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The Chemiluminescence of Indole Derivatives. I

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The chemiluminescence of forty-one indole derivatives has been investigated by using two types of reaction mixtures: (A) the EtOH-KOH-H_2O_2-$K_3Fe(CN)_6$-Sample and (B) the DMSO-KOH-Sample. In both systems the reactions were carried out in the presence of air. The light intensity of chemiluminescence was measured by the deflection of the microammeter of the photometer or recorded by a connected automatic recorder. The relative light intensity (RLI), the ratio of the maximum intensity of the chemiluminescence of the sample to that of indole, was also measured. The electron-repelling group, such as hydroxyl and substituted aminovinyl group, at the 3- or 5-position enhanced the RLI, but alkyl, acetyl, formyl groups or chlorine at the various positions had little effect or a negative effect on the RLI. Some of the compounds showed a maximum intensity soon after the reagents were mixed, while some others slowly showed a maximum, which then decayed slowly.

Among many types of chemiluminescent compounds, indole derivatives are especially interesting because of their relation to the bioluminescent substances. Though the firefly luciferin is a thiazole derivative,[1] the Cypridina luciferin has recently been determined to be an indole derivative,[2] and sea pansy luciferin has some characteristics of 2- or 3-substituted indole.[3] Balanoglossus luciferin is also an indole derivative, probably *N*-substituted one.[4]

It has been reported that various indole derivatives showed chemiluminescent reactions.[5] When these derivatives were oxidized with potassium peroxydisulfate in an aqueous solution containing sodium hydroxide, indole showed the strongest intensity, while they were oxidized in the presence of oxygen in a dimethyl sulfoxide (DMSO) solution containing potassium hydroxide, 3-methylindole showed the strongest intensity. The mechanism of the chemiluminescence of these indole derivatives was not, however, studied in detail.

Because we have been interested in the bioluminescence and have studied the indole derivatives for several years, we were prompted to study the mechanism of the chemiluminescence of indole derivatives. In this paper we want to present the results of the measurements of chemiluminescence and discuss those results.

Experimental

Materials. 3-[2′-Benzoylaminovinyl]-indole was prepared as follows. A suspension of 1.0 g of β-[3′-indolyl]-α-benzoylamino-acrylic acid[6] in 2.0 g of quinoline was heated with 0.1 g of copper chromite in an oil bath (150°C) for 15 min. After this mixture had then cooled, 100 m*l* of ethyl ether was added, and the mixture was washed three times with 20 m*l* of 2 N hydrochloric acid and then 50 m*l* of water. The ether solution was dried over anhydrous sodium sulfate. After the solvent had been removed, the residue was chromatographed in an alumina column with ethyl ether. 3-[2′-Benzoylaminovinyl]-indole was thus obtained as fine yellow needles (from $CHCl_3$). Mp 194°C, yield 0.14 g.

Found: C, 77.85; H, 5.15; N, 10.71%. Calcd for $C_{17}H_{14}N_2O$: C, 77.88; H, 5.38; N, 10.68%.

λ_{max}^{EtOH} mμ (ε): 234 (26600) 340 (16800), IR (KBr disk): 3420, 1630, 1540, 935, 740, 695 cm^{-1}.

The following derivatives were synthesized in our laboratory by the respective methods described in the literature.[7-30]

N-Methylindole[7]; faintly yellow oil; bp 70—75°C/2 mmHg.

2, 3-Dimethylindole[8]; recrystallized from ligroin; colorless leaflets; mp 107—108°C.

2, 3-Dimethylindolenyl-3-hydroperoxide[9]; colorless prisms; mp 113°C (decomp.).

2-Ethylindole[10]; recrystallized from ligroin; colorless

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plates; mp 43°C.

2, 3-Diethylindole[11]; recrystallized from ligroin; colorless leaflets; mp 29°C.

2, 3-Diethylindolenyl-3-hydroperoxide[12]; recrystallized from ethyl acetate - petroleum ether; colorelss needles; mp 110°C.

1, 3-Diacetylindole[13]; recrystallized from ethanol; colorless needles; mp 150°C.

3-Acetylindole[14]; recrystallized from ethanol; colorless needles; mp 190—191°C.

2-Ethyl-3-acetylindole[11]; recrystallized from benzene; faintly yellow needles; mp 159°C.

Tetrahydrocarbazole[9]; recrystallized from ethanol-water; colorless leaflets; mp 113°C.

3-Formylindole[15]; recrystallized from methanol; colorless crystals; mp 197—199°C.

Indole-3-acetaldehyde[16]; a colorless, syrupy liquid.

Gramine[17]; recrystallized from acetone; colorless crystals; mp 134°C.

Indole-3-glyoxylic acid[18]; yellow crystals; mp 215°C (decomp.).

5-Hydroxyindole[19]; recrystallized from chloroform-petroleum ether; colorless needles; mp 104.5—105°C.

5-Chloroindole[20]; recrystallized from petroleum ether; silvery plates; mp 70—71°C.

6-Chloroindole[20]; recrystallized from petroleum ether; colorless leaflets; mp 86—87°C.

5-Chloroindole-2-carboxylic acid[20]; recrystallized from ethanol-water; fine, colorless needles; mp 288°C (decomp.).

6-Chloroindole-2-carboxylic acid[20]; recrystallized from ethanol-water; fine, colorless needles; mp 240°C (decomp.).

Indole-2-carboxylic acid[21]; recrystallized from benzene; colorless leaflets; mp 203—204°C.

5, 6-Dimethoxyindole[22]; fine, colorless needles; mp 142—144°C.

2-Phenylindole[23]; recrystallized from ethanol; colorless leaflets; mp 186°C.

3-Phenylindole[24]; recrystallized from ligroin; colorless leaflets; mp 88—89°C.

3-[2′-Diisopropylaminovinyl]-indole[25]; light yellowish-brown crystals; mp 129—130°C.

3-[2′-Dibenzylaminovinyl]-indole[25]; colorless crystals; mp 128.5—130°C.

3-[2′-Nitrovinyl]-indole[26]; recrystallized from methanol; dark red prisms; mp 170—171°C.

Indole-3-acrylic acid[27]; recrystallized from ethyl acetate; pale yellow plates; mp 191°C.

N-Hydroxy-2-phenylindole[28]; recrystallized from chloroform; yellow needles; mp 175°C.

N-Methoxy-2-phenylindole[29]; recrystallized from ethanol; colorless crystals; mp 49—50°C.

N-Acetoxy- 2 -phenylindole[29]; recrystallized from ethanol; colorless crystals; mp 79—80°C.

N-Hydroxy - 2 - [4′ - dimethylaminophenyl]-indole[29]; dark green prisms; mp 165°C.

N-Hydroxy-2-[α-furyl]-indole[29]; a dark brown solid.

N-Hydroxyindole-2-carboxylic acid[30]; recrystallized from acetone-water; colorless prisms; mp 159.5°C (decomp.).

The following compounds were obtained commercially; indole (Wako Pure Chemical Industries, Ltd., G. R.) purified by vacuum sublimation, mp 52°C; isatin (Wako Pure Chemical Industries, Ltd., G. R.) mp 202°C; tryptophane (Nippon Rikagaku Yakuhin K. K., G. R.), mp 227°C; 2-methylindole (Tokyo Chemical Industry Co., Ltd., G. R.), recrystallized from hot water as needles, mp 61°C; 3-methylindole (Tokyo Chemical Industry Co., Ltd., G. R.), recrystallized from ligroin as leaflets, mp 95°C; 5-methylindole (Tokyo Chemical Industry Co., Ltd., G. R.), recrystallized from water as needles, mp 58°C; tryptamine (Tokyo Chemical Industry Co., Ltd., G. R.), recrystallized from ligroin as needles, mp 146°C; and indole-3-acetic acid (Tokyo Chemical Industry Co., Ltd., G. R.), recrystallized from chloroform as plates, mp 164°C.

Measurements of the Intensity of the Light. The maximum intensity of the light which was emitted when a solution of the sample was mixed with a solution of the oxidizing agents, as will be described below, was measured by studying the deflection of the microammeter of the photometer, a modified Model 139 Hitachi photometer. The change in the intensity of light with time was recorded with a QPD 53 Hitachi recorder. All measurements were carried out at about 20°C.

Measurements were carried out in the two systems as follows.

(A) One milliliter of a solution (10^{-2} and 10^{-3} mol/*l*) of the sample in 0.1 N potassium hydroxide in ethyl alcohol was mixed with 1 m*l* of a mixture of 3% hydrogen peroxide and a 3% aqueous solution of potassium ferricyanide (1 : 1 v/v).

(B) One milliliter of a solution of the sample in dimethyl sulfoxide (10^{-3} mol/*l*) was mixed with 1 m*l* of 5 N aqueous potassium hydroxide in the presence of air.

A test tube (11×75 mm) containing the sample solution was attached to the photometer. With a pipet, the oxidizing solution was then added to the sample solution as quickly as possible in order to mix it well with the sample solution. The results are shown in Table 1. The relative light intensity (RLI) is the ratio of the maximum deflection of the sample to that

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TABLE 1. RELATIVE LIGHT INTENSITY (RLI) OF THE CHEMILUMINESCENCE OF INDOLES

Compound	Concentration, mol/*l* RLI in the system		
	(A) the sample-EtOH-KOH-H_2O_2-$K_3Fe(CN)_6$		(B) the sample-DMSO-KOH
	10^{-2}	10^{-3}	10^{-3} (mol/*l*)*
Indole	1(4.3)**	1(0.4)**	1(5.9)**
Methylindole	—	1.5	7.9
2-Methylindole	5.9	0.5	0.05
3-Methylindole	—	0.5	13
5-Methylindole	—	2.0	2.1
2, 3-Dimethylindole	0.8	—	31
2-Ethylindole	1.5	—	0
2, 3-Diethylindole	2.5	—	9.0
Tetrahydrocarbazole	0.3	—	1.0
3-Acetylindole	0.3	—	0.5
2-Ethyl-3-acetylindole	1.0	—	0.3
1, 3-Diacetylindole	3.8	—	0.7
3-Formylindole	0.1	1.7	0
Indole-3-acetaldehyde	5.1	—	13
Indole-3-acetic acid	1.3	1.5	0.4
Gramine	1.5	—	0
Indole-3-glyoxylic acid	3.0	—	0.8
Tryptamine	2.0	—	3.3
Tryptophane	0.6	—	0
Isatin	—	0.5	0.3
5-Hydroxyindole	—	68	0
5-Chloroindole	1.8	—	0
6-Chloroindole	13	—	0
5-Chloroindole-2-carboxylic acid	1.3	—	0
6-Chloroindole-2-carboxylic acid	1.3	—	0
Indole-2-carboxylic acid	—	0.1	0.03
5, 6-Dimethoxyindole	—	14	0.3
2-Phenylindole	—	0.5	0.1
3-Phenylindole	—	90	0
3-[2′-Diisopropylaminovinyl]-indole	—	590	—
3-[2′-Piperidinovinyl]-indole	—	130	—
3-[2′-Dibenzylaminovinyl]-indole	—	100	—
3-[2′-Benzoylaminovinyl]-indole	—	80	6.8
β-[3-Indolyl]-α-benzoylamino-acrylic acid	5.3	—	—
3-[2′-Nitrovinyl]-indole†	3.5	—	0
Indole-3-acrylic acid†	76	—	0
N-Hydroxy-2-phenylindole	—	0.7	0
N-Methoxy-2-phenylindole	1.3	1.3	0
N-Acetoxy-2-phenylindole	—	1.3	0.6
N-Hydroxy-2-[4′-dimethylaminophenyl]-indole	—	42	1.7
N-Hydroxy-2-[α-furyl]-indole	—	67	0
N-Hydroxy-2-indole-carboxylic acid	0.1	0.7	0.1

* Concentration. ** Deflection of the microammeter.

of indole measured at the same concentration.

Results and Discussion

Table 1 shows that, in the A system, almost all of the indole derivatives investigated emit a detectable light. Regarding the B system, since no detectable light was emitted even when the measurements were carried out at the concentration of 10^{-2} mol/*l*, the data for concentrations other than 10^{-3} mol/*l* will be omitted here. The RLI corresponds to the maximum intensity, but it does not correspond to the total amount of emitted light. As is shown in Fig. 1, in some cases the intensity rose to a maximum soon after the reagents had been mixed and then quickly decreased, whereas in the other cases (indicated by † in Table 1) the intensity slowly rose to a maximum and then decreased slowly. Although the absorptions of the emitted light by reaction mixtures may be different, the RLI values will be of use for the study of the chemiluminescence.

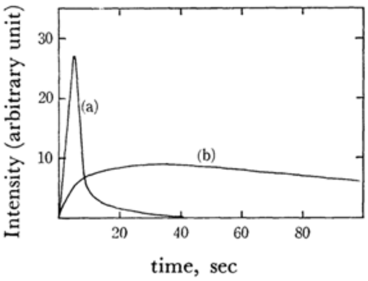


Fig. 1. Light intensity of indole derivatives in (A) system.

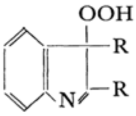
(a) 6-Chloroindole
(b) 3-[2′-Nitrovinyl]-indole

The fact that there is no parallelism between the RLI values in the A and B systems may be explained by a difference between the chemiluminescent reactions in the two systems. The strongest intensity was shown by 3-[2′-diisopropylaminovinyl]-indole in A, and by 2, 3-dimethylindole in B.

As the intensity of the chemiluminescence of indole derivatives is not as strong as luminol, it is better to measure the light intensity at a high concentration. However, their solubilities are somewhat limiting. It was found that the maximum intensity of the emitted light is proportional to the concentration of the sample. For example, in the case of indole, the deflections of the microammeter were 45, 4.3 and 0.4 at the concentrations of 10^{-1}, 10^{-2} and 10^{-3} mol/*l* of indole, respectively. Therefore, in the A system the RLI were measured at the concentration of 10^{-2} mol/*l* except in cases where solubility limitations were present, while in the B system, all the measurements were carried out at the concentration of 10^{-3} mol/*l*.

It was found that, in the A system, the electron-repelling groups, such as the hydroxyl group at the 5-position and substituted amino vinyl groups at the 3-position, enhanced the intensity, whereas chlorine at the 5-position, and acetyl or formyl groups at the 3-position exert little effect or a negative effect on the intensity; alkyl groups directly attached to the ring also exert little effect on the intensity. In order to deduce the general relationship between the substituents, their positions, and the RLI, further investigations will be required.

As it is presumed that peroxide or hydroperoxide may be formed during the course of the chemiluminescent reaction, the chemiluminescence of 2, 3 - dialkylindolenyl - 3 - hydroperoxides (Ia and Ib)[9,12] was also studied. It was found that, in the B system, the RLI of the dimethyl derivative (Ia) was 2.6×10^5, while that of the diethyl derivative (Ib) was 3.1×10^4. These compounds were suitable for a study of the mechanism of the chemiluminescence of indole derivatives in the B system.



Ia: $R=CH_3$
Ib: $R=C_2H_5$

Our study of the chemiluminescent reaction of indole derivatives will be reported on in another paper.

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