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The Chemiluminescence of Indole Derivatives. III. On the Mechanism of the Chemiluminescence of 2,3-Dimethylindole and 5-Substituted-2,3-dimethylindoles in the Dimethyl Sulfoxide-Alkali System

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A bright green light is observed on the treatment of a solution of 2,3-dimethylindole (I) and its hydroperoxide (II) in dimethyl sulfoxide (DMSO) with alkali (potassium *t*-butoxide or granular potassium hydroxide) under streams of oxygen and hydrogen respectively. The change in their ultraviolet and fluorescent spectra with the time and the chemiluminescent spectra is measured. The compound II is concluded to be the reaction intermediate of I; it decomposes to yield an excited singlet state of an *o*-acetamidoacetophenone anion (III*), a light emitting species in this reaction. The investigation, then extended to four 5-substituted-2,3-dimethylindoles, revealed that three of them displayed chemiluminescence through the same mechanism as in the case of I.

In relation to the luciferin of the bioluminescent marine organisms,1-3) the chemiluminescence of indole derivatives is an especially interesting phenomenon; that of various simple indoles has recently been reported.4-6)

We studied the relative light intensities of forty-one indole derivatives in the following two systems.7)

(A) Sample-EtOH-KOH-H_2O_2-$K_3Fe(CN)_6$

(B) Sample-DMSO-KOH-AIR

We found that 2,3-dimethylindolenyl-3-hydroperoxide (II) emitted a bright flash of light in the B system. Although McCapra *et al.* suggested

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1) Y. Kishi, T. Goto, Y. Hirata, O. Shimomura and F. H. Johnson, "Bioluminescence Conference at Hakone, 1965," ed. by F. H. Johnson and Y. Haneda, Princeton University Press, Princeton, New Jersey (1966), p. 88.

2) M. J. Cormier and C. B. Eckrode, *Biochim. Biophys. Acta*, **64**, 340 (1962).

3) M. J. Cormier and L. S. Dure, *J. Biol. Chem.*, **238**, 790 (1963).

4) G. E. Philbrook, J. B. Ayers, J. F. Garst and J. R. Totter, *Photochem. Photobiol.*, **4**, 869 (1965).

5) A. W. Berger, J. N. Driscoil and J. A. Pirog, *ibid.*, **4**, 1123 (1965).

6) E. H. White and M. J. C. Harding, *ibid.*, **4**, 1129 (1965).

7) N. Sugiyama, M. Akutagawa, T. Gasha, H. Yamamoto and Y. Saiga, This Bulletin, **40**, 347 (1967).

the intermediacy of a indolyl-3-hydroperoxide and a hydroperoxide decomposition mechanism accompanying a light emission,8,9) the mechanism of the chemiluminescence has remained obscure.

In a preceding paper, we proposed a mechanism of the chemiluminescence of the indole derivative involving the hydroperoxide as an intermediate.10) In the present paper, the mechanisms of 2,3-dimethylindole and 5-substituted-2,3-dimethylindoles will be discussed in detail in the light of the spectral studies.

Experimental

2,3,5-Trimethylindolenyl-3-hydroperoxide (VIIa). VIIa was prepared as follows. 2,3,5-Trimethylindole (200 mg) was dissolved in warm light petroleum (bp 60—80°C, 20 ml), and then a trace of benzoyl peroxide was added to the solution in the presence of atmospheric oxygen. After an hour the solution became opaque and then began to deposit crystalline hydroperoxide (140 mg), which was collected and recrystallized from a mixture of ethyl acetate and light petroleum to produce almost colorless prisms.

Mp 111.5°C (decomp.) Found: C, 69.34; H, 7.09; N, 7.44%. Calcd for $C_{11}H_{13}O_2N$: C, 69.09; H, 6.85; N, 7.33%.

UV: λ_{max}^{EtOH} mμ (log ε) 223 (4.24) 267 (3.59).

IR: ν_{KBr} cm^{-1} 3440, 3070, 2750, 1595, 1470, 860, 830

Attempted Preparation of 5-Chloro-2,3-dimethylindolenyl-3-hydroperoxide (VIIc). An ether - light petroleum solution of 5-chloro-2,3-dimethylindole (VIc) was oxidized with atmospheric oxygen as has been described above. After 20 hr a brown amorphous solid deposited out. This solid could not be recrystallized from any solvent, but it showed the characteristics of indolenine (*e. g.*, λ_{max} at approx. 230 and 260 mμ in the UV spectrum, and a positive starch-iodide test.).

Materials. The following derivatives were synthesized in our laboratory by the methods described in the literature.11-15)

*2,3-Dimethylindole (I)*11): Recrystallized from ligroin, colorless leaflets, mp 107—108°C (Found: C, 82.69; H, 7.70; N, 9.63%).

UV: λ_{max}^{EtOH} mμ (log ε) 283 (3.8) 290 (3.75).

IR: ν^{KBr} cm^{-1} 3380, 2900, 1590, 1460, 760.

*2, 3, 5-Trimethylindole (VIa)*12): Recrystallized from alcohol, colorless plates, mp 120°C (Found: C, 82.51; H, 8.30; N, 8.78%).

UV: λ_{max}^{EtOH} mμ (log ε) 231 (4.39) 288 (3.82).

IR: ν^{KBr} cm^{-1} 3380, 2900, 2840, 1590, 1475, 870, 790.

*5-Methoxy-2,3-dimethylindole (VIb)*13): Recrystallized from alcohol, mp 109°C (Found: C, 75.54; H, 7.51; N, 7.99%).

UV: λ_{max}^{EtOH} mμ (log ε) 230 (4.41) 278 (3.90).

IR: ν^{KBr} cm^{-1} 3390, 2920, 2850, 1590, 1475, 870, 790.

*5-Chloro-2,3-dimethylindole (VIc)*13): Recrystallized from benzene, glistening white plates, mp 140—141.5°C Found: C, 66.15; H, 5.64; N, 7.72%.

UV: λ_{max}^{EtOH} mμ (log ε) 235 (4.49) 293 (3.80) 301 (sh.) (3.74).

IR: ν^{KBr} cm^{-1} 3400, 2900, 1575, 1470, 860, 800, 750.

*5-Nitro-2,3-dimethylindole (VId)*13): Recrystallized from benzene, golden needles, mp 187°C (Found: C, 63.35; H, 5.55; N, 14.65%).

*2,3-Dimethylindolenyl-3-hydroperoxide (II)*14): Recrystallized from ethyl acetate-light petroleum, colorless prisms, mp 103°C (Found: C, 67.7; H, 6.3; N, 7.9%).

UV: λ_{max}^{EtOH} mμ (log ε) 225 (4.11) 260 (3.46).

IR: ν^{KBr} cm^{-1} 3400, 3060, 2800, 1600, 1455, 780.

*5-Methoxy-2,3-dimethylindolenyl-3-hydroperoxide (VIIb)*14): Recrystallized from ethyl acetate - light petroleum, colorless needles, mp 109°C (decomp.) (Found: C, 63.96; H, 6.59; N, 6.49%).

UV: λ_{max}^{EtOH} mμ (log ε) 223 (4.19) 281 (3.82).

IR: ν^{KBr} cm^{-1} 3450, 3050, 2800, 1595, 1470, 870, 810.

*o-Acetamidoacetophenone (III)*15): Recrystallized from ethanol-water, long colorless needles, mp 74—75°C.

2-Acetamido-5-methylacetophenone (VIIIa): VIIa was dissolved in boiling water and the solution was kept at room temperature; then VIIIa slowly separated. Recrystallized from methanol, slightly yellow leaflets, mp 123.5—124.5°C.

VIIIa was also prepared by treating the indole with ozonized oxygen.12) Recrystallized from methanol, mp 123.5—124.5°C.

*2-Acetamido-5-methoxyacetophenone (VIIb)*14): Recrystallized from light petroleum, long yellow needles, mp 91—91.5°C.

2-Acetamido-5-chloroacetophenone (VIIIc): VIc (0.3 g in AcOH (27 ml) + H_2O (2 ml)) was treated with ozonized oxygen at room temperature until the indole spot disappeared on T. L. C. The product was isolated by basification and extracted with ether. Recrystallized from methanol, almost colorless silky needles, mp 134—134.5°C.

Measurement of the Total Amount of Chemiluminescence. The total amount of light which was emitted when a solution of the sample was mixed with a solution of the alkali in the presence of oxygen was measured by a modified Moldel 139 Hitachi Photometer.

TABLE 1. TOTAL LIGHT EMISSION OF I AND II

Compd.	Concn.	Atmosphere	Total emission*
I	10^{-4} mol/l	Oxygen	31.1
II	10^{-4}	Hydrogen	36.1

* Area, arbitrary unit

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8) F. McCapra, D. G. Richardson and Y. C. Chang, *Photochem. Photobiol.*, **4.**, 1111 (1965).

9) F. McCapra and Y. C. Chang, *Chem. Commn.*, **1966**, 522.

10) N. Sugiyama and M. Akutagawa, This Bulletin, **40**, 240 (1967).

11) H. R. Snyder and C. W. Smith, *J. Am. Chem. Soc.*, **65**, 2452 (1943).

12) D. W. Ockenden and K. Schofield, *J. Chem. Soc.*, **1953**, 612.

13) D. W. Ockenden and K. Schofield, *ibid.*, **1957**, 3175.

14) R. J. S. Beer, T. Donavanik and A. Robertson, *ibid.*, **1954**, 4139.

15) N. J. Leonard and S. N. Boyd, Jr., *J. Org. Chem.*, **11**, 405 (1946).

TABLE 2. CHEMILUMINESCENCE AND FLUORESCENCE MAXIMA OF I AND II

Compound	Concn.	Atmosphere	Chemiluminescence maximum	Fluorescence* maximum
I	10^{-2} mol/*l*	Oxygen	520 mμ	520 mμ
II	10^{-3}	Hydrogen	520	520

* Fl. of reaction mixture

TABLE 3. RELATIVE TOTAL LIGHT EMISSION OF 5-SUBSTITUTED-2,3-DIMETHYLINDOLES

Compound	Total emission*
I	10
VIa	11
VIb	5.6
VIc	3.0
VId	10^{-5}

* Area, arbitrary unit
Condition: 10^{-3} mol/*l*, DMSO soln. 1 m*l* + 10^{-2} mol/*l*, *t*-BuOK DMSO soln. 1m*l* + O_2 stream

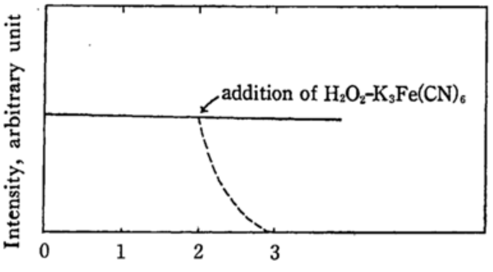


Fig. 1. Chemiluminescent curves of II.
10^{-3} mol/*l* in 0.1N ethanolic potassium hydroxide
--- added 1 m*l* of 3% H_2O_2 - 3% $K_3Fe(CN)_6$ mixture

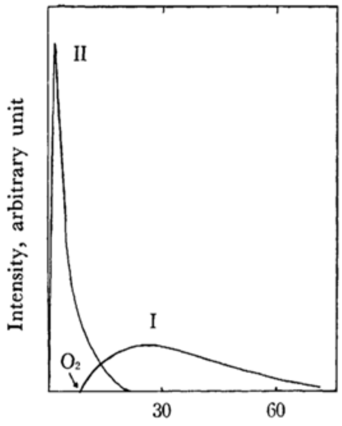


Fig. 2. Chemiluminescent curves of I and II.
10^{-4} mol/*l*, DMSO soln. 1 m*l* + 10^{-3} mol/*l*, BuOK DMSO soln. 1 m*l* + O_2(I) or Ar(II) stream

The change in the intensity of light with the time was recorded with a QPD 53 Hitachi Recorder, and the total emission was determined by the area of the emission curve. All measurements were carried out at 20°C.

One milliliter of a solution of a sample in DMSO (10^{-3} or 10^{-4} mol/*l*) was mixed with 1 m*l* of a potassium *t*-butoxide DMSO solution (10^{-2} or 10^{-3} mol/*l*) in an oxygen or argon stream, or 2 m*l* of a sample solution was added to 0.5 g of granular potassium hydroxide. The results are shown in Tables 1 and 3 and in Fig. 2.

Emission Spectra. In front of the slit of a spectrometer was set a test tube containing about 3 g of granular potassium hydroxide. Ten milliliters of the sample solution was at once added to this test tube. By means of a fine syringe needle, tiny bubbles of oxygen were continuously passed through this solution from the bottom of the test tube. Suddenly a brilliant emission of light was took place; it continued for a period of time. The emission spectrum of this light was measured.

Fluorescence Spectra. The fluorescence spectra were measured with a Hitachi Recording Spectrophotometer, Model EPS-2, equipped with an attachment for measuring the fluorescence spectrum. The 365 mμ line of a high-pressure mercury lamp was used for excitation. The chemiluminescent reaction was carried out by adding 10 m*l* of a solution of 2,3-dimethylindole (10^{-3} mol/*l*) in DMSO to an excess of granular potassium hydroxide in the oxygen stream. After periods of 1, 5, 10, and 30 min, 0.2 m*l* portions of this solution (solution A) were placed in a small Erlenmyer flask and diluted with 3.5 m*l* of the blank solution, which had been made by letting a quantity of DMSO stand with an excess of granular potassium hydroxide. These solutions were then used for the measurement of the fluorescence spectra.

In the case of 2,3-dimethylindolenyl-3-hydroperoxide (II), the chemiluminescent reaction was carried out in an argon stream and the fluorescence spectra was measured by the same procedure as above. The results are given in Fig. 4.

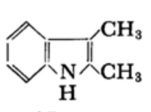
Ultraviolet Spectra. 0.2 m*l* of the solution A was diluted with 3 m*l* of ethanol after 0, 1/2, 5, and 30 min periods. These solutions were then used for the measurement of the ultraviolet spectra; the results are shown in Fig. 3.

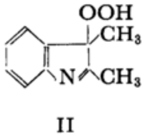
Results and Discussion

Mechanism of the Chemiluminescence. In order to get accurate information about the chemiluminescence mechanism of indole derivatives, it is necessary to find a compound which posesses such desirable properties as being easily accesible in the pure state and showing a demonstrable intensity of light emission in a measureable period of time.

In addition, since the oxidation reaction is involved in the chemiluminescent reaction, it is even more important to be able to obtain an oxidation intermediate with the same properties.

It was found that 2,3-dimethylindole (I) and its hydroperoxide, 2,3-dimethylindolenyl-3-hydroperoxide (II), satisfy these requirements.


I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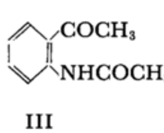

III

When II was dissolved in 0.1 N ethanolic potassium hydroxide, a weak light emission, detectable only by a photometer, continued for about six hours. However, when an oxidizing agent of the system A, a hydrogen peroxide-potassium ferricyanide solution, was added to the light-emitting ethanolic potassium hydroxide solution of II, the emission spontaneously disappeared, as shown in Fig. 1. Therefore, it is probable that, in the system A, II is not substantially connected with the light emission.

On the contrary, in the system B, II showed a bright flash of light, as has been previously reported.[7,10] Thus, it was assumed that II is an intermediate of the chemiluminescence of I in the system B.

This assumption was proved to be reasonable by the following results. When, under an oxygen atmosphere, 2-ml solutions of I and II in dimethylsulfoxide of a concentration of 10^{-4} mol/l were added to 0.5 g of granular potassium hydroxide, approximately equal amounts of light were emitted (Table 1). The emission period of I was longer than that of II, however, and the maximum intensity of I was lower than that of II (Fig. 2). Further, under a hydrogen atmosphere, the light emission was not observed in the case of I, although, in the case of II, a brilliant emission was observed. From these results, it seems probable that II is an intermediate and that the oxidation of I to II by oxygen is the rate-controlling step in the chemiluminescent reaction of I.

The emission spectra of both the solution of I (10^{-2} mol/l) and that of II (10^{-3} mol/l) appeared at 520 mμ, as shown in Table 2. This fact suggests that the same emitting species are concerned in the chemiluminescent reaction of I and II.

This was further supported by the following facts. After the light emission of the dimethylsulfoxide solution of I or II by potassium hydroxide was over, the ultraviolet absorption maxima of the

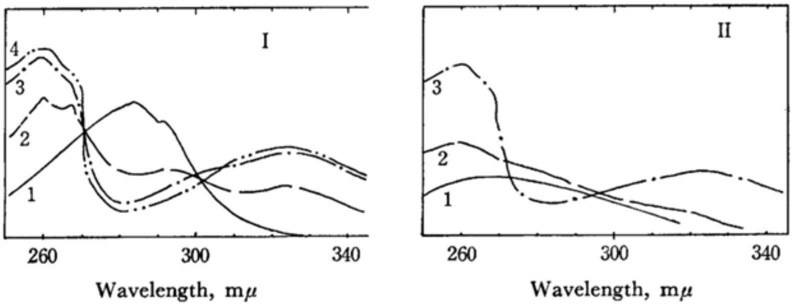


Fig. 3. The change of UV spectra of I and II in chemiluminescent reaction.
1: 0 min 2: 1/2 min 3: 5 min 4: 30 min

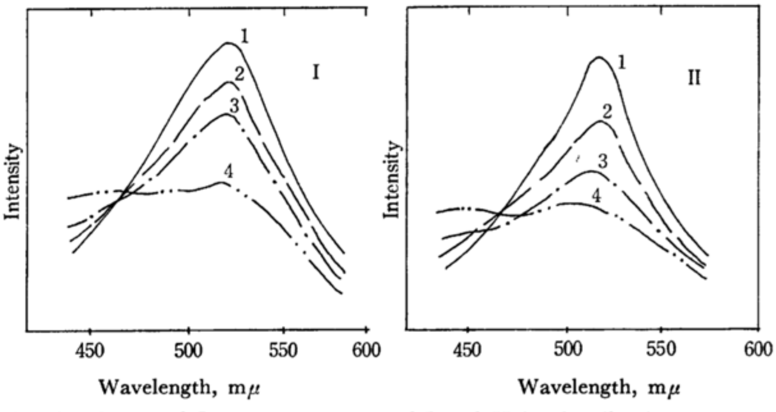


Fig. 4. The change of fluorescence spectra of I and II in chemiluminescent reaction.
1: 1 min 2: 5 min 3: 10 min 4: 30 min

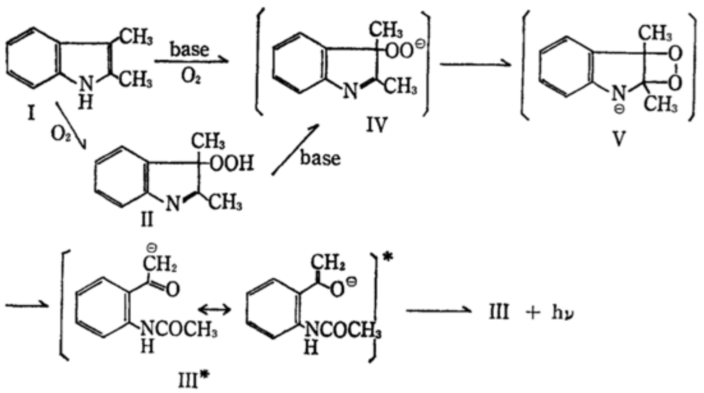


Fig. 5. Reaction scheme for the chemiluminescence of I.

solution of I (284 and 292 mμ) and that of II (260 mμ) both shifted to 259, 267 and 325 mμ, values which are consistent with those of *o*-acetamidoacetophenone (III).

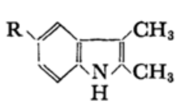
Furthermore, the fluorescence spectra of I and II varied similarly with the time and showed a maximum at 520 mμ, which completely coincided with that of III. The fluorescence spectra and ultraviolet spectra in the chemiluminescent condition are summarized in Figs. 3 and 4.

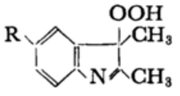
Now it can be concluded that II is the intermediate of the chemiluminescent reaction of I, that the product of this chemiluminescent reaction is III, and that the emitting species of the chemiluminescent reaction of I and II are the same. The emitting species may be the excited singlet state of the *o*-acetamidoacetophenone anion (III*), because the maximum of the fluorescence spectra of III in the same condition as chemiluminescence coincided completely with that of the emission spectra of I and II, and with their fluorescence spectra after the chemiluminescent reaction.

From these results the mechanism of the chemilueminscent reaction of 2,3-dimethylindole in the dimethylsulfoxide-potassium hydroxide oxygen system shown in Fig. 5 may reasonably be formulated.

Compound I is changed to the hydroperoxide anion, IV, by oxygen and a base, such as potassium *t*-butoxide or granular potassium hydroxide. On the other hand, Compound II requires only a base to change to IV. IV converts to the four-membered cyclic peroxide V, a short-lived intermediate, which then decomposes to an excited *o*-acetamidoacetophenone anion (III*). An excited state of III falls to the ground state with the emission of light.

Chemiluminescence of 5-Substituted-2,3-dimethylindoles. We prepared four 5-substituted-2,3-dimethylindoles and measured the total amount of light, they emitted by treating 10^{-3} mol/*l* solutions in DMSO with potassium *t*-butoxide and oxygen. The indoles were found to decrease in chemiluminescence efficiency in the order; VIa>I>VIb>VIc>VId (Table 3).


VI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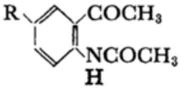
VIII

R=a: CH_3, b: CH_3O, c: Cl, d: NO_2

According to our experiment, 5-nitro-2,3-dimethylindole (VId) could not afford the hydroperoxide as II, and the chemiluminescence of VId was extremely weak.

The chemiluminescence maxima of indoles

TABLE 4. CHEMILUMINESCENCE MAXIMA OF VI AND VII

R of VI or VII	Chemiluminescence maxima of	
	VI	VII
H	520 mμ	520 mμ
CH_3	532	532
CH_3O	548	546
Cl	532	—

TABLE 5. FLUORESCENCE MAXIMA

R of VI, VII or VIII	Fluorescence maxima of		
	VI	VII	VIII
H	520 mμ	520 mμ	520 mμ
CH_3	531	531	532
CH_3O	549	546	546
Cl	532	—	533

* Under the same condition as chemiluminescent reaction.

(VIa, b, c) and the hydroperoxide (VIIa, b) are shown in Table 4. The hydroperoxide (VIIc) was too unstable to be obtained in the crystalline state. The fluorescence maxima of VI and VII after chemiluminescence and that of 5-substituted-2-acetamidoacetophenones (VIII) are shown in Table 5. After the chemiluminescent reaction of the solution of VI or VII, the solution was proved by T. L. C. to contain mainly VIII, which was also identified by a T. L. C. of the authentic samples. Thus VIII was the major product of the chemiluminescent reactions of both indoles (VI) and hydroperoxides (VII).

A good match was found between the chemiluminescence maxima of indoles (VI), those of the hydroperoxides (VII), and the fluorescence maxima of the major product (VIII). Therefore, it may be reasonably concluded that 5-substituted-2,3-dimethylindoles emitted light by the same mechanism as 2,3-dimethylindole.