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The Chemiluminescence of Acyl Luminols

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From a study of the chemiluminescence spectra of acyl luminols (e. g., 3-acetylaminophthalhydrazide, 3-propanoylaminophthalhydrazide and 3-undecenoylaminophthalhydrazide) and of the corresponding fluorescence spectra of the reaction products, it is tentatively assumed that the emitting species of the chemiluminescence of acyl luminols in dimethylformamide-water (3 : 1 in vol.) is the corresponding 3-acylaminophthalate ion in the excited state.

Since Albrecht[1] first described the bright chemiluminescence of luminol (3-aminophthalhydrazide) (I), the synthetic approach to a clarification of the mechanism of the chemiluminescence had been attempted by several groups of workers.[2] Recently White et al.[3] showed that the 3-aminophthalate ion might be the light emitting species in the chemiluminescent reaction of luminol in water or in dimethylsulfoxide.

As a part of the investigation of the synthesis of chemiluminescent polymers,[4] the present authors have studied the synthesis and chemiluminescence of acyl luminols. The object of this paper is to present a convenient modified method for the acylation of luminol and to describe the relation between the chemiluminescence spectra of acylated luminols and the corresponding fluorescence spectra of the chemiluminescent reaction products.

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[1] H. O. Albrecht, *Z. Phys. Chem.*, **136**, 321 (1928).

[2] a) H. D. K. Drew and R. F. Garwood, *J. Chem. Soc.*, **1937**, 1841. b) H. D. K. Drew and R. F. Garwood, ibid., **1938**, 791. c) E. H. White, O. Zafiriou, H. H. Kägi and J. H. M. Hill, *J. Am. Chem. Soc.*, 86, 940 (1964). d) M. A. Bernanose, *Bull. soc. chim. France*, **18**, 329 (1951).

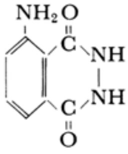
[3] E. H. White and M. M. Bursey, *J. Am. Chem. Soc.*, **86**, 941 (1964).

[4] N. Sugiyama et al., to be published.

Results and Discussion

The Acylation of Luminol.—It has been found in this laboratory that dimethylformamide is a suitable solvent for the acylation of luminol, which is sparingly soluble in most organic solvents. Monoacyl derivatives of luminol were prepared by the action of several acyl chlorides on luminol dissolved in dimethylformamide in the presence of pyridine.

The ultraviolet absorption spectra of luminol and its three acyl derivatives, 3-acetylaminophthalhydrazide (II), 3-propanoylaminophthalhydrazide (III) and 3-undecenoylaminophthalhydrazide (IV), are shown in Fig. 1. The absorption maximum about 330 nm* was useful for the identification of the acyl derivatives.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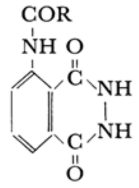
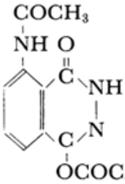

R: CH_3- II
CH_3CH_2- III
CH_2=$CH(CH_2)_8$- IV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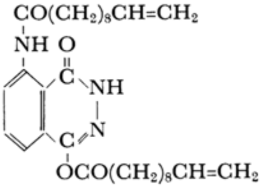
VI

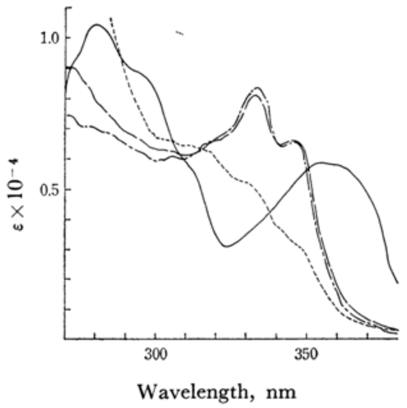

Fig. 1. Ultraviolet absorption spectra of luminol and acyl luminols.
luminol (I) ——, 3-acetylaminophthalhydrazide -·—, 3-propanoylaminophthalhydrazide —·—, 3-undecenoylaminophthalhydrazide ---- in dimethylformamide.

When luminol was treated with an excess of several acyl chlorides, diacyl derivatives of luminol were obtained. Those were tentatively deduced to be V and VI from their infrared absorption spectra, which have the strong absorption at 1760 cm^{-1} characteristic of the carbonyl group of the enol ester type.

The Chemiluminescence Spectra of Luminol and Acyl Luminols.—The color of the chemiluminescence of luminol was bright blue, with a spectral maximum at 470 nm, while acyl derivatives showed a greenish blue chemiluminescence, with a maximum at about 480 nm. The chemiluminescent spectra spread over a broad region between about 400 nm and 600 nm.

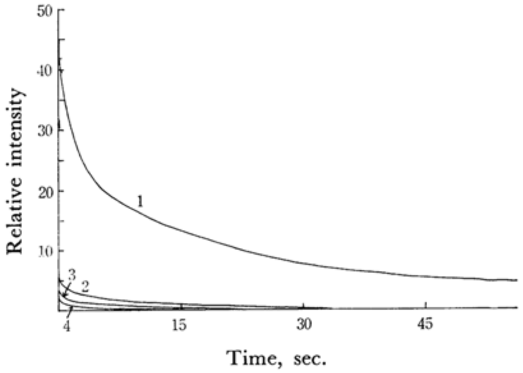

Fig. 2. Change of chemiluminescence intensity at the emission maximum.
luminol (I) (470 nm) …… 1, 3-acetylaminophthalhydrazide (II) (480 nm) …… 2, 3-propanoylaminophthalhydrazide (III) (480 nm) …… 3, 3-undecenoylaminophthalhydrazide (IV) (480 nm) …… 4
Concn.: 7.5×10^{-4} mol. in DMF-water mixture (vol. 3 : 1) respectively.

Figure 2 shows the change in chemiluminescence intensity with time. The chemiluminescence intensity strikingly decreases with the acylation of the amino group of luminol. This suggests that both the inductive and steric effects of the acylamino group serve to decrease the chemiluminescence of luminol, as has been reported by earlier investigators.[5] The spectra of the chemiluminescence of acyl luminols show the emission maximum at a little longer wavelength than luminol, as is shown in Table I. This fact is likely to show that the energy difference between the emitting species and the emission products of acyl derivatives is smaller than that of luminol. Moreover, a comparison of the wavelengths and intensities at the maximum of emission spectra of

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* Following the IUPAC rule, the new notation nm (nanometer) is used instead of mμ.

[5] a) H. D. K. Drew and F. H. Pearman, *J. Chem. Soc.*, **1937**, 586. b) C. N. Zellner and G. Dougherty, *J. Am. Chem. Soc.*, **59**, 2580 (1937). c) B. E. Cross and H. D. K. Drew, *J. Chem. Soc.*, **1949**, 638. d) K.-D. Gundermann and M. Drawert, *Chem. Ber.*, **95**, 2018 (1962).

TABLE I. FLUORESCENCE AND CHEMILUMINESCENCE OF LUMINOL (I), 3-ACETYLAMINOPHTHALHYDRAZIDE (II), 3-PROPANOYLAMINOPHTHALHYDRAZIDE (III), 3-UNDECENOYLAMINOPHTHALHYDRAZIDE (IV)

Compound		Fluorescence λ_{max},nm(*)		Chemiluminescence λ_{max},nm(*)		Ultraviolet absorption λ_{max},nm
Luminol(I)	A	410	(100)	470	(100)	353
	B	475				358
2-Acetylaminophthalhydrazide(II)	A	412	(3)	480	(10)	333
	B	500				344
3-Propanoylaminophthalhydrazide(III)	A	412	(3)	480	(8)	333
	B	500				344
3-Undecenoylaminophthalhydrazide(IV)	A	412	(5)	480	(5)	300(sh.)
	B	495				346

* Relative intensity
A Fluorescence in dimethylformamide.
B Fluorescence after chemiluminescent reaction in dimethylformamide water-mixture (Vol. 3 : 1).

II, III and IV shows that the acylamino group with a longer alkyl chain is more unfavorable to the emission than that with a shorter one (Table I and Fig. 2).

The Relation of Chemiluminescence and Fluorescence.—The fluorescence spectra in various conditions are useful in deciding the emitting species in the chemiluminescent reaction.⁶⁾ First, the authors have examined the fluorescence spectra of luminol and its acyl derivatives in dimethylformamide or in the mixed solvent of dimethylformamide and water (3 : 1 in vol.). The results are summarized in Table I. During the measurement of the fluorescence spectrum of luminol, it was found that the intensity at the maximum of 410 nm decreased with the increase in the intensity at the new maximum, 470 nm, as is shown in Fig. 3. The spectral change seemed to be accelerated by adding water. Also, acyl luminols showed a similar phenomenon, which is likely to be related to the photochemical reaction with oxygen in the solvent.⁶ᵈ⁾ A more precise experiment investigating this phenomenon is now in progress.

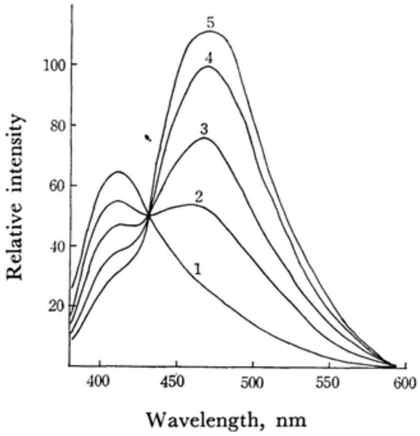

Fig. 3. The change of fluorescence spectrum of luminol in dimethylformamide with time 1—5, 5 min. interval.

Next, the fluorescence spectrum of the total reaction product of the chemiluminescent reaction of each compound was measured after chemiluminescence. The results are summarized in Table I, while the typical chemiluminescence spectra of I and II are shown in Figs. 4 and 5 respectively.

White et al.³⁾ reported, that in the chemiluminescence of luminol in dimethylsulfoxide and also in water, a good match had been found in the wavelength distribution in chemiluminescence, the

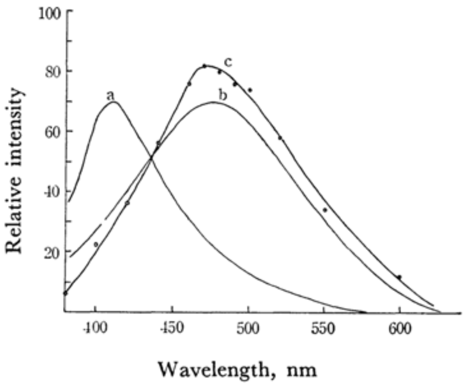

Fig. 4. Fluorescence and chemiluminescence spectra of luminol.

a. Fluorescence in DMF, 2×10^{-7} mol.
b. Fluorescence after chemiluminescent reaction, 7.5×10^{-6} mol.
c. Chemiluminescence in DMF-water (3:1 in vol.) in 7.5×10^{-4} mol.

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6) In addition to luminol (cf. Ref. 3), other chemiluminescent compounds, such as lucigenin (a), lophine (b, c, d) and 7-dialkylamino-naphthalin-1, 2-dicarboxylic acid hydrazide (e) have been recently treated by the similar approach. a) F. McCapra and D. G. Richardson, *Tetrahedron Letter*, **1964**, 3167. b) J. Sonnenberg and D. M. White, *J. Am. Chem. Soc.*, **86**, 5686 (1964). c) E. H. White and M. J. C. Harding, ibid., **86**, 5686 (1964). d) K. Maeda, H. Ojima and T. Hayashi, This Bulletin, **38**, 76 (1965). e) K.-D. Gundermann and W. Horstmann, *Ann.*, **684**, 127 (1965).

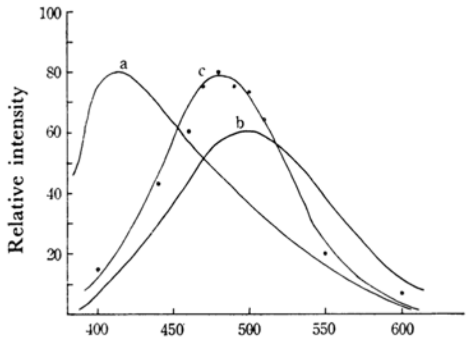

Fig. 5. Fluorescence and chemiluminescence spectra of 3-acetylaminophthalhydrazide.
- a. Fluorescence in DMF, 1.4×10^{-5} mol./l.
- b. Fluorescence after chemiluminescent reaction, 7.5×10^{-6} mol.
- c. Chemiluminescence in DMF-water (3 : 1 in vol.), 1.5×10^{-2} mol./l.

fluorescence of the total reaction product, and the fluorescence of sodium aminophthalate. The present observation of luminol in a dimethylformamide-water mixture (3 : 1 in vol.), shown in Fig. 4, led to the same conclusion as that of White et al.,[3] who concluded that the 3-aminophthalate ion is the light-emitting species.

In the case of acyl luminols a rough coincidence between the maximum of the chemiluminescence spectrum and the fluorescence maximum after chemiluminescence was observed (Table I). Considering that the wavelength distribution of the chemiluminescence spectrum is similar to that of the corresponding fluorescence spectrum after chemiluminescence, the authors tentatively assume that 3-acylaminophthalate ion in the excited state is mainly the light emitting species of the chemiluminescent reaction of 3-acylaminophthalhydrazide.

Experimental*

3-Acetylaminophthalhydrazide (II).—To the solution of luminol (0.9 g.) in dimethylformamide (20 ml.), pyridine (0.4 ml.) was added; then acetyl chloride (0.4 ml.) in dimethylformamide (1 ml.) was slowly stirred in under cooling. After the reaction mixture had been allowed to stand overnight, water was carefully poured into the mixture. The fine crystals which formed were collected, washed with water, and recrystallized from dimethylformamide and water successively to give faint yellow needle crystals, m. p. 315°C. The authentic sample, which was synthesized from 3-acetylaminophthalic anhydride and hydrazine, showed a m. p. of 320°C (decomp.).[2a]

Found: C, 54.38; H, 4.32; N, 19.20. Calcd. for $C_{10}H_9O_3N_3$: C, 54.79; H, 4.14; N, 19.17%.

UV: λ_{max}^{DMF} 333 nm ($\varepsilon=8400$), IR: ν^{KBr} 3420, 1530 cm^{-1} (-NH).

3-Acetylamino-*O*-acetylphthalhydrazide (V). — When the treatment as described in the preparation of II was carried out with an excess of acetyl chloride (4 ml.), 3-acetylamino-*O*-acetylphthalhydrazide was obtained. The compound was soluble in dimethylformamide to a much smaller extent than luminol, and was reprecipitated from dimethylformamide and water as light yellow crystals, m. p. 251°C (decomp.).

Found: C, 54.75; H, 4.22; N, 16.16. Calcd. for $C_{12}H_{11}O_4N_3$: C, 55.17; H, 4.21; N, 16.09%.

UV: λ_{max}^{DMF} 332 nm ($\varepsilon=9600$), IR: ν^{KBr} 3420, 1760, 1530 cm^{-1}.

3-Propanoylaminophthalhydrazide (III).—Luminol (0.9 g.) in dimethylformamide (20 ml.), pyridine (0.4 ml.), and propanoyl chloride (0.5 ml.) were treated as in the case of II. The product formed was then reprecipitated from dimethylformamide and water as a pale yellow powder, m. p. 278°C (decomp.).

Found: C, 56.18; H, 4.74; N, 18.21. Calcd. for $C_{11}H_{11}O_3N_3$: C, 56.55; H, 4.75; N, 18.02%.

UV: λ_{max}^{DMF} 333 nm ($\varepsilon=8200$), IR: ν^{KBr} 3420, 1530 cm^{-1}.

3-Undecenoylaminophthalhydrazide (IV). — Luminol (0.9 g.) and undecenoyl chloride (0.6 ml., prepared from undecenoic acid and thionyl chloride) brought to reaction according to the procedure used in the preparation of II. A light yellow powder resulted, m. p. 275°C.

Found: C, 66.73; H, 7.13; N, 12.22. Calcd. for $C_{19}H_{25}O_3N_3$: C, 66.45; H, 7.34; N, 12.24%.

UV: λ_{max}^{DMF} 300 nm ($\varepsilon=6700$, shoulder), IR: ν^{KBr} 3420, 2925, 2850, 1530 cm^{-1}.

3-Undecenoylamino-*O*-undecenoylphthalhydrazide (VI).—The procedure used in the preparation of IV but with an excess of undecenoyl chloride (about 6 ml.) gave a diundecenoyl derivative as a faint yellow powder, m. p. 142°C.

Found: C, 70.56; H, 8.22; N, 8.30. Calcd. for $C_{30}H_{43}O_4N_3$: C, 70.69; H, 8.50; N, 8.25%.

UV: λ_{max}^{DMF} 329 nm ($\varepsilon=8300$), IR: ν^{KBr} 3420, 2950, 2850, 1760, 1540 cm^{-1}.

The Chemiluminescence Spectra.—The chemiluminescence spectra of luminol and its acyl derivatives in the dimethylformamide-water system were measured with an Hitachi recording spectrophotometer, EPS-2 type. The procedure for the measurement of the chemiluminescence spectrum was as follows: To a 10^{-3} mole. solution of luminol or acyl luminols in dimethylformamide (3 ml.), 1 ml. of a 1 *N* sodium hydroxide solution containing potassium ferricyanide (10^{-2} mole./1.) was added. The final concentrations of the components in the chemiluminescent system were: luminol or acyl luminols, 7.5×10^{-4} mol., the hydroxide ion, 2.5×10^{-1} mol., and the ferricyanide ion, 2.5×10^{-3} mol./1. The mixture gave a brilliant chemiluminescence. The decrease in the chemiluminescence of luminol and acyl luminol with time was measured in a dimethylformamide-water mixture (3 : 1 in vol.), at the maximum of the chemiluminescence spectrum at 38—40°C.

The Fluorescence Spectra.—The fluorescence spectra were measured at 30°C with a spectrophotometer equipped with an attachment for measuring the fluorescence spectrum. The 365 nm line of a high-pressure mercury lamp was used for excitation, and the spectra were measured at concentrations of 10^{-5}—10^{-4} mol./l.

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* All melting points are uncorrected.