The Chemiluminescence, Fluorescence and Absorption Spectra of 2, 4, 5-Triphenylimidazole

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In 1877 Radziszewski[1] found that 2, 4, 5-triphenylimidazole (lophine) exhibits chemiluminescence in ethanolic potassium hydroxide with oxygen at room temperature, yielding benzoic acid and ammonia. Trautz[2] later reported that lophine exhibited a brighter chemiluminescence with such ordinary oxidizing agents as aqueous solutions of hydrogen peroxide, chlorine, bromine and potassium ferricyanide. Cottman, Moffet and Moffet[3] and Cook and Jones[4] investigated the chemiluminescence of several substituted derivatives of lophine. However the mechanism of the chemiluminescence of lophine has never been studied. We have, therefore, studied the mechanism of the chemiluminescence of lophine for several years and have published[5] a mechanism involving the triphenylimidazolyl radical. molecular oxygen and peroxide. The details of our study of the mechanism of the chemiluminescence of lophine will be reported in the following papers.

Cottman and his collaborators[3] measured the chemiluminescence spectra of lophine and several of its substituted compounds with a photographic method and reported that those spectra were spread over 480—600 mμ, with a single maximum near 530 mμ. No details of the spectra, however, were shown. We measured the chemiluminescence spectra of lophine and several of its substituted compounds with a recording spectrophotometer and also measured the spectra of fluorescence and absorption. The present paper deals with the results obtained on those spectra of lophine.

1) B. Radziszewski, *Ber.*, **10**, 70 (1877).
2) M. Trautz, *Z. Physik. Chem.*, **53**, 86 (1905).
3) E. W. Cottman, R. B. Moffet and S. M. Moffet, *Proc. Indiana Acad. Sci.*, **47**, 133 (1938); *Chem. Abstr.*, **32**, 9081 (1938).
4) A. H. Cook and D. G. Jones, *J. Chem. Soc.*, **1941**, 278.
5) T. Hayashi and K. Maeda, This Bulletin, **35**, 2057 (1962).

Experimental

Lophine was prepared by the method of Davidson, Weiss and Jelling.[6] It was recrystallized from ethanol as colorless fine needles, m. p. 271—272°C.

The Chemiluminescence Spectrum. — The chemiluminescence spectrum of lophine in 1 N ethanolic potassium hydroxide and oxygen was measured with the Hitachi recording spectrophotometer, EPS 2 type, equipped with an apparatus for measuring the chemiluminescence spectrum of a solution. The equipment and the procedure for measuring the chemiluminescence spectrum are as follows: lophine (0.5 g.) was added to 12 ml. of 1 N ethanolic potassium hydroxide in a quartz tube 1.5 cm. inner diameter and 25 cm. long. A cork at the upper end of the tube was provided with a small reflux condenser, a thermometer and a narrow glass tube for streaming gas into the solution in the tube. When the mixture in the tube was stirred by a nitrogen stream for a while, a pale yellow solution of lophine ($C=2.81\times10^{-1}$ mol./l.) was obtained. Then the tube containing the solution was put in a metallic tube, about 2 cm. in inner diameter, fixed in the center of a small metallic cylindrical thermostat which had been set in place of a light source. Hot water was circulated throughout this thermostat from another one in order to heat the solution in the tube at 70°C. Small windows both on an outer wall of the cylindrical thermostat and on a wall of the metallic tube containing the quartz tube which contained a solution of lophine, were connected with a narrow horizontal metallic tube, through which a light of chemiluminescence was introduced into the spectrophotometer. When the temperature of the solution of lophine became 70°C, a gentle stream of heated oxygen was passed into the solution. A chemiluminescence was exhibited at once; the spectrum was immediately measured by the recording spectrophotometer.

The Fluorescence Spectra. — The fluorescence spectra of lophine were measured with the spectrophotometer equipped with an apparatus for measuring fluorescence spectra. The 365 mμ line of a high

6) D. Davidson, M. Weiss and M. Jelling, *J. Org. Chem.*, **2**, 319 (1937).

pressure mercury lamp was used for the excitation.

The Absorption Spectra.—The absorption spectra of lophine were measured with the spectrophotometer.

Results and Discussion

The Chemiluminescence Spectra of Lophine.—An ethanolic potassium hydroxide solution of lophine was used for measuring the chemiluminescence spectrum, since lophine was not dissolved in water. Like luminol and lucigenin, lophine exhibited chemiluminescence only in an alkaline medium. The light of the chemiluminescence of lophine with oxygen or with an aqueous solution of hydrogen peroxide was greenish blue, and the light could be detected even at the concentration of 10^{-5} mol./l. However, the intensity of chemiluminescence at room temperature was so weak that the chemiluminescence spectrum could not be measured by the recording spectrophotometer.

The intensity of the chemiluminescence with oxygen increased with a rise in the temperature. For example, a 2 N ethanolic potassium hydroxide solution of lophine ($C=1.6\times10^{-2}$ mol./l.) did not emit luminescence at -15°C. It did, however, emit a very weak luminescence at 0°C; the intensity increased with a rise in the temperature to about 80°C, at which point the solution began to boil, resulting in a rapid decrease in the intensity of the luminescence. Since the intensity was recovered by passing oxygen into the boiling solution, the rapid decrease in luminescence by boiling was obviously due to the loss of oxygen from the solution. These results indicated that the optimum temperature for measuring the chemiluminescence spectrum of lophine was 70—75°C.

In the chemiluminescence of lophine (concentration 10^{-1}—10^{-2} mol./l.) in 1—2 N ethanolic potassium hydroxide at room temperature, with oxygen which had previously been saturated, it was found that the logarithum of the total intensity of the chemiluminescence on an arbitrary scale linearly decreased with time for about ten minutes, that is at a rate probably of the first order with respect to the concentration of oxygen, whereas in the chemiluminescence with oxygen continuously passed into the solution, it was found that the total intensity hardly decreased at all for several minutes.

In view of these results, the chemiluminescence spectrum was measured by passing oxygen into a solution ($C=2.81\times10^{-1}$ mol./l.) in 1 N ethanolic potassium hydroxide at 70°C. The spectrum is shown in Fig. 1. It spreads over only a visible region, about 465—660 mμ, with a maximum at about 545 mμ. The time of the sweeping of the recorder from 450 to 680 mμ was 24 sec. Although it was found that the intensities of chemiluminescence just before and after measurement hardly decreased, resulting in no shift in the maximum, a part of the spectrum between about 520—590 mμ, centering about the maximum, was repeatedly measured with a sweeping time of 7 sec.; thus it was ascertained that the maximum was at 545 mμ.

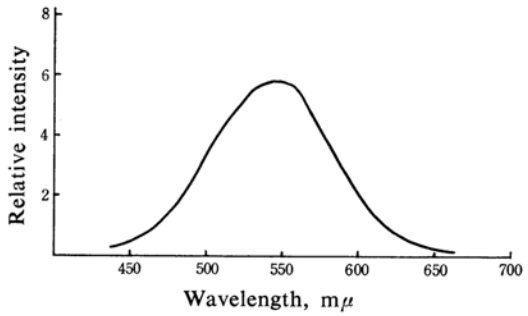

Fig. 1. The chemiluminescence spectrum of lophine in 1 N ethanolic potassium hydroxide ($C=2.81\times10^{-1}$ mol./l.) exhibited by passing oxygen through at 70°C.

The maximum wavelength, 545 mμ, in the chemiluminescence spectrum is more than 100 mμ longer the maximum wavelength, 441mμ, in the fluorescence spectrum of lophine and that, 440 mμ, of the fluorescence spectrum of benzoic acid, which is a product of the chemiluminescence of lophine. Therefore, the chemiluminescence of lophine is neither the fluorescence of lophine nor the fluorescence of benzoic acid. It is most likely a radiation emitted from some substance in an excited state which is probably produced by reactions involving the 2,4,5-triphenylimidazolyl radical produced from lophine and molecular oxygen. We now study the constitution of the intermediate compound emitting luminescence.

When a 1 N ethanolic potassium hydroxide solution of lophine was heated at 70°C on a water bath while being stirred by a stream of oxygen in the light, fine colorless crystals gradually appeared in the solution and ammonia was detected with a Nessler reagent in 0.1 N sulfuric acid by which the oxygen which had passed through the solution of lophine was washed. The fine crystals which separated were identified as potassium benzoate. In addition to benzoate and ammonia, sometimes a small quantity of fine colorless needles was isolated and then recrystallized from ethanol as fine needles, m. p. 231—232°C. This compound was identified as 2,4,6-triphenyl-1,3,5-triazine (cyaphenine). It was also obtained when chemiluminescence was exhibited with

ethanolic solution of bromine, its yield increasing when the solution of lophine was saturated with ammonia before the addition of bromine. These facts, and the formation of lophine by the reduction of cyaphenine with zinc dust in acetic acid,[4] suggest the formation of cyaphenine on the chemiluminescence is likely to be due to a reaction involving lophine and ammonia produced by the oxidation of lophine on the cheminescence.

The chemiluminescence spectrum with a 3% aqueous solution of hydrogen peroxide at 70°C was also measured with the recording spectrophotometer. Two milliliters of hydrogen peroxide heated at 70°C was quickly stirred into a solution of 0.72 g. of lophine in 12 ml. of 1 N ethanolic potassium hydroxide ($C=2.04\times10^{-1}$ mol./l.) heated at 70°C in the tube used for the measurement of the spectrum with oxygen; the spectrum was then immediately recorded. It spreads over 480—650 mμ, with a maximum at 555 mμ. When a 1 N ethanolic potassium hydroxide solution containing 0.5 g. lophine was heated at 70°C with 3% hydrogen peroxide for about 30 min. in another experiment, benzoic acid (0.3 g.) and ammonia were produced.

The measurement of the chemiluminescence spectrum with bromine was unsuccessful because of coloration by bromine. However, an important clue to the mechanism of chemiluminescence of lophine was obtained. When an ethanolic solution of bromine was added very slowly, drop by drop, to a hot solution of lophine in ethanolic potassium hydroxide without stirring, a reddish-purple color at once appeared around the part where a drop of a bromine solution was added; this color rapidly disappeared however, with the formation of a white precipitate, which was identified as potassium bromide. When a similar experiment was carried out in a dark room, chemiluminescence was observed around the part where a drop of a solution of bromine was added. The part where chemiluminescence was observed obviously corresponded to that where a reddish-purple color had appeared. In the chemiluminescence of lophine with a 2% aqueous solution of potassium ferricyanide, a reddish-purple color also appeared. When the dissolved oxygen in the solutions was removed, no chemiluminescence was observed, although the reddish-purple color appeared with the addition of bromine or potassium ferricyanide. Therefore, molecular oxygen was necessary for the chemiluminescence with bromine or potassium ferricyanide. From these facts and some other investigations concerning this coloration,[7] it was established that the reddish-purple color was due to the 2, 4, 5-triphenylimidazolyl radical produced by the oxidation of the lophine anion with bromine or potassium ferricyanide, and that the disapperance of the color was due to the combination of the free radical with oxygen in the solution to form an adduct which was probably in the following illuminating stage. The 2, 4, 5-triphenylimidazolyl radical was reasonably considered to be the intermediate also in the chemiluminescence with oxygen or hydrogen peroxide, although no reddish-purple color was observed in these cases.

The Fluorescence Spectra of Lophine. — The fluorescence spectra of lophine were measured at 20°C in ethanolic potassium hydroxide, in ethanol, and in ethanol containing acetic acid. In order to exclude a shift of the maximum of a spectrum by the oxidation of lophine, the dissolved oxygen in the solution was removed by a stream of nitrogen previously. The fluorescence spectra of lophine are shown in Fig. 2.

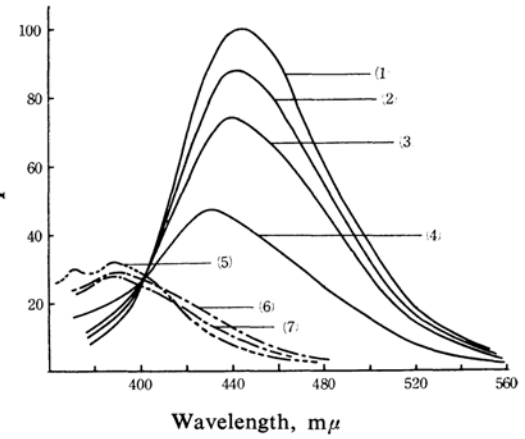

Fig. 2. The fluorescence spectra of lophine in ethanolic potassium hydroxide, in ethanol, and in ethanol containing acetic acid at 20°C.
I: relative intensity of fluorescence
Concn. of lophine: $C=4.05\times10^{-5}$ mol./l.
(1) 2 N KOH-C_2H_5OH, λ_{max} 444 mμ
(2) 1 N KOH-C_2H_5OH, λ_{max} 441 mμ
(3) 0.2 N KOH-C_2H_5OH, λ_{max} 439 mμ
(4) 0.02 N KOH-C_2H_5OH, λ_{max} 432 mμ
(5) C_2H_5OH, λ_{max} 371 mμ, 388 mμ
(6) 1 N CH_3COOH-C_2H_5OH, λ_{max} 391 mμ
(7) 0.01 N CH_3COOH-C_2H_5OH, λ_{max} 389 mμ

The maximum intensity at 440 mμ of the fluorescence spectrum of lophine in 0.4 N ethanolic potassium hydroxide varied with the increase in the concentration of lophine at 25°C, as is shown in Fig. 3.

During the measurement of the fluorescence spectrum of lophine, a logarithum of the

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

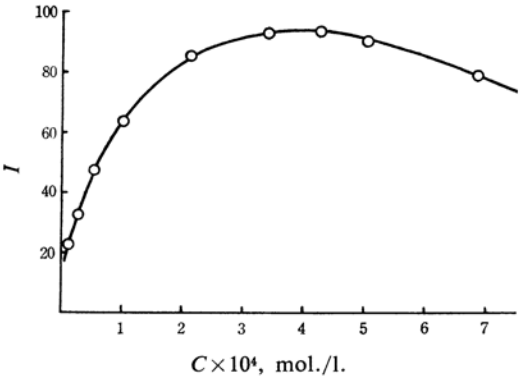

Fig. 3. The effect of the concentration of lophine on the intensity at 440 mμ of the fluorescence spectrum of lophine in 0.4 N ethanolic potassium hydroxide at 25°C.
I: relative intensity of fluorescence at 440 mμ
C: the concn. of lophine in a solution of 0.4 N ethanolic potassium hydroxide

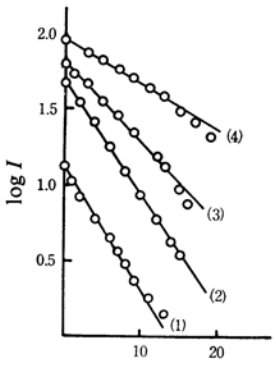

Fig. 4. The decrease with time in relative intensity at 440 mμ of the fluorescence spectrum of lophine in 0.4 N ethanolic potassium hydroxide at 25°C.
I: relative intensity of fluorescence at 440 mμ
Concn. of lophine:
(1) 5.05×10^{-6} mol./l. (3) 1.01×10^{-4} mol./l.
(2) 5.05×10^{-5} mol./l. (4) 3.40×10^{-4} mol./l.

maximum intensity at 440 mμ linearly decreased with the time of irradiation, as is shown in Fig. 4. When the dissolved oxygen in the solution was removed by a stream of nitrogen, no decrease in the intensity of fluorescence was observed, but when a stream of oxygen was passed into the solution, the rate of the decrease of fluorescence increased. Hence, the decrease of fluorescence is obviously involved with the oxidation with oxygen, and the rate of decrease is first order with respect to the concentration of oxygen in the solution.

The rate of the decrease of fluorescence was increased when the quantity of light illuminated was augmented by an increase in the area of a window on the wall of a cell containing the solution, but it was hardly affected by a rise in the temperature from 10 to 30°C. Therefore, the oxidation of the lophine responsible for the decrease in fluorescence was likely to be photochemical.

The tendency for the rate of the decrease of fluorescence to decrease with an increasing concentration of lopine, as shown in Fig. 4, can be understood from the relation between the relative intensity of fluorescence and the concentration of lophine which is shown in Fig. 3.

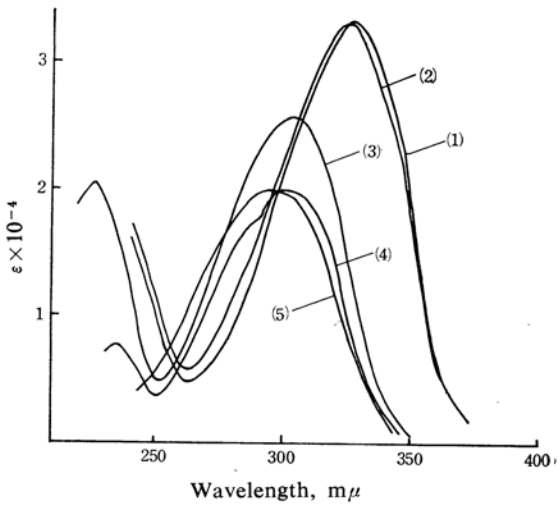

Fig. 5. The absorption spectra of lophine in ethanolic potassium hydroxide, and in ethanolic solutions of acetic acid at 20°C.
Concn. of lophine:
(1) 4.08×10^{-5} mol./l. in 1 N KOH-C_2H_5OH
(2) 4.18×10^{-5} mol./l. in 0.1 N KOH-C_2H_5OH
(3) 3.91×10^{-5} mol./l. in ethanol
(4) 4.12×10^{-5} mol./l. in 0.1 N CH_3COOH-C_2H_5OH
(5) 4.13×10^{-5} mol./l. in 1 N CH_3COOH-C_2H_5OH

When the fluorescence of lophine in such a dilute ethanolic potassium hydroxide as 0.5 N was exhibited with or without a gentle stream of oxygen at room temperature, a small quantity of cyaphenine was isolated from a solution in which fluorescence had nearly ended. When fluorescence was exhibited in a rather concentrated ethanolic potassium hydroxide such as 1.8 N with a stream of oxygen at room temperature, a small quantity of benzoic acid was isolated and ammonia was detected. Cyaphenine was probably formed from lophine and ammonia which had been formed by the oxidation of lophine. Considering the facts that cyaphenine was very weakly fluorescent in the solution and that benzoic acid was also

weakly fluorescent, the decrease in the intensity of the fluorescence of lophine with time may be attributed to the photochemical oxidation of lophine with oxygen to give benzoic acid and ammonia.

The Absorption Spectra of Lophine. — The absorption spectra of lophine were measured in ethanol, in ethanolic potassium hydroxide, and in ethanolic solution of acetic acid at 20°C. The spectra are shown in Fig. 5.

The absorption maximum, λ_{max} 303 mμ ($\varepsilon = 2.56\times10^4$), in ethanol was shifted to 326 mμ ($\varepsilon = 3.33\times10^4$) in 1 N ethanolic potassium hydroxide; conversely, 303 mμ was shifted to 295 mμ ($\varepsilon = 2.03\times10^4$) in a 1 N ethanolic solution of acetic acid. The shift in ethanolic potassium hydroxide is probably due to the dissociation of the NH of an imidazole ring.

Summary

The chemiluminescence spectra of 2, 4, 5-triphenylimidazole (lophine) in 1 N ethanolic potassium hydroxide exhibited with oxygen and with hydrogen peroxide at 70°C have been measured. Both spectra spread over about 470—660 mμ; the maximum wavelengths are 545 mμ and 555 mμ respectively.

The fluorescence spectra of solutions of lophine have been measured at 70°C. The maximum wavelengths are as follows: 371 mμ, 388 mμ (ethanol), 441 mμ (1 N KOH–C_2H_5OH) and 391 mμ (1 N CH_3COOH–C_2H_5OH).

The absorption spectra of lophine have also been measured: λ_{max} 303 mμ ($\varepsilon = 2.56\times10^4$) in ethanol: λ_{max} 326 mμ ($\varepsilon = 3.33\times10^4$) in 1 N ethanolic potassium hydroxide, and λ_{max} 295 mμ ($\varepsilon = 2.03\times10^4$) in a 1 N solution of acetic acid in ethanol.

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