The infinity absorbance (per cent acyl–enzyme at equilibrium) and the rate of approach to infinity [k_3 + $k_5/(1 + K_m/(S))$] are identical for reactions starting with both the acid and the ester (Fig. 1).

Thus an acyl–enzyme is formed from a specific acid substrate of α-chymotrypsin. An excellent preparative method for specific acyl–enzymes may be based on the data of Table I.

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Chemiluminescence of Luminol: The Chemical Reaction[1]

Sir:

Chemiluminescence, the production of light in excess of black body radiation by chemical reactions, is not well understood for systems in the condensed phase. A large number of chemiluminescent reactions have been discovered,[2] but virtually nothing is known about the chemistry involved. We report here an investigation of the chemiluminescence of luminol[3] (5-amino-2,3-dihydro-1,4-phthalazinedione, I), one of the most efficient and probably the best known of the chemiluminescent compounds. Light is produced when basic aqueous solutions of luminol containing oxygen are treated with an oxidizing agent such as potassium ferricyanide, although an increased amount of light is obtained if hydrogen peroxide is included in the system. The oxidizing agents used cause extensive degradation of the reaction products, however, and consequently the only product identified prior to our work was nitrogen.[4] The chemiluminescence in aprotic solvents, such as dimethyl sulfoxide and dimethylformamide, is considerably simpler,[2e] and in these solvents only oxygen and a base are required.

$$ \text{I} + 2\text{NaOH} + \text{O}_2 \longrightarrow \tag{1} $$

$$ \text{N}_2 + 2\text{H}_2\text{O} + \text{II} + h\nu $$
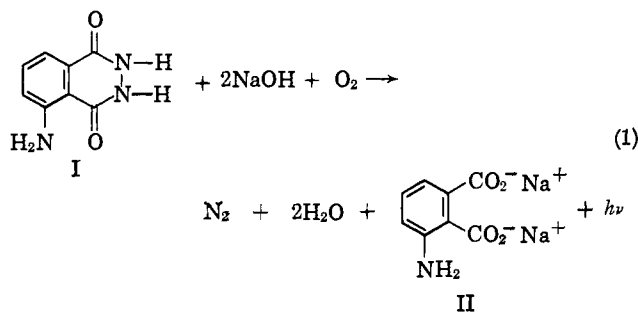
The products of the reaction are nitrogen and sodium aminophthalate (II) (plus traces of the aminobenzoate); these compounds were identified by gas–liquid and paper chromatography and by their spectra. The sodium aminophthalate was converted, furthermore, into acetamidophthalic anhydride and into dimethyl aminophthalate; yields of up to 90% of these compounds were obtained. As will be shown elsewhere,[5] a recovery of this magnitude, coupled with the quantum yields (which require that about 50% or more of the luminol molecules lead to an excited state of II) ensures

(1) Abstracts, 134th National Meeting of the American Chemical Society, Chicago, Ill., Sept., 1958; p. 94P. This work was supported by the National Institutes of Health (Grant A-2399).

(2) (a) R. S. Anderson, *Ann. N. Y. Acad. Sci.*, **49**, 337 (1948); (b) W. Vaughan, *Chem. Rev.*, **43**, 447 (1948); (c) E. N. Harvey, "A History of Luminescence," The American Philosophical Society, Philadelphia, Pa., 1957; (d) A. Bernanose, *Bull. soc. chim. France*, 567 (1950); 39D (1952); (e) E. H. White, "Light and Life," W. D. McElroy and B. Glass, Ed., The Johns Hopkins Press, Baltimore, Md., 1961, p. 183; E. H. White, *J. Chem. Educ.*, **34**, 275 (1957); (f) H. Stork, *Chemiker Ztg.*, **85**, 467 (1961).

(3) H. O. Albrecht, *Z. Physik. Chem.* (Leipzig), **136**, 321 (1928).

(4) F. H. Stross and G. E. K. Branch, *J. Org. Chem.*, **3**, 385 (1938).

(5) E. H. White and M. M. Bursey, *J. Am. Chem. Soc.*, **86**, 941 (1964).
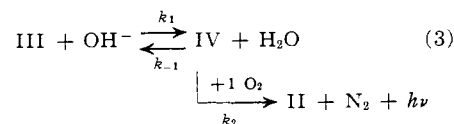
that the aminophthalate ion is a product of the chemiluminescence proper, and that it does not stem from some accompanying "dark" reaction. Oxygen (1.04 moles) and 2.00 moles of NaOH were consumed in the reaction, and 0.99 mole of nitrogen was formed per mole of luminol, thus indicating the stoichiometry shown in eq. 1.

The reaction of 1 mole of a base with luminol (pK_a = 6)[4] yields a mononegative ion[6] (III), which does not react with oxygen at an appreciable rate. The addition of slightly more than 1 mole of base to solutions of luminol containing oxygen leads to chemiluminescence. The dinegative ion (IV) is undoubtedly the critical intermediate formed by the action of this second molecule of base; the amino group is not directly involved, since the same dependence of chemiluminescence on base was noted for phthalic hydrazide. In the absence of oxygen, basic solutions of luminol are stable indefinitely. The chemiluminescence of luminol, therefore, involves simply the reaction of oxygen with the dinegative ion of luminol (eq. 2).

$$ \text{IV} + \text{O}_2 \longrightarrow h\nu + \text{products} \tag{eq. 1} $$

$$ \tag{2} $$
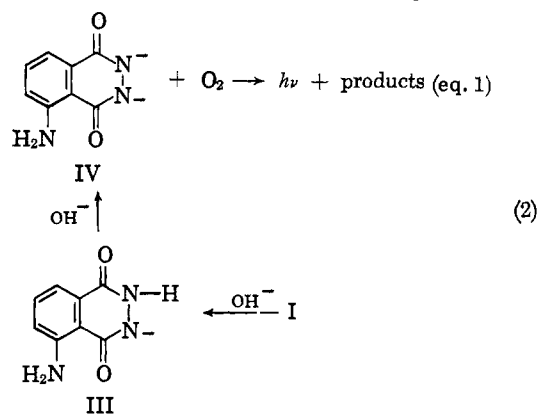
To confirm this sequence of events, and also to show that the two oxygen atoms introduced into the aminophthalic acid framework came from the oxygen gas and not from hydroxide ion or from adventitious water (*e.g.*, in "anhydrous" dimethyl sulfoxide), the chemiluminescence was carried out with O[18] enriched oxygen gas in a system of 70 mole % dimethyl sulfoxide, 30 mole % water, and an excess of sodium hydroxide. It was found that over 85% of the label ended up in aminophthalate ion II (the 15% loss of O[18] was shown by suitable blanks to result from exchange reactions occurring during the analysis).

The kinetics of the chemiluminescence has also been measured in this system. The second ionization constant for luminol is estimated[4] to be about 10^{-13}, and only a low concentration of the dinegative ion (IV) is present in the reaction mixture. Substitution of amino or methoxy groups on the phthalic hydrazide skeleton increases the rate of the reaction, indicating that the oxidation is the slow step in the chemiluminescence. The reaction, therefore, involves an equilibrium with base leading to the dinegative ion, followed by a slow oxidation step (eq. 3).[7]

$$ \text{III} + \text{OH}^- \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{IV} + \text{H}_2\text{O} \tag{3} $$

$$ \xrightarrow[k_2]{+1\ \text{O}_2} \text{II} + \text{N}_2 + h\nu $$

Using the steady-state approximation,[8] and with the logical assumption that $k_{-1}(\text{H}_2\text{O}) \gg k_2(\text{O}_2)$, the ex-

(6) The sodium salt of luminol has been isolated by E. H. Huntress, L. N. Stanley, and A. S. Parker [*ibid.*, **56**, 241 (1934)].

(7) For the reaction in protic solvents see ref. 2e.

(8) J. Hine, "Physical Organic Chemistry," 2nd Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 66.

pression for the rate of photon emission becomes $(k_1 k_2 / k_{-1}[H_2O])[III][OH^-][O_2] = k[III][OH^-][O_2]$. Experimentally, in the presence of an excess of base, and kept saturated with oxygen at 35° ([III] $\approx$ concentration of luminol = 4.0×10^{-4} M, [OH$^-$] = 6.0×10^{-2} M, [O$_2$] = 1.6×10^{-3} M), the reaction was strictly pseudo-first order, $k' = 2.5 \times 10^{-3}$ sec.$^{-1}$. Changing the concentration of luminol 10-fold, the concentration of base 40-fold, and the concentration of oxygen 10-fold (independently) showed that the reaction was first-order in each of these reactants.

Intermediates have not been detected to date (by e.p.r., for example) in the oxidation step (eq. 3), which proceeds at a reasonable rate even at −60°. An oxidation of dinegative ion IV to the azo state is un-likely since 1,4-phthalazinedione (V) is nonchemi-luminescent under conditions which lead to the produc-tion of considerable light from phthalic hydrazide. Cyclic intermediates[9] such as VI are attractive from the standpoint of visualizing the formation of nitrogen and the aminophthalate ion (II). We have found, how-ever, that certain acyclic hydrazides such as 1-hydroxy-2-anthroic acid hydrazide (VII)[10] are practically as
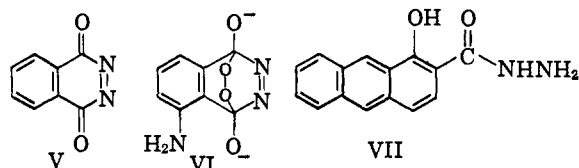
chemiluminescent as luminol (quantum yield = $ca.$ $^1/_3$ that of luminol); cyclic adducts or intermediates, therefore, are not necessary features of the reaction. Evidence is being sought at present for multistep reaction paths which lead to the aminophthalate ion (II), the emitter of light in the chemiluminescence of luminol.[5]

(9) P. C. Wilhelmsen, R. Lumry, and H. Eyring, "The Luminescence of Biological Systems," F. H. Johnson, Ed., American Association for the Ad-vancement of Science, Washington, D. C., 1955, p. 96.

(10) We thank Mr. David F. Roswell for preparing this compound.

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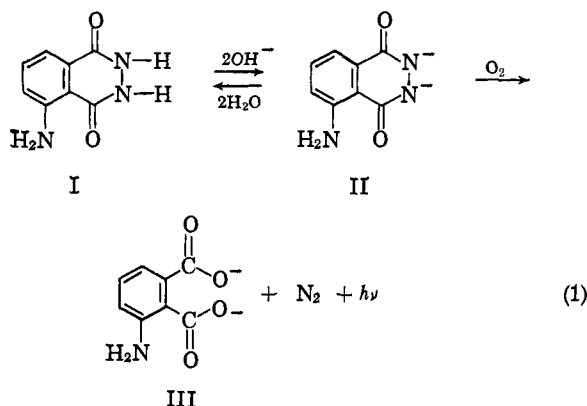   JOHN H. M. HILL
RECEIVED NOVEMBER 1, 1963

Chemiluminescence of Luminol and Related Hydrazides: The Light Emission Step

Sir:

The chemiluminescence of luminol (I) in relatively nonacidic solvents has been shown to involve the fol-lowing chemical reactions.[1]

(1)

(1) E. H. White, O. Zafiriou, H. H. Kägl, and J. H. M. Hill, *J. Am. Chem. Soc.*, **86**, 940 (1964).
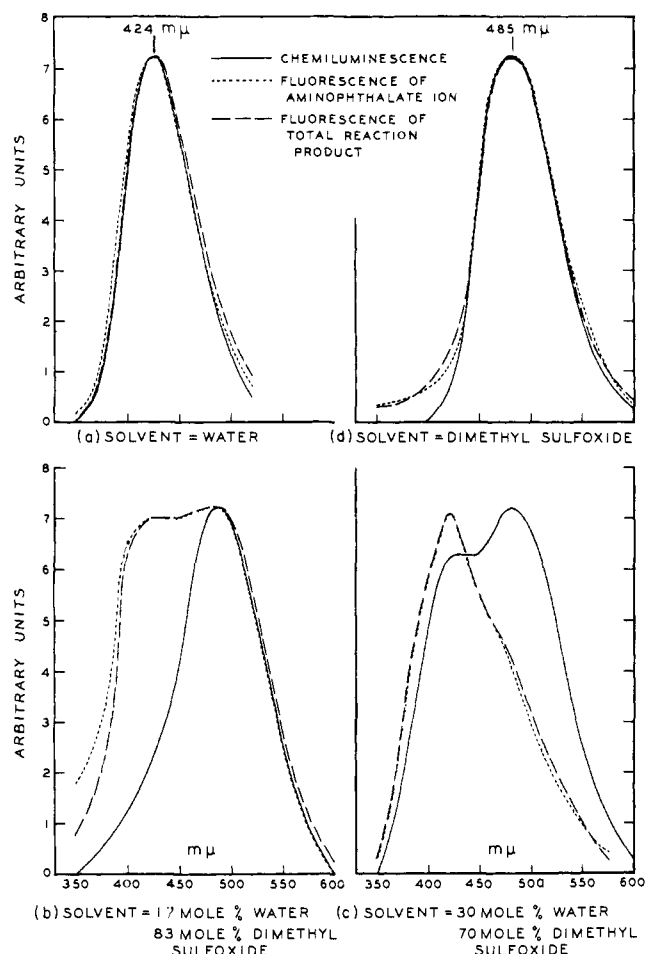
Fig. 1.—Fluorescence and chemiluminescence emission spectra.

In this communication, we will consider the produc-tion of light in the reaction and show that the amino-phthalate ion (III) is the light emitting species.

In the chemiluminescence of liquid solutions, the conversion of chemical energy into light energy in-volves the formation of a reactant, intermediate, or product molecule in an excited electronic state (di-rectly or by energy transfer), and the emission of a photon by that species.[2] Since excited states are also reached by the absorption of light, it should be possible to correlate the wave length distribution in chemi-luminescence with that in phosphorescence, if a triplet state were the emitter, or in fluorescence,[3a] if a singlet state were involved.

In the chemiluminescence of luminol in dimethyl sulfoxide and also in water,[4] a good match has been found for the wave length distribution in chemilumi-nescence, the fluorescence of the total reaction product, and the fluorescence of sodium aminophthalate (Fig. 1a and d). In mixtures of dimethyl sulfoxide and water (or other protic solvents), furthermore, a double emission occurs in *both* chemiluminescence and fluo-rescence (Fig. 1b and c), with peak positions unchanged from the values in the pure solvents. The occurrence of two emission peaks is attributed to the formation of two different kinds of excited state molecules: an ex-cited aminophthalate ion hydrogen bonded to water—

(2) Certain of these steps have been established for gas phase reactions: (a) M. G. Evans, and M. Polanyi, *Trans. Faraday Soc.*, **35**, 178, (1939), also pp. 185-226; (b) M. G. Evans, H. Eyring, and J. F. Kincaid, *J. Chem. Phys.*, **6**, 349 (1938); (c) F. Kaufman and J. R. Kelso, *ibid.*, **27**, 1209 (1957).

(3) "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961: (a) H. Linschitz, p. 173; (b) E. H. White, p. 183.

(4) The system used was luminol, NH₃, H₂O₂, and Cu⁺⁺ (at 0°) (see ref. 3b, for the mechanism in water solutions); see also H. Seliger in ref. 3.