

THE CHEMILUMINESCENCE OF LUCIGENIN

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Abstract

The electrochemical behavior of lucigenin has been studied in both aqueous and non-aqueous systems. The initial reduction of lucigenin has been shown to proceed by a one-electron charge transfer step leading to the mono-cation radical of lucigenin. The charge transfer step is followed by a rapid disproportionation of the radical yielding dimethyl biacridene (DBA) as the end product. The rate constant of the following disproportionation step has been estimated as 8×10^3 l/M-sec. The oxidation of DBA has been shown to proceed via two, consecutive one-electron oxidations, occurring at the same potential, yielding lucigenin as the product.

The electrochemically generated chemiluminescence (ECL) of lucigenin has been studied in both aqueous and non-aqueous media. The non-aqueous ECL has been shown to arise from the reaction of superoxide with lucigenin. The spectral characteristics of the ECL, when compared with the fluorescence spectra and quantum efficiencies of N-methyl acridone (NMA), DBA and lucigenin allow identification of the emitting species. NMA is the primary emitter formed in the excited state by the reaction of superoxide with lucigenin. In several systems a second, longer wavelength component of emission was observed. This was the fluorescence of either DBA or lucigenin, depending on their respective concentrations and quantum efficiencies. Both DBA and lucigenin act as energy acceptors from excited NMA via singlet-singlet energy transfer.

Chemiluminescence has been observed in three non-aqueous solvents (DMSO, DMF and acetonitrile) from the reaction of potassium tert-butoxide with lucigenin and oxygen. Spectra of this chemiluminescence show the same gross behavior as those observed in the ECL studies. The same considerations as to extent of reaction, quantum efficiencies of acceptor fluorescence and energy transfer probabilities may be used to explain these spectra as were used for the ECL spectra. At high base and lucigenin concentrations a third yet unidentified product is formed.

A study has been made of the quenching of lucigenin's fluorescence by various anions, notably chloride. As in the case of amine quenching, a direct relationship between ionization potential of the quencher and its quenching efficiency is seen. Rate constants for the quenching are diffusion controlled and a charge-transfer mechanism is proposed. Photoreaction of lucigenin has been observed and studies have shown this process to occur from the first excited singlet state of lucigenin.

Thesis Supervisor: David M. Hercules
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"THERE IS NOTHING - ABSOLUTELY NOTHING - HALF SO MUCH WORTH
DOING AS SIMPLY MESSING ABOUT IN BOATS."

The Water Rat

from

"The Wind in the Willows"

by

Kenneth Grahame

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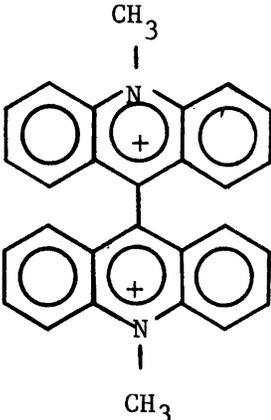
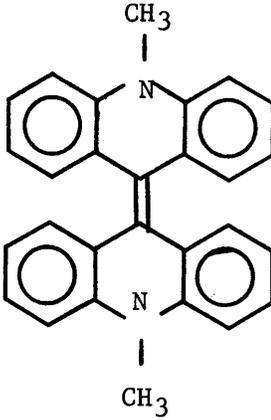
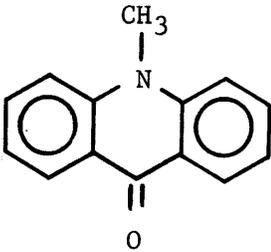
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Commonly Occurring Structures and their Abbreviations

<u>Structure</u>	<u>Name</u>	<u>Abbreviation</u>
	Lucigenin	L ⁺⁺
	Dimethyl biacridene	DBA
	N-methyl acridone	NMA
(CH ₃) ₂ SO	Dimethylsulfoxide	DMSO
CH ₃ CN	Acetonitrile	AN
(CH ₃) ₂ N-COH	Dimethyl Formamide	DMF
KOC(CH ₃) ₃	Potassium tert-butoxide	KOtBu

III Introduction

Chemiluminescence (CL), the production of light from a chemical reaction, is not an unusual phenomenon. Indeed, light emission, albiet low level, has been shown to occur in a seemingly endless number of reactions. The phenomenon is not new since CL was observed from the oxidation of Lophine in 1877 (1). Luminol (2) and lucigenin (3) are two of the best known examples of chemiluminescent reactions and have received a great deal of attention because of their intense emission. Most solution CL studied to date falls into one of four major categories: reactions involving molecular oxygen or peroxides (4), oxidation of anion radicals (5), alternating current electrolysis of aromatic hydrocarbons (6,7) and reduction of ruthenium chelates (8).

The chemiluminescence of lucigenin (dimethyl biacridinium ion) is a reaction involving peroxide, and was first reported in 1935 by Gleu and Petsch (3). They observed intense CL when lucigenin was treated with hydrogen peroxide in basic solution. It was also observed that the emission was either green or blue, depending on the conditions under which the reaction was run. Addition of alcohols, reducing agents or heating caused the emission to change from green to blue (3, 9, 10). N-methyl acridone, the major product of the reaction, was tentatively (10) and later conclusively (11) identified as the primary emitter in the blue CL reaction. Kautsky and Kaiser (9) attributed the green emission to lucigenin fluorescence excited by emission from N-methyl acridone. This was later shown not to be the case by Spruit-van der Berg (10). Until now the green emitter had not been identified.

Since the discovery of the lucigenin CL, numerous studies have been carried out concerning its mechanism (9-16). The subject has been reviewed by McCapra (17) and Gundermann(4). The reaction has been shown to be of a redox type (2, 11, 18) but the exact nature of the redox step has not yet been ascertained. The electrochemistry of lucigenin has been briefly treated by Totter (11) but an extensive analysis of its behavior has not been reported.

The electrochemical generation of lucigenin CL at a platinum electrode in basic solution was reported by Tammamushi and Akiyama (19). They observed light at the platinum cathode, along the path of hydrogen evolution. No further work has been reported concerning the electrochemically generated CL (ECL) of lucigenin.

Driscoll et. al. (20) have reported the observation of lucigenin CL in methanol by reaction with potassium t-butoxide. Observation of this CL in other non-aqueous solvents has not been reported.

The purpose of the present investigation was two-fold. First, it was evident that a detailed study of the electrochemical behaviour of lucigenin would be of interest as applied to redox nature of the CL reaction. The second objective was the production of lucigenin CL in non-aqueous media. It was hoped that, like luminol, the CL process in non-aqueous media would be simpler than that seen in water. The production of lucigenin CL by electrochemical means seemed to be the logical starting place for such studies.

IV. Experimental

A. Chemicals

Lucigenin (nitrate salt) was obtained from Columbia Organic Chemicals and was recrystallized twice from 1:1 methanol-ethanol before use.

N-methyl acridone was obtained as a sample synthesized in the MIT Organic Laboratories (21) and was recrystallized from ethanol until a constant melting point of 202° - 203° C was obtained.

Dimethyl biacridene (DBA) was prepared according to Decker and Petsch (22). 5 grams of lucigenin were dissolved in 75 ml of Glacial Acetic acid. 25 grams of zinc pellets were added and the solution was allowed to stand overnight. The DBA produced is insoluble in the reaction medium and the bulk precipitates out on the zinc surface. The zinc-DBA precipitate was washed thoroughly with water and dried in a vacuum oven at 100°. The DBA was then dissolved in chloroform and, after concentration in a rotary evaporator, recrystallized twice from chloroform. 2.5 grams of DBA were obtained from the recrystallization.

Tetrabutyl ammonium perchlorate was prepared from 1 M tetrabutyl ammonium hydroxide (Southwestern Analytical). 70% perchloric acid (G. Frederick Smith) was diluted 1:1 with water and was added dropwise with stirring to the hydroxide until the solution was acidic (pH ~2). The precipitated perchlorate was collected by filtration and washed several times with hot water to remove all traces of acid. The perchlorate was then recrystallized twice from 70% H₂O -30% methanol and dried in a vacuum oven at 100°.

Potassium t-butoxide was used as obtained from MSA Research Corporation. It was stored in a dry nitrogen atmosphere to ensure no re-

action with water.

Baker Analyzed Reagent 30% Hydrogen Peroxide was used as obtained.

All other organic and inorganic chemicals used were reagent grade or better.

B. Solvents.

Absolute ethanol (U.S. Industrial Chemicals Co.) was used as obtained.

Dimethyl Sulfoxide, Dimethyl Formamide (Matheson, Coleman and Bell, spectroscopic grade) and Acetonitrile (Eastman Chemicals, spectroscopic grade) were dried over molecular sieves before use.

All other solvents used were of spectroscopic grade when available or reagent grade or better.

C. Solutions.

Solutions of most reagents were made by weighing the reagent and dissolving it in the appropriate volume of solvent. Solutions of DBA were prepared by saturating the solvent with DBA and determining its concentration by its absorption at 420 nm (ϵ_{max} at 420 nm = 1.66×10^4). H_2O_2 solutions were prepared by appropriate dilution of 30% H_2O_2 . Solutions of HCl or NaOH were prepared by dilution of 1 M stock solutions of Accu-Lute reagent.

D. Apparatus.

1. Electrochemical instrumentation.

Potentiostat. A potentiostat constructed by D. W. Shive was used in the direct electrochemical studies. This instrument and its use are described in detail elsewhere (23).

Recorder. A Houston HR-97 X-Y recorder was used to record the current-voltage curves for scan rates up to 0.333 V/sec.

Wave Generator. A Wavetek triangular wave generator was used to drive the potentiostat for scan rates greater than 0.333 V/sec.

Oscillograms. Oscillograms were obtained by use of a Tektronix Model 536 oscilloscope equipped with a Type 53A plug-in unit. The traces were photographed on polaroid film using a Tektronix Model C-12 camera with projected graticule.

Current Integrator. A current integrator (24) was used in the coulometric measurements. This consisted of a Nexus SQ-3 operational amplifier connected to the current amplifier of the potentiostat through a 5 meg ohm resistor. A 10 μ F capacitor was in the feedback loop of the integrator. The output voltage of this network is a direct function of the coulombs passed and is equal to $5 \times 10^{-2} E_0$ (in Volts) coulombs. The output voltage of the current integrator was measured on a Hewlett Packard Model 344A digital volt meter.

2. Electrochemical cell.

A standard three electrode cell was used in all electrochemical measurements. The reference electrode was Ag/AgCl in 0.1 M aqueous KCl. The reference electrode was separated from the bulk of the solution in the cell with a porous Vycor plug. The counter electrode consisted of a 2 cm² platinum foil and was isolated from the test solution by a fine glass frit. The indicator electrode was a platinum sphere of 5.53×10^{-2} cm² area. 0.1 M tetrabutyl ammonium perchlorate was used as supporting electrolyte in non-aqueous solutions and 0.1 M KCl in aqueous media. For coulometric measurements a 2 cm² platinum foil working electrode was used. Provision for bubbling with N₂ was provided.

3. Spectroscopic Instrumentation.

Aminco-Bowman Spectrofluorometer. This instrument is described by Lytle (8) and was used without modification. It employs two Aminco grating monochromators (4-8401) mounted on an optical bench. The excitation monochromator is blazed for 300 nm while the emission monochromator is blazed for 500 nm. The sample chamber may be used for either fluorescence or chemiluminescence measurements. The detector is an E.M.I. 9558QA photomultiplier of S-20 response. This particular tube yielded a sensitivity of 300 amps/lumen. Spectra were recorded on a Hewlett Packard Model 7005B X-Y recorder.

Absolute SpectroFluorometer. For routine fluorescence studies and quantum efficiency measurements a Turner Model 210 absolute spectrofluorometer was used. This instrument and its use are described by the Turner manual (25) and by Turner (26).

Spectrophotometer. Absorption spectra were recorded on a Cary Model 14 spectrophotometer.

Image Intensifier Spectrograph (IIS). An IIS was used to measure ECL spectra. The optical system was constructed according to Bass and Kessler (27), in conjunction with an RCA C70021 HP2 Image Intensifier tube, and is described by Ness and Hercules (28). A Polaroid camera using type 107 film was used to record the spectra. This has the advantage that the results are immediately available. However, since this film is available only in positive form, negatives have to be made before the spectra can be analyzed on a recording densitometer.

Recording Densitometer. A Leeds and Northrup recording microphotometer (L & N 6700 A2) was used to record the IIS spectra. This instrument and its use is described in the L & N manual DB-1325 (29).

Lifetimes. Lifetimes of fluorescence were obtained on a TRW Model

31A nanosecond fluorometry system.

Electrochemical. The potentiostat used consisted of Heath EUA-14-A operational amplifiers along with a Heath EUA-19-2 polarography module. A three electrode cell was used with a platinum counter electrode isolated from the bulk of the solution by a fine glass frit. A reference electrode of Ag/AgCl in 0.1 M KCl was used and was isolated from the test solution by a porous Vycor plug. Both platinum foil and mercury pool indicator electrodes were employed. Provision for bubbling N₂ or O₂ through the solution was included.

The cell used for taking ECL spectra is shown in Figure IV-1. A platinum wire was used as a reference electrode in this cell due to space limitations.

Syringe Drive. The syringe drive-"T" mixer combination used in the flow measurements has been described in detail by Lytle (8).

E. Procedures

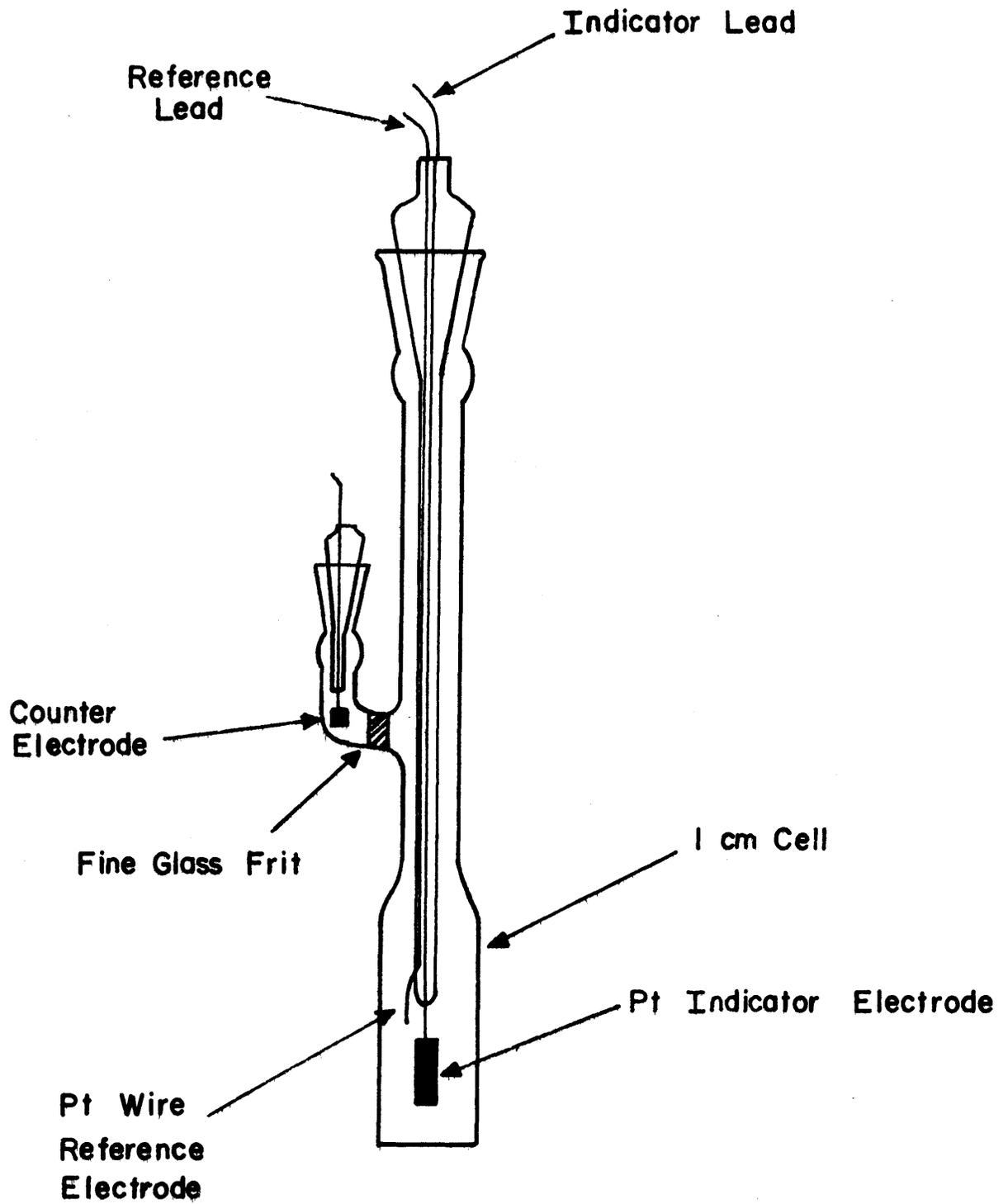
1. Electrochemical

a) Current-voltage curves. Standard techniques were used in obtaining current voltage curves for scan rates up to 0.333 V/sec.

b) Oscillograms. For scan rates greater than 0.333 V/sec the Wavetek triangular wave generator was used to drive the potentiostat and the current voltage curves were recorded on an oscilloscope. The scan rates for a given run were calculated by noting the voltage range per cycle and the value of the cycles per second applied from the Wavetek. Residual currents were estimated to subtract capacity currents in obtaining peak currents from these data.

c) Coulometry. The current integrator described above was used in the coulometry measurements to determine n_c for the reduction of

Figure IV-1
Cell Used For Taking ECL Spectra



ECL CELL

lucigenin and n_a for the oxidation of DBA, n being the number of electrons involved in the charge transfer step. 50 ml of a known concentration of lucigenin were introduced into the electrochemical cell. DMSO was used as the solvent to minimize evaporation of solvent during bubbling with N_2 . A current-voltage curve was taken using the small spherical indicator electrode. A proportionality constant was then calculated by dividing the known moles of lucigenin present initially by the initial peak current in cm.

The current integrator was then connected, the large working electrode substituted for the platinum sphere and bubbling with nitrogen started. A constant potential of -0.35 V vs Ag/AgCl was then applied. After sufficient electrolysis (~ 10 min.) the applied potential was shut off and the voltage output of the current integrator was measured with a digital volt meter. The number of coulombs passed during the electrolysis was calculated by the relationship noted above. A second current-voltage curve was then recorded, using the small sphere as the indicator in quiescent solution. The difference in initial and final peak currents, when multiplied by the calculated proportionality constant gives the moles of substance reduced or oxidized. The moles of electrons passed was calculated by dividing the coulombs passed by 9.65×10^4 . Dividing the moles of electrons passed by the moles reduced (or oxidized) gives n_c or n_a . The procedure was repeated and n was taken as the average of several runs.

d) ESR. A Varian Model E-3 ESR instrument was used to detect the presence of radicals in the lucigenin reduction. A two electrode cell was used consisting of a platinum wire indicator electrode and a mercury drop reference electrode. Standard techniques were used in observing the ESR signal obtained.

2. ECL

a) Qualitative Observation of ECL. The three electrode cell with Ag/AgCl reference electrode was used in these measurements. Current-voltage curves were recorded on a Houston Model HR-100 X-Y recorder. Solutions were approximately 10^{-3} M in lucigenin and were saturated with oxygen by bubbling for five minutes.

A mercury pool was used as the indicator electrode for aqueous solutions while a platinum foil indicator electrode was used in non-aqueous media. A current-voltage curve for the solution to be studied was recorded and a constant potential was then applied corresponding to the potential at the peak of the oxygen reduction wave. Light emission was observed visually.

b) Measurement of ECL Spectra. Initial measurements were made using the Aminco-Bowman spectrofluorometer in conjunction with the ECL cell shown in Figure VI-1. The excitation source was not used. The slits of the emission monochromator were opened to 3 mm. The photomultiplier was operated at 1750 V for greatest sensitivity. At these settings the signal to noise ratio was 20 to 1. The results of these measurements have been reported by Legg and Hercules (30).

ECL spectra of higher resolution and less distortion were obtained by use of the Image Intensifier Spectrograph (IIS). Exposure times from 5 to 20 seconds gave satisfactory results. Over-exposure of the Polaroid plate resulted in a flattening of the spectra and such spectra were discarded. Since the intensity of the ECL varied over a wide range during and between measurements several pictures of varying exposure times were taken and the best exposure was used.

The Polaroid Camera was mounted on a variable position rack and up to nine exposures could be taken on one photograph. A small mercury

