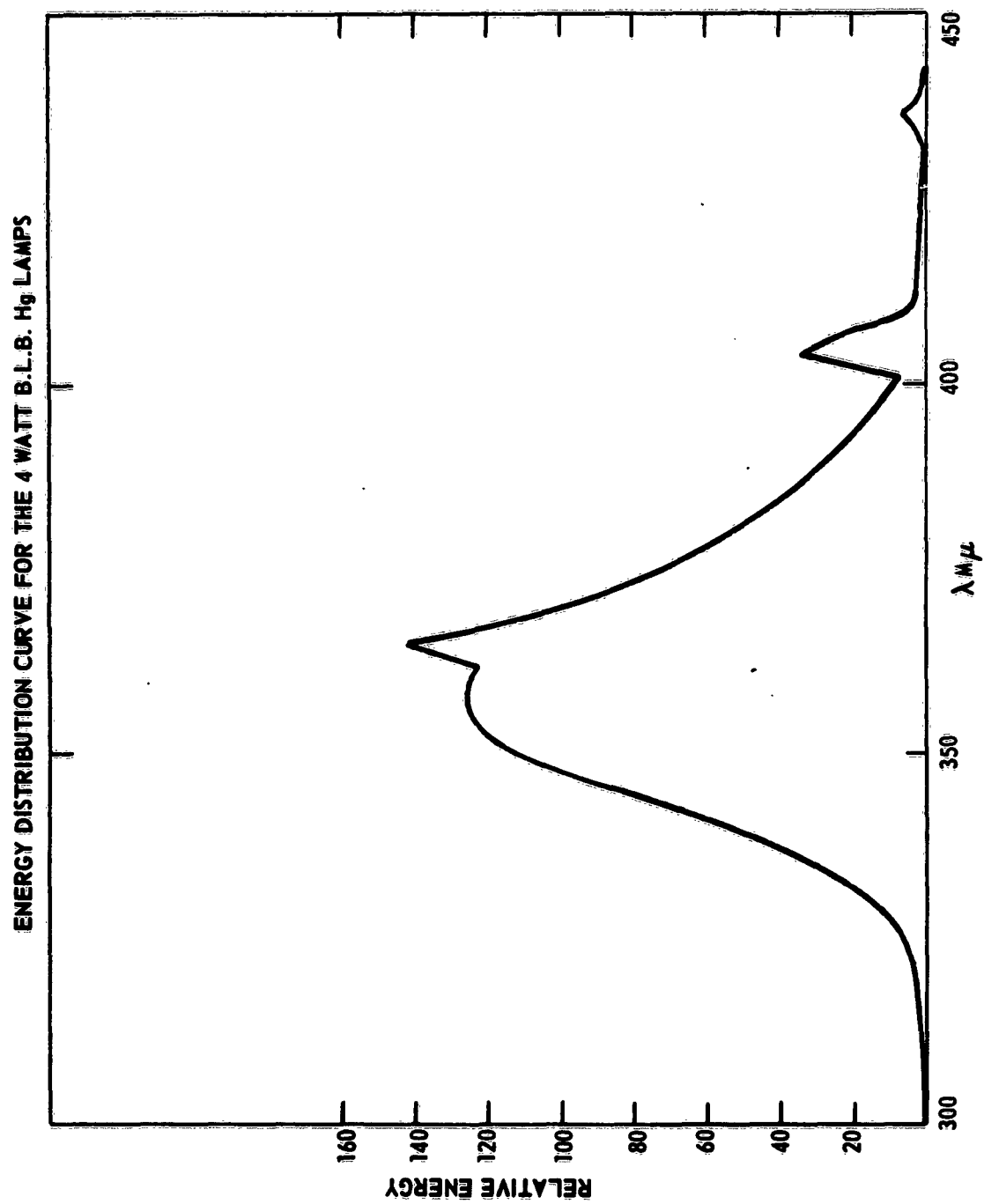
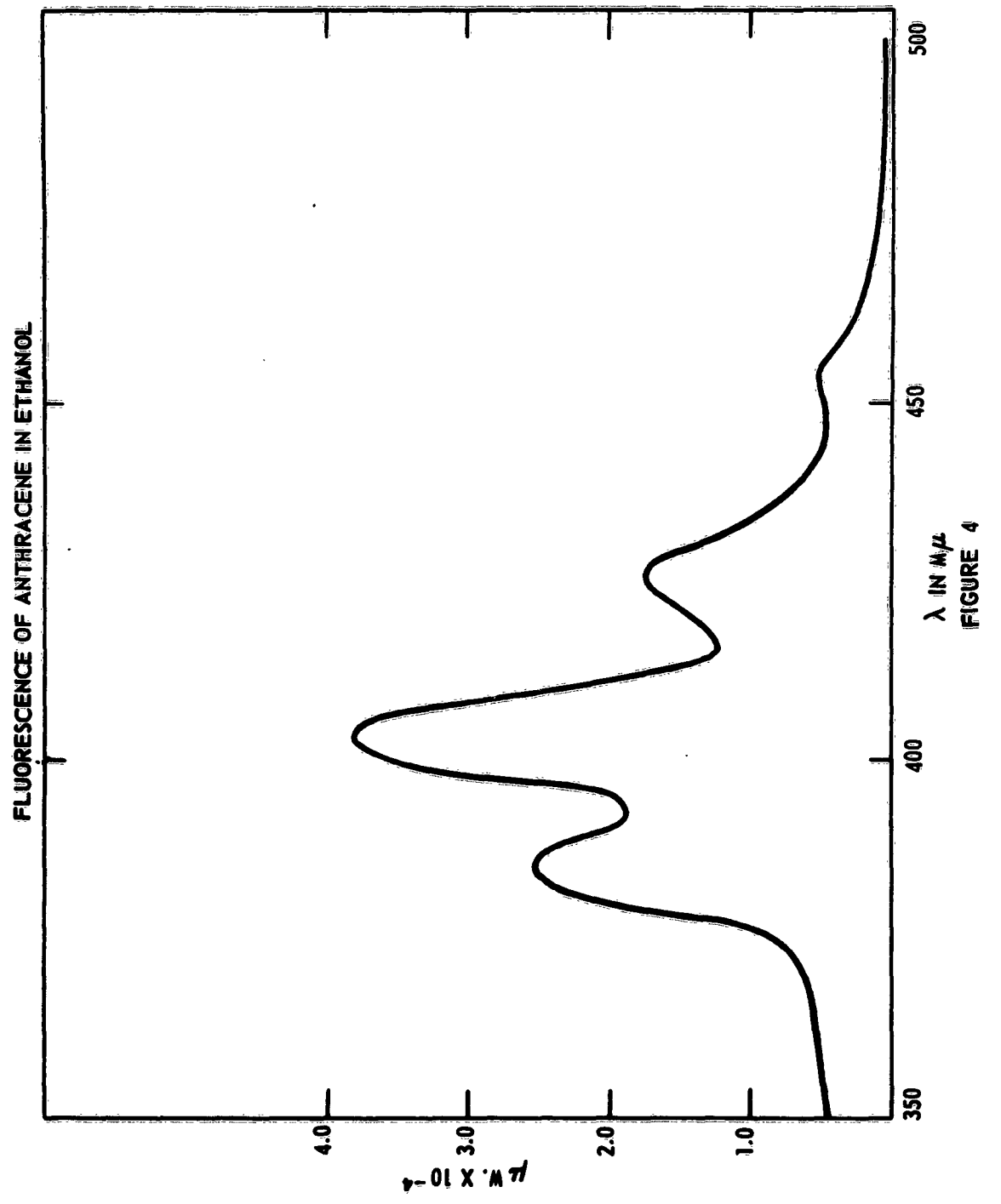


FIGURE 3

In order to obtain a value for the photomultiplier reading (at a specific wavelength) in terms of total emission of a light source in the position of the cell containing a chemiluminescent system, a tungsten source of known candlepower and color temperature was used. This unit was a "Quantalumen" regulated power supply and tungsten light of 2.3 candlepower with a color temperature of 2460° K. To obtain its total ultraviolet emission, the bulb was inverted and immersed in a ferrioxalate solution for a fixed time. With the lamp in the position of the cell on the spectroradiometer-fluorimeter, a actinometer solution was exposed behind the entrance slit of the monochromator. The photomultiplier output was recorded before and after the test. The results showed that 1×10^{-4} of the total spherical output ($2.94 \times 10^3 \text{ uW}$) was entering the monochromator; this is in good agreement with the slit area as compared to that of a sphere of the radius equal to the lamp to slit distance.

The fluorescence spectrum of anthracene was obtained, and found to be in good agreement with spectra published²⁹. The fluorescence spectrum of anthracene is shown in Figure 4.



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