75

CHEMILUMINESCENCE

by

KENNETH HENRY ASHCROFT, B.Sc., A.R.P.S.

A Project

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

(Teaching)

McMaster University

April 1979

MASTER OF SCIENCE (TEACHING) 1979
(Chemistry)

McMaster University
Hamilton, Ontario

TITLE: Chemiluminescence

AUTHOR: Kenneth Henry Ashcroft, B.Sc., A.R.P.S.

SUPERVISORS: Dr. C. Beattie, Dr. R. A. Bell

NUMBER OF PAGES: v, 54

ABSTRACT:

Many of the chemiluminescent systems published to date have been re-investigated. They have been presented in a manner that is more suited to use by teachers. A historical introduction and a summary of the theoretical background has been provided, so that all the information required for beginning work on the topic is contained herein.

Details of a new chemiluminescent system are also provided.

C O N T E N T S
****

Page

ACKNOWLEDGMENTS

The suggestion that led to this project was made by Dr. R. A. Bell, and his advice, assistance, and encouragement made its completion possible. The members of his research group, Messrs. R. McLeod, A. Castelanho, and A. Wong acted as mentors in the laboratory.

Preparation of the material in a form suitable for inclusion in a chemistry curriculum was made possible by the scrutiny of the work by Dr. C. Beattie.

Dr. J. J. McCullough was the source of information regarding the structures of triptycene and related compounds, and he also supervised spectral analysis.

The manuscript was typed by Mrs. Irene Johnston and the diagrams drawn by Miss Susan Ashcroft.

CHEMILUMINESCENCE

Chapter 1

Introduction to the topic, its relation to the science curriculum, and justification of the work undertaken.

CHEMILUMINESCENCE

Introduction

Although chemiluminescence was the subject of experiments done a century ago, and has been part of organic chemistry courses for decades, considerable interest in the topic has developed in the last two years. This makes it a worthy subject for research, with a view to including more of the material in the chemistry curriculum. Furthermore, the phenomena which triggered the initial questions in the minds of nineteenth-century scientists also have an inherent fascination for students. Also, the theoretical interest to scientists is supplemented by practical developments which impinge on everyday life.

A philosophy of scientific inquiry

The scientific method of inquiry implies a mechanism whereby the results of experiments and the judgments based upon them may be checked by other investigators after the initial work has been published. This checking must be done before any findings are incorporated into the science curriculum. Science has been defined by Campbell as *"the study of those judgments concerning which universal agreement can be obtained."* (4) It would not be difficult to achieve consensus on the following statements:

- there are systems, some living and some non-living, which glow in the dark,
- light and heat are forms of energy which are produced, simultaneously, by a number of radiant systems,
- though there are many systems other than radiant bodies which produce heat alone, it is far less common for a system to generate light at room temperature.

The questions arise out of the third statement: "How do the luminescent systems produce light alone? Can new luminescent systems be discovered or developed?" In principle, sufficient work will uncover answers to these questions which are capable of being checked, whereas questions of a more philosophical nature may be answerable only in ways which do not lend themselves to empirical testing.

Thus, the study of luminescence satisfies the first requirement of the most recent theory concerning scientific knowledge, as propounded by Polanyi (28) and developed by Ziman (47). *"All true scientific research starts with hitting on a deep and promising problem, and this is half the discovery."* The problem then becomes the explanation of known luminescent systems and the search for new ones. After the initial questions about luminescence had been posed, the interest was divided according to the nature of the light-producing system:

bioluminescence - produced by living organisms,

chemiluminescence - produced in vitro from non-living systems.

There are chemical systems which are luminescent, and are apparently unrelated to the biological compounds. However, once it had been recognised that all luminescent systems involve oxygen, attempts were made to relate the two types of systems together. Another aspect of the philosophy of science is hereby illustrated; although a problem of apparently narrow scope may be initially investigated on the basis of its inherent interest, the results of the research may give understanding of a process which is of much wider dimensions or of a more fundamental nature. Though not all projects are capable of doing so, a research programme may often result in a marketable product which has some effect on the mode of life of the general public, even though the initial thurst of the research was theoretical, and not technological.

In this project, there is one example of the fallibilism principle. An announcement of the successful completion of a new synthetic route was followed by the checking of the assumptions, results, and conclusions by other investigators (see page 11). The success proved short-lived. This checking process has been mentioned already as a constant feature of scientific endeavour.

Luminescence and the science curriculum

There is enough sound, understandable theory in this project that teachers should be able to avoid the "magician" approach to science, in which phenomena are presented for their power to amaze. Thus, even students in Grade 4 have acquired some facts which are related to chemical kinetics (that reaction rates are dependent on temperature, for instance) whilst observing processes

in the kitchen. They can be asked to consider three ways of cooking eggs, namely frying, boiling, and placing on a hot pavement. They can then predict the behaviour of a "light-stick"[1] when changed from room temperature to that of a container of hot water or that of an ice-bath. This is the type of methodology advocated in science by the Nuffield Committee, based on the work of van Praagh on the heuristic method. Science is to be presented, not as a collection of facts to be mastered, but as a series of puzzle-solving and predicting exercises, based on information acquired by the students, which leads to testable predictions based on generalisations formulated by the student. (26)

The scientific method is illustrated in classic simplicity by the preparation of dioxetanes (see page 48). The biological luminescent systems are very complex, involving enzymes as well as the molecules which cause excitation and emission. Until the work of Kopecky, the simplest systems involved at least two types of molecule. Dioxetanes had been proposed only as possible "intermediate" structures, which formed during the luminescent reactions. However, once dioxetanes had been isolated, and shown to be luminescent, the number of types of molecule was reduced to one. That this result is significant can be deduced from the fact that further work on dioxetanes has produced more than thirty compounds, all of which are luminescent. Further, the investigation of dioxetanes, coupled with that into diacyl peroxides (see page 12) has led to an overall theory which explains the interactions taking place in luminescent systems most satisfactorily. Thus, the investigation of chemical systems has provided insight into the originally-discovered biological systems.

Pragmatic justification for teaching luminescence

That this material is most appealing to teachers has been deomonstrated by appearances and requests for lectures, at three major conferences and two teacher workshops. A summary of the initial experiments was published in Chem. 13 News (University of Waterloo) January, 1979, and this resulted in considerable correspondence. There are several of the experiments which have been evaluated using three separate groups of Grade 4/5 students. Chemiluminescence is already in use as a project option in Grades 12 and 13,

1. A commercial chemiluminescent product: a cylindrical plastic tube containing sufficient material to produce light for several hours.

and is mentioned in at least one text. There is every reason to expect that the syntheses of triptycene and diphenoyl peroxide will significantly supplement the luminol synthesis which is already a feature of most undergraduate chemistry courses.

Initial findings and objectives of the research project

A survey of the periodicals, and reading of three reviews, showed that there are several classes of chemiluminescent compounds of historical significance, and that there are many branches of current research. Apart from workers in Russia and Japan, the majority of the publications originate from groups led by the following:

K. Kopecky, University of Alberta

F. McCapra, University of Sussex

M. M. Rauhut, American Cyanamid Co.

G. Schuster, University of Illinois

E. H. White, Johns Hopkins University.

Attempts were to be made to repeat as many of the original experiments as possible, to evaluate them for use by teachers. The accounts of research in Russia and in Japan indicated that the elucidation of any new chemiluminescent system would not be likely in the time available for this project. The experiments would be collated and simplified so that they would be directly usable by teachers.

The following list delineates some of the problems which were obvious at the outset, or which arose during the investigation. Finding solutions to these problems became the central objective of the project.

1. The information required to duplicate the observations of previous investigators is not readily accessible. Any one set of experiments may have been used for the production of several reports, and much "detective work" may be needed to extricate the essentials. This is especially (and perhaps understandably) so in the case of the Cyalume "light-sticks". Though a series of indirect statements is made, it has not been possible to find the precise composition of the liquids inside the tubes.
2. The information in the literature is often incomplete. It is common

for reference to be made to some previous paper for details of a synthesis; examination of the previous paper reveals that it, too, contains inadequate data. The only recourse is to make intelligent guesses to try to establish what the original procedure might have been.

3. Several reported syntheses involve as starting materials chemicals which are either expensive, or not available from laboratory suppliers.
4. There are some critical errors in the literature, which appear to be typographical. If a concentration is listed as 0.2 M instead of 0.02 M, experiments using a solution of the wrong concentration may work unsatisfactorily or not at all.
5. Two or more sets of instructions may be given for the same reaction; one example is:

   - "Mix A and B in the ratio 1:12. Heat for 4 hours."
   - "Mix A and B in the ratio 1:28. Heat for 6 hours."

   In this case, the reaction carried out using the first set of instructions gave very poor yields.
6. There may be two or more synthetic routes for producing one compound from another (see page 22). The most favourable route is selected on the basis of reliability, cost of reagents, time required, and safety/toxicity considerations.
7. Some problems arising in the syntheses are not mentioned in the literature - these may be matters as simple as methods of crystallisation, but would cause problems for a teacher lacking access to advice from more experienced research workers.

Summary of achievements and failures in the preparation of this project.

There were two unexpected events during the project.

(a) In an attempt to track down four references quoted in the literature, the American Cyanamid Company was contacted by telephone. Almost immediately the message was directed to Dr. M. M. Rauhut, and a lengthy and valuable conversation ensued. As a result, work on oxalate esters was simplified, and the work on triptycene pursued further.

(b) In investigating a range of different materials to act as light-emitters in the oxalate ester system, it was decided to synthesise and evaluate triptycene. That light was, in fact, produced is a very interesting theoretical problem, details of which are given on page 49. As far as can be ascertained, the chemiluminescence of triptycene has not previously been observed.

Apart from unsuccessful procedures and errors in syntheses which are described below, there were only two objectives that were not achieved.

(a) Spectroscopic analysis of the chemiluminescence of triptycene could not be completed. This is due to the fact that light emission in this experiment is weak when reaction rate is moderate, and the instruments available were insufficiently sensitive. When reaction rate is increased, gas evolution is so rapid that damage may result to the equipment. Further tests could be done to establish the orange colour of the luminescence, and also to compare the luminescence spectrum with the fluorescence spectrum. The standard cell of the spectrometer would have to be modified to provide a light-tight continuous-flow system.

(b) The only method available for the preparation of dioxetanes that has proved to be reliable is that of Kopecky and Mumford. This involves the use of a 98% hydrogen peroxide solution, which is an extremely hazardous reagent. This preparation was not attempted.

The material that has been elucidated is presented in such a way that it is accessible, detailed, and concise. A number of errors have been eliminated, and all syntheses use materials that are available in suppliers' catalogues.

There is no mention of methodology in this project - to develop it into a curriculum unit would be a project in itself. It is intended to arouse and extend interest in the material, and to cater to the needs of the large number of teachers who have scant knowledge of, and even less plentiful information on, the topic. It should thus be regarded, not as a Teacher's Guide, but as a Laboratory Manual.

Chapter 2

Historical perspective

A short history of chemiluminescence

It is important to differentiate between luminescence and two other related phenomena.

Fluorescence occurs when certain gases are placed in a discharge tube, and a high voltage is applied across the tube, or when certain materials are irradiated. As soon as the current is switched off, or irradiation ceases, light output rapidly ceases.

Phosphorescence is shown by certain substances which continue to emit light even after the external source of energy is removed.

Luminescence is the property of a system to emit light at low temperatures without any external energy source. The energy for the light production originates from the interaction between molecules within the system itself.

Luminescent systems have been observed to occur in nature. Examples are the "phosphorescence" of the sea, pieces of wood undergoing bacterial decay, fireflies, glow-worms, and certain marine animals including crustaceans and deep-sea fish.

The first laboratory investigation into chemiluminescence was carried out in 1877 by Razizewski. Alkaline solutions of lophine in ethanol were seen to glow on shaking with air. The glow was intensified by addition of hydrogen peroxide or haemoglobin.

Whilst using photographic developers, in 1887 Eder detected a glow produced by alkaline pyrogallol solutions. This was thoroughly investigated by Trautz (41) in 1905.

E. Newton Harvey carried out a vast number of investigations into luminescent systems, both chemical and biological (17). Hindsight reveals that three of his trials, with seemingly "alchemical" materials, are of considerable

significance:

- that raw leather (fat-free) is luminescent, gives evidence for the involvement of protein-based material (such as riboflavin) in luminescence,

- that lobster blood can be made part of a luminescent system gives a hint for further work on haemoglobin in particular, and heavy metals in general,

- that turnip juice can be used for luminescence is a fact that became significant after experiments using enzymes such as horseradish peroxidase in 1977.

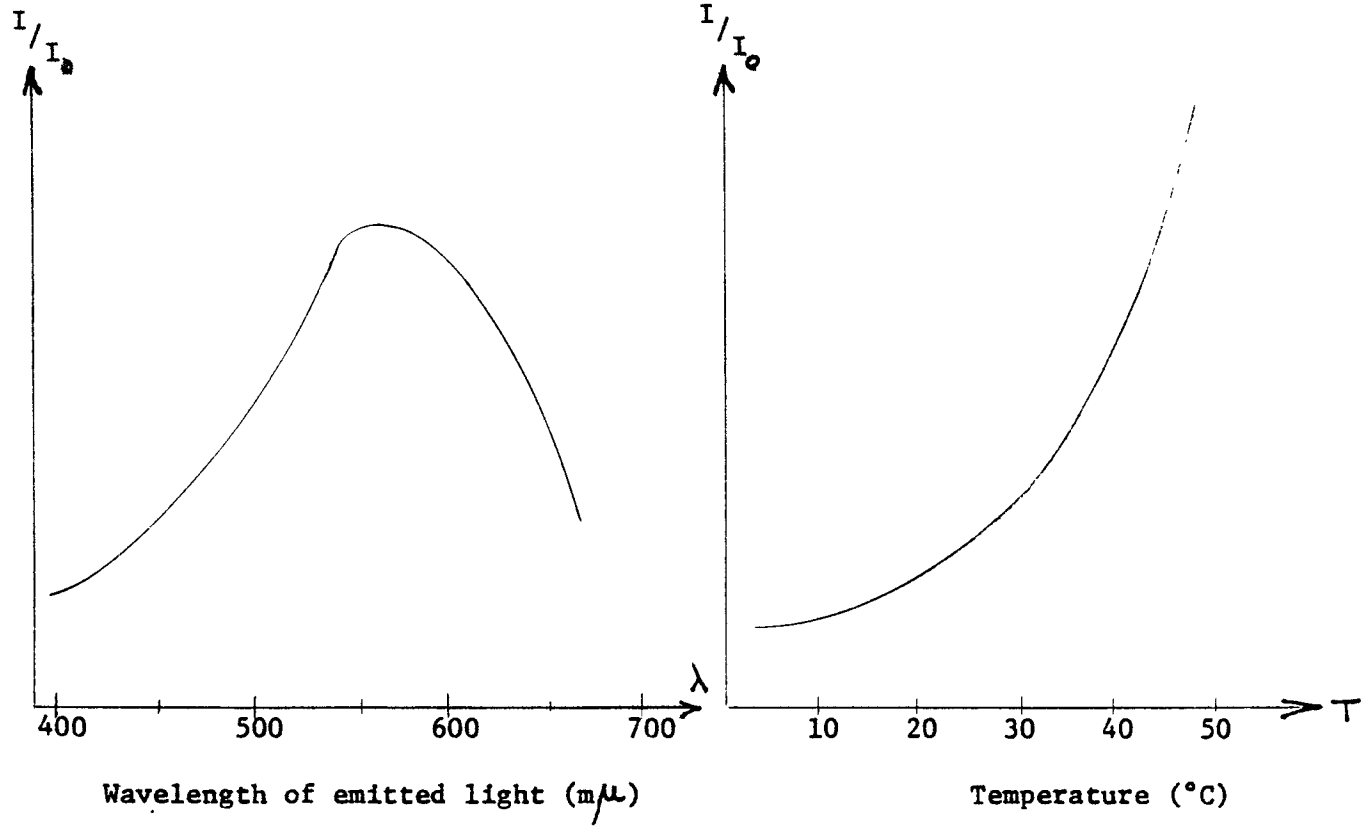
Mallet, in 1927, produced the first report of the interaction between chlorine and hydrogen peroxide. This was not pursued until the work of Gattow and Schneider in 1954 (16); the mechanism was not investigated fully until the work of Khan and Kasha in 1966 (20).

Luminol was first reported by Albrecht in 1928, and its luminescence was so spectacularly bright that a great number of investigations have been carried out using this material. Two principal contributions were by Drew in 1937 and 1939 (8,9).

The luminescence of riboflavin is very dim, but it was investigated thoroughly because it is a fluorescent substance, and as riboflavin 5'-phosphate, had been shown to be involved in the bioluminescence of certain bacteria. Strehler and Shoup (40) in 1953 reported a detailed investigation of the riboflavin luminescence. Electronic equipment had been developed, such as photomultipliers and scintillation counters, which permitted spectral analysis of the dim glow. Variation of light intensity is dependent on a number of factors, four of which are shown in Figure 1.

A second type of bright luminescence was observed in the reactions of oxalyl chloride and its derivatives in 1961. A great deal of work was carried

Figure 1 - Variation of relative light intensity with four reaction parameters in the luminescence of riboflavin.



out by Chandross (6) and Rauhut (3, 33) which had a practical outcome in the production of Cyalume "light-sticks". A significant discovery, which is easy to demonstrate, was that the vapours from the oxalyl chloride reaction will also cause materials such as anthracene to emit light.

Since there are correlations[1] between the structures of certain acridine derivatives and of some molecules involved in bioluminescence, work was undertaken in 1965 to elucidate chemiluminescent acridine systems. Many were discovered (of which three are shown in Figure 3), including aldehydes, ketones, and hydrazides. One compound, 9-chlorocarbonyl 10-methylacridinium chloride is a very bright emitter. The synthesis of ethyl and methyl dehydroluciferin was also achieved (5, 30, 31, 34).

In 1970, White (43, 45) published a thorough investigation of the luminol mechanism, and also of substituted luminol compounds. In the same year, Slabaugh (38) reported the use of violanthrone as a light-emitter in the chlorine-hydrogen peroxide system. The mechanism of this reaction was investigated by Abbott (1).

Many investigators expected that, if a dioxetane could be isolated, it would be an unstable compound, since it had been proposed as an intermediate in certain luminescent reactions. However, Kopecky and Mumford, in 1969, showed that, although the preparation of dioxetanes is fraught with danger, the products are relatively stable (21). This initiated a group of investigations which are still being pursued (25). One occurrence of note is an unsuccessful attempt to find an alternative dioxetane synthesis. Story et. al. (39) reported that such a method had been found, using photolysis as a means of producing the dioxetane ring. The results were evaluated independently by Bailey (2) and Kopecky (22) and subsequently disproved.

There are several promising avenues of investigation that have been

1. Lucigenin is 10, 10'- dimethyl-9, 9'- biacridinium nitrate

explored in the last two or three years, and in which work is still in progress.

- the luminescence of Schiff bases was first reported by McCapra (23) based on the synthetic method of Irving and Leermakers (18). Though Schiff bases are synthetic, they have been shown to give luminescence when treated with an enzyme, horseradish peroxidase, by Duran, et. al. (10).

- a new class of luminescent compounds was prepared using the method of Ramirez, et. al. (29) and reported by Schuster and Yoo (36). These are the diacyl peroxides. Though not brightly luminescent, the compounds are theoretically important, since investigation of the reaction mechanism has given rise to a new, general theory of wide application. This theory (see page 51) may be capable of explaining many, if not all, of the luminescent reactions that have been investigated before. A simple summary of this theory was given by Fox (13).

- there was reported an inorganic chemiluminescence between the ruthenium complex, $Ru(bipy)_3Cl_3$, and sodium borohydride, by Gafney and Adamson (15).

- the chemiluminescence of triptycene was investigated for this project. The synthesis of the compound, for which two methods are reported (12, 14) involves the reactive intermediate, benzyne, which is discussed by Panar (27). The electron interactions during reactions of triptycene are of theoretical interest, and the subject of current articles such as that by Iwamaru and Makino (19). That triptycene does, in fact, luminesce with oxalyl chloride, shows that there must be electron exchange between this molecule and the oxalyl complex.[2]

Applications of Chemiluminescence (37, 42)

1. The work of Rauhut and his colleagues has resulted in large-scale production of Cyalume "light-sticks", which have numerous applications

2. The nature of this interaction is the subject of a proposed article by Dr. R. A. Bell. (see p. 49)

as safety lights. The closing ceremonies of the Montreal Olympiad involved the use of these devices.

2. The fact that heavy metals catalyse chemiluminescent reactions can be used as a method of analysing for the metals when present in very low concentrations. Iron, vanadium, nickel, cobalt, chromium, and manganese can be detected at levels as low as 10^{-8} - 10^{-11}M. The method has been applied in pollution studies, and by the oil and automotive companies to investigate metal friction and to analyse exhaust gases.
3. That adenosine triphosphate (ATP) is involved in luminescence can be used to detect quantities of ATP as low as 10^{-12} g. The method was proposed as a method of detecting extra-terrestrial life, since this quantity of ATP is contained in 10 000 cells of an organism such as E. coli.
4. Much work has been done in Russia to use chemiluminescence as a method of probing the kinetics of reactions, since the intensity and spectral distribution of the light gives readily assimilable information about the molecular processes occurring (see page 48).

Chapter 3

Instructions to Teachers

General warning to instructors:

Before using any of the materials listed below, ascertain the conditions under which it may be safely handled:

chlorine	ozone
dimethylsulphoxide	oxalyl chloride
organic peroxides	potassium tert-butoxide

Part I

The set of instructions for carrying out the procedures is classified thus:

A. recommended for students, relatively inexpensive, safe and easy to perform,

B. recommended for teachers, on basis of cost, hazard, or time required: demonstration only,

C. recommended as individual projects for 3rd/4th year college chemistry students under proper supervision.

Note: literature melting-points were obtained from Albert, A., The Acridines, E. Arnold Ltd., London 1966, unless otherwise designated.

A1 - In a 1000 mL flask, place the following solutions. 100 mL of 35% formaldehyde, 100 mL of 10% Na_2CO_3, then 5 g of pyrogallol freshly dissolved in 100 mL water. In the dark, pour in 100 mL of 3% H_2O_2. A deep red glow results.

A2 - The current cost of luminol is about $2.00 per gram. The following method enables students to carry out experiments individually with minimal expense: instead of test tubes, use 1 dram stoppered vials, such as Kimble #60975-L. In each vial, place 5 pellets of KOH, 0.1 g of Na_2O_2, and 1 mg of luminol. Immediately add 10 drops of solvent, stopper the vial, and shake vigorously. Students may be allowed to select a solvent from the following: ether, methanol, acetone, hexane, chloroform, dimethylsulphoxide (DMSO). DMSO gives a very bright glow which lasts many hours.

A3 - After doing experiment *A2*, students may repeat it, using DMSO as solvent, but adding fluorescent dyes, such as 1 mg samples of solid fluorescein, dichlorofluorescein, or Rhodamine B, before shaking the tube.

A4 - Dissolve 2.5 g NaOH in 200 mL water. Add 0.2 g luminol and 15 mL 30% H_2O_2. Distribute this to students in 2.5 mL portions in test-tubes. Catalysis of the reaction can be demonstrated by adding a few crystals of each of various metal salts, such as: (a) $CuSO_4$ (b) $CrCl_3$ (c) KCl (d) $MgSO_4$ (e) $K_3Fe(CN)_6$. (e) is most spectacular.

B1 - Make a mixture of the following substances:

0.2 g luminol, 4 g hemoglobin, 4 g sodium perborate, 30 g Na_3PO_4, and 30 g powdered sucrose (icing sugar). Keep it in an air-tight jar.

Prepare three 1000 mL graduated cylinders, containing distilled water. To each cylinder, add 2.5 g of the mixture, and stir with a glass rod. Leave one sample, to another add 2 mL of 2% fluorescein, to the other add 2 mL of 1% Rhodamine B, and stir. Luminol alone glows purple, fluorescein glows yellow and Rhodamine B glows pink.

B2 - Luminol clock experiment. Dissolve 1.5 g $CuSO_4 . 5H_2O$ in 100 mL water then add 1.5 g NaCN or 2.0 g KCN.

Take 5 mL of this stock solution, add 100 mL concentrated ammonia and 0.25 g luminol. Place in a 500 mL flask, and swirl constantly, or stir with a magnetic stirrer. In the dark, add 40 mL 10% H_2O_2. Darkness continues for 2-3 minutes, then blue luminescence suddenly begins, together with evolution of oxygen. Dark time depends on $[CN^-]$. The stock solution slowly oxidises on standing; after a few days, dark time decreases to 30 seconds. (44)

B2 - *Continued...*

The reaction is interesting in that luminol oxidation is pH dependent and catalysed by heavy metal ions (see experiment A4). The ammonia is not alkaline enough to promote visible light production on its own, and the Cu^{2+} ions are initially complexed by CN^-. The CN^- is oxidised, to release $Cu(NH_3)_4^{2+}$ into the solution, which then catalyses the oxidation of the luminol.

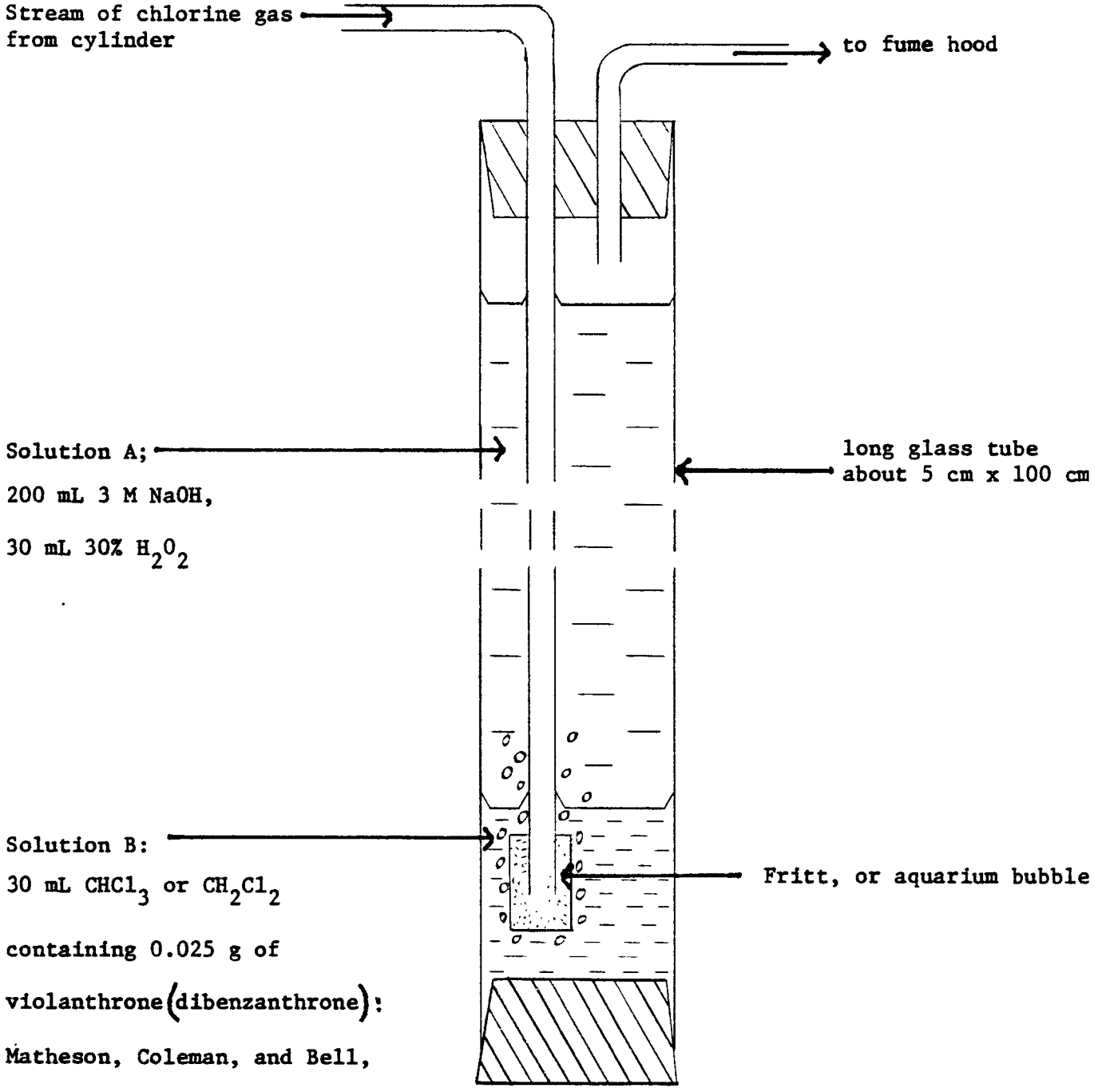
B3 - Set up apparatus which resembles that shown. (Fig. 2) (A large gas washing bottle is an elegant substitute.) Pass a slow stream of chlorine through the tube, and a fiery red luminescence is produced, which is carried up the tube in the interface between the bubbles and the solution.

Solution A: 200 mL 3M NaOH, 30 mL 30% H_2O_2

Solution B: 30 mL $CHCl_3$, or CH_2Cl_2 containing 0.025 g Violanthrone (Matheson, Coleman and Bell #P9346).

This is the most spectacular chemiluminescence published to date.

Figure 2 - Apparatus for demonstrating luminescence using violanthrone.



B4 - Dissolve 1 mL oxalyl chloride in 10 mL of 1,4-dioxane and add a few crystals of anthracene or Rhodamine B. Place in a 250 mL flask, and add 3 mL 30% H_2O_2 dissolved in 10 mL dioxane. Oxalyl chloride reacts vigorously in other solvents; at room temperature the reaction lasts only seconds. The reaction can be controlled in two ways:

(i) dissolve the oxalyl chloride and luminescer in 10 mL ether, and cool in an ice/salt bath. Add cooled 30% H_2O_2 from an eyedropper to form an interface, where the reaction takes place. (Keep the vessel cold).

(ii) Modify the oxalyl chloride molecule by replacing the chlorine atoms by other electron-attracting species, such as 2,4,6-trichlorophenol or 2,4-dinitrophenol. This is the basis of experiments B5, B6 and C2.

B5 - Prepare one of the esters described in C2. Dissolve 0.1 g of ester in 10 mL dimethylphthalate, and add 1 mg anthracene. To this, add 3 mL 30% H_2O_2. A bright glow is produced which is long-lasting. Other materials may be used to replace anthracene; rubrene and 9,10-diphenylanthracene work well. The effect of changing the solvent may also be investigated: dioxane works well.

B6 - Purchase a "Cyalume" light-stick, produced by Les Promotions Safari Inc., 706 Lenormand, Boucherville,PQ, J4B 5B0, at $1.50. Start the luminescence reaction according to the directions, then show the effect of temperature on reaction rate by immersing the tube in an ice/salt bath, then in water at 60° C.

B7 - Make a solution of riboflavin of concentration 35 mg L^{-1}. (The phosphate may also be used). To 100 mL of this solution, add 20 mL of 30% hydrogen peroxide solution buffered to pH = 7. The light output can be increased by adding heavy metal salts:

1 mg $FeSO_4$ = 2x 1 mg $CuSO_4$ = 4x

B8 - The chlorine/oxygen luminescence may be easily demonstrated thus: fill a 10 mL hypodermic syringe with sodium hypochlorite solution (household bleach). Place 10 mL of 30% hydrogen peroxide solution in a large test tube, and hold the syringe vertically above the liquid. Squirt in the liquid vigorously, and a red glow is produced. A dim green glow is produced when the experiment is repeated with Rhodamine B; with violanthrone, a bright red glow results.

Cl - (Fig. 2)

Many derivatives of acridine are chemiluminescent. Three were investigated, and acridine 9-aldehyde proved the simplest. Two starting materials are available.

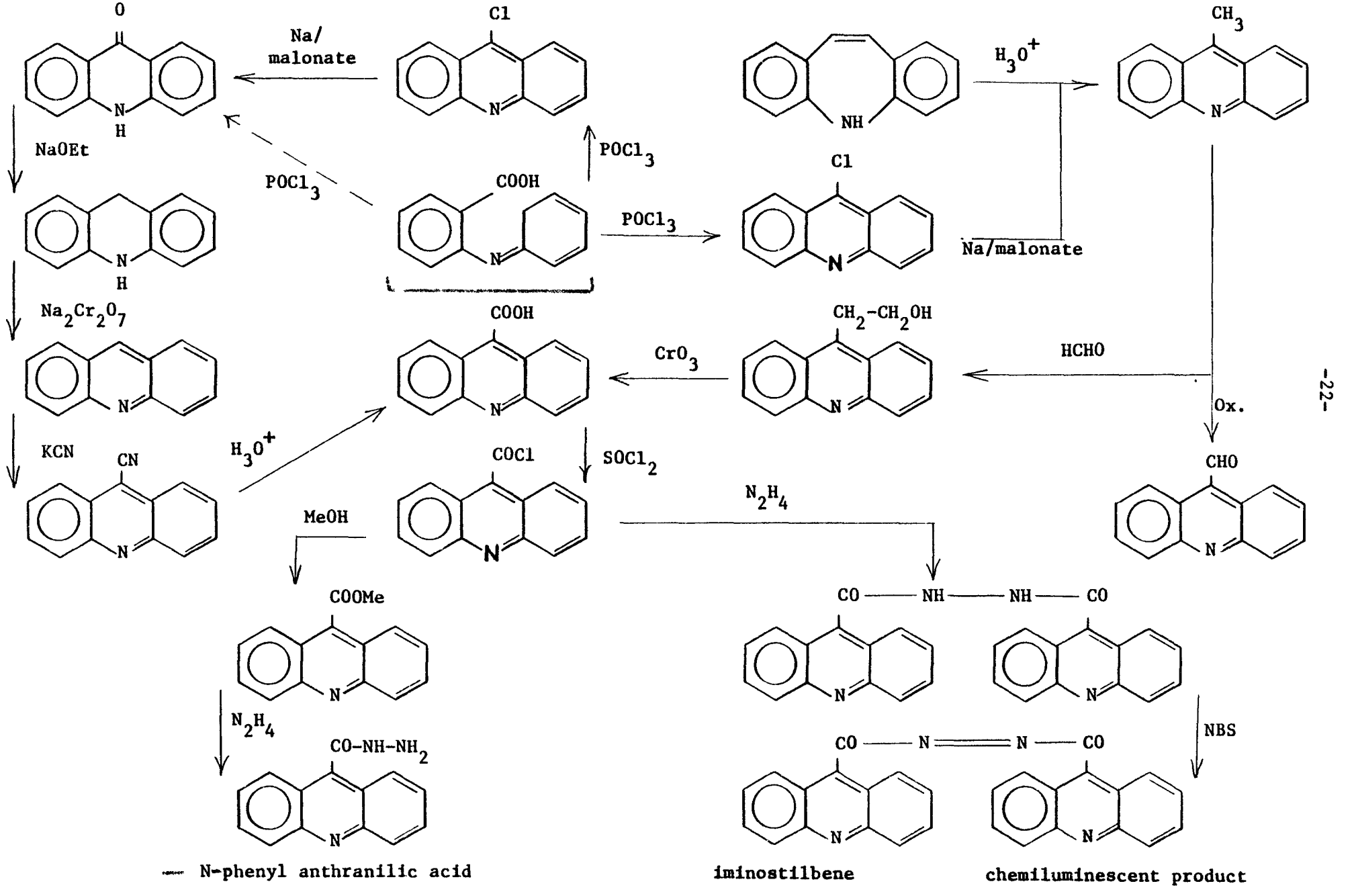
(a) N-Phenylanthranilic acid is treated with $POCl_3$ to give 9-chloroacridine, which in turn reacts with sodium and diethyl malonate to give 9-methylacridine. This is standard procedure, and is described by Albert in his text *The Acridines*[1]. Details are given on page 31.

(b) The following rearrangement is not so efficient, but is more interesting: 200 mL concentrated HCl is stirred at room temperature, and 5 g iminostilbene is added gradually (to prevent coagulation). After 1 hour, heat the flask, and reflux for 15 minutes. Add 100 mL hot water, and filter the hot solution. 9-methylacridine is obtained from the hydrochloride solution by adding enough 3M NaOH to make the solution alkaline. Filter, and recrystallise from ethyl acetate. (Yield ~2 g MP 117°C) (See Fig. 6)

The product from (a) or (b) is oxidised to the aldehyde: 2 g of 9-methylacridine is dissolved in hot ethanol, and 2 drops of 2M NaOH added. 1.5 g of freshly prepared (see p. 31) p-nitrosodimethylaniline are added, and the solution refluxed for 4 hours then cooled. The red crystals of the nitrone are filtered off, suspended in 5 mL of water, and 2.5 mL of

1. A. Albert, ibid.

Figure 3 - Chemiluminescent compounds derived from acridine.



C1 - *Continued...*

6M HCl added. When heated on a water-bath, the nitrone dissolves, hydrolyses, and forms the aldehyde. This is cooled, filtered, and washed with 1:3 saturated NaCl/HCl. The product is purified by suspension in 20 mL of hot water, then adding 30 mL of 2M sodium acetate. The precipitate is filtered hot, washed with water, and vacuum dried. Yield, 1.5 g of yellow-green crystals MP 150° C. The chemiluminescence is demonstrated by adding 0.1 g of product to 2 mL dimethylsulphoxide, and stirring in 0.05 g of potassium t-butoxide . The light is a bright green.

C2 - Sufficient oxalate ester for many demonstrations can be prepared as follows: (see p. 42)
To 20 g of 2,4,6-trichlorophenol,[2] add 250 mL benzene. Dry the mixture azeotropically by distilling off 50 mL. Cool in an ice bath, add 14 mL of freshly distilled triethylamine,[3] stir, and add 5 mL of oxalyl chloride dropwise. Allow the mixture to attain room temperature, and stand overnight. Remove the solvent under reduced pressure, and stir for 15 minutes with 50 mL petroleum ether. Filter, dry under vacuum, and recrystallise from benzene. Yield, 18 g, MP 190° C. Chemiluminescence is demonstrated as in B5, and then catalysis of the reaction can be demonstrated by sprinkling a few sodium salicylate crystals on the liquid surface.

2. Preparation of this material is given on p. 42.

3. This material must be free of water and ethanol. If in doubt, stand overnight over molecular sieves.

C3 - (Fig. 4) *A diacyl peroxide preparation*.

The luminescence mechanism in the following experiment is of considerable theoretical interest. Place 4.16 g of 9,10-phenanthrenequinone in a flask with 100 mL of CH_2Cl_2 and add 2.0 mL of trimethyl phosphite.

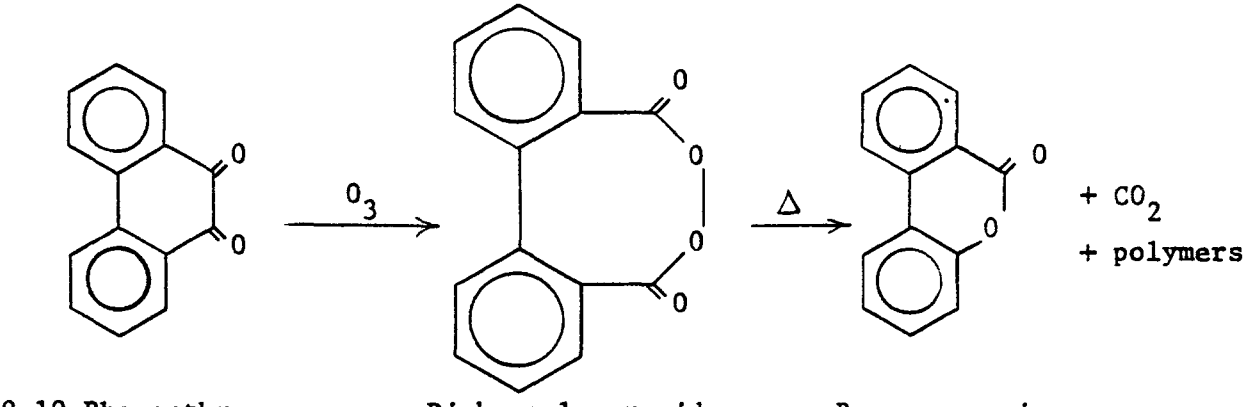
Flush with N_2 or Ar, and allow to stand for 1 hour. Cool the flask in a dry ice/methanol bath, and maintain this temperature while passing ozone from apparatus such as the Welsbach T-408. The ozonolysis takes 1-2 hours, until the initial dark brown colour of the solution ceases to lighten any further.

Sweep out residual ozone in a stream of N_2 or Ar. Filter through cold apparatus, and place the solution in a flask cooled in ice. Remove the solvent under vacuum, and collect the bright orange crystalline produce (4 g). Recrystallise by dissolving in 1:1 CH_2Cl_2: methanol at 0° C, and removing some solvent under vacuum. Two recrystallisations produce pale yellow needle crystals. These may be stored at -20° C.

To show the chemiluminescence, dissolve 0.1 g of peroxide in 5 mL CH_2Cl_2 at 0° C. Add 0.001 g of 9,10-diphenylanthracene, then, in the dark, transfer the test tube from the ice bath to a hot water bath. Decomposition of the peroxide is accompanied by a dim purple glow.

Warning: do not subject the *solid* peroxide to shock or temperatures above 60° C. It will explode violently.

Figure 4 - Preparation and luminescence reaction of diphenoyl peroxide.



9,10-Phenanthrene quinone

Diphenoyl peroxide

Benzocoumarin

C4 - Dioxetanes are relatively stable once prepared. However, the only published preparations involve 98% H_2O_2, in the method of Kopecky and Mumford from the University of Alberta. Anyone wishing to investigate these compounds, fundamental to understanding the chemiluminescence mechanism, should read the article in *Accounts of Chemical Research,* Volume 7, Number 4 (1974), pages 97-105. (The author has not investigated this preparation.)

C5 - Preparation of Triptycene (12)

Technique for slow addition of a solid.

Place 2 g. of anthracene,[4] 2 mL of isobutyl nitrite, and 20 ml. of 1,2-dimethoxyethane in a 125-mL round-bottomed flask mounted over a microburner and fitted with a short reflux condenser. Insert a filter paper into a 55-mm. short stem funnel, moisten it with 1,2-dimethoxyethane, and rest the funnel in the condenser. Weigh 2.6 g. of anthranilic acid[5] on a folded paper, scrape it into the funnel with a spatula, and pack it down. Bring the mixture in the reaction flask to a gentle boil and note that the anthracene does not all dissolve. Measure 20 mL of 1,2-dimethoxyethane into a graduate and use a capillary dropping tube to add small portions of the solvent to the funnel, to slowly leach the anthranilic acid into the reaction flask. If you make sure that the condenser is exactly vertical and the top of the funnel is centered, it should be possible to arrange for each drop to fall free into the flask and not dribble down the condenser wall. Once drip from the funnel has started, add fresh batches of solvent only 2-3 drops at a time.

Reaction time: 1 hour

Aim to complete leaching the first charge of anthranilic acid in a period of not less than 20 minutes, with use of about 10 mL of solvent. Then add a second 2.6 g. portion of anthranilic acid to the funnel, remove the burner and run in 2 mL of isoamyl nitrite through the condenser, resume heating, and leach in the anthranilic acid as before in about 20 minutes. Reflux for 10 minutes more and then add 10 mL of 95% ethanol and a solution of 3 g.

4. Eastman Practical Grade anthracene, although, yellow, is satisfactory.
5. Eastman Practical Grade anthranilic acid.

of sodium hydroxide in 40 mL of water to produce a suspension of solid in a brown alkaline liquor. Cool thoroughly in ice, and also cool a 4:1 methanol-water mixture for rinsing. Collect the solid on a small Buchner funnel and wash it with the chilled solvent to remove brown mother liquor. Transfer the moist, nearly colorless solid to a tared 125-ml. round-bottomed flask and evacuate on the steam bath until the weight is constant; the anthracene-triptycene mixture (m.p. about 190-230°) weighs 2.1 g. Add 1 g. of maleic anhydride and 20 mL of triethylene glycol dimethyl ether ("triglyme", b.p. 222°), heat the mixture to the b.p. under reflux, and reflux for 5 minutes. Cool to about 100°, add 10 m of 95% ethanol and a solution of 3 g. of sodium hydroxide in 40 mL of water, cool in ice, and cool 4:1 methanol-water for rinsing. Triptycene separates as nearly white crystals from the slightly brown alkaline liquor. The washed and dried product weighs 1.5 g., appears to be colorless, and melts at 255°, but it contains a trace of black insoluble material. Dissolve the material in excess methylene chloride (10 mL /g.), filter from specks of black solid, and rinse the flask and paper with a liberal amount of methylene chloride. Then add a boiling stone, heat on the hot plate to boiling, slowly add about two volumes of methanol (b.p. 65°), and boil on the hot plate to eliminate the solvent of lower b.p. (41°) and higher solvent power. Concentrate until crystals just begin to separate, and let the flask stand for crystallization. Still better crystals can be obtained by recrystallization from methylcyclohexane (23 mL /g.), which gives flat, rectangular, laminated prisms. (See Fig. 7)

The chemiluminescence of triptycene may be demonstrated by methods *B4* and *B5*. It is a dim orange. *(This experiment should appeal to instructors, since any anthracene remaining in the crystals will produce a bright purple glow, which will mask that of triptycene).*

Part II

Further syntheses of chemiluminescent acridine derivatives.

Note: These syntheses are suitable for students, and many of them are standard procedures. Some of the steps were repeated more than once. However, because of the sequential nature of the scheme, during the development of this project it was performed in its entirety only once.

Other acridine syntheses (See Fig. 3, p. 22)

Preparation of 9-chloroacridine

20 g. of diphenyl anthranilic acid were mixed with 60 mL of phosphoryl chloride and refluxed for 1 hour at 115°C. The product was cooled to 50° C, and 80 mL of chloroform were added. The mixture was poured onto a stirred mixture of 80 mL of concentrated ammonia and 20 g. of ice chips. The vessel was kept in an ice bath for 30 minutes, and then removed. (It is most important to keep the temperature below 40° C). After removal of the vessel from the ice bath, the remaining ice chips were allowed to melt, and the mixture was warmed to 40° C while being stirred. The solution was extracted twice with 10 mL of chloroform, the extracts dried with calcium chloride, and the solvent removed using a rotary evaporator at reduced pressure. The crude yellow-green product (M.P. 119° C) was recrystallised from benzene, and yielded 13.5 g. of yellow needles (68%) of melting-point 119°C. (Literature 120° C).

Synthesis of 9-methyl acridine from 9-chloroacridine

9 g. of diethyl malonate was dissolved in 40 mL of absolute ethanol, and 1.2 g. of sodium were added in 0.1 g. quantities. 7 g. of 9-chloroacridine dissolved in 40 mL of toluene were added, and the mixture heated under reflux for 16 hours, with constant stirring. The resulting mixture was treated with 75 mL of 1:1 concentrated hydrochloric acid:water, and then the organic solvents removed by distillation (this is done by distilling until the temperature suddenly rises above 72° C).

Refluxing of the material was continued for 4 hours, then 75 mL of water were added. The mixture was filtered through a bed of charcoal while hot. The filtrate contains 9-methyl acridine hydrochloride, and was treated with 2 M

sodium hydroxide until pH > 10 to precipitate the free product. This was filtered and dried, and recrystallised from ethyl acetate.

Yield = 6.25 g. (75%) M.P. = 117° C

Preparation of p-nitroso dimethylaniline (*N, N, dimethyl-4-nitrosoaniline*)

3 g. of N, N,-dimethylaniline were mixed with 10.5 mL of concentrated hydrochloric acid and cooled below 5° C. The mixture was stirred, and 1.8 g. of sodium nitrite dissolved in 3 mL of water were added over 1 hour. After standing 1 hour, the mixture was filtered and washed, first with 40 mL of 1:1 concentrated hydrochloric acid:water and then with 10 mL of 95% ethanol. The product was the hydrochloride, a yellow powder of M.P. > 120° C.

NOTE: It is essential that the tip of the tube, through which the nitrite solution is added, shall be below the surface of the reaction vessel.

The hydrochloride was suspended in 40 mL of water, cooled below 5° C and stirred. 6 mL of 10% sodium carbonate was added dropwise over 1 hour. The green precipitate was filtered, washed three times with 2.5 mL deionised water, then three times with 2.5 mL 50% ethanol.

Yield = 3.0 g. (80%) M.P. = 91° C

Preparation of Acridine-9-aldehyde (9-formylacridine) from 9-methylacridine

1.93 g. of 9-methylacridine and 1.5 g. of freshly prepared p-nitroso dimethylaniline were dissolved in hot 95% ethanol, and made alkaline with a few drops of 2 M sodium hydroxide. The mixture was refluxed for 4 hours, and filtered, giving a red nitrone which was washed with 95% ethanol until the washings were colourless.

Yield: 0.44 g. M.P. = 231-2° C (literature = 233° C).

0.4 g. of the nitrone were mixed with 1 mL of water, then 0.5 mL of 8 M

hydrochloric acid were added. The mixture was stirred and heated on a water bath. The nitrone dissolved and then formed yellow crystals of the hydrochloride. These were cooled, filtered, and washed with a 1:3 brine: 2 M hydrochloric acid mixture. The residue was dissolved in 4 mL of hot water, and the aldehyde precipitated by adding 0.75 mL of 2 M sodium acetate dropwise. The mixture was filtered hot, and washed with hot water, yielding 0.21 g. of yellow-green product (82%) M.P. = 143° C (literature = 145-6° C). After recrystallisation from absolute ethanol, the melting-point was 150° C (literature = 150° C).

Synthesis of acridone

10 g. of N-phenyl anthranilic acid were refluxed with 25 mL of concentrated sulphuric acid for 4 hours. A trap was placed at the top of the reflux condenser to prevent steam from returning to the reaction vessel. 250 mL of water were boiled, and the refluxed mixture was trickled down the side of the beaker. (This method is used because cold water tends to spatter. Care is still needed, however). After boiling for 5 minutes longer, the mixture was filtered. The residue was suspended in 100 mL of water and 7.5 g. of anhydrous sodium carbonate added. After boiling and stirring for 5 minutes, the mixture was filtered, and the residue was dried, extracted with water to remove sodium sulphate, filtered and dried again.

Yield 8.0 g. ($>$90%) M.P. = 344-6°C (literature 348° C)

Recrystallisation from 5:2 acetic acid:aniline was attempted, but could not be satisfactorily completed. In fact, on standing, an intensely coloured red substance formed.

Preparation of acridan from acridone

5 g. of acridone were refluxed with 200 mL amyl alcohol, and 15 g. of sodium were added down the condenser over 30 minutes in pieces no larger than 0.25 g. Refluxing was carried on for a further 20 minutes, then 200 mL of water were cautiously added. The alcohol was removed by steam distillation. The mixture was filtered and the residue dried.

Yield 3.8 g (80%) M.P. = 164° C (literature = 170° C)

Preparation of acridine from acridan

3.62 g. of acridan were stirred while refluxing with 430 mL of water and 20 mL of 20% sulphuric acid. Two aliquots of 1.96 g. of potassium dichromate dissolved in 30 mL of boiling water were added at 5-minute intervals. 5 g. more dichromate were dissolved in 50 mL of boiling water, and added to the reaction mixture to precipitate the acridine as the dichromate. The mixture was boiled for exactly 5 more minutes, then cooled and refrigerated overnight. The precipitate was suspended in 140 mL of hot water, 35 mL of hot water added, heated to 100° C and then rapidly cooled. After filtering and washing with water, the residue was dissolved in 0.5 M hydrochloric acid, filtered and reprecipitated with dilute ammonia. The product was filtered and dried over calcium chloride in a vacuum desiccator.

Yield 2.1 g. (60%) M.P. = 105° C (literature = 110° C)

Preparation of 9-cyano acridine from acridine

2 g. of potassium cyanide were dissolved in 12 mL of water and 0.9 g. acridine added. 3 g. of benzoyl chloride were added dropwise, and the suspension shaken in a stoppered flask. The resultant yellow oil solidified after standing for 2 hours. The residue was filtered, dissolved in methanol, filtered through charcoal and recrystallised, giving yellow needles.

Yield 0.6 g. (65%) M.P. = 185° C (literature = 181° C)

Preparation of acridine 9-carboxylic acid from 9-cyano acridine

0.5 g. of 9-cyano acridine were stirred and heated with 5 mL of 90% sulphuric acid for 2 hours on a boiling water-bath. After cooling, 1.4 g of sodium nitrite were slowly added. After 2 hours of further heating on the water-bath, 85 mL of water were added. The residue was filtered, and extracted with 3 M sodium hydroxide. After being reprecipitated with dilute sulphuric acid, the product was filtered and recrystallised from 95% ethanol.

Yield 0.22 g. (40%) M.P. = 271° C (literature = 289° C)

Preparation of acridine 9-carboxylic acid from 9-methyl acridine

20 g. of 9-methyl acridine was heated under reflux with 20 mL of 20% formaldehyde on a boiling water-bath for 4 hours, then allowed to stand overnight. The residue was filtered, washed with water, and recrystallised from 75% ethanol. This gave 7.4 g. of 9-(beta-hydroxyethyl) acridine, M.P. = 155° C (= literature)

7.25 g of the crystals were dissolved in 20% sulphuric acid, and heated on a boiling water, with stirring. 9 g. of chromium trioxide were dissolved in a mixture of 15 mL concentrated sulphuric acid and 35 mL water. This solution was added dropwise to the stirred mixture over 1 hour. (In order to prevent boiling-over, the addition rate was decreased near the end). After heating for 1 hour further, the mixture was diluted with water, cooled, and filtered. The residue was extracted with 3 M sodium hydroxide, filtered, and reprecipitated by treatment with dilute hydrochloric acid until pH < 3, filtered and dried.

Yield 5 g. (67%) M.P. = 281° C (literature = 289° C)

Preparation of 9-chlorocarbonyl acridine

1.75 g. of acridine 9-carboxylic acid was added to 25 mL of thionyl chloride and refluxed for 6 hours. After removing solvent under vacuum until the solution was at the point of crystallising, heptane was added dropwise to precipitate the product.

Yield 1.6 g. (80%) M.P. = 213° C (literature [30] = 218° C)

Preparation of 9-carbomethoxy acridine

0.5 g. of 9-chlorocarbonyl acridine was added to 10 mL of methanol, and saturated sodium bicarbonate solution added dropwise to neutrality. After extraction with dichloromethane, the produce was obtained by removal of solvent under vacuum, and recrystallisation from heptane.

Yield 0.3 g. (68%) M.P. = 123° C (literature [30] = 127° C)

Preparation of 9-acridine carboxylic acid hydrazide

0.15 g. of 9-carbomethoxy acridine was mixed with 0.3 mL of 95% hydrazine and 10 mL of 100% ethanol, and refluxed under argon for 6 hours. 5 mL of solvent were removed under vacuum, a further 0.3 mL of 95% hydrazine added, and the mixture refluxed for a further 20 minutes. Then the addition of 0.3 mL hydrazine and refluxing was repeated. The solution was cooled, and the product then precipitated. (If no precipitate forms, but an oil, repeat the addition of 3 mL hydrazine and refluxing until a precipitate is obtained.) After filtration, it was recrystallised from methanol.

Yield 0.10 g. (75%) M.P. = $\Delta >$ 180° C (literature [30] = 244° C)

Preparation of azodi-9-acridonyl

0.05 g. of 9-chlorocarbonyl acridine was dissolved in 1.5 mL dichloromethane and added dropwise to a stirred solution of 0.014 g. hydrazine and 0.063 g. triethylamine[6] in 1 mL of pyridine, and stirring was continued for 1 hour. The mixture was left to stand overnight. After removal of the solvent under vacuum, the residue was treated with 1.5 mL of 5% sodium bicarbonate solution, and collected on a sintered funnel. The product was orange-brown 1,2-bis-9-acridonyl hydrazine.

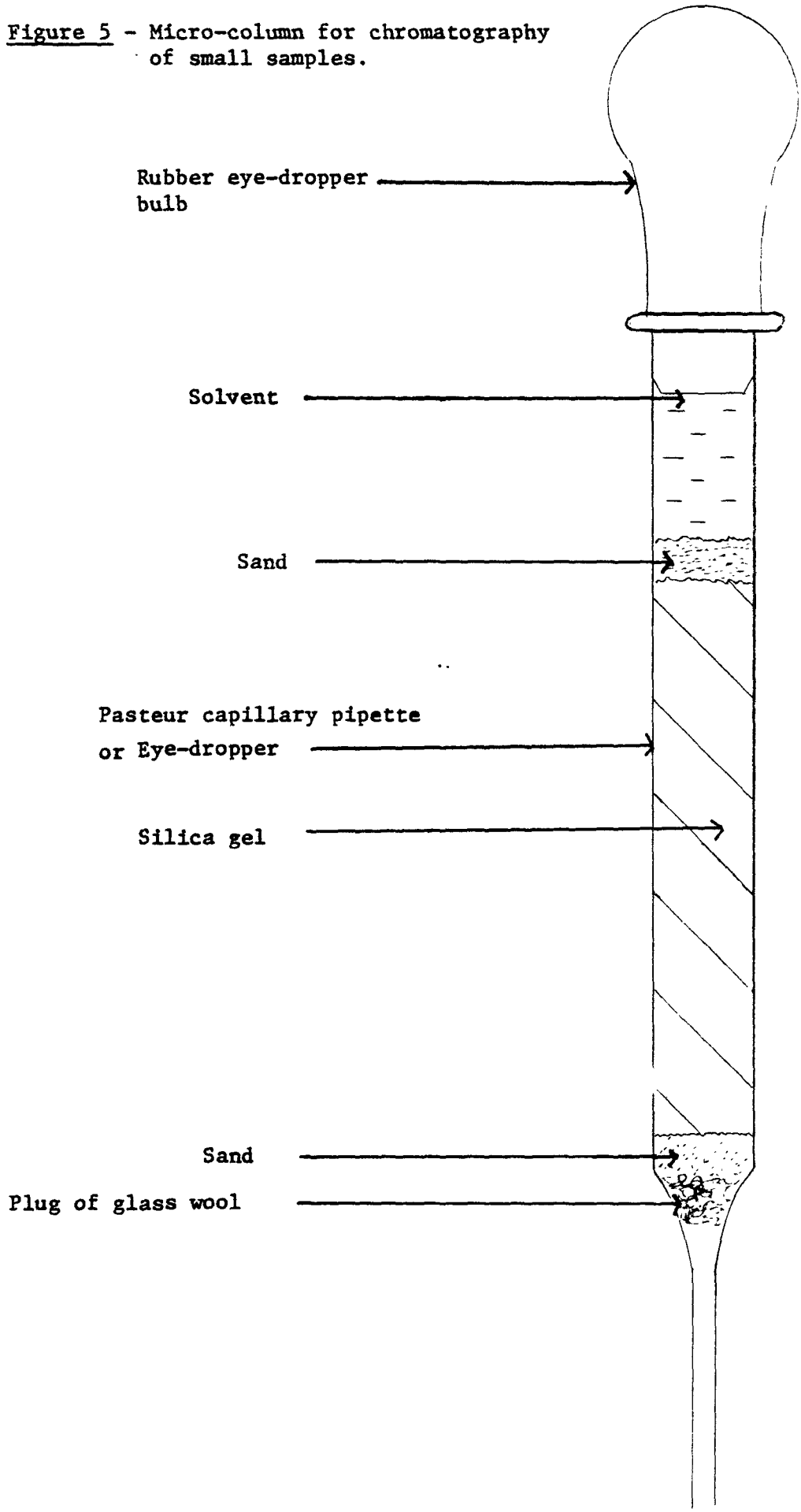
Yield 0.032 g. (65%)

5 mg. of this material was suspended in 4 mL of dichloromethane, and 1 drop pyridine added. The mixture was cooled to 0°C and 5 mg. of N-bromosuccinimide was gradually added. The solution was stirred for 2 hours at room temperature, washed with water, with 10% sodium carbonate, and again with water. The solution was dried over sodium sulphate, and the solvent removed under vacuum. The crude red product was purified on a microcolumn of silica gel, (Fig.5), using chloroform as eluent. The impurities eluted, and on removal of the chloroform gave a yellow oil, smelling of pyridine. The desired product remained on the gel, and was eluted with methanol, giving orange needles on crystallisation.

Yield 3 mg. (60%) M.P. Δ> 210° C (literature 30 = 267° C)

6. See p. 23

Figure 5 - Micro-column for chromatography of small samples.



Preparation of chemiluminescent Schiff bases

The literature method is extremely simple - isobutyraldehyde was mixed with an aromatic amine and allowed to stand over a drying agent for 12 hours. 9-amino anthracene was the reported amine. This material was not available, so 4-amino pyridine and 1-amino naphthalene were substituted.

1. Attempts with 4-amino pyridine, by three different methods, gave colourless resins on removal of excess solvent. The products were not chemiluminescent. It would appear that the basicity of the amine may have caused polymerisation of the aldehyde. This substance was analysed by N.M.R., and the method rejected.
2. (a) 1 g. of 1-amino naphthalene were mixed with 10 mL of isobutyraldehyde, and allowed to stand over molecular sieves for 12 hours.
   (b) 1 g. of 1-amino naphthalene were mixed with 10 mL of isobutyraldehyde, and 10 mL of dichloromethane, then allowed to stand over molecular sieves for 12 hours.
   (c) 1 g. of 1-amino naphthalene were mixed with 10 mL of isobutyraldehyde and 10 mL of dichloromethane and heated under reflux for 12 hours.

In all three cases, solvent was removed under vacuum, and a pink-brown viscous liquid formed. N.M.R. analysis showed that all three products were the same substance, with (c) containing a trace of polymeric material. All three samples were chemiluminescent, thus it appears that the synthesis method is not critical.

Chemiluminescence was demonstrated by placing in a test tube, 3 drops of Schiff base, 1 mL of DMSO, and shaking with 0.1 g. of alkali. Potassium hydroxide gave a dim luminescence lasting 50 seconds. Potassium t-butoxide gave a brighter luminescence lasting 5 seconds.

Chapter 4

Further Experiments

These experiments consist of the background work used to establish the material in Chapter 3. The alternate oxalate ester synthesis (p. 42) may be offered to students instead of that given in Experiment *C2*.

FURTHER EXPERIMENTS

Luminol reactions.

For many years, all luminol experiments were conducted using water as solvent. These reactions were of short duration. Dimethyl sulphoxide was shown to be a much better solvent, but all reported experiments quoted the use of rather large quantities of luminol. A recent sharp rise in the cost of this material prompted the development, as part of this project, of a much smaller-scale demonstration. In an attempt to prolong the reaction, it was decided to investigate a two-phase system.

The hydrogen peroxide solution normally quoted as the oxidising agent was, therefore, replaced by solid sodium peroxide, and the alkali (Potassium or sodium hydroxide) used in the form of pellets.

The method proved successful - the glow is bright, and is visible even in a large room, since it takes place on the surface of the pellets and the walls of the tube. It lasts for many hours, even days. When the glow becomes dim, vigorous shaking of the tube brings more reactant into solution, and the brightness increases. The method is also not critical; variations in proportions of the reacting materials have little effect on the initial brightness.

Since the method is so economical, and the tubes used are so small, it is easy to set up a large number of trials to investigate the effect of adding different fluorescent materials or different catalysts.

Preparation of oxalate esters.

The original reference gives preparation of esters from both 2, 4,-dinitrophenol and 2, 4, 6-trichlorophenol. The dinitro ester produces a brighter luminescence, but the yield in the preparation is lower.

The method[1] of preparation of the dinitro ester is as given on page 25, using 18.5 g of 2, 4-dinitrophenol. The purification step, however, required different solvents: after removal of the benzene, the mixture was stirred with 15 mL of chloroform. After filtering, the residue was washed with ethyl acetate, and then recrystallised from ethyl acetate.
Yield 10.0 g (50%) M.P. = 198° C (literature 1 = 195° C)

2, 4, 6-trichlorophenol is a standard laboratory reagent, but was not available. It was, therefore, synthesised by bubbling chlorine through a solution of 30 g phenol in 300 mL of water. A vigorous exothermic reaction occurred, and the material abruptly crystallised when excess phenol was chlorinated. The residue proved very difficult to crystallise from the quoted ethanol/water mixture, so that the crude product was used for the preparation of the oxalate ester.
M.P. of crude product = 59-61°C (literature 1 = 61° C)
(M.P. of phenol = 42° C)

Since 2, 4-dinitrophenol and 2, 4, 6-trichlorophenol both yield chemiluminescent esters, it was decided to try to make an ester using 2, 4, 6-trinitrophenol (picric acid). Three attempts were made, one using the method on page 25, one mixture was refluxed for 2 hours, and one mixture was refluxed for 12 hours. The product in all three cases was not chemiluminescent, and its melting point and NMR spectrum were indistinguishable from those of picric acid. This synthesis was, therefore, considered unsuccessful.

Using the two oxalate esters that were obtained, trials were made with different luminescers (anthracene, Rhodamine B, fluorescein, dichlorofluorescein, violanthrone, 9, 10-diphenyl anthracene, and rubrene) and with different

1. Mohan, A.G., and Turro, N.J., J. Chem. Ed. 51 528 (1974)

solvents, (ether, dioxane, dimethylsulphoxide, diethyl phthalate, and dibutyl phthalate.) The results of these trials illustrate the point made on page 49, that there is a specific interaction between the components of a chemiluminescent system, thus, for example:

- dimethyl sulphoxide is an excellent solvent for the luminol reaction, but gives no appreciable luminescence if used for oxalate esters.

- violanthrone gives an intense red glow when used in the chlorine/ hydrogen peroxide system, but with an oxalate ester, the glow is a dim green.

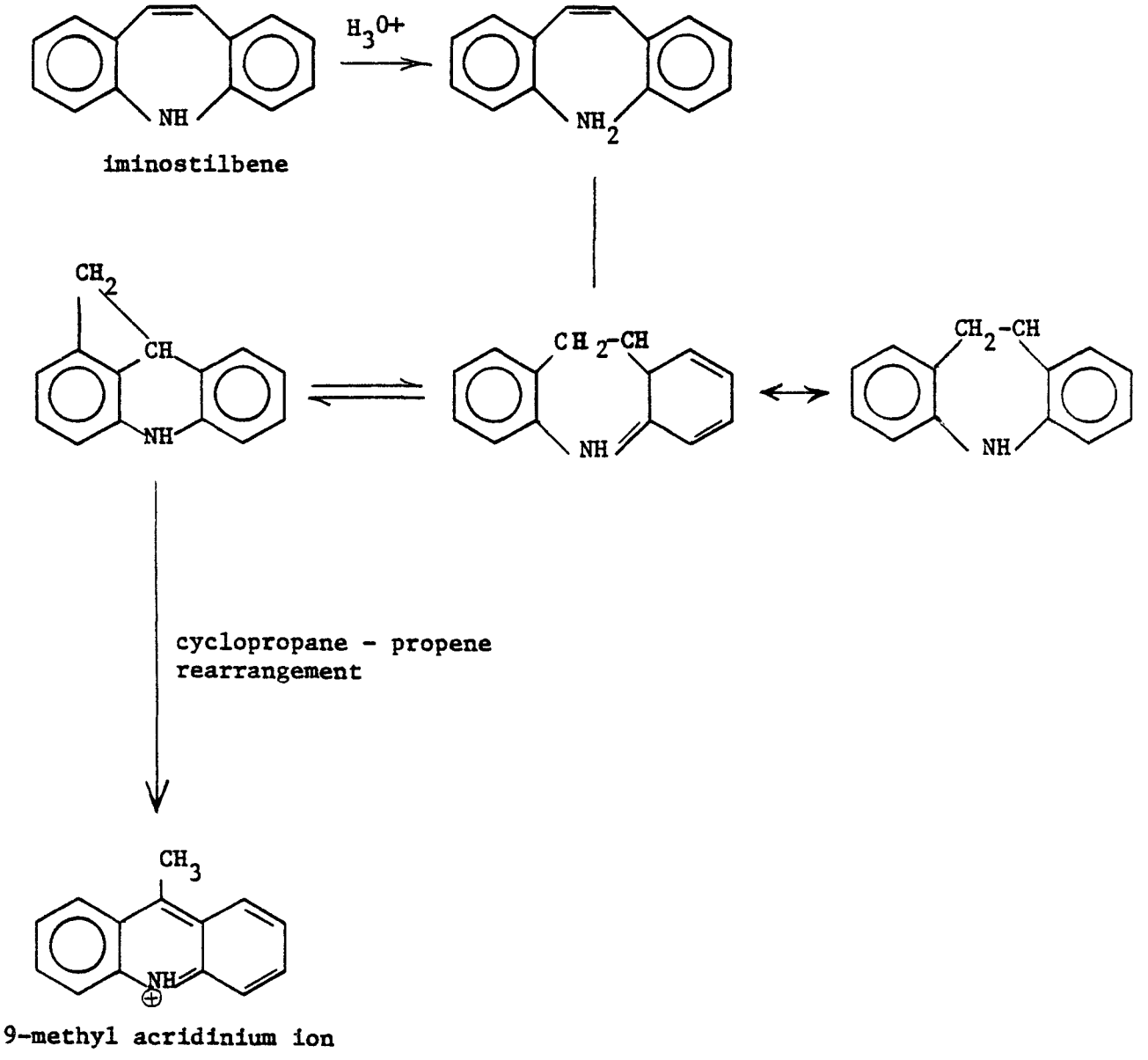
Interfacial luminescence.

Different luminescers were evaluated for the chlorine/hydrogen peroxide system, with little success. The specificity of the interaction between the exciter and the emitter molecules was thereby confirmed. Thus, although Rhodamine B gives a bright red light with oxalyl chloride, in chlorine/ hydrogen peroxide it gives a dim green glow.

Synthesis of 9-methyl acridine from iminostilbene. (35)

The original reference gives the mechanism shown in Fig. 6; the only details of the experimental method give the acid concentration and the time, but no details of quantities! Experiments were thus conducted to find the optimum ratio of imine to acid. The first used 1 g of imine, 8 mL concentrated hydrochloric acid, and 16 mL of water. Yield was 0.1 g. The reaction was difficult to control, because the imine coagulated, and it was obvious that there were lumps of unreacted starting material. The procedure was, therefore, modified, and the refluxing preceded by sufficient stirring at room temperature to prevent coagulation in the initial stages. The yield increased on refluxing and recrystallisation, using the method given by Albert. The effect of changing the concentration was also investigated,

Figure 6 - Mechanism proposed by Rumpf and Reynaud for the isomerisation of iminostilbene. (35)



and yields were increased by using concentrated (18 M) acid. However, none of the trials resulted in yields greater than 30%.

Selection of synthetic routes to acridine 9-carboxylic acid.

The current prices of n-phenyl anthranilic acid, iminostilbene and acridine are in the ratio 1:4:8. Thus, the route from the cheapest starting material is desirable, since the yield from the iminostilbene is so low. However, the latter route may be selected because of inherent interest in the mechanism, or to save time. The route via acridine is not recommended, since the conversion to acridine 9-carboxylic acid via 9-cyano acridine proved difficult to perform satisfactorily using the literature method. It was also determined that the route to 9-chloroacridine via acridone was not as satisfactory as the one described.

The most obvious choice is to obtain the carboxylic acid directly from some supplier. Perhaps this will be made possible if enough interest is shown in preparing chemiluminescent acridine compounds. Then the ab initio syntheses given above will be required only for preparing acridine 9-aldehyde.

Chapter 5

Theory

A simple introduction which may be used as a basis for further reading.

Theory of chemiluminescent reactions.

There are two requirements for any chemiluminescent reaction to occur in solution.

1. "Sufficient energy in a chemical reaction must become available in a single step to leave the product or intermediate molecule in a state corresponding to its first electronically excited state or vibrationally excited state.

2. The product or intermediate molecule must be a reasonably fluorescent molecule so that de-excitation by fluorescence is a probable event, in which case we obtain direct chemiluminescence, or else efficient energy transfer must occur between the excited, non-fluorescent molecule and a second, fluroescent species, giving rise to sensitised chemiluminescence". (37)

Note that this second type of luminescence is also referred to as electron transfer luminescence by many authors, and in the new theory by Schuster as chemically initiated electron-exchange luminescence.
Direct luminescence is shown by fluorescein and the dioxetanes. It can be represented by the scheme:

$$A \longrightarrow B^* \longrightarrow B + h\nu$$

Sensitised chemiluminescence is shown by the luminol/fluorescein system, or the oxalyl chloride/ anthracene system. It can be represented by the scheme:

$$A \longrightarrow B^*$$

$$B^* + C \longrightarrow B + C^*$$

$$C^* \longrightarrow C + h\nu$$

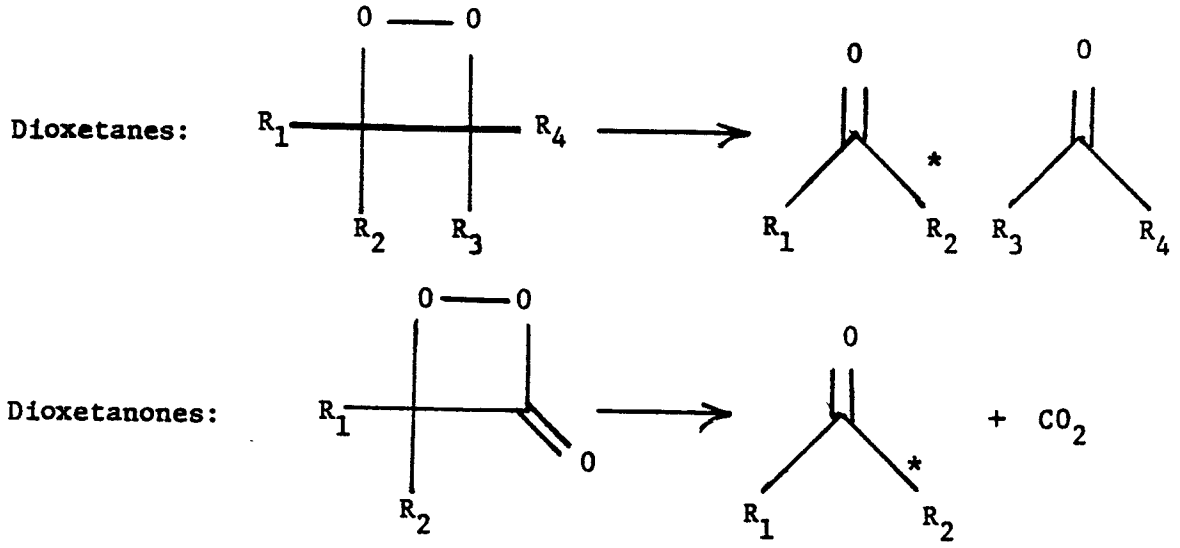
This scheme may be simplified for systems that produce a short-lived emitter that is rapidly destroyed when light emission has occurred:

$$A \longrightarrow B^* \longrightarrow B + h\nu$$

$$\downarrow \text{ dark reaction}$$

$$C$$

The theoretical interest in the direct luminescence of dioxetanes lies in the fact that the reaction is monomolecular. Therefore, the kinetics of the reaction can be readily investigated: the energy difference between the initial and final structures in the light-producing step is related to the frequency of the emission. The intensity of the emission is governed by the rate of reaction. The fact that the molecules are small limits the number of structures that can be suggested as intermediates. The initial and final states can be represented thus:



Similar equations can be written for Schiff base intermediates. It will be seen that the decompositions involve only the rearrangement of four electrons. If one of the product molecules is in the singlet excited state, light emission can occur. Light emission will be reduced if the major proportion of the product is excited to the triplet state. Thus, the dioxetane system lends itself, not only to the generation of electronically excited molecules, but also to investigation of the rules dictating their spin multiplicities. The method thus simplifies luminescence to its fundamentals; not only is the reaction independent of enzyme action, which

imposes temperature restrictions, but the number of reacting molecules is reduced to one.

That there is a specific interaction between exciter and emitter in sensitised chemiluminescence has been postulated for some time, and can be inferred from the following chart:

OBSERVATIONS OF LIGHT PRODUCED IN CHEMILUMINESCENT SYSTEMS				
	Fluorescein	Rhodamine B	Violanthrone	Anthracene
Oxalyl/chloride	nil	intense red	dim green	bright purple
Luminol	bright yellow-green	bright salmon pink	dim blue-green	dim yellow-green
Chlorine/ oxygen	dim green	very dim green	intense red	nil

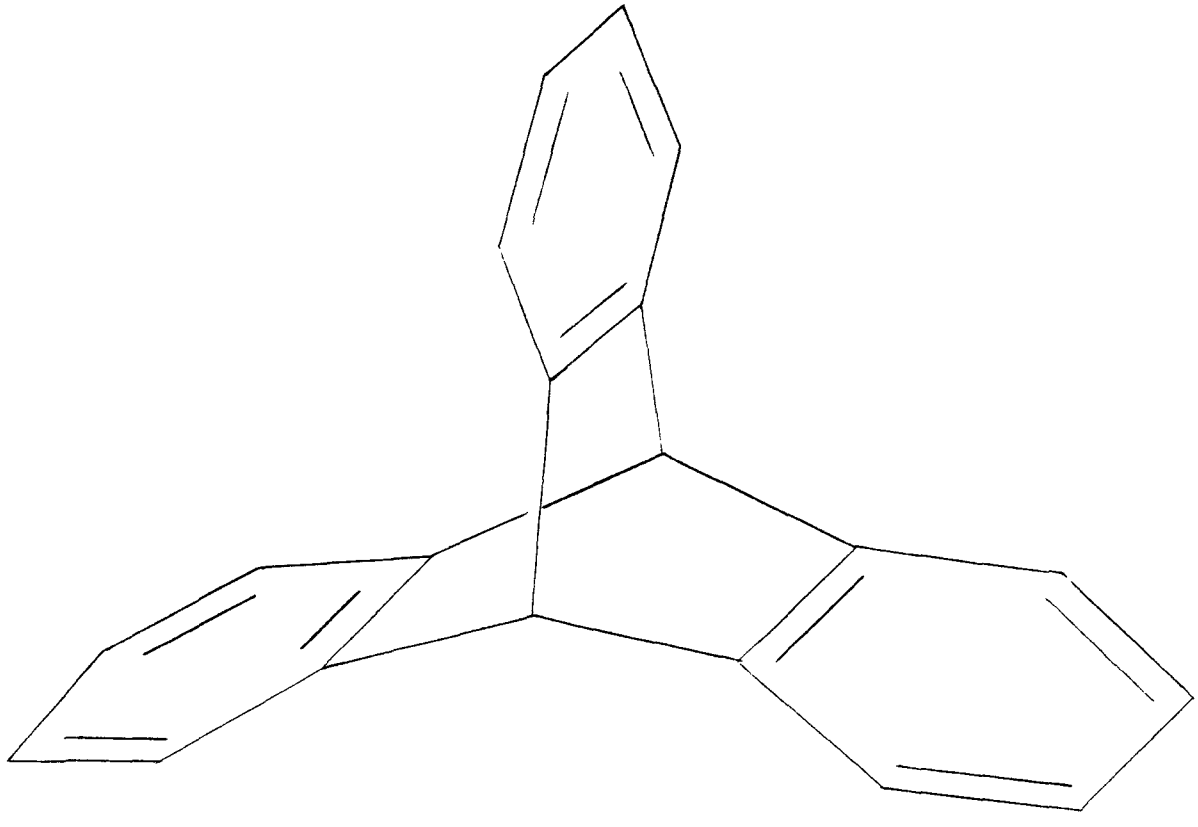
It has been shown that many of the sensitised luminescent systems, such as oxalyl chloride/anthracene, involve transfer of one electron from the π-orbital of one molecule to the π-orbital of another identical molecule, thus, forming an anion/cation radical pair. If one of these radicals is produced in the singlet excited state, light emission can occur. Not every luminescence occurs in this fashion - the mechanism for the inorganic luminescence quoted on page 12 is:

$$R^{3+} + R^{+} \longrightarrow (R^{2+})^{*} + R^{2+}$$

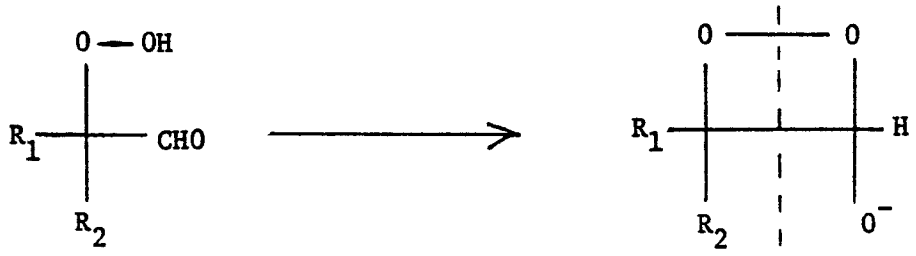
where R = $Ru(bipy)_3$, the tris(bipyridyl) ruthenium group

The elucidation of the electronic states of the excited species has evinced much recent interest, and the fact that there is no π-bond system extending through the three-dimensional triptycene molecule means that some unusual mode of electron delocalisation must be taking place. (See Fig. 7).

Figure 7 - Structure of triptycene showing spatial arrangement.



McCapra (23) suggested two alternative routes by which molecules could undergo oxidation in basic media and produce light. The essential step in one of these is the formation of a hydroperoxide, which then loses a proton to form the dioxetane ring; for example:



The ring structure then cleaves along the axis shown and the energy of this reaction creates the excited species. The structures of such species, known as *exciplexes,* have been the subject of much recent work.[1]

The precise nature of the interaction between the exciter molecule and the hydrocarbon is the basis of Schuster's theory: an electron is transferred from one hydrocarbon molecule to the exciter molecule. The hydrocarbon molecule then becomes a positive ion, and the exciter molecule a negative ion. The negative ion undergoes reaction, and becomes a negative ion of different composition. This second negative ion transfers an electron back to the hydrocarbon positive ion, and the transfer occurs with sufficient energy to excite the hydrocarbon to its singlet excited state.

Schuster proposed this theory in 1977 on the basis of the diacyl peroxide experiment described earlier. The proposal has been well received by other workers. The lure of the theory is that it may be applicable to the processes involved in bioluminescence, thus explaining the mechanisms of the reactions which triggered the initial questions a century ago.

1. Zachiariasse, K.A., Proc. Int. Exciplex Conference, London, Ontario, 1974

SUGGESTED READINGS

1. Seliger and McElroy (37)
   - a survey of the whole field of luminescence, both biological and chemical systems, with suggested mechanisms and theory up to 1965.

2. Schneider, H.W., J. Chem. Ed. 47 #7, 519 (1970)
   - a review of the work by White on luminol, and suggestions for commercial applications.

3. McCapra (24)
   - a 40-page review by one of the principal contributors to the subject.

4. Chemiluminescence and Bioluminescence , Hercules, D.M. and Lee, J., editors, Plenum Press, N.Y. (1973) (7)
   - anecdotal reports and papers given at a conference attended by all the principal workers in the field. Very useful detail of the work by Rauhut for the Cyanamid Company.

5. Hercules, D.M., in Physical Methods of Organic Chemistry , Weissberger, A., and Rossiter, B., editors, Academic Press, N.Y. (1971), Part II, Chapter 13
   - contains details of the early work on electron-transfer luminescence.

6. Rauhut (32) and Bollyky (3)
   - suggestions that dioxetanes are the intermediates responsible for luminescence.

7. Faulkner (11) and Wilson (46)
   - two 50-page reviews of the most recent progress in electron transfer and dioxetane luminescence, respectively.

8. Schuster (36)
   - experimental evidence for the electron exchange theory.

BIBLIOGRAPHY AND REFERENCES

1. Abbott S.R., Ness, S., and Hercules, D.M., J. Am. Chem. Soc. 92 1128 (1970)

2. Bailey, P.S., Carter, T.P., Fisher, C.M., and Thompson, J.A., Can. J. Chem. 51 1278 (1973)

3. Bollyky, L.J., Whitman, R.H., Roberts, B.G., and Rauhut, M.M., J. Am. Chem. Soc. 89 6523 (1967)

4. Campbell, N., What is Science? , Methuen, London (1921) p. 27

5. Cass, M.W., Rapaport, E. and White, E.H., J. Am. Chem. Soc. 94 3168 (1972)

6. Chandross, E.A., Tetrahedron letters 12 761 (1963)

7. Cormier, M.J., Hercules, D.M., and Lee, J. (eds.), Chemiluminescence and Bioluminescence , Plenum Press, N.Y. (1973)

8. Drew, H.K.D., and Pearman, F.H., J. Chem. Soc. (1937) 586

9. Drew, H.K.D., and Garwood, R.F., J. Chem. Soc. (1939) 836

10. Duran, N., Faria Oliviera, O.M.M., Haun, M., and Cilento, G., J.C.S. Chem. Comm. (1977) 442

11. Faulkner, L.R., in Int. Review of Science, Phys. Chem. Series 2, Vol. 9 , Butterworths, London, (1976) pp. 213-264

12. Fieser, L.F., Organic Experiments , 2nd ed., Raytheon Co. Mass. (1968) pp. 307-309

13. Fox, J.L., Chem. and Eng. News, (March 6, 1978) 17

14. Friedman, L., and Logullo, F.M., J. Am. Chem. Soc. 85 1549 (1963)

15. Gafney, H.D., and Adamson, A.W., J. Chem. Ed. 52 481 (1975)

16. Gattow, G., and Schneider, A., Naturwissenschaften 41 116 (1954)

17. Harvey, E.N., Jour. Biol. Chem. 31 311 (1917)

18. Irving, C.S., and Leermakers, P.A., Photochem. Photobiol. I 665 (1968)

19. Iwamuru, H., and Makino, K. J.C.S. Chem. Comm. (1978) 720

20. Khan, A.U. and Kasha, M., J. Am. Chem. Soc. 88 1579 (1966)

21. Kopecky, K.R., and Mumford, C., Can. J. Chem. 47 709 (1969)

22. Kopecky, K.R., Lockwood, P.A., Filby, J.E., and Reid, R.W., Can. J. Chem. 51 468 (1973)

23. McCapra, F., and Burford, A., J.C.S. Chem. Comm. (1976) 607, 608 and (1977) 874

24. McCapra, F., Progr. Org. Chem. 8, 231 (1973)

25. McCapra, F., Beheshti, I., Burford, A., Hann, R.A., and Zalika, K.A. J.C.S. Chem. Comm. (1977) 944, 946

26. Nuffield Foundation, Nuffield Chemistry, Introduction and Guide Longmans Green and Penguin Books Ltd., London (1966) pp. 1-8 17-104, 145-155

27. Panar, M., and Roberts, J.D., J. Am. Chem. Soc. 82 3629 (1960)

28. Polanyi, M., The Tacit Dimension , Anchor Books, N.Y. (1967) pp. 23-25

29. Ramirez, F., Desai, N.B., and Mitra, R.B., J. Am. Chem. Soc. 83 492 (1960)

30. Rapaport, E., Cass, M.W., and White, E.H., J. Am. Chem. Soc. 94 3153 (1972)

31. Rapaport, E., Cass, M.W., and White, E.H., J. Am. Chem. Soc. 94 3160 (1972)

32. Rauhut, M.M., Acc. Chem. Res. 2 80 (1969)

33. Rauhut, M.M., et. al., J. Am. Chem. Soc. 89 6515 (1967)

34. Rauhut, M.M., Sheehan, D., Clarke, R.A., Roberts, B.G., and Semsel, A.M., J. Org. Chem. 30 3587 (1965)

35. Rumpf, P., and Reynaud, R., Bull. Chim. Soc. France 2241 (1962)

36. Schuster, G.B., and Koo, J-Y., J. Am. Chem. Soc. 99 6107 (1977)

37. Seliger, H.H., and McElroy, W.D., Light-Physical and Biological Action , Academic Press, N.Y., (1965) Chapter 3

38. Slabaugh, W.H., J. Chem. Ed., 47 552 (1970)

39. Story, P.R., Whited, E.A., and Alford, J.A., J. Am. Chem. Soc. 94 2144 (1971)

40. Strehler, B. L., and Shoup, C.S., Arch. Biochem. Biophys. 47 815 (1953)

41. Trautz, M., and Schorigin, P., Zeitschr. Physik. Chem. 53 1-111 (1905)

42. Vassil'ev, R.F., Russ.Chem. Rev. 39 529 (1970)

43. White, E.H., and Bundrett, R.B., in Cormier, Hercules, and Lee, op, cit. p. 232

44. White, E.H., J. Chem. Ed. 34 275 (1957)

45. White, E.H., and Roswell, D.F., Acc. Chem. Res. 3 54 (1970)

46. Wilson, T., in Int. Review of Science, Phys. Chem. Series 2, Vol. 9 , Butterworths, London, (1976) pp. 265-322

47. Ziman, J., Public Knowledge , Cambridge U.P. (1960) pp. 35-55