Luminol and Cyalume: Syntheses of Chemiluminescent Substances

Prelab Exercise: Write a balanced equation for the reduction of nitrophthalhydrazide to aminophthalhydrazide using sodium hydrosulfite, which is oxidized to bisulfite.

Chemiluminescence is the process whereby light is produced by a chemical reaction with the evolution of little or no heat. The periodic flashes of the male firefly in quest of a mate and the glow of light seen in the wake of a boat, under a rotten log, or among the many organisms found at great depth in the ocean are examples of natural chemiluminescence. The interaction of luciferin from the firefly, the enzyme luciferase, adenosine triphosphate (ATP), and molecular oxygen is the most carefully studied of these reactions. In this experiment, both luminol (1) and Cyalume (13) can be synthesized.

Luminol (3-aminophthalhydrazide) and Cyalume are both oxidized with hydrogen peroxide, albeit by rather different mechanisms, to excited intermediates that can directly or indirectly emit light. Cyalumes, products of the American Cyanamid Company, are sold as toys in the form of light sticks, as emergency flares, and even as fishing lures or golf balls for playing at night! Luminol is not a commercial product.1

Luminol

Oxidation of luminol is attended with a striking emission of blue-green light. An alkaline solution of the compound is allowed to react with a mixture of hydrogen peroxide and potassium ferricyanide. The diion (2) is oxidized to the singlet excited state (two unpaired electrons of like spin) of the aminophthalate ion (3). This slowly undergoes intersystem crossing to the triplet excited state (two unpaired electrons of opposite spin) 4, which decays to the ground state ion (5) with the emission of one quantum of light (a photon) per molecule. (See Chapter 58 for a more detailed discussion of photochemistry.)

Luminol (1) is made by reduction of the nitro derivative (8) formed on thermal dehydration of a mixture of 3-nitrophthalic acid (6) and hydrazine (7).

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An earlier procedure for effecting the first step called for addition of hydrazine sulfate to an alkaline solution of the acid, evaporation to dryness, and baking the resulting mixture of the hydrazine salt and sodium sulfate at 165°C, and it required a total of 4.5 h for completion. Louis Fieser reduced this working time drastically by adding high-boiling triethylene glycol (bp 290°C) to an aqueous solution of the hydrazine salt, distilling the excess water, and raising the temperature to a point where dehydration to 8 is complete within a few minutes. Nitrophenolhydrazide (8) is insoluble in dilute acid but soluble in alkali, by virtue of enolization; it is conveniently reduced to luminol (1) by sodium hydrosulfitite (sodium dithionite) in alkaline solution. In dilute, weakly acidic or neutral solution luminol exists largely as the dipolar ion (9), which exhibits beautiful blue fluorescence.

An alkaline solution contains the doubly enolized anion (10) and displays particularly marked chemiluminescence when oxidized with a combination of hydrogen peroxide and potassium ferricyanide.

**Cyalume**

Another type of chemiluminescent substance is exemplified by the esters of oxalic acid that are commercial products marketed by the American Cyanamid Co. under the name Cyalume. They are the most efficient non-enzyme fluorescent substances known, with quantum efficiencies of up to 26%.

In this experiment, 2,4,6-trichlorophenol (11) reacts with the diacid chloride, oxalyl chloride (12), in the presence of triethylamine to give the desired oxalic acid ester (13) and the amine hydrochloride. A number of different oxalic acid esters will work in the luminescent reaction.

In the light-producing reaction, the ester undergoes nucleophilic attack by hydrogen peroxide to produce a peroxyxalic acid, which, by an intramolecular displacement reaction, generates a small intermediate molecule, perhaps the 1,2-dioxetanedione (14). This molecule transmits its energy to a fluor escer, such as 9,10-diphenylanthracene (15), through a charge-transfer complex that produces the excited singlet state of the fluor escer when it decomposes to two molecules of carbon dioxide. trans-9-(2-Phenylethyl)anthracene, synthesized by the Wittig reaction in Chapter 43, is also an excellent fluor escer. The observed color depends on the structure of the fluor escer.

The structure of the intermediate molecule, written here as 14, has not been established with certainty. The intermediate is known to be volatile, and carbon dioxide is a product of the reaction but carbon monoxide is not. Efforts to detect this intermediate by Fourier transform infrared, carbon NMR, and mass spectroscopy have all been unsuccessful.

The light-producing reaction of the oxalate esters differs from luminol in that the ester decomposes to a small molecule that transfers its energy to a different fluorescent molecule, while in luminol, the molecule itself is fluorescent and gives off light from the excited state. In addition, the luminol reaction is over in a few minutes, but the peroxyxalate reaction can last for a day or more.
2,4,6-Trichlorophenol
MW 197.45
mp 64–66°C

Oxalyl chloride
MW 126.93
bp 63–64°C
den 1.455

Triethylamine
MW 101.19
bp 89°C
den 0.726

\[
2\text{Cl} - \text{C} - \text{Cl} + \text{O} - \text{C} - \text{Cl} \quad + \quad \text{HN(CH}_2\text{CH}_3}_2 \quad \rightarrow \\
\text{O} - \text{C} - \text{O} - \text{C} - \text{O} + \text{H}_2\text{O}_2 \\
\text{Cl} - \text{C} - \text{Cl} + \text{Cl} \quad + \quad \text{HN(CH}_2\text{CH}_3}_2 \quad \rightarrow \\
\text{O} - \text{C} - \text{O} - \text{C} - \text{O} + \text{H}_2\text{O}_2 \\
\]

Bis(2,4,6-trichlorophenyl)oxalate
(A Cyalume)
MW 448.9
mp 162–163°C

Triethylamine hydrochloride

**MICROSCALE**

Hydrazine is a carcinogen. Handle with care. Wear gloves and carry out this experiment in the hood.

The two-step synthesis of a chemiluminescent substance can be completed in 25 min.

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**Chapter 65  Luminol and Cyalume**

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\text{O} - \text{C} - \text{O} + \text{NH}_2 \quad \rightarrow \quad \text{Charge transfer complex} \\
\]

\[
\text{1,2-Dioxetanedione (?) \quad 14} \\
\]

\[
\text{2 CO}_2 + 9,10\text{-Diphenylanthracene Single excited state} \\
\]

\[
\text{hv} + 9,10\text{-Diphenylanthracene Ground state} \\
\]

9,10-Diphenylanthracene
MW 330.43
mp 245–248°C

or

trans-9-(2-Phenylethenyl)anthracene
(from Chapter 43)

**Experiments**

1. **Synthesis of Luminol**

First heat a tube containing 3 mL of water on the steam bath. Then heat a mixture of 200 mg of 3-nitrophthalic acid and 0.4 mL of an 8% aqueous solution of hydrazine\(^2\) (Caution) in a 10 × 100 mm reaction tube over a hot sand bath until the solid is dissolved. Add 0.6 mL of triethylene glycol, and clamp the tube in a vertical position above the hot sand bath. Insert a boiling chip and a thermometer, and boil the solution vigorously to distill the excess water. Intermittently remove the thermometer and replace it with an aspirator tube to facilitate this. There will be a period during which the solution will boil at 110°C, and then over a 3- or 4-min period it will rise to 215°C. Lift the tube from the hot sand and by intermittent gentle heating maintain a temperature of 215 to 220°C for 2 min. Remove the tube, cool to about 100°C (crystals of the product often appear), add the 3 mL of hot water, cool the tube in cold water, and collect the light yellow granular nitro compound (8) by vacuum filtration on the Hirsch funnel.\(^3\) The dry weight should be about 140 mg. The nitro compound need not be dried and can be transferred at once, for reduction, to the uncleaned tube in which it was prepared. Add 1.0 mL of 3 M sodium hydroxide solution, stir with a rod, and to the resulting deep brown-red

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2. Dilute 3.12 g of the commercial 64% hydrazine solution to a volume of 25 mL.

3. The reason for adding hot water and then cooling rather than adding cold water is that the solid is then obtained in more easily filterable form.
solution add 0.6 g of fresh sodium hydrosulphite dihydrate (not sodium hydrogen sul fate or sodium bisulfit e). 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 0.4 mL of acetic acid, cool the tube in a beaker of cold water, and stir; collect the resulting precipitate of light-yellow lumino (1) by vacuum filtration on the Hirsch funnel. The filtrate on standing overnight usually deposits a further crop of lumino (20–40 mg).

Cleaning Up Combine the filtrate from the first and second reactions, dilute with a few milliliters of water, neutralize with sodium carbonate, add 3 mL of household bleach (5.25% sodium hypochlorite solution), and heat the mixture to 50°C for 1 h. This will oxidize any unreacted hydrazine and hydrosulphite. Dilute the mixture and flush it down the drain.

2. Synthesis of Lumino

First heat a flask containing 15 mL of water on the steam bath. Then heat a mixture of 1 g of 3-nitrophenolic acid and 2 mL of an 8% aqueous solution of hydrazine (Caution) in a 20 × 150 mm test tube over a thermowell until the solid is dissolved, add 3 mL of triethylene glycol, and clamp the tube in a vertical position in a hot sand bath. Insert a thermometer, a boiling chip, and an aspirator tube connected to an aspirator, and boil the solution vigorously to distill the excess water (110–130°C). Let the temperature rise rapidly until (3–4 min) it reaches 215°C. Remove the burner, note the time, and by intermittent gentle heating maintain a temperature of 215 to 220°C for 2 min. Remove the tube, cool to about 100°C (crystals of the product often appear), add 15 mL of hot water, cool under the tap, and collect the light yellow granular nitro compound (B). Dry weight 0.7 g.

The nitro compound need not be dried and can be transferred at once, for reduction, to the uncleared test tube in which it was prepared. Add 5 mL of 3 M sodium hydroxide solution, stir, and cool to room temperature, and the resulting deep brown-red solution add 3 g of fresh sodium hydrosulphite dihydrate. 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; collect the resulting precipitate of light yellow lumino (1). The filtrate on standing overnight usually deposits a further crop of lumino (0.1–0.2 g).

Cleaning Up Combine the filtrate from the first and second reactions, dilute with a few milliliters of water, neutralize with sodium carbonate, add 40 mL of household bleach (5.25% sodium hypochlorite solution), and heat the mixture to 50°C for 1 h. This will oxidize any unreacted hydrazine and hydrosulphite. Dilute the mixture and flush it down the drain.

3. The Light-Producing Reaction

This reaction can be run on a scale five times larger. Dissolve the first crop of moist lumino (dry weight about 40–60 mg) in 2 mL of 3 M sodium hydroxide solution and 18 mL of water; this is stock solution A. Prepare a second stock solution, B, by mixing 4 mL of 3% aqueous potassium ferricyanide, 4 mL of 3% hydrogen peroxide, and 32 mL of water. Now dilute 5 mL of stock solution A with 35 mL of water, and in a dark place, pour this solution and solution B simultaneously into an Erlenmeyer flask. Swirl the flask and, to increase the brilliance, gradually add further small quantities of alkanil and ferricyanide crystals.

Ultrasonic sound also can be used to promote this reaction. Prepare stock solutions A and B again but omit the hydrogen peroxide. Place the combined solutions in an ultrasonic cleaning bath or immerse an ultrasonic probe into the reaction mixture. Spots of light are seen where the ultrasonic vibrations produce hydroxyl radicals.

And the sanguinary-minded can mix solutions A and B, omitting the ferricyanide from solution B. Light can be generated by adding blood dropwise to the reaction mixture.

Cleaning Up Add 2 mL of 3 M hydrochloric acid, dilute the solution with water, and flush the mixture down the drain.

4. Synthesis of Bis(2,4,6-trichlorophenyl) Oxalate, a Cyalume

Dissolve 0.395 g of 2,4,6-trichlorophenol in 3 mL of toluene in a 5-mL round-bottomed flask equipped with a magnetic stirring bar. Add to this solution 0.28 mL (0.2 g) of triethylamine, cool the container in ice and add, in two portions, 0.10 mL (0.14 g) of oxalyl chloride. There will be an immediate voluminous precipitate of triethylamine hydrochloride. Add an air condenser to the flask and reflux the mixture gently, with stirring, for 30 min to complete the reaction. Cool the mixture well in ice and collect the solid on a Hirsch funnel. Complete the transfer of material with a small volume of hexane, which is also used to wash the solid on the filter.

Press the material as dry as possible on the filter and then suspend it in about 5 or 6 mL of water in a 10-mL Erlenmeyer flask. Stir the solid well with the water, using a spatula to break up any lumps, then cap the flask and shake it vigorously. In this process, the amine hydrochloride dissolves in water and leaves the product as a suspension. Empty the filtrate in the filter flask into the flammable waste container and then collect the solid product from the aqueous solution on the Hirsch funnel. Wash the solid well with water and press it as dry as possible on the filter. Spread the product out to dry, determine its weight,
and then recrystallize it from the minimum volume of boiling toluene. Allow the product to crystallize slowly and then cool the tube in ice. Collect the product on the Hirsch funnel (use hexane to complete the transfer of material) and wash the crystals with a small volume of hexane. Determine the weight and melting point of the dry material and calculate the percentage yield.

**Cleaning Up** Discard the aqueous filtrate down the drain and the organic filtrates in the flammable waste container.

5. **The Chemiluminescent Reaction**

Dissolve 50 mg (or less, if that is all you have) of bis(2,4,6-triphenyl) oxalate and about 3 mg of 9-(2-phenylethenyl)anthracene (Chapter 43) or 9,10-diphenylanthracene in 5 mL of diethyl phthalate by warming on the steam bath. In another container, shake 0.2 mL of 30% hydrogen peroxide (or, less satisfactory, 2 mL of 3% peroxide) with 5 mL of diethyl phthalate. Add the peroxide suspension dropwise to the solution of the oxalate ester and the fluorescer in a darkened room. Try cooling and warming the mixture after initial mixing and note the effect of temperature on the rate of a reaction. Also try adding one or two more mg of the fluorescer (9,10-diphenylanthracene is expensive) and more hydrogen peroxide.

**Cleaning Up** Dispose of the mixture in the halogenated waste container.