

## [42] Demonstrations of Chemiluminescence

By FRANK McCAPRA

### Introduction

Although the chemist may envy the biologist with his descriptions of the dramatic displays of bioluminescence in nature, exciting a sense of wonder in the beholder, the chemist can provide complementary displays, much more easily summoned up, by reactions emitting bright chemiluminescence. The value of such displays, of course, lies not only in the pleasure they give, but in the way in which the underlying chemistry can be linked to the chemistry of bioluminescence. In addition, there are a number of scientific principles such as entropy, energy conversion, the nature of light, the electromagnetic spectrum, and so on inherent in the reactions so demonstrated, providing an excellent basis for a variety of pedagogic purposes.

This article does not cover all possible demonstrations, but should provide a means of devising those appropriate to the reader's purpose, whether it is merely to excite some admiration or to enlarge upon the principles already mentioned. Each description will include a suggestion for mounting the display, a summary of the chemical reactions involved, and, where applicable, a link to the relevant bioluminescence. It is written for interested scientists in general (from a chemical or biochemical background) and specialists must look elsewhere for more definitive treatments. It is intended to be largely self-contained, and there is plenty of literature elsewhere for those digging more deeply. The chemistry of chemiluminescent and bioluminescent reactions is discussed in an earlier article in this volume.<sup>1</sup>

Some demonstrations can be mounted with readily available materials from the laboratory and chemical supply companies, but others require some effort in synthesis. Even where the materials are available commercially, the scale of a demonstration required to seduce a large audience makes it an expensive affair. Synthetic procedures for the key reagents are therefore included. If the reader is not skilled in practical organic chemistry, a useful stratagem is to persuade the organizer of an undergraduate organic chemistry practical class to include the synthesis (and of course the light reaction) in the syllabus for the course. Many of the examples here contain lessons in synthesis (most procedures are well within the skills of a second- or third-year undergraduate student, with supervision) and they will provide

<sup>1</sup> F. McCapra, *Methods Enzymol.* **305** [1] 2000 (this volume).

useful pegs on which to hang instruction in concepts in photochemistry, thermodynamics, and so on. The descriptions are given with a variety of demonstrators in mind, ranging from those in a high school, who would presumably confine themselves to commercially available materials, to those with access to an organic chemistry laboratory. Safety is of course paramount, especially if the audience is young, and although in over 200 demonstration lectures the author has harmed neither himself nor the watchers, the only suitable demonstrator is one who has had practical scientific experience, and can be expected to interpret the warnings given here, as befits his or her own circumstances. That being said, there are few chemical demonstrations, with the exceptions to be indicated, that are as safe. The few hazardous reagents are noted.

Although some of the reactions are bright enough to be seen in low-level ambient light, it has to be said at the outset that all will benefit from being shown in as near total darkness as can be arranged. I have often been invited to lecture in schools with quite inadequate means of blackout of the windows, on bright June days, with disappointment evident among all concerned. Each chemiluminescent compound is suited to a particular form of demonstration, but except where noted, it would be possible, with a little experimentation, to substitute another if availability is a problem. The demonstrations are grouped according to the chemiluminescent compound, rather than by type of display. It is to be expected that in assembling a demonstration lecture, a variety of effects will be drawn from reactions of several compounds. Finally, these demonstrations carry with them the age old warning attached to any public display—rehearse, rehearse, rehearse. The experiments are all certain to work in the proper circumstances, but the exact effect and the maximum light yield given the user's own conditions of apparatus, solvents, and so on can only be determined by a degree of trial and error. The quantities and proportions are not immutable, and experimentation around the parameters given here is recommended.

## Luminol

This compound, whose chemiluminescence was discovered in 1928, has been featured in many demonstrations.<sup>1a</sup> Although it is synthesized easily,<sup>2</sup> it is inexpensive enough to purchase and use in quantity if desired.<sup>3</sup>

<sup>1a</sup> B. Shakhshiri, "Chemical Demonstrations: A Handbook for Teachers of Chemistry," Vol. 1, p. 125. University of Wisconsin Press, Madison, WI, 1981.

<sup>2</sup> L. F. Fieser, "Experiments in Organic Chemistry," p. 199. Heath, Boston, 1957.

<sup>3</sup> Aldrich Chemical Co., 3-aminophthalhydrazide 12,307-2.

### *Synthesis*<sup>2</sup>

This is a typical second-year undergraduate laboratory experiment. First put a flask containing 15 ml of water on the steam bath to get hot. Then heat a mixture of 1 g of 3-nitrophthalic acid and 2 ml of an 8% aqueous solution of hydrazine in a test tube until the solid is dissolved, add 3 ml of triethylene glycol, and clamp the tube in a vertical position about 2 in above a Bunsen burner or hot plate with heat transfer. Insert a thermometer and an aspirator connected to a suction pump and boil the solution vigorously to distill off the excess water (110–130°). Let the temperature rise rapidly until (3–4 min) it reaches 215°. Remove the burner, or from the hot plate, note the time, and, by intermittent gentle heating, maintain a temperature of 215–220° for 2 min. Remove the tube, cool to about 100° (crystals of the product often appear), add the 15 ml of hot water, cool under the tap, and collect the light yellow granular nitro compound (dry weight, 0.7 g). The nitro compound need not be dried and can be transferred at once, for reduction, to the uncleaned test tube in which it was prepared. Add 5 ml of 10% sodium hydroxide solution, stir with a rod, and, to the resulting deep brown-red solution, add 3 g of sodium hydrosulfite dihydrate (molecular weight 210.15). Wash the solid down the walls with a little water. Heat to the boiling point, stir, and keep the mixture hot for 5 min, during which time some of the reduction product may separate. Then add 2 ml of acetic acid, cool under the tap and stir, and collect the resulting precipitate of light yellow luminol. The filtrate on standing overnight usually deposits a further crop of luminol (0.1–0.2 g). The luminol is sufficiently pure for the demonstrations. The synthesis can be scaled up very easily.

### *Demonstrations*

The easiest of all the demonstrations is that produced by dissolving luminol in a dipolar aprotic solvent such as dimethyl formamide (DMF), dimethyl sulfoxide, or *N*-methyl acetamide (hexamethylphosphoramide is in this class but there have been adverse reports on its toxicity) and adding a very strong base. The more usual solvent is dimethyl sulfoxide<sup>4</sup> (DMSO). While it is true that the DMSO should be relatively dry and free from its major impurity, dimethyl sulfide, the solvent obtained from freshly opened containers as sold, is best for our purposes. The base used is most conveniently potassium hydroxide pellets, with sodium hydroxide being less effective. These should be fresh and relatively free from surface carbonate, although this is only a problem when the deposit is excessive. Sodium hydroxide can be satisfactory, but in general it is inferior, probably as a

<sup>4</sup> Aldrich Chemical Co., methyl sulfoxide M8, 108-2.

result of a lower aqueous solubility and the varying amounts of water in the "dry" DMSO. Another base that can be used is potassium *tert*-butoxide,<sup>5</sup> producing the brightest light, but not actually the best demonstration. The luminol need only be reasonably pure, and there is no need to recrystallize it (a rather difficult operation). Commercial material used "as is" is usually excellent. The most convenient container is a 1 liter Erlenmeyer (conical) flask with a fairly wide neck and secure stopper, either a rubber bung or ground glass. If the latter is used, beware the seizure of the stopper, which can occur in the presence of strong base, if the flask is put aside for any length of time. Make sure there is no leakage, as the flask will be shaken vigorously.

### *Safety*

Solid KOH, NaOH, and potassium *tert*-butoxide are powerful, corrosive alkalis. Handle only with gloves. DMSO and DMF are absorbed through the skin, but are not particularly toxic. Luminol has no known hazards, but should obviously not be ingested.

*Materials.* KOH pellets; potassium *tert*-butoxide; DMSO; luminol; and a conical flask with good stopper.

### *Procedure*

1. KOH pellets (100 g or sufficient to cover the bottom of the flask) are placed in the flask and barely covered with fresh DMSO (about 60–100 ml). Luminol [200 mg or enough to cover (heaped) a 25 cent coin] is added and the flask is stoppered. Vigorous shaking in the dark will result in the DMSO becoming luminescent with a blue-green light. The time to maximum brightness is variable (depending on water content, purity, etc.), but provided not too much luminol has been used and the other materials are fresh, the interval is never much more than about 1 min. An opportunity is provided for comments, serious or otherwise, while shaking. No heat is generated (except in the demonstrator!) and, provided a hand is kept over the stopper, it is completely safe.
2. Dissolve/suspend potassium *tert*-butoxide (2.0 g) in DMSO (200 ml) in a suitable container (e.g., the 1-liter flask mentioned earlier) and add luminol (100 mg). Shake vigorously. The light is brighter and appears more quickly, but the drama is less!

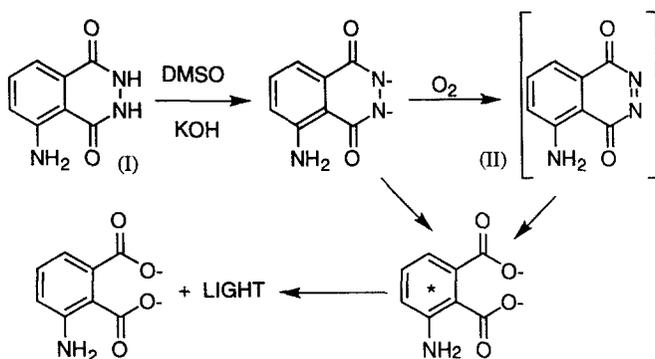
<sup>5</sup> Aldrich Chemical Co., potassium *tert*-butoxide 15,667-1.

### Comments

Using twice the amount of DMSO increases the time to maximum brightness, but the light is more visible. The requirement for oxygen is demonstrated by leaving the flask with the smaller amount of DMSO to settle. A dark solution with a luminescent meniscus (of a depth depending on the amount of luminol used) shows the diffusion of oxygen. Filling the flask with pure oxygen before stoppering gives an enhanced light emission. Increasing the amount of luminol (say twofold) requires more shaking and may give quenching of the light, but the light will last for about 24 hr, provided the shaking is seldom and brief.

### Mechanism

Luminol(I) forms the dipotassium salt on the surface of the KOH and probably reacts with oxygen to form superoxide ion and the luminol radical anion. Cage recombination gives a peroxide that rearranges to form the peroxide precursor to the excited state. An outline reaction scheme is shown, but the last word on the mechanism of this, the first of the strongly chemiluminescent compounds, has not been said. An excellent analysis of the possibilities has been given by White and Roswell.<sup>6</sup> Transition metals such as copper(II) and iron(III) are required in aqueous systems to achieve the same oxidation. The diazaquinone(II) is an intermediate in both media.



### Additional Effects

Fluorescent energy acceptors can be added to change the greenish blue light to the color of the fluorescence of the additive. Some of these are

<sup>6</sup> E. H. White and D. F. Roswell, in "Chemiluminescence" (J. G. Burr, ed.), p. 215. Dekker, New York, 1985.

expensive (although synthesized easily<sup>7</sup>) and it may be as well to reduce the scale to fit the budget. Predissolve the fluorescer (some are not easily soluble) in the DMSO and, if reducing the scale, add the base as a saturated solution of KOH in water, as described later.

The fluorescer (see later,<sup>7</sup> 1 mg) is dissolved in DMSO (10 ml), and luminol (50 mg) is added and dissolved. Several drops of a saturated KOH solution [about 40% (w/v)] are then added and the well-stoppered container is shaken as before to provide the color of light indicated:

Green: 9,10-bis(phenethynyl)anthracene

Yellow: 1,8-dichloro-9,10-bis(phenethynyl)anthracene

Salmon: Rubrene

Red: 5,12-bis(phenethynyl)naphthacene

## Other Experiments

### *Safety*

Sodium hypochlorite (domestic bleach) is caustic. The oldest demonstration involving luminol is also one of the easiest to prepare.<sup>8</sup> Luminol (100 mg, a large spatulaful) is dissolved in NaOH (0.1 M, 200 ml), and the solution is added to 200 ml of commercial chlorine bleach (about 5% sodium hypochlorite). Blue light is produced. It is not the most efficient reaction, but it is very easy to do. Be careful with the bleach, which in this undiluted form is very caustic to the eyes and can damage clothing.

Another old experiment<sup>9</sup> reflects one of the uses of luminol: the detection and analysis of transition metals. Potassium ferricyanide was used in the early examples, but a more sustained light is produced using the cuprammonium ion.<sup>1</sup>

### *Materials*

Sodium carbonate; ammonium carbonate; copper sulfate; sodium bicarbonate; hydrogen peroxide (30% i.e. 100 vol); water; luminol. The water need not be distilled if the supply is reasonably clean; the salts can be any of the hydrated forms available to hand. The hydrogen peroxide can be 3%, requiring a proportionate change in the amount used, but the higher

<sup>7</sup> Obtainable from the Aldrich Chemical Co. or synthesized in P. Hanhela and D. Paul, *Aust. J. Chem.* **34**, 1669 (1981) II. Violet and blue emitters, *Aust. J. Chem.* **34**, 1687 (1981). III. Yellow and red fluorescent emitters, *Aust. J. Chem.* **34**, 1701 (1981)

<sup>8</sup> H. O. Albrecht, *Z. Physik. Chem.* **136**, 321 (1928); W. H. Fuchsman and W. G. Young, *J. Chem. Educ.* **53**, 548 (1976).

<sup>9</sup> E. H. Huntress, L. N. Stanley, and A. S. Parker, *J. Chem. Educ.* **11**, 142 (1934).

concentration is more usually found in the chemistry laboratory and is in fact more stable, almost indefinitely so, in a refrigerator at 4°. It is in any case best for several of the later experiments described here.

### *Safety*

Copper sulfate is toxic if ingested, and sodium carbonate is moderately caustic and dangerous to the eyes. Thirty percent hydrogen peroxide causes skin burns: a white, painful burn, which on small areas is transient. Absorption onto combustible surfaces with high surface areas such as paper towels can later result in spontaneous combustion. Always soak any spills with water before disposal.

### *Procedure*

To make solution A: Dissolve  $\text{Na}_2\text{CO}_3$  (5.0 g) in water (500 ml) and add luminol (0.25 g) with stirring, until dissolved. Add  $\text{NaHCO}_3$  (25.0 g),  $\text{CuSO}_4$  (0.5 g), and  $(\text{NH}_4)_2\text{CO}_3$  and stir until all is dissolved. Make up to a final volume of 1 liter with water.

To make solution B: Add hydrogen peroxide [5.0 ml of 30% (v/v)] to water (1 liter)

*Demonstration.* The volumes in this demonstration have been made deliberately high so that a variety of mixing effects can be obtained. These effects are limited only by the ingenuity of the demonstrator, and only pointers are given here. The simplest involves pouring both A and B from a height so that the streams mix before reaching a very large beaker or other glass vessel. Alternatively, two large (500-ml) dropping funnels can be arranged to drip solutions A and B into a large glass filter funnel to which is attached a spiral of translucent Tygon or polyethylene tubing 1 cm in diameter before exiting into a collecting vessel. A glass spiral produces a marginally better effect, but is of course somewhat more difficult to make.

### *Additional Effects*

Make up two separate solutions of fluorescein and rhodamine (any version) by adding 0.5 g of each to 100 ml of 0.1 M NaOH. Small amounts of these solutions (to be determined by experiment) are added *alternately* to the flowing luminol solution via the filter funnel, giving a gorgeous three-color effect in the Tygon spiral. The quantum yield is enhanced by the transfer of energy to the more efficient fluorescers, and the increase in light intensity adds to the overall effect. The unused fluorescer solution can be kept for several weeks.

## Clock Reactions and Other Curiosities

Chemists have been fascinated by periodic, usually color, reactions for a very long time. Various clock and oscillating reactions<sup>10</sup> have challenged theorists to describe the sometimes bizarre effects in kinetic terms. Perhaps the most well known of these is the Balousov-Zhabotinski oscillating reaction. An extra dimension is added by coupling these reactions to light emission. Not all are suitable for demonstration to a large audience, but they are certainly sufficiently intriguing to include on the right occasions. E. H. White, probably the most influential of chemists with a major interest in the mechanism of chemiluminescence, described<sup>11</sup> the first clock reaction using luminol.

### *A Luminol Clock Reaction*

An aqueous solution of luminol, hydrogen peroxide, ammonia, and a complex cyanide starts colorless and dark, and after a short and variable delay, in the dark, produces a flash of light. The delay in producing the light—the clock effect—is mirrored by a change in the solution from colorless to blue when the reaction is viewed in daylight.

### *Safety*

Potassium cupric cyanide is as toxic as potassium cyanide, and acid must *never* come into contact with the solutions. If complex cyanide is unavailable, it can be made by adding the stoichiometric amount of cupric chloride and potassium cyanide.

### *Procedure*

Use distilled or deionized water for all solutions. Dissolve luminol (2 g) in 100 ml concentrated ammonia (15 M) in a 1-liter volumetric flask and make up to the mark. Make up 50 ml of a 0.1 M solution of potassium cupric cyanide  $[K_2Cu(CN)_3]$  by dissolving the salt (1.1 g) in exactly 50 ml of distilled water. Make up 500 ml of a 3% hydrogen peroxide solution from 50 ml of 30% hydrogen peroxide.

For each of the clock reactions, place 100 ml of the luminol solution in a suitable flask and add 5.0 ml of the complex cyanide solution, followed by 30 ml of the 3% hydrogen peroxide solution. Mix by stirring while adding the hydrogen peroxide to avoid high local concentrations, which can cause a premature start to the reaction. Light will be emitted in a 2-sec flash after

<sup>10</sup> M. Orban, *J. Am. Chem. Soc.* **108**, 6983, (1986).

<sup>11</sup> E. H. White, *J. Chem. Educ.* **34**, 275 (1957).

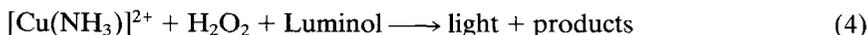
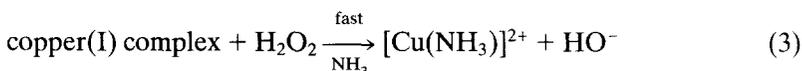
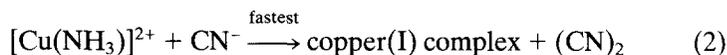
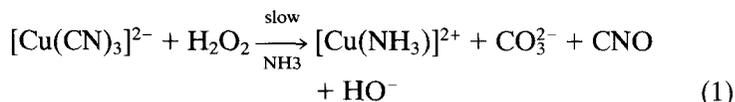
a delay of 10 sec; by varying the hydrogen peroxide concentration,<sup>1</sup> the delay can be increased roughly as shown.

Delay (seconds)	Volume of H <sub>2</sub> O <sub>2</sub> (ml of 3%)
10	30
15	20
25	10
60	5

With precision, speed, and a fair amount of practice, it is possible to have a pattern of light both in space and in time on the demonstration bench. It is, of course, not necessary to use all of the different delays, as the "clocks" are started in sequence by the addition of the hydrogen peroxide, which obviously provides a pattern in time on its own.

The effect is enhanced if, for example, five different individually shaped glass containers are used (*not* wine glasses, Coke bottles, etc.) and if the audience is prevented from seeing the preparations by a simple screen. The presenter should distance himself from the bench when the display starts, meanwhile describing the chemistry in simple terms. Because the induction period is essentially determined by the time it takes to oxidize the complexing cyanide ion, to give free catalytic cuprammonium ion (see the description of the mechanism), the delay can be manipulated by changing the concentration of the complex cyanide or adding KCN (there should be no free copper ion). There may be some value in looking at these alterations if this demonstration is featured. Experimentation can be carried out in the light, without luminol, as the solutions turn bright blue in coincidence with light emission.

#### *Mechanism*<sup>11</sup>



Luminol is acting as an indicator for the catalytic-free cuprammonium ion; reactions (1), (2), and (3) account for the "clock," and an increase in cyanide concentration "ties up" this ion. Conversely, an increase in H<sub>2</sub>O<sub>2</sub> concentration destroys cyanide ion and decreases the time to the appear-

ance of the blue color of the free cuprammonium ion and the accompanying flash of light.

### Other Clock Reactions

Shakhashiri<sup>1</sup> has described variations on the reaction, and if the use of the very toxic cyanides is a concern, an alternative method requiring the synthesis of a deuterioferriheme has been described.<sup>12</sup> More elaborate versions,<sup>13</sup> such as the oscillating reactions of the Belousov-Zhabotinski type, may be of interest to kineticists looking for an unusual and arresting undergraduate laboratory experiment. On the whole, they are less suitable for demonstration to larger audiences due to their smaller scale.

### A Chemiluminescent Ammonia Fountain

Although most chemiluminescent reactions are attractive when demonstrated in simple flasks on the bench, extending the display with movement and the use of "classical" chemistry apparatus such as glass tubing provides a sense of occasion. One such demonstration is obtained by combining the ammonia fountain with luminol chemiluminescence.<sup>1,14</sup> It is based on the partial vacuum created by the introduction of water into a very large flask of ammonia vapor, the vacuum so formed then sucking in the two solutions, A and B, of the classical luminol reaction described earlier.

The apparatus consists of a round flask, preferably of 2.0-liter size, although a 1-liter flask can be used, with reduction of the volumes of solutions A and B to 500 ml (see Fig. 1; it is assumed that proper support and clamping of the flask are provided). This is fitted with a rubber stopper (this must fit well), bored to accept 6-mm glass tubing, and a length of glass tubing pushed through to within about 5 cm of the wall of the flask. A long syringe needle is inserted alongside the tubing (best done first). A short length of Tygon tubing is placed over the end of the glass, and the flask is filled with ammonia gas from a lecture bottle.<sup>15</sup> Keep the flow rate low so as not to overload the venting from the needle. Quickly attach the flask to the rest of the apparatus (see Fig. 1) and connect a syringe containing 10

<sup>12</sup> P. Jones, J. E. Frew, and N. Scowen, *J. Chem. Educ.* **64**, 70 (1987).

<sup>13</sup> H. Brandl, S. Albrecht, and T. Zimmermann, in "Chemiluminescence and Bioluminescence: Molecular Reporting with Photons" (J. W. Hastings, L. J. Kricka, and P. E. Stanley, eds.), p. 196. J. Wiley, Chichester, 1996.

<sup>14</sup> N. C. Thomas and J. H. Dreisbach, *J. Chem. Educ.* **67**, 339 (1990).

<sup>15</sup> The ammonia can also be produced by heating 10 g of ammonium chloride with 10 g of calcium hydroxide in 10 ml of water according to B. Z. Shakhashiri, "Chemical Demonstrations: A Handbook for Teachers of Chemistry," Vol. 2, p. 202.

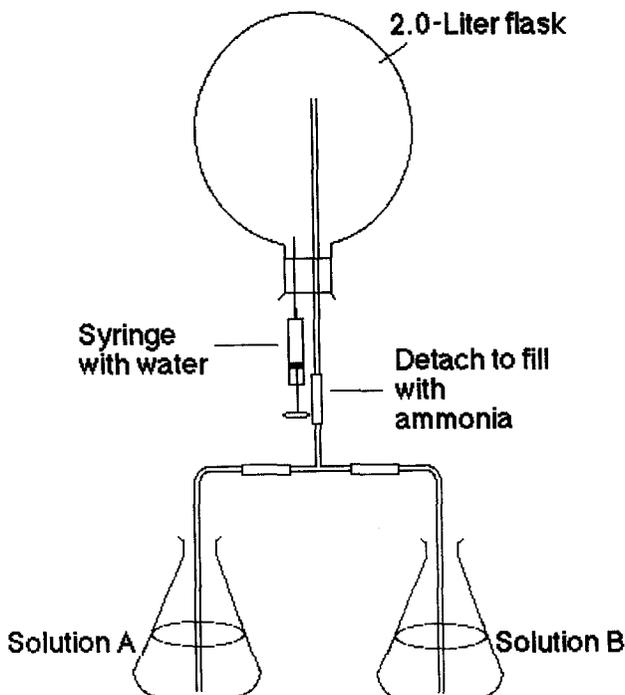


FIG. 1. Apparatus for the chemiluminescent ammonia fountain.

ml water to the needle. Leave an air bubble to avoid premature injection of the water during connection. It is a good idea to have a few trial runs to see how long the setup remains viable as the ammonia may slowly dissolve or leak. Wrapping the mouth of the flask in Parafilm will increase security. Inject the water in total darkness. A fountain of blue light starts up and should run for 1–2 min. This certainly requires practice. Be sure to dry the flask between runs.

#### *Additional Effects*

Add 10 mg of dry fluorescein or rhodamine to the large empty flask before setting up for a multicolor effect.

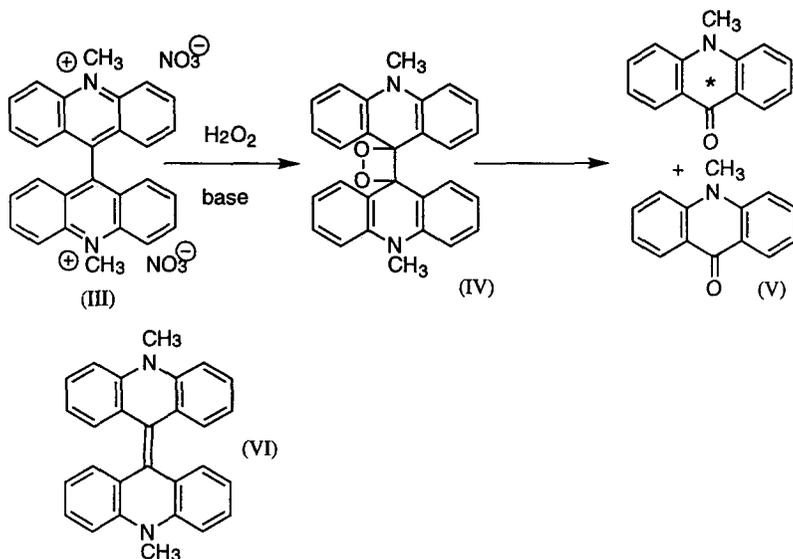
#### **Lucigenin**

Like luminol, the chemiluminescence of this compound (10,10'-dimethyl-9,9'-biacridinium dinitrate) was examined in the 1930s, and it

is equally available from commercial sources.<sup>16</sup> Oxidation by hydrogen peroxide in alkaline aqueous ethanol results in a blue-green light of moderate intensity, usually lasting about 15 min depending on conditions. The reaction is not a clean one, and the green light derives from energy transfer to fluorescent by-products. This confuses the investigation of the mechanism,<sup>17</sup> but is of no consequence for the demonstration. The complexity of the reaction is actually an advantage in this context in that changes in color by the addition of fluorescein (yellow) and rhodamine (red) are intensified by the efficient energy transfer from the by-products.

The intensity of light emission is lower than that from the aqueous luminol reaction, but it is longer lasting, and the yellow and red light from energy transfer is more striking. These attributes make it particularly suitable for chemiluminescent "sign writing," although one can mount an effective static demonstration if desired.

### Mechanism



The mechanism is considerably more complex than that shown here, and the literature<sup>17</sup> should be consulted by the specialist. To explain the demonstration, the outline is sufficient. The addition of hydrogen peroxide to the ring system gives a dioxetane [which incidentally can be synthesized

<sup>16</sup> Aldrich Chemical Co., bis-*N*-methylacridinium nitrate B4920-3.

<sup>17</sup> R. Maskiewicz, D. Sogah, and T. C. Bruice, *J. Am. Chem. Soc.* **101**, 5347 (1979); (b) R. Maskiewicz, D. Sogah, and T. C. Bruice, *J. Am. Chem. Soc.* **101**, 5355 (1979).

by the addition of singlet oxygen to biacridylidene(VI)]. This decomposes to give one *N*-methylacridone(V) molecule in the excited state and one in the ground state. There is insufficient energy to populate both. The biacridylidene(VI), among other by-products of the lucigenin reaction, accepts energy from the excited *N*-methylacridone (NMA)(V) to give the overall greenish light observed. Compare this color with the clean blue emission from NMA itself in the acridinium ester experiment.

### “Cold Light”

For over 20 years, the author has started his lecture demonstrations, especially those for lay audiences, with what amounts to a chemical neon sign, with the words “cold light” fashioned from 1-cm glass tubing. This older term for chemiluminescence emphasizes the intriguing central feature of the phenomenon.

### Materials

Although lucigenin chemiluminescence can be catalyzed by solutions of sodium hydroxide, the by-products generate a dark solution and milky precipitate. A much cleaner result is obtained as follows.

*Solution A.* Make up a *saturated* solution of lucigenin by shaking an excess (about 1 gr) in 1 liter of ethanol (any grade). The excess solution can be stored for many months.

*Solution B.* 100 ml of ammonia (0.880, 15 *M*) add ethanol to make 1 liter. Solutions A and B are used in equal proportions. To the selected portion of solution A is added 30% hydrogen peroxide in the ratio of 2 ml per liter of A. This is stable enough to be made up several hours before the demonstration. The same volume of solution B is then added to give light. Lesser volumes of B can also be used. Two further solutions are prepared. Fluorescein (500 mg) is dissolved in 2 *M* NaOH (100 ml), and rhodamine (200 mg) is dissolved in ethanol (100 ml).

### Apparatus

Many alternatives to the apparatus to be described can be devised, but the basic design used by the author can serve as a model. To a sheet of blockboard or plywood about 1 cm thick and about 60 cm square are screwed two “wings” 60 × 30 cm. Two pieces of laboratory scaffolding long enough to bridge the approximately 58-cm gap between the wings are each furnished with two right angle bosses, with each one placed 15 cm from each end. The two lengths of scaffolding are attached across the rear of these wings about 15 cm from the top and bottom of the wings, acting

as braces. Two long pieces of the scaffolding are threaded through the bosses, and rings supporting two 500-ml dropping funnels are placed about 30 cm above the top of the wood. The ends of the dropping funnels just enter a large filter funnel clamped between and below them. The author was fortunate in having the services of an expert glass blower, Mr. Kenneth Pyke of Sussex University, who constructed the words "cold light" in continuous script in 1-cm-wide glass tubing. The entry to the "c" emerged from the back of the 60-cm square front, and the exit from the "t" went into the back of the apparatus to take a plastic or rubber tube, leading to a container for the effluent. A glass stop cock placed here allows one to stop the display at the best view of the colors, if desired, but remember to close off the supply from the two funnels. The lettering is supported at intervals by twists of copper wire pushed through two small holes on either side of the tubing. A plastic tube leads from the filter funnel to the entry to the "c" in the back.

The dropping funnels are almost filled with the solutions A and B (including the hydrogen peroxide addition) and the flow is adjusted to fill the lettering. The front of the apparatus should be covered in a black cloth, which is removed as soon as the taps are opened, all in total darkness. (Hint: use an earlier display such as the luminol-DMSO flask as a "torch" to see what you are doing, meanwhile complaining about the power cut or outage). Small quantities (ca. 5 ml) of the fluorescer solutions are added, one after the other to the filter funnel, from time to time, creating a triple color chemiluminescent sign. If a good glass blower cannot be found, it is possible to make the sign from Tygon tubing. In this case the wire twists have to be fairly frequent, and a better effect is obtained by blacking out the interconnecting nonscript portions of the text by black adhesive tape.

A similar device using luminol has been described,<sup>18</sup> but the author has found that lucigenin (equally available) gives a better result. Of course, a simpler, but still effective display can be constructed using a long spiral of Tygon tubing, running from the filter funnel to a large beaker, as before. The addition of the concentrated fluorescer solutions during the experiment is also effective in this method of presentation.

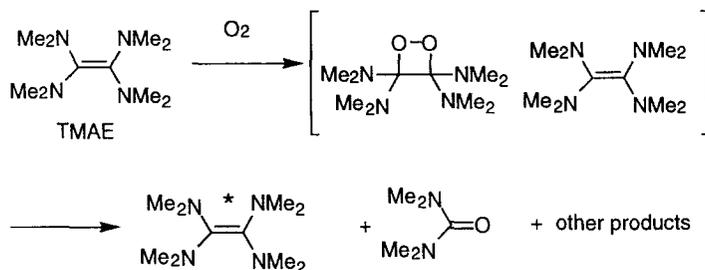
### TMAE [tetrakis(dimethylamino)ethylene]

The abbreviation for tetrakis(dimethylamino)ethylene<sup>19</sup> is usually pronounced "Tammy" and is regarded with affection by demonstrators of chemiluminescence, as one only needs to take the top off the readily purchased bottle to have this spontaneously chemiluminescent material react

<sup>18</sup> D. B. Phillips, *J. Chem. Educ.* **70**, 773 (1993).

<sup>19</sup> Aldrich Chemical Co., tetrakis(dimethylamino)ethylene 23,423-0

with oxygen to give an unearthly green glow! A moderately interesting demonstration is obtained by pouring about 10 ml into a large spherical flask and rotating the flask so that the liquid coating glows. (Keep the flask stoppered. There will be enough oxygen, and TMAE smells powerfully of dimethylamine.) TMAE is an extremely electron-rich olefin with a very low ionization potential, causing it to react spontaneously with oxygen. The involvement of the dioxetane shown has not been fully substantiated, but is very likely. The strong electron-donating properties of TMAE allow the formation of a complex between it and the dioxetane. It is presumed that the charge donation catalyzes the decomposition of the dioxetane, with excitation of the fluorescent TMAE. Thus the light you see is from the fluorescence of the starting material.



However, there really is only one way to demonstrate TMAE chemiluminescence, and that is to write a luminescent message on paper, on a firm backing, facing the audience, in the total darkness of the lecture room. Among well-educated audiences, "Fiat Lux" goes down well. A truly magic marker can be made easily by taking a 5- or 10-ml glass vial with a screw cap and narrow neck and *very tightly* wedging a rolled wick in the neck. The wick should project about 1 mm. Everything depends on the skill with which this is done and on the selection of the paper or preferably cloth material for the wick. Several materials are suitable, but varying local availability precludes a specific recommendation. Some TMAE is then injected with a syringe through the wick and the cap is screwed on to provide a most effective chemiluminescent pen. Keep the wick end lowest while writing. If the cap fitting is good, and the wick well made, the pen can be stored and reused dozens of times. Note that only glass vials and polypropylene or phenol-formaldehyde closures should be used, as TMAE will dissolve plastics such as polystyrene.

### Acridinium Esters

These are available commercially, but are extremely and unreasonably expensive for demonstrations. Their synthesis is easy, and because they

are used extensively in clinical diagnostics, being detectable down to the attomole range with the simplest chemistry, their demonstration helps to answer<sup>20</sup> a frequently asked question: What use is chemiluminescence? The light is an attractive clear royal blue and can be made to flash on a very short time scale, unlike many other demonstrations. The effect can be startlingly bright.

## Synthesis

### Safety

Acridine, as its name implies, can irritate the skin and mucous membranes. Potassium cyanide is highly toxic. Dimethyl sulfate, like all alkylating agents, is potentially carcinogenic.

The least expensive route starts with acridine, but a reasonable compromise is to purchase acridine-9-carboxylic acid. The last step, methylation, has been considered a problem in that the methylating agents are toxic. However, the involatile dimethyl sulfate is handled easily and is effective.

*Acridine-9-carboxylic acid.* Acridine (25 g, 0.14 mol) is dissolved in ethanol (110 ml) and the solution is added to glacial acetic acid (8 ml). A solution of KCN (12 g, 0.31 mol) in 20 ml water is added and the mixture is stirred under reflux for 1 hr. After cooling, the precipitate is filtered off and washed with 2 M NaOH solution and water. It is dissolved in chloroform and dried over anhydrous  $Mg_2SO_4$ . Evaporation of the filtrate to dryness and recrystallization from *n*-propanol yields acridine-9-nitrile (19.6 g, 70%) (mp 185–186°, ir 2222  $cm^{-1}$ , CN).

The acridine-9-nitrile (15 g, 70 mmol) is added to concentrated sulfuric acid (120 ml) and heated on a steam bath for 2.5 hr. It is cooled to 0° in an iced water bath and sodium nitrite (55 g, 0.8 mol) is added slowly. It is important to allow time for each portion of sodium nitrite to react as otherwise there will be excessive frothing during the next stage. Brown fumes are evolved. The mixture is then heated carefully until no more gas is given off. It is then stirred at 100° for 2 hr, cooled, and poured slowly into iced water to precipitate the yellow product. This is filtered off, washed with water, and sucked dry. The product is then dissolved in the minimum of 2 M NaOH solution and filtered through a sintered funnel. The deep red filtrate solution is treated with concentrated HCl until the

<sup>20</sup> A. K. Campbell, "Chemiluminescence, Principles and Application in Biology and Medicine." Horwood/VCH, Chichester, 1988.

yellow precipitate has permanently reformed. This is filtered off, washed with water, and sucked dry. It is dried further at 55° under reduced pressure for 24 hr (yield 14.7 g, 89%) (ir 1650  $\text{cm}^{-1}$ , acid C=O).

*Acridine-9-carbonyl chloride.* Acridine-9-carboxylic acid (10 g, 41 mmol) is refluxed in a large excess of redistilled thionyl chloride under dry conditions until solution is complete (ca. 3 hr). The reagent is evaporated off (any residue of thionyl chloride being removed by coevaporation with dry benzene) to give a yellow powder that is used without further purification (ir 1780  $\text{cm}^{-1}$ , Cl—C=O).

*Phenyl acridine-9-carboxylate.* Acridine-9-carbonyl chloride (about 10 g, from the previous experiment) is stirred in dry pyridine, forming a brown suspension after a few minutes. Phenol (4 g, about 10% molar excess) is dissolved in a few milliliters of pyridine and added to the suspension. The reaction mixture is stirred for 8–12 hr at room temperature and is then poured into a stirred mixture of ice and concentrated hydrochloric acid to precipitate the product. This is filtered off, washed with water, and sucked dry. Recrystallization gives the pure product in 85% yield. Recrystallization from toluene gave beige needles, mp 186–188°. Nmr  $\delta$  7.3–8.42 complex aromatic pattern, ir 1740  $\text{cm}^{-1}$ .

*Phenyl 10-methylacridinium-9-carboxylate methosulfate.* Phenylacridine-9-carboxylate (500 mg, 1.7 mmol) is dissolved in 40 ml dry toluene. Dimethyl sulfate (4 ml, 42 mmol) is added and, after stirring at 100° for 5 hr, the cooled precipitated salt is filtered and washed with toluene to dissolve excess dimethyl sulfate. The solid is then washed with diethyl ether and allowed to suck dry. The product is a bright yellow powder (635 mg, 89%). Recrystallization from ethanol affords a pure sample.

A reproducible melting point could not be obtained. Acridinium salts often show this behavior. Nmr ( $d_6$ -DMSO)  $\delta$  4.97 (3H, s; N—CH<sub>3</sub>).  $\delta$  7.42–9.07 (12H, m; aromatic H's) ir 1608  $\text{cm}^{-1}$  (aromatic C=C), 1760  $\text{cm}^{-1}$  ester C=O ms 315 (M<sup>+</sup> + H), 299 (M<sup>+</sup> + H—CH<sub>3</sub>), 178 (206-CO), 206 (299-C<sub>6</sub>H<sub>5</sub>).

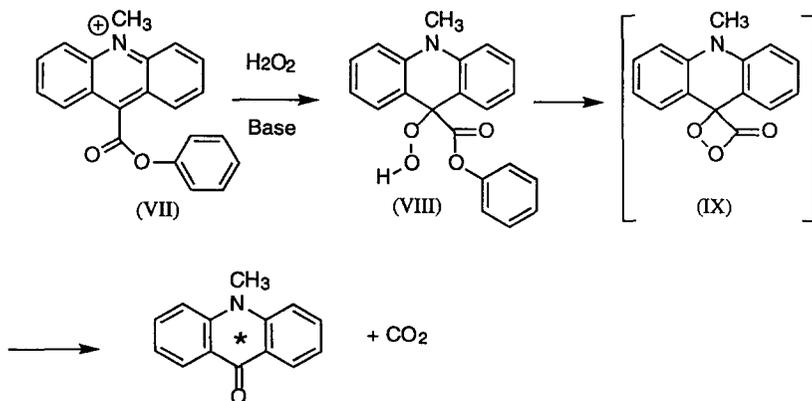
### *Demonstrations*

1. Advantage can be taken of the rapid rate of reaction by dissolving 50–100 mg of acridinium phenyl ester(VII) in 100 ml of 95% ethanol in a 250-ml conical flask and adding 100  $\mu\text{l}$  of 30% hydrogen peroxide or its more dilute equivalent. The slow, regular addition of drops of 5% NaOH from a burette while lecturing or performing other tasks in near darkness creates a worthwhile effect. The reaction is almost independent of the quantities given and it is an extremely easy demonstration to set up. The burette can be replaced by a pipette with a teat for a brief demonstration.

2. This is also an easy experiment and like the previous one, can be arranged very quickly with guaranteed success. The author uses a 1000-ml measuring cylinder, but any long glass tube container will do. Dissolve about 1 g of NaOH in 100 ml distilled or deionized water in the cylinder by swirling and fill to the top mark with 95% ethanol. Add about 0.5 ml of 30% hydrogen peroxide (or its equivalent). Mix thoroughly by inversion. The cylinder can be prepared ahead of time. In the dark, add, by gentle tapping from a vial, a stream of fine crystals of acridinium 9-phenyl ester (20–50 mg is plenty) to create a drift of scintillations. After a suitable wait for appreciation, invert the whole cylinder, covered by your hand, to give a remarkably bright display. This experiment is very tolerant of quantities, and estimates are acceptable. The crystals should be as fine as possible, but should *not* be powdered, as they must descend by gravity, yet be small enough to dissolve readily. A fast recrystallization with cooling and shaking usually does the job. Do, however, try it out first. For reasons not yet examined, energy transfer to other fluorescers is poor.

### Mechanism

Hydrogen peroxide is more nucleophilic than hydroxyl ion and the peroxide(VIII) is formed in about 90% yield. The dioxetanone(IX) is only a transient and decomposes in a fast step to excited *N*-methylacridone. The fluorescence of this is blue (442 nm). Its formation is made possible by the acidic phenol acting as a good leaving group. Many other leaving groups with similar properties have been used. It is interesting (and almost certainly significant) that the same “rules” apply to the oxalate esters.



### Acridan 9-phenyl Carboxylate

The light from the reaction of this compounds has the same color as that from the acridinium ester, but the mechanism is identical in principle to that for the reaction of firefly luciferin and coelenterazine. These two compounds give beautiful chemiluminescent reactions, but are very difficult to synthesize, so that the acridan becomes a good substitute. The simplicity and power of the demonstration, and the exact correspondence with the light-emitting step of the biological reactions, make it a good, easily transported, single demonstration for any lecture on bioluminescence, where a full-scale demonstration lecture is inappropriate.

#### *Demonstration*

Extremely small quantities give a very bright light and demonstrate the simplicity underlying the biochemical reactions in that removal of a proton by a strong base results in a spontaneous reaction with atmospheric oxygen to give the light. Cover the bottom of a 100-ml conical flask with a single layer of KOH pellets and add about 20 ml of DMSO. This mixture is stable indefinitely if stoppered tightly. A few specks of the acridan ester added from a spatula, followed by shaking, give an instantaneous bright light. The experiment can of course be scaled up to resemble the luminol reaction in DMSO described already, but with a much brighter and bluer light.

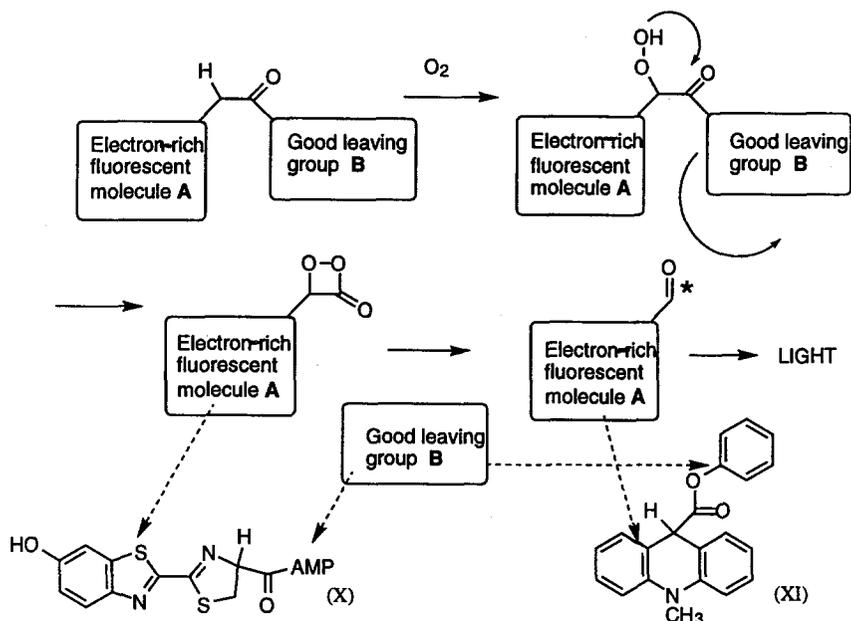
#### *Synthesis*

*Phenyl 10-methylacridan-9-carboxylate.* Phenyl acridinium-9-carboxylate (1 g) is refluxed with zinc dust (0.3 g) in an acetic acid solution (10 ml) until the intermediate purple color vanishes. Any unreacted zinc dust is removed by filtration, and the product is precipitated by the addition of water. If the precipitate is difficult to filter, extraction with ether followed by drying over  $Mg_2SO_4$  followed by evaporation of the solvent gives good recovery. Recrystallization from ethanol gives white needles, mp 116–118°, in 60% yield. Nmr  $\delta$  3.42 (3H, s;  $NCH_3$ ); 5.17(1H, s; C9-H). 6.84–7.40 (13H, m; aromatic H's).

#### Connection between Chemiluminescence and Bioluminescence

The *acridan* phenyl ester reacts to form the dioxetanone, but by an autoxidation mechanism, as it is reduced compared to an acridinium ester. This is a very general mechanism and, in terms of the light-generating step, identical in principle to the mechanism pertaining in the firefly and those

marine organisms using coelenterazine as their luciferins. The repetition of the following general reaction scheme can be seen in the reactions of both firefly luciferin(X) and the acridan(XI).



### Active Oxalate Esters<sup>21</sup>

These compounds were developed by a team at American Cyanamid, led by Dr. Michael Rauhut,<sup>22</sup> as the most effective exploitation of chemiluminescence as a source of illumination. He had calculated that with a fully efficient reaction (quantum yield = 1.0) and a highly soluble compound to give a high power density and using 1 liter of a 5.0 M solution, a light source equivalent to a 40-W incandescent bulb burning for up to 2 weeks could be devised. Devices based on this system are now familiar worldwide, mainly in the form of "light sticks" of various sorts, justifying this suggestion, and standing as a tribute to the detailed development work carried out by his team. The remarkably high efficiency ( $\phi_{es} = 60\%$ ) of the basic reaction provides really effective illumination with a much longer lifetime than

<sup>21</sup> A. G. Mohan, in "Chemi- and Bioluminescence" (J. G. Burr, ed.), p. 245. Dekker, New York, 1985.

<sup>22</sup> M. M. Rauhut, *Acc. Chem. Res.* **2**, 80 (1969).

any other reaction. Although the refinements introduced by American Cyanamid are of necessity missing from the more convenient approach described here, this is still an impressive display. The principal reactant, bis(2,4,6-trichlorophenyl)oxalate, can be purchased, but its synthesis is very straightforward and desirable if it is to be used on any significant scale.

## Preparation of Chemiluminescent Oxalate Esters

### *Safety*

Oxalyl chloride is volatile and toxic. Use a fume hood until it is all added to the flask. Nitrobenzene is an unpleasant toxic solvent, absorbable through the skin. Avoid using (see below for an alternative) unless the operators are skilled. The phenols are corrosive, and dinitrophenol stains the skin. Use gloves. Benzene is potentially carcinogenic.

### *Synthesis*<sup>23</sup>

*Bis(2,4-dinitrophenyl)oxalate (DNPO)*. 2,4-Dinitrophenol (9 g) is placed in a three-necked flask with toluene<sup>24</sup> (150 ml) and the phenol is dried by azeotropic distillation (usually requires the removal of 30–50 ml). The solution is cooled in an ice bath to 10° after adding a dropping funnel with drying tube to the flask. Dry triethylamine (6.5 ml) is added to the mixture in the flask, and oxalyl chloride (4.5 ml) in dry toluene (50 ml) is added via the dropping funnel over 20 min. The flask is stirred by a magnetic stirrer during this operation. Remove the ice bath and stir at room temperature for 2 hr. A thick suspension forms during these operations. Concentrate (Buchi apparatus or other means of removal of solvent under reduced pressure) this suspension to a still filterable mass, and filter under vacuum. Chloroform (see next step) can be used to effect the transfer of residues to the funnel. Wash, on the filter, with a total of 100 ml of chloroform, in portions, to remove triethylamine hydrochloride. This material is suitable for demonstrations, but residual triethylamine hydrochloride is an inhibitor of the reaction if present in quantity. For the very best material, recrystallization from ethyl acetate (the ester is not very soluble) or nitrobenzene can be

<sup>23</sup> M. M. Rauhut, J. J. Bollyky, G. B. Roberts, M. Loy, H. Whitman, and A. V. Iannotta, *J. Am. Chem. Soc.* **89**, 6515, (1967). A. G. Mohan and N. J. Turro, *J. Chem. Educ.* **51**, 528 (1974).

<sup>24</sup> Benzene is much more convenient, but there are restrictions on its use in undergraduate laboratories.

carried out. The dinitrophenyloxalate is tedious to recrystallize, for the average demonstration, use crude. The yield is about 5 g (50%); mp 191–195°. Store in a desiccator.

*Bis-2,4,6-trichlorophenyloxalate (TCPO)*. Use the stoichiometric amount of trichlorophenol in the experiment just described, but this time, make sure that the transfer to the funnel can be made without chloroform, as the ester is soluble in this solvent. Wash rapidly with water and suck dry. Transfer to a freshly charged vacuum desiccator and dry thoroughly. Surprisingly, this easily hydrolyzed compound can be used directly for the demonstration after the water wash, but for storage it must be very dry. The yield is about 7 g (60%).

The trichlorophenyloxalate is recrystallized easily from toluene, with excellent results. This is the ester of choice for demonstrations, and recrystallization is worthwhile in this case. The mp is 192–194°.

### *Demonstrations*

Small amounts of light are detectable in the absence of an added fluorescer, probably as a result of energy transfer to fluorescent impurities. However, on the addition of one of a large number of mainly aromatic hydrocarbon-based fluorescers, bright light of any desired color is obtained. The fluorescers can even be mixed to give white light! Make up three chemiluminescing solutions blue, yellow, and green (see later). These can be mixed to achieve a perfect white light without difficulty, simply judging by eye.

Several requirements of the very efficient commercial system have been abandoned in the interests of safety and convenience, but the difference is barely noticeable on the time scale of a lecture demonstration. The changes mainly affect lifetime, and there is clearly no virtue in a display lasting 13 hr during a lecture! The recommended solvents are dialkyl phthalates, and although other solvents do work, the increase in efficiency in the phthalates is so great as to make them essential. In addition, they are among the safest, although it is essential to dispose of the spent solvent in properly designated waste disposal containers as they should not be allowed to contaminate water supplies.

*Solution A.* Bis(2,4,6-trichlorophenyl) oxalate (500 mg) and 9,10-diphenylanthracene (50 mg) are dissolved in diethyl (or dialkyl) phthalate (100 ml).

*Solution B.* Add 20 ml of *tert*-butanol to 80 ml diethyl phthalate and add 1 ml of 30% hydrogen peroxide. Add sodium salicylate (10 mg) and mix.

Mix solutions A and B. Both separate solutions are stable for about

12 hr and even longer if moisture is excluded from A. Blue light appears and reaches a maximum intensity in about 5 min. The light should last for at least 30 min at a useful intensity, but if this is not attained, simply increase the concentration of the oxalate (undissolved oxalate in the flask will act as a reservoir, with shaking from time to time). Blue is the weakest color for two reasons. The human eye is more sensitive in the green and yellow regions of the spectrum, and the catalytic effect of fluorescers with lower ionization potentials is less. However, the light is still impressive.

Note that water is a competitor with hydrogen peroxide for the oxalate, and the hydrolysis of the oxalate destroys the light. The commercial devices use 98% peroxide, a very dangerous and corrosive material in its undiluted state and difficult to obtain. This, and the rigorous exclusion of water generally, together with a much more soluble ester, explains the much better results from the light sticks. Professional chemists can handle it safely, however, and demonstrations have been described using it.<sup>23</sup> The original literature<sup>22,23</sup> can also be consulted for procedures and explanation. Note that it is present in a completely safe, highly diluted form in *tert*-butanol in the inner glass tubes of the light sticks.

#### *Alternative Procedure*

A simpler procedure for use when time is short, or when portability is required, involves adding 1 ml of 30% hydrogen peroxide to 100 ml of the phthalate solvent in a glass container and adding 100 mg of the oxalate which has been premixed in a mortar with 10 mg sodium salicylate and 20 mg of the fluorescer. Vigorous shaking gives strong light that is a little slow to develop to its maximum intensity. Vials of the solid mixture can be prepared ahead of time, and although the mixture does deteriorate, it can be kept for several months in a desiccator.

#### Other Fluorescers (Brighter Light)

Most of the classical fluorescers such as fluorescein and rhodamine are poor sensitizers of this reaction, with best results undoubtedly being obtained with the polynuclear aromatic hydrocarbons. The brightest (green) light is obtained using 9,10-bis(phenylethynyl)anthracene. Rubrene gives a very bright yellow orange light (it is not very soluble, and best results are obtained by predissolving as much as possible in the phthalate solvent). It is not used for very long-lived displays, as it photobleaches, and is degraded by the hydrogen peroxide. However, it is remarkably effective for lecture demonstrations, where this disadvantage can be ignored. The very

stable 1,8-dichloro-9,10-bis(phenylethynyl)anthracene gives a clear yellow light. The efficient emission of red light is much harder to obtain, and in some of the commercial light sticks, the plastic outer tube is itself red fluorescent and acts as a filter for the yellow-green light actually produced by the reaction. Rhodamine will give a relatively short-lived, but strong, red light, but if you are going to use this, the oxalate should be bis(2,4-dinitrophenyl) oxalate and sodium salicylate or any other basic catalyst should not be used. Violanthrone gives weak red light, with solubility being a problem, and 5,12-bis(phenylethynyl)naphthacene (see the discussion on luminol effects) is preferable even if the light does not have the blood red character of rhodamine.

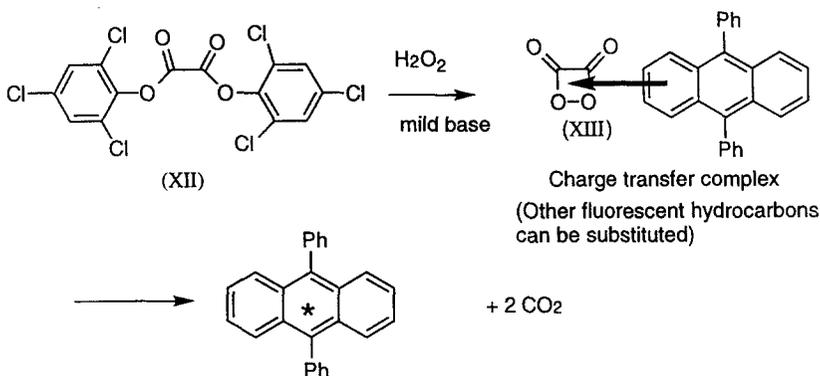
### Magic Light

All of the descriptions of the demonstrations of chemiluminescence are open to improvement and innovation by the user, and as an indication of the possibilities, the author suggests the following little bit of magic. In a mortar and pestle grind three *separate* mixtures, each containing 50 mg of bis(2,4,6-trichlorophenyl) oxalate(XII), 10 mg of sodium salicylate, and 10 mg of, respectively diphenylanthracene, bis(phenylethynyl)anthracene, and rubrene. You can use any more convenient multiple of these quantities. Transfer the material, using portions of about 50 mg of each, if more was prepared, to three separate 10-ml conical flasks. Add about 5 ml of *dry* acetone to each (ignore the undissolved material). Take a good-quality paper napkin or serviette and, using a Pasteur pipette, cover the serviette in random splotches or spot a pattern, using all three colors, and covering all the paper. Fold very tightly, when dry, and place in the bottom of a small opaque glass or ceramic container. During the demonstration, display a clean serviette and a flask of water-white solution B (the phthalate/butanol/hydrogen peroxide mixture; see general oxalate demonstration described earlier). Claim the total absence of ingredients. By sleight of hand, "lose" the clean serviette in the container (or up your sleeve if you really are a magician), pour on enough solution B to just wet the paper, and unfold the Jacob's coat of many colors, switching off the house lights at the same time. The last might require an assistant.

### Mechanism

Details of the mechanism are still under investigation, and several peroxy esters are implicated as the energy-rich intermediate. No direct evidence

for the dioxetanone(XIII) exists. However, provided that one is aware of the complexity, the simple scheme shown will satisfy most of the audience. The dioxetanone and other peroxy esters may form a charge transfer complex with the hydrocarbons and then decompose to give the excited state of the hydrocarbon directly within the complex. The reaction is the most efficient synthetic chemiluminescent example discovered to date, having a quantum yield of 60%, much greater than any bioluminescent reaction, except that of the firefly.



### Why Do These Reactions Produce Light?

In the expectation that these demonstrations will be presented by the general chemist or biochemist, the details of the mechanisms have been kept to a minimum. The question of light emission has not yet been answered to everyone's satisfaction. Sadly, the reactions that comprise the most convenient demonstrations are among the hardest to explain. This gives an unnecessarily gloomy picture, and it is certainly possible to provide some solid ground on which to stand. The acridan esters and, by extension, the acridinium esters react by the same mechanism as do firefly luciferin and coelenterazine. In its broad outline, this is a proven mechanism. The scheme given earlier for these compounds does not include the final step: that of the population of the excited state. This is more complicated, but can be addressed simply by saying that the four-membered ring has electronic properties that lead to an avoidance of the ground state. Provided there is enough energy in the decomposition of this ring, then an electronically excited state will be generated, and the electron-rich, aromatic portion of the molecule will ensure fluorescence from this state. The other luciferins (including the

bacterial system) cannot be described in the same terms, but progress is being made.

### Other Light-Emitting Reactions

Because one of the attractions of chemiluminescence is the unusual nature of the source of the light, it is interesting to compare it with other uncommon light sources. Easily visible examples include fluorescence, phosphorescence, electroluminescence, and triboluminescence. Most of these phenomena can be used to interrelate many basic photophysical and chemical concepts, as pointed out in the introduction. Each lecturer can be expected to bring their own preferred examples to illustrate, and the following is only an indication of the possibilities.

Fluorescence can be excited after the chemiluminescent reaction is over by shining a "black lamp"—a source of ultraviolet light—on the flasks. Phosphorescence can be demonstrated using the many adhesive stars and planets sold in most toy shops for use on the ceilings of children's rooms, with the same lamp. Keep them in the dark in a container until the demonstration. The "instantaneous" nature of the prompt fluorescence (excited state lifetime about  $10^{-8}$  secs) can be contrasted with the very much longer lived phosphorescence, lasting for several minutes, if not hours. This long life exposes the excited triplet state to quenching in fluid solution, whereas the short-lived fluorescent singlet state emits before it can be quenched. It is worth pointing out that triplet states are indeed formed in chemiluminescent reactions, but that the foregoing circumstances explain why emission from them is rarely visible. These considerations can lead to discussions of singlet and triplet states, kinetic (collision) theory, spin conservation, the spectral sensitivity of the human eye versus electronic detection, and many other topics. An additional demonstration of the influence of temperature on the kinetics of reactions is provided by the strong quenching effect of cooling on the intensity of the longer lived chemiluminescence demonstrations. A commonly asked question (even by mature scientists!) is whether the spent reaction can be reactivated. Comparison with the ashes of a fire and a reminder of the second law of thermodynamics and the concept of entropy are timely at this point!

Triboluminescence<sup>25</sup> (the emission of light from stressed solids) can also be demonstrated, but only from relatively weak point sources, so that it is not a suitable topic for large lecture rooms. However, questions are often

<sup>25</sup> R. Angelos, J. I. Zink, and G. E. Hardy, *J. Chem. Educ.* **56**, 413 (1979) and references cited; A. J. Walton, *Adv. Phys.* **26**, 887 (1977).

raised about it because it is much more common than is usually realized. A related example is the blue light observed when a piece of Scotch tape (Sellotape) is stripped from a surface in the dark, and although not triboluminescence by the strictest definition, the light is produced by a gas discharge in air (the emission is from excited  $N_2$  molecules), excited by the recombination of electrostatically produced positive and negative charges. A similar, but not identical, mechanism operates in triboluminescence proper. Apparently, almost half of all compounds are triboluminescent, but only a few have been investigated.

The earliest report is that by Sir Francis Bacon, who noticed the light produced on scraping loaf sugar. A modern, brighter form is that of the sweet or candy known as Lifesavers. If either a sugar cube or a (opaque, white) Lifesaver is crushed, a flash of light is seen. It is blue in the case of sugar and green from the candy. In the latter case, there is energy transfer from the excited nitrogen molecule to the fluorescent flavoring agent, methyl salicylate, or oil of wintergreen. Uranyl nitrate also shows quite bright green flashes when crystals are crushed, and again energy transfer to the very fluorescent uranyl ion in the solid is responsible. This may well be the most effective demonstration of triboluminescence and is relatively inexpensive, especially if the powder produced is recrystallized. *N*-Acetyl-anthranilic acid crystals<sup>26</sup> also show the phenomenon, with a similar mechanism. The last two compounds have crystals soft enough to be crushed between two substantial watch glasses or glass plates, making them easier to demonstrate to a small audience. There is more than one mechanism, but the commonest and best understood is that, under stress, the crystal acquires two opposite charges on opposite sides of an imminent fracture and, on separation of the surfaces, an electrical gas discharge occurs, exciting the nitrogen molecules of the air, with emission in the blue and ultraviolet regions of the spectrum. If the medium is fluorescent, energy transfer results in light of the expected color.

In electroluminescence, electrons are promoted to the conduction band of a phosphor, such as zinc sulfide, by alternating current, which then ionizes a fluorescent atom. Recombination of an electron with the atom forms the excited state, and the emission of light ensues. These phosphor screens are commonly used to backlight liquid crystal displays, and examples are easy to obtain for demonstration purposes. Note that the active displays used in more recent lap-top computers use p-n transistor or semi-conducting junction materials and are considerably more sophisticated. The principles are, however, the same.

<sup>26</sup> J. Erikson, *J. Chem. Educ.* **55**, 688 (1972).