

Synthesis and Characterization of Luminol

Maria C. Nagan, Eric V. Patterson and James M. McCormick

Department of Chemistry, Truman State University, 100 East Normal, Kirksville, MO 63501

Abstract

Luminol (5-amino-2,3,-dihydrophthalazine-1,4-dione) is synthesized and its chemiluminescent properties are explored. Both spectral and physical data indicate that the luminol is sufficiently pure, despite a low yield. Chemiluminescence of luminol is observed in the presence of H₂O₂ and an oxidant enhancer. The quantum yield of the luminol chemiluminescence is determined to be moderate (0.10 ± 0.03) when compared to other chemiluminescent compounds.

Introduction

The chemiluminescence of luminol, 5-amino-2,3-dihydrophthalazine-1,4-dione, was first discovered by Albrecht in 1928.¹ Since then, the luminescent properties of luminol and other hydrazines have been thoroughly investigated^{2,3} and have found applications in such diverse areas as the detection of ion concentrations in aqueous solutions,⁴⁻⁶ monitoring the progress of H₂O₂-dependent reactions⁷ and even the detection of blood at crime scenes.⁸ In the current study, luminol, one of the more widely studied chemiluminescent molecules, is synthesized and its spectroscopic properties are examined.

The mechanism of luminol light emission and how it can be enhanced is still not well understood. However, a few pioneering studies have explored the chemiluminescent properties of luminol in both aprotic and protic solvents. In aprotic solvents such as dimethylsulfoxide, luminol has been shown to chemiluminesce via an excited 3-aminophthalate (**2**) dicarboxylate anion (Scheme 1).^{9,10} The reaction requires two equivalents of base, such as NaOH, to deprotonate the amide nitrogens before subsequent reaction with O₂ gives **2**. In protic solvents, the reaction is more complex and the chemiluminescent reaction involves H₂O₂, whose exact role is undetermined, and some additional oxidant such as potassium ferricyanide in addition to luminol.² The emission of light requires both H₂O₂ and the oxidant enhancer to produce **2**.^{2,11} A free-radical mechanism has been proposed,¹² but the mechanism of oxidant enhancement, increased luminescence in the presence of the oxidant, is still not well understood. Enhanced

luminescence can be achieved with a number of different ferric enzymes including horseradish peroxidase and luciferin.^{13,14}

Chemiluminescence is distinct from fluorescence and phosphorescence in that a chemical is used to excite a molecule and an external source of photons is not needed. In chemiluminescence, a molecule is excited through a set of chemical reactions. The excited molecule, usually in the lowest excited singlet state,¹⁰ can spontaneously lose its electronic energy in the form of a photon. If the electronic spin ($\Delta S=0$) is preserved, this process is referred to as fluorescence.¹⁵ The radiant intensity of chemiluminescence, I_{CL} , can be expressed as¹⁶

$$I_{CL} = \phi_{CL} \frac{dC}{dt} \quad (1)$$

and is dependent upon both the rate of chemical reaction, dC/dt , and the chemiluminescent quantum yield ϕ_{CL} . The chemiluminescent quantum yield, the number of photons emitted per chemiluminescing molecule, provides a means to quantify the efficiency and degree of chemiluminescence.

In this study, luminol was synthesized and the chemiluminescent properties of it were explored. The luminescence was first qualitatively observed in a hydrogen peroxide-dependent reaction, using a Cu(II) species as the oxidant. Then the chemiluminescent emission spectrum of luminol in aqueous solution was obtained to determine the chemiluminescent quantum yield of luminol in water and the extent of chemiluminescence.

Experimental

Melting point data was obtained on a Thomas-Hoover apparatus. Infrared (IR) spectra were collected on a Beckman Coulter IR spectrophotometer. Proton and carbon nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer. All chemicals were obtained from Sigma (St. Louis, MO) and used without further purification.

5-Amino-2,3-dihydrophthalazine-1,4-dione (luminol). Luminol was synthesized from 3-nitrophthalic acid (Scheme 2) according to previously published procedures.¹⁷ In a test tube, 3-nitrophthalic acid (1.0024 g, 4.748 mmol) and 8 % aqueous hydrazine (2.05 mL) were combined. Triethyleneglycol (3.02 mL) was then added to the mixture and excess water was distilled away by heating. The mixture was refluxed at 215 °C for 2 minutes, and quenched with hot water (15 mL). Cooling the reaction mixture in an ice bath afforded a precipitate, which was collected by vacuum filtration. To the isolated solid was added 3 M sodium hydroxide (5 mL) and then sodium hydrosulfite (2.510 g). The mixture was refluxed for 5 minutes at 180 °C and quenched with glacial acetic acid (2 mL). Upon cooling on ice, a yellow solid was collected and recrystallized from 95% ethanol: 35% yield; m. p. 326-334 °C; IR (KBr) 1496 (s), 1603 (s), 1667 (s), 3029 (s-broad), 3442 (m), 3464 (m) cm^{-1} ; ^1H NMR (400 MHz, DMSO-d_6) δ 6.939 (m;1), 6.999 (d;1), 7.360 (d; 2), 7.490 (m; 1), 11.200 (s;2) ppm.

Luminescence. To a solution (250 mL) containing 0.3 M sodium bicarbonate, 4 mM anhydrous ammonium carbonate, 1.8 mM copper (II) sulfate, 37.5 mM sodium

carbonate, product (0.1002 g) from the above synthesis was added, followed by an equal volume to a 0.15% aqueous hydrogen peroxide.

Luminescence Spectroscopy. The chemiluminescence emission spectrum of 99.5% pure luminol (Sigma) was obtained using a Perkin-Elmer luminescence spectrometer (model LS-5B). The previously determined maximum emission wavelength of luminol in water (424 nm) was used in the present study.¹⁰ The chemiluminescence spectra for luminol were obtained for three different samples (25 mM) in water (Fig. 1).

Results

Synthesis. The yield of the luminol synthesis used here was somewhat disappointing at only 35% and the melting point range was somewhat larger than expected for a pure substance. However, the IR spectrum, and in particular the NMR spectrum, are consistent with those expected for luminol and indicated that the target molecule was indeed synthesized.

Luminescence. The expected blue fluorescence^{2,3} was observed upon combination of the solutions as described above for most luminol preparations. However, some batches showed anomalous behavior. With some the intensity appeared to the eye to be somewhat less than the majority of the samples and with others it took longer to reach full intensity, again judged by eye, when compared to the majority of the samples. This variation was in spite of very similar melting points and NMR spectra. Samples from these preparations were not used in the luminescence spectroscopy studies.

Luminescence Spectroscopy. The luminescent spectra of luminol shown in Fig. 1 closely matches that found in previous investigations.^{2,3} From this spectrum the chemiluminescent quantum yield was determined to be 0.10 ± 0.03 .

Discussion

Both physical and spectral data indicate that luminol was indeed synthesized. The IR spectrum (See Supporting Information) shows the characteristic amide vibrational stretch at about 3400 cm^{-1} . No carboxylic acid stretch (1700 cm^{-1}) or nitro group stretch (1550 cm^{-1}) was present in the IR spectrum but instead an amine at 1603 cm^{-1} , indicating successful conversion of the starting material to the desired product. The proton NMR spectrum (See Supporting Information) indicates that both aromatic amine ($\delta = 7.360\text{ ppm}$; 2) and amide protons ($\delta = 11.200\text{ ppm}$; 2) are present in addition to three aromatic protons ($\delta = 6-8\text{ ppm}$). Lastly, the yellow solid's melting point of $326-334\text{ }^\circ\text{C}$, although a little low and the range rather broad, is similar to the literature melting point of luminol ($332\text{ }^\circ\text{C}$).¹⁷

The 35 % overall yield for the reaction was low. This may be due to an intrinsically low reaction-efficiency, which is expected since the overall reaction is not entropically favorable. The reaction requires two consecutive condensations, the first intermolecular and the second an intramolecular cyclization, both of which are likely to have $\Delta S < 0$. With a high reaction temperature and a net negative ΔS , the overall free energy of reaction is therefore less favorable. A modification of the synthesis, by replacing the carboxylic acid groups of 3-nitrophthalic acid with more reactive group

(e.g. acid chlorides) may increase overall yield. Lastly, the acidity of the solution is a critical component because the carbonyls need to be activated in the first step of the reaction (Scheme 1) but too many protons may lead to protonation of hydrazine. As this reaction is conducted in one pot, careful control of the pH in future experiments may increase yield.

The chemiluminescence of luminol was examined both in a qualitative and quantitative manner. Luminol chemiluminesced blue in the presence of H_2O_2 and Cu(II) . In addition, the chemiluminescent quantum yield of luminol was determined (0.10 ± 0.03) and shown to be moderate relative to most chemiluminescent compounds which have quantum yields in the range of 0.01 to 0.2.¹⁶ Other derivatives of luminol have been found to luminesce more intensely,² specifically, changing the 5-amino substituent to an alkoxide or an alkyl amino group has been shown to increase light emission.^{18,19} As well, enhancers such as ferric enzymes greatly increase luminescence intensity.^{13,14} It is therefore not surprising that many applications of chemiluminescence employ either a luminol derivative or an oxidant enhancer.

Conclusion

Luminol has been successfully synthesized in pure form. The yield of 35% was rather low but expected since the reaction is not entropically favorable. The chemiluminescent quantum yield was determined to be 0.10, which is indicative of a rather moderate luminescence intensity.

Supporting Information Available

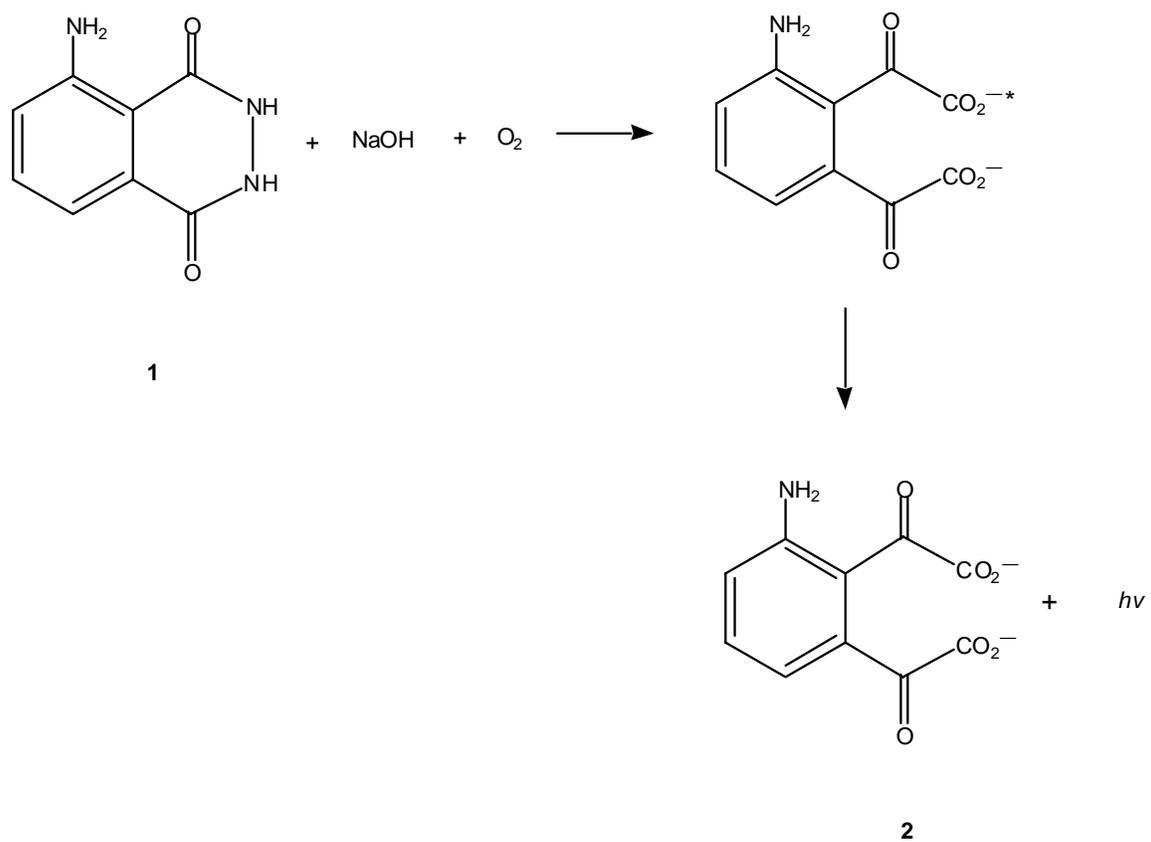
Full synthetic details and characterization are available in this section (PDF). The material is available free of charge via the Internet at <http://pubs.acs.org>.

References

1. Albrecht, H. O. *Z. Phys. Chem. (Leipzig)* **1928**, *136*, 321.
2. White, E. H.; Roswell, D. F. *Acc. Chem. Res.* **1970**, *3*, 54-62.
3. Gundermann, K. D. *Chimia* **1971**, *25*, 261.
4. Kurihara, M.; Hasebe, T.; Kawashima, T. *Bunseki Kagaku* **2002**, *51*, 205-233.
5. Meseguer-Lloret, S.; Campins-Falco, P.; Tortajada-Genaro, L. A.; Blasco-Gomez, F. *Int. J. Env. Anal. Chem.* **2003**, *83*, 405-416.
6. Nieman, T. In *Chemiluminescence and Photochemical Reaction Detection in Chromatography*; Birks, J. W., Ed.; VCH: New York, 1989, pp 99-123.
7. Yuan, J.; Shiller, A. M. *Anal. Chem.* **1999**, *71*, 1975-1980.
8. Kent, E. J. M.; Elliot, D. A.; Miskelly, G. M. *J. Foren. Sci.* **2003**, *48*, 64-67.
9. White, E. H.; Zafirou, O.; Kagi, H. H.; Hill, J. H. M. *J. Am. Chem. Soc.* **1964**, *86*, 940-941.
10. White, E. H.; Bursey, M. M. *J. Am. Chem. Soc.* **1964**, *86*, 941-942.
11. Lind, J. S.; Merényi, G.; Eriksen, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 7655-7661.
12. White, E. H. In *Light and Life*; McElroy, W. D., Glass, B., Eds.; Johns Hopkins Press: Baltimore, MD, 1961, p 183.
13. Whitehead, T. P.; Thorpe, G. H. G.; Carter, T. J. N.; Groucutt, C.; Kricka, L. J. *Nature* **1986**, *305*, 185-189.

14. Easton, P. M.; Simmonds, A. C.; Rakishev, A.; Egorov, A. M.; Candeias, L. P. *J. Am. Chem. Soc.* **1996**, *118*, 6619-6624.
15. Levine, I. N. *Physical Chemistry*; 3rd ed.; McGraw-Hill: New York, 1988.
16. Skoog, D. A.; Holler, F. J.; Nieman, T. A. *Principles of Instrumental Analysis*; 5th Ed.; Thomson Learning, 1998.
17. Williamson, K. L. In *Macroscale and Microscale Organic Experiments*; 2nd ed.; D.C. Heath: Lexington, MA, 1994, pp 153-162.
18. White, E. H.; Bursley, M. M. *J. Org. Chem.* **1966**, *31*, 1912.
19. Gundermann, K. D.; Horstmann, W.; Bergmann, G. *Ann.* **1965**, *684*, 127.

Scheme 1. Chemiluminescence of luminol (5-amino-2,3-dihydrophthalazine), **1**, in aprotic solvents.¹⁻³



Scheme 2. Preparation of luminol (**1**) from 3-nitrophthalic acid (**3**) via a nitrohydrazine intermediate (**4**).

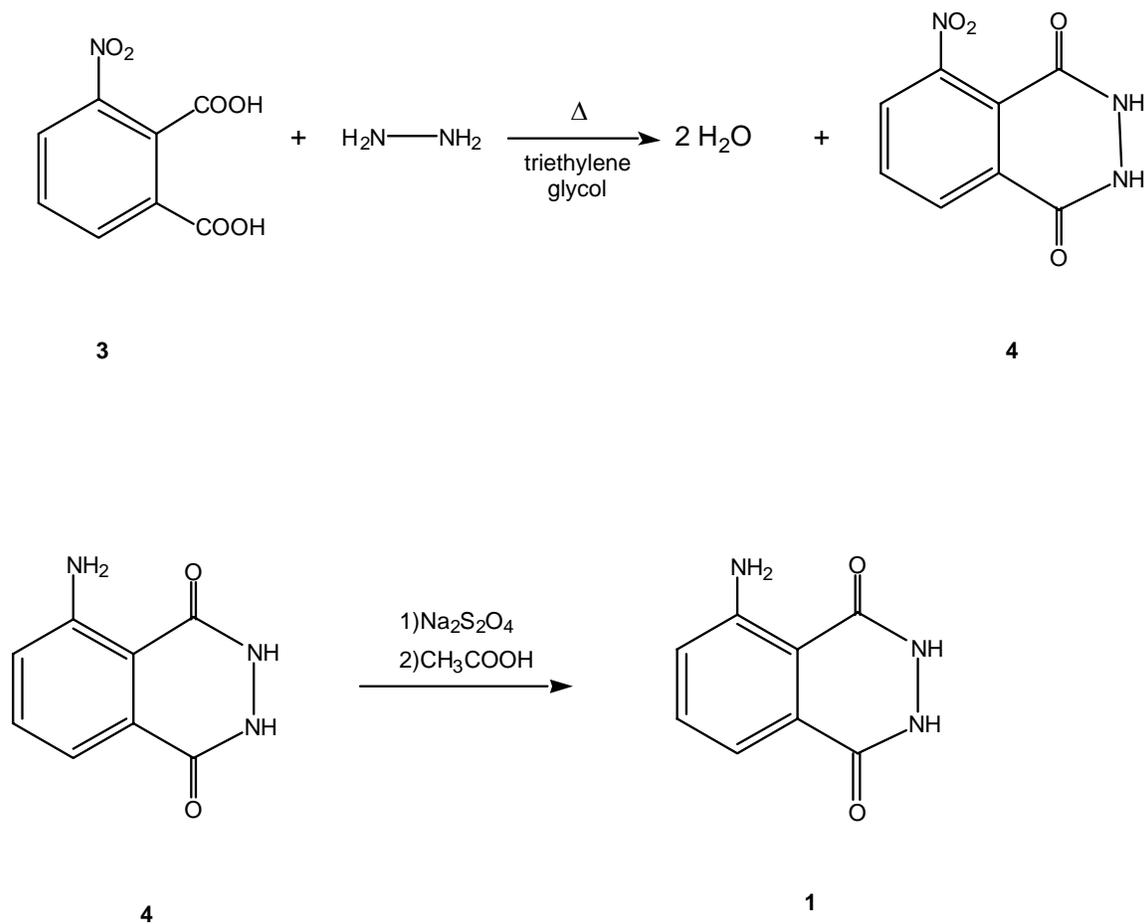


Figure Legends

Figure 1. Chemiluminescence emission spectra of luminol obtained as described in the text. Each spectrum was obtained in triplicate and each spectrum is indicated by a separate line (—, - - - or ...).

LUMINESCE SPECTRUM GOES HERE.

ALL AXES WOULD BE APPROPRIATELY LABELED WITH UNITS.