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The Spectra of the Chemiluminescence, Fluorescence and Absorption of Lucigenin and Its Electron Spin Resonance*[1]

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In order to study the mechanism of the chemiluminescence of lucigenin which was emitted with hydrogen peroxide in an alkaline solution, the color and the spectrum of the chemiluminescence and fluorescence, the spectrum of absorption, and the electron spin resonance were investigated. The color of the chemiluminescence was found to vary from green to blue with a decrease in the concentration of lucigenin. The spectrum of the blue chemiluminescence spread over only a visible region of 420—650 mμ, with a peak at 485 mμ. With an increase in the concentration of lucigenin, the peak shifted to 510 mμ or longer, corresponding to the green color of the chemiluminescence. This shift is due to self-absorption by lucigenin. The fluorescence spectrum of lucigenin in aqueous sodium hydroxide showed a peak at 508 mμ. The peak gradually disappeared, and the spectrum eventually showed new peaks at 435 and 450 mμ which almost agreed with the peaks of the fluorescence spectrum of 10-methylacridone. Lucigenin exhibited an electron spin resonance of a single peak (g-value 2.004) in a solid state. These results show that the chemiluminescence of lucigenin is neither of the fluorescence of lucigenin nor that of 10-methylacridone yielded in the chemiluminescent solution of lucigenin and that the mechanism of the chemiluminescence is likely to involve a free radical intermediate.

In 1935 Gleu and Petsch[1] reported that 10, 10'-dimethyl-9, 9'-biacridinium dinitrate (lucigenin)[2] emitted a bright chemiluminescence in an alkaline solution with hydrogen peroxide; they proposed an interesting mechanism which involves peroxide and diol of dimethylbiacridane as intermediates. Subsequently mechanisms of the chemiluminescence of lucigenin were proposed by several investigators.[3] Some of the mechanisms involved a free radical; Tamamushi[4] proposed a mechanism involving a biradical of dimethylbiacridene which combines with molecular oxygen to form a peroxide responsible for the emission of light, and Kariakin[5] proposed a mechanism involving a biradical responsible for chemiluminescence and concluded that the chemiluminescence was the phosphorescence. However, the mechanism remains to be established.

As part of a study of the mechanism of the chemiluminescence of lucigenin, the spectra of the chemiluminescence, fluorescence and absorption were measured. Furthermore, the electron spin resonance was measured, because, on considering the proposed mechanism mentioned above[4,5] and the free radical mechanism of the chemiluminescence of lophine,[6-9] the mechanism of the chemiluminescence of lucigenin seemed likely to be the free radical mechanism.

Experimental

Material. Lucigenin was prepared by the method of Decker and Dunant[10] from 10-methylacridone, which had been obtained from acridine. It was recrystallized from water and dried at 80°C under reduced pressure, thus obtaining yellow ocher leaflets which did not melt below 350°C; λ_{max} (water) 261 mμ (ε 2.2×10^5), 353 mμ (ε 2.0×10^4), 369 mμ (ε 4.5×10^4), 432 mμ (ε 1.2×10^4).

Found: C, 65.40; H, 4.31; N, 10.94%. Calcd for $C_{28}H_{22}O_6N_4$: C, 65.87; H, 4.34; N, 10.98%.

Measurements. The spectrum of the chemiluminescence of lucigenin which had been produced in an aqueous solution of sodium hydroxide with hydrogen peroxide at room temperature was measured by a

*1 This work was reported at the 15th Symposium of the Reaction Mechanism of Organic Reactions of the Chemical Society of Japan, Nagoya, October, 1964.

1) K. Gleu and W. Petsch, *Angew. Chem.*, **48**, 57 (1935).

2) H. Decker and W. Petsch, *J. Prakt. Chem.*, **143**, 211 (1935).

3) R. M. Acheson, "Acridines," Interscience Publisher, New York (1956), p. 277.

4) B. Tamamushi and H. Akiyama, *Trans. Faraday Soc.*, **35**, 491 (1939). These authors found that a solution of lucigenin showed chemiluminescence with only molecular oxygen dissolved in the solution; this chemiluminescence was marked at higher temperatures.

5) A. V. Kariakin, *Opt. Spectry. USSR (English Transl.)*, **7**, 75 (1959).

6) T. Hayashi and K. Maeda, This Bulletin, **35**, 2057 (1962).

7) K. Maeda, H. Ojima and T. Hayashi, *ibid.*, **38**, 76 (1965).

8) J. Sonnenberg and D. M. White, *J. Am. Chem. Soc.*, **86**, 5685 (1964).

9) E. H. White and M. J. C. Harding, *ibid.*, **86**, 5686 (1964).

10) H. Decker and G. Dunant, *Ber.*, **42**, 117 (1909).

Hitachi recording spectrophotometer, EPS 2 type, equipped with an apparatus for measuring the spectra of the fluorescence and chemiluminescence of a solution. The details of the apparatus and the procedure for the measurement have been described in a previous paper[7] in which the spectra of the chemiluminescence, fluorescence and absorption of 2, 4, 5-triphenylimidazole (lophine) were reported. Because the intensity of the chemiluminescence scarcely decreased for about 1 min after the reagents had been mixed in order to obtain an emission at concentrations of about 10^{-6}—10^{-3} mol/*l* of lucigenin, the chemiluminescence spectrum was measured with a sweeping time of about 25 sec for a wavelength range of about 420—650 mμ over which the spectrum spread. In order to confirm the wavelength of the peak of the spectrum, a part of the spectrum, about 470—500 mμ, where the spectrum showed a peak, was measured by recording it with a sweeping time of about 5 sec.

The spectra of the fluorescence and the absorption of lucigenin were measured by the same spectrophotometer in both an aqueous solution and an alkaline solution at room temperature. The excitation was carried out with light of 365 mμ of a high-pressure mercury-vapor lamp.

The electron spin resonance of lucigenin was measured in a solid state at room temperature with an electron spin resonance spectrophotometer (JES-3B type[11]) of the Japan Electron Optics Laboratory.

Results and Discussion

The Color and Spectrum of the Chemiluminescence. Solutions of lucigenin in aqueous sodium hydroxide emitted a green chemiluminescence at rather high concentrations of lucigenin, such as 0.1% (2×10^{-3} mol/*l*) or higher, with hydrogen peroxide at room temperature. Although the intensity of the chemiluminescence at the concentrations mentioned above decreased slowly as a result of a decrease in the concentration of lucigenin caused by the chemiluminescent reaction, the color scarcely changed during about a 5-min period. However, when the concentration of lucigenin was varied from about 2×10^{-3} to 1×10^{-6} mol/*l*, the color of the chemiluminescence changed from green to light blue.[12]

The chemiluminescence spectrum of lucigenin which was emitted with hydrogen peroxide in a 0.12 N aqueous solution of sodium hydroxide was measured at various concentrations of lucigenin, 1.0×10^{-6}—2.0×10^{-4} mol/*l* at 23°C. A 0.5 N aqueous solution of sodium hydroxide (2 m*l*) and a 3% aqueous solution of hydrogen peroxide (2 m*l*) were added to 4 m*l* of aqueous solutions of lucigenin of 2.0×10^{-6}—4.0×10^{-4} mol/*l*[13] respectively at room temperature, and the mixture was quickly mixed well, and then poured into a cell of the spectrophotometer in order to measure the chemiluminescence spectrum immediately.

The chemiluminescence spectrum spread over the visible region of only about 420—650 mμ, with a peak. The wavelength of the peak varied from 485 to 510 mμ[14] as the concentration of lucigenin increased from 1.0×10^{-6} to 2.0×10^{-4} mol/*l*, as is shown in Fig. 1.

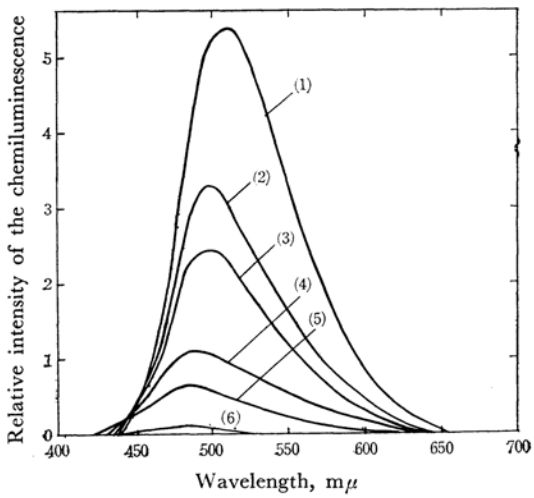

Fig. 1. The chemiluminescence spectra of lucigenin.
The concentration of lucigenin (the wavelength of the peak):
(1) 2.0×10^{-4} mol/*l* (510 mμ)
(2) 1.0×10^{-4} mol/*l* (498 mμ)
(3) 5.0×10^{-5} mol/*l* (498 mμ)
(4) 1.0×10^{-5} mol/*l* (488 mμ)
(5) 5.0×10^{-6} mol/*l* (485 mμ)
(6) 1.0×10^{-6} mol/*l* (485 mμ)
Temp.: 23°C

The shift of the peak of the chemiluminescence spectra which correspons to the change in the chemiluminescence color mentioned above is likely to be due to the partial self-absorption of the

11) X band; modulation frequency, 100 KC; maximum field intensity, 10000 gauss.

12) Although it is difficult to express the color correctly just as observed and although, furthermore, the decrease in the intensity makes the expression of the color difficult, it is certain that the color at higher concentrations varies from that at lower concentrations.

13) At concentrations higher than about 2×10^{-3} mol/*l*, reddish-brown precipitates were formed soon after a solution of lucigenin had been mixed with a 0.12 N sodium hydroxide solution.

14) B. Tamamushi (Scientific Papers of the Institute of the Physical and Chemical Research, **41**, 166 (1943)) reported that the spectrum of the chemiluminescence of lucigenin which was emitted at concentrations of 2.5×10^{-3}—2.0×10^{-2} mol/*l* in a sodium hydroxide solution with hydrogen peroxide in the presence of osmium tetraoxide at room temperature had been measured by a photographic method to show a peak at about 520 mμ. Kariakin[5] reported that the chemiluminescence spectrum of lucigenin (dibromide) measured by a spectrophotometric method showed a peak at 515 mμ and a small shoulder at 480 mμ. These peaks of the spectra are those of the apparent spectra distorted by self-absorption at rather high concentrations.

chemiluminescence spectrum by a yellow solution of lucigenin in aqueous sodium hydroxide, because the yellow solution showed strong absorption over a wavelength region shorter than about 490 mμ, a region which overlaps the peak of the chemiluminescence spectrum of lucigenin, as will be described in the following section. Consequently, it may be concluded that the 5 and 6 spectra of Fig. 1, showing a peak at 485 mμ, are the true chemiluminescence spectra of lucigenin, whereas the 4—1 spectra which showed the peak at 488 and 510 mμ respectively, are the apparent spectra absorbed by lucigenin.[15]

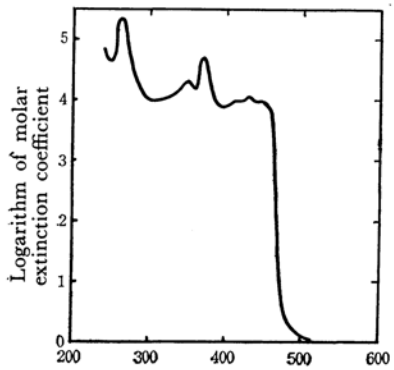

Fig. 2. The absorption spectrum of lucigenin in an aqueous solution.[16]
Concentration: 1.06×10^{-5} mol/l

The Absorption Spectrum. The absorption spectrum of lucigenin in an aqueous solution is shown in Fig. 2 and its absorption maxima are given in Table 1. The aqueous solution of lucigenin obeyed Beer's law in a concentration range of 1.1×10^{-5}—1.1×10^{-2} mol/l.

TABLE 1. THE ABSORPTION MAXIMA OF LUCIGENIN IN AN AQUEOUS SOLUTION (CONCENTRATION: 1.06×10^{-5} mol/l) AND THEIR MOLAR EXTINCTION COEFFICIENTS

λ_{max}, mμ	ε
261	2.2×10^{5}
353	2.0×10^{4}
369	4.5×10^{4}
412 (sh)	9.2×10^{3}
432	1.2×10^{4}
435 (sh)	9.3×10^{3}

The absorption spectrum in an aqueous solution did not change at room temperature with time, whereas the spectrum in aqueous sodium hydroxide changed slowly.

The absorption spectrum in a 0.12 N sodium hydroxide solution at a concentration of 3.8×10^{-5} mol/l which was measured immediately after the preparation of the solution showed absorption maxima at 261, 353, 369, 410(sh), 432 and 453(sh) mμ, as is shown in Fig. 3(a).

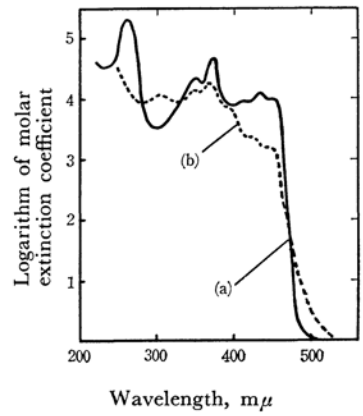

Fig. 3. The absorption spectra of lucigenin in a sodium hydroxide solution.
Concentration of lucigenin: 3.8×10^{-5} mol/l in a 0.12 N sodium hydroxide solution
(a) Immediately after the preparation of the solution
(b) One hour after the preparation of the solution

The absorption maxima scarcely changed when the concentration of sodium hydroxide was varied.

The spectrum shown in Fig. 3(a) slowly changed to the spectrum shown in Fig. 3(b) after about 1 hr. The change in the spectrum was accelerated by irradiation.

The spectrum shown in Fig. 3(b) is likely to be the spectrum of the dark brown component of the reddish-brown precipitate,[17] which were yielded when a sodium hydroxide solution with a rather

15) The peaks of the chemiluminescence spectrum, 520 and 515 mμ, which were measured by Tamamushi[14] and Kariakin[5] respectively, are likely to be further shifted from 510 mμ because of such high concentrations of lucigenin as 10^{-2}—10^{-3} mol/l.

16) The absorption spectra of lucigenin measured at concentrations of 1.0×10^{-6}—1.0×10^{-5} mol/l in 0.1 N nitric acid were almost identical with the spectrum measured in the aqueous solution, and the molar extinction coefficients of the absorption maxima in the solution in 0.1 N nitric acid almost agreed with those in the aqueous solution.

17) Gleu and Petsch[1] reported that the solution of lucigenin in aqueous sodium hydroxide yielded reddish precipitates; they are likely to be the same as the reddish-brown precipitates mentioned above. The precipitates were separated into a yellow ocher solid, melting at 352—355°C, slightly soluble in ethanol and insoluble in water, and a dark brown solid, melting at about 220—240°C (eff.), soluble in ethanol and water. The relative amount of the latter appeared to be a little smaller than that of the former. Both compounds contain nitrogen; the former does not contain oxygen, whereas the latter does. The formation of these solids did not require molecular oxygen, because the solid are yielded in an oxygen-free solution.

high concentration was added to the solution of lucigenin.

The Color and Spectrum of the Fluorescence. In an aqueous solution lucigenin emitted a strong fluorescence. Its color was green, irrespective of the concentration, and it did not change with time.

The fluorescence spectra measured in aqueous solutions of 2.46×10^{-5}—4.93×10^{-3} mol/l at room temperature showed a maximum at 508—510 mμ and a shoulder at 485 mμ, as is shown in Fig. 4.[18]

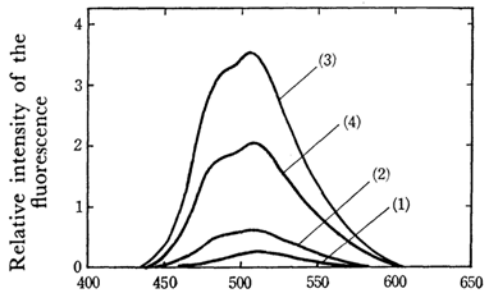

Fig. 4. The fluorescence spectra of lucigenin in aqueous solutions.
Exciting light: 365 mμ
Concentration of lucigenin (wavelength of the peak):
(1) 4.93×10^{-3} mol/l (510 mμ)
(2) 4.93×10^{-4} mol/l (509 mμ)
(3) 4.93×10^{-5} mol/l (508 mμ)
(4) 2.46×10^{-5} mol/l (508 mμ)

The color of the fluorescence scarcely changes when the concentration of lucigenin is varied, because the absorption spectrum of lucigenin scarcely overlaps at all with the wavelength region where the peak of the fluorescence spectrum lies.

In aqueous sodium hydroxide lucigenin also emitted fluorescence. The color was green just after the beginning of irradiation with the exciting light, irrespective of the concentration of lucigenin, although the intensity was far weaker than that in the aqueous solution and the color of the fluorescence rapidly changed from green to blue.

The rate of the change in the color was accelerated by increasing the concentration of sodium hydroxide, decreasing the concentration of lucigenin, and increasing the quantity of the exciting light. The change in the color is due to the photooxidation of lucigenin to 10-methylacridone with oxygen under irradiation, as will be described later.

The fluorescence spectra of lucigenin at various concentration of 3.8×10^{-6}—3.8×10^{-3} mol/l in a 0.12 N sodium hydroxide solution were measured immediately after the solutions had been prepared by mixing aqueous solutions of lucigenin with a 0.5 N aqueous solution of sodium hydroxide. The spectra showed a peak at 508 mμ, as Fig. 5 shows.

The fluorescence spectra in both aqueous solutions and aqueous sodium hydroxide are covered by the absorption spectra of lucigenin in a wavelength region shorter than about 490 mμ where

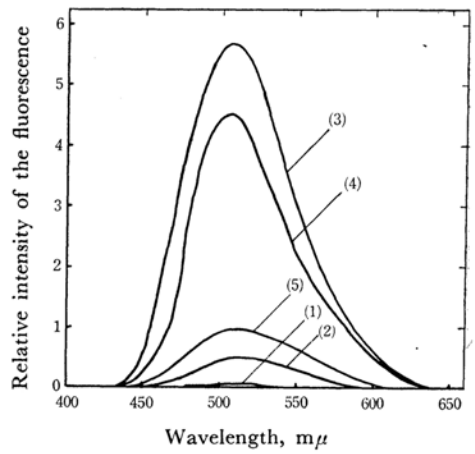

Fig. 5. The fluorescence spectra of lucigenin in a 0.12 N aqueous solution of sodium hydroxide just after the preparation of the solution.
Exciting light: 365 mμ
Concentration of lucigenin:
(1) 3.8×10^{-3} mol/l
(2) 3.8×10^{-4} mol/l
(3) 3.8×10^{-5} mol/l
(4) 1.9×10^{-5} mol/l
(5) 3.8×10^{-6} mol/l

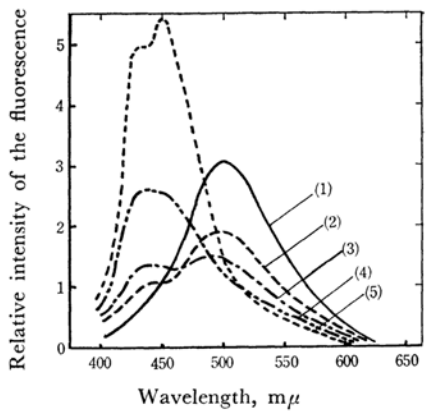

Fig. 6. The variation in the fluorescence spectra of lucigenin in 0.12 N aqueous sodium hydroxide with time.
(1) just after the preparation of the solution
(2) about 1 min after the preparation
(3) about 4 min after the preparation
(4) about 10 min after the preparation
(5) about 20 min after the preparation

18) Kariakin[5] reported that lucigenin (dibromide) showed maxima at 515 and 480(sh) mμ in a 0.5% aqueous solution.

a distortion of the fluorescence spectra occurs as a consequence of self-absorption. However, because the peaks of the fluorescence spectra in the solutions do not lie in that region, the peaks are considered to change scarcely at all with an increase in the concentration of lucigenin.

In aqueous sodium hydroxide the fluorescence spectrum changed with time, as is shown in Fig. 6. The intensity of the peak at 508 mμ rapidly decreased with a shift of the wavelength to a shorter wavelength, and simultaneously a new peak appeared at about 440 mμ, with a rapid increase in the intensity. After about 10 min the original peak disappeared and a shoulder appeared at about 450 mμ. After about 20 min the spectrum showed the peaks at 435 and 450 mμ.

The new peaks at 435 and 450 mμ almost agreed with the peaks at 438 and 453 mμ of the fluorescence spectrum of 10-methylacridone.[19] Because this compound has been isolated[20] from the solution used in the measurement of the fluorescence spectrum in sodium hydroxide, the new peaks may be said to be due to 10-methylacridone.[21] This shows that the changes in the fluorescence color and in the fluorescence spectrum of lucigenin in aqueous sodium hydroxide with time are due to the oxidation of lucigenin to 10-methylacridone under irradiation, probably with oxygen dissolved in the solution.

From these results, it may be concluded that the chemiluminescence of lucigenin is neither the fluorescence of lucigenin nor that of 10-methylacridone.

The Electron Spin Resonance. Because the free radical mechanisms of the chemiluminescence had been proposed in the cases of lucigenin[4,5] and lophine,[6-9] the electron spin resonance of lucigenin was investigated in a solid state. Crystals of lucigenin which had been recrystallized three times from water and dried at about 80°C under reduced pressure exhibited an electron spin resonance of a single peak with a line width of about 10 gauss. Its g-value was 2.004. The spin concentration was about 1×10^{14} spin/g; this scarcely varied at all with further, repeated recrystallization. The signal intensity increased under irradiation with sunlight; it was, for example, 2.4 times larger after irradiation for 4 hr. When the irradiated solid was set aside in the dark for 2 days after irradiation, the intensity gradually subsided to the original intensity.

The reddish-brown precipitates mentioned above[17] exhibited an electron spin resonance of a single peak, while the yellow ocher solid[22] and the dark brown solid into which the reddish-brown precipitates were separated, as has been mentioned above,[17] also exhibited an electron spin resonance in a solid state. The spin concentrations of the reddish-brown precipitates and both the solids mentioned above were much larger than that of lucigenin.

These results suggest a free radical mechanism of the chemiluminescence of lucigenin.

The authors wish to express their hearty thanks to Dr. Hiroshi Midorikawa of the Institute of Physical and Chemical Research for his kind help, and to the Ministry of Education for the financial support granted this research.

19) The fluorescence spectrum of 10-methylacridone was measured in aqueous sodium hydroxide containing ethanol, because it is nearly insoluble in aqueous sodium hydroxide.

20) Considering the formation of 10-methylacridone by the photooxidation of lucigenin with oxygen, the yield of this compound was almost quantitative.

21) From the chemiluminescent solution of lucigenin, this compound was isolated by Gleu and Petsch[1] and also by the present authors.

22) By repeated recrystallizations from pyridine, the yellow ocher solid became bright yellow crystals, mp 357—359°C, which were confirmed to be identical with 10, 10′-dimethylbiacridene; they exhibited no electron spin resonance.