An Intermediate of the Chemiluminescent Reaction of Luminol

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The involvement of 5-amino-1, 4-phthalazinedione (IV) in the chemiluminescence of luminol (II) was first suggested by Bernanose,⁽¹⁾ while IV has been reported by Stross and Branch⁽²⁾ to be a nonchemiluminescent product. Other investigators have suggested that IV reacts with diimide to generate excited luminol in the chemiluminescent process.⁽³,⁴⁾ It has also been pointed out that the intermediate IV cannot be involved in the chemiluminescence of luminol because no chemiluminescence is observed with 1, 4-phthalazinedione (III)

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1) M. A. Bernanose, *Bull. soc. chim. France,* **18**, 329 (1951).

2) F. H. Stross and G. E. K. Branch, *J. Org. Chem.*, **3**, 385 (1938).

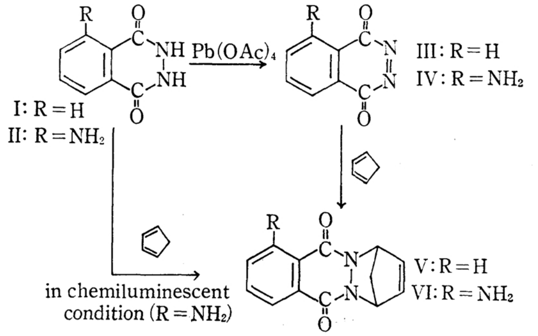
3) H. O. Albrecht, *Z. phys. Chem.*, **136**, 321 (1928).

4) W. S. Metcalf and T. I. Quickenden, *Nature*, **206**, 506 (1965).

itself under the conditions where chemiluminescence was observed with phthalhydrazide (I).[5] Recently, Rauhut *et al.*[6] have interpreted their kinetic data on the chemiluminescence of I by assuming the intermediate IV. There has, however, ultimately been no evidence for the formation of IV in the chemiluminescence of luminol.

The extraordinary reactivity of cyclic diazaquinone as a dienophile[7] stimulated the present authors to attempt the reaction with cyclopentadiene under the chemiluminescent conditions.*[1] When the oxidation of I with one mole of lead tetraacetate was carried out in acetonitrile, there was obtained a green solution, to which cyclopentadiene was added. The green color disappeared immediately, and the adduct (V) was produced. The proof for the V structure is based on the IR and UV absorption spectra. The IR spectra show a strong carbonyl peak in the 1630 cm^{-1} region. The UV band at 310 nm does not shift upon the addition of alkali.

When the same reaction was carried out with II, the oxidation was completed in a shorter time than in case of I, and a violet solution was obtained.



The violet substance was unstable and was not isolated, but the structure was presumed to be IV because of the formation of the VI adduct with cyclopentadiene. The spectral data were consistent with the VI structure.

Next, the chemiluminescent reaction was carried out in the presence of cyclopentadiene. After the oxidizing agent had been added to a DMF solution of luminol containing cyclopentadiene, the reaction mixture was neutralized. During the oxidation process no chemiluminescence was observed. The thin-layer chromatography of the reaction mixture is shown in Fig. 1. A remarkable difference can be noticed between the reaction product of luminol chemiluminescence in the presence of cyclopentadiene and that of phthalhydrazide chemiluminescence under the same conditions. The adduct was obtained from the former, but not from the latter. This indicates that the chemiluminescence of luminol proceeds through the formation of IV, but that this is not true for the phthalhydrazide chemiluminescence. It may, therefore, be that the chemiluminescence mechanism of unsubstituted phthalhydrazide is substantially different from that of luminol.

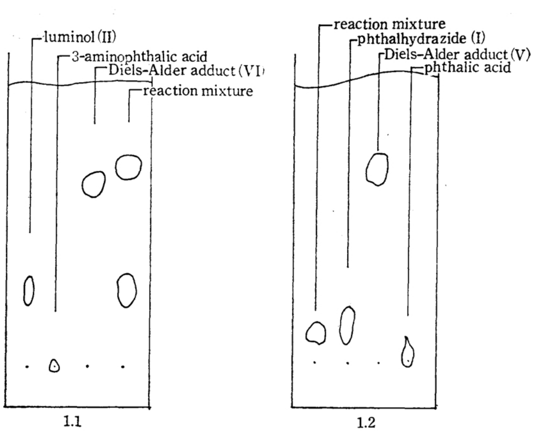


Fig. 1. Thin-layer chromatography of reaction products of luminol chemiluminescence (1.1) and phthalhydrazide chemiluminescence (1.2) in the presence of cyclopentadiene.

Experimental

All melting points are uncorrected.

Oxidation of Phthalhydrazide with Lead Tetraacetate and Subsequent Addition of Cyclopentadiene. To a slurry of phthalhydrazide (I) (1.62 g) and purified acetonitrile (50 m*l*) in a flask provided with a loose-fitting stopper and cooled in an ice-bath, lead tetraacetate (4.44 g) was added, and then the heterogeneous mixture was stirred. As the reaction proceeded, the solution became thick with precipitated lead acetate, and the color of the solution changed from brown to green. After about one hour, a test for lead tetraacetate was negative; the reaction mixture was immediately filtered, and to the clear green filtrate cyclopentadiene was added. The solution immediately became colorless, and a white precipitate appeared. The solvent was removed from the reaction mixture under reduced pressure to afford a light yellow solid, which was then dissolved in ethanol and recrystallized to give colorless crystals (V), mp 236—238°C.

Found: C, 68.83; H, 4.45; N, 12.38%. Calcd for $C_{13}H_{10}N_2O_2$: C, 69.01; H, 4.46; N, 12.38%.

UV: λ_{max}^{EtOH} nm (ε) 310 (5300).

IR: λ^{KBr} cm^{-1} 1630 (C=O).

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[5] E. H. White, O. Zafiriou, H. H. Kägi and H. M. Hill, *J. Am. Chem. Soc.*, **86**, 940 (1964).

[6] M. M. Rauhut, A. M. Semsel and B. G. Roberts, *J. Org. Chem.*, **31**, 2431 (1966).

[7] R. A. Clement, *ibid.*, **25**, 1724 (1960); T. J. Kealy, *J. Am. Chem. Soc.*, **84**, 966 (1962); R. A. Clement, *J. Org. Chem.*, **27**, 1115 (1962).

*[1] Chemiluminescence system: luminol, NaOH, $K_3Fe(CN)_6$ in DMF-H_2O (3 : 1 in vol.). *Cf.* Y. Omote, T. Miyake, S. Ohmori and N. Sugiyama This Bulletin, **39**, 932 (1966).

Oxidation of Luminol with Lead Tetraacetate and Subsequent Addition of Cyclopentadiene. The reaction was carried out as above with 1.77 g of luminol (II), 50 m*l* of acetonitrile, and 4.44 g of lead tetraacetate. After 5 min the color of the solution changed from brown to violet, and the reaction mixture was immediately filtered. Then to the filtrate cyclopentadiene was added. After the solvent had been removed, the residual yellow solid was washed with water and then dissolved in ethanol. When the alcohol solution was concentrated, unchanged II and an unknown substance were produced; further concentration gave the VI adduct, which was recrystallized from ethanol to afford light yellow crystals, mp 234°C (decomp.).

Found: C, 64.06; H, 4.75; N, 16.94%. Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42%.

UV: λ_{max}^{EtOH} nm (ε) 298 (6800), 370 (9200).

IR: ν^{KBr} cm^{-1} 3350, 3450 (NH_2), 1630, 1600 (C=O).

NMR: τ (in DMS) 4.25 (q.), 3.25 (m.), 2.53—2.74 (m.).

The Reaction of Phthalhydrazide with Cyclopentadiene under Chemiluminescent Conditions. To a 15 m*l* solution of I (10^{-3} mol/*l*) in dimethylformamide, cyclopentadiene was added, and then immediately 5 m*l* of a 1 N sodium hydroxide solution containing potassium ferricyanide (10^{-2} mol/*l*). After the mixture had been shaken, it was neutralized with 1 N hydrochloric acid. The reaction products were detected by thin-layer chromatography (silica gel, ethanol) and compared with the V adduct.

The Reaction of Luminol with Cyclopentadiene under Chemiluminescent Conditions. The same reaction was run with luminol, and the reaction products were examined with the VI adduct.