Dyes and Pigments, Vol. 27, No. 1, pp. 1–7, 1995

0143-7208/95 $9.50 + 0.00

0143-7208(94)00030-1

Chemiluminescent Properties of Some Luminol Related Compounds

Junichi Ishida, Maki Takada, Tomohiro Yakabe
& Masatoshi Yamaguchi*

Faculty of Pharmaceutical Sciences, Fukuoka University,
Nanakuma Johnan-ku, Fukuoka 814-80, Japan

(Received 7 June 1994; accepted 12 July 1994)

ABSTRACT

The chemiluminescent properties of three new luminol related compounds, viz. 6-isothiocyanatobenzo[g]phthalazine-1,4(2H,3H)-dione (IPO), 3-propyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione (PDIQ) and 3-benzyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione (BDIQ), were examined, and compared with other luminol analogs. The compounds produced chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate (III) in sodium hydroxide solution. Chemiluminescence intensities were affected by the concentrations of hydrogen peroxide, potassium hexacyanoferrate (III) and sodium hydroxide. Chemiluminescence reaction conditions, and the time dependence of the chemiluminescence of the compounds and luminol as a reference, were studied. IPO, PDIQ and BDIQ were found to produce chemiluminescence 1·7–5·5 times larger than luminol.

1 INTRODUCTION

Luminol is a well-known chemiluminescent compound, and has been widely used for various sensitive analyses.[1–3] 3-Aminophthalic acid has been shown to be a light emitter of the chemiluminescence from luminol.[4,5] The fluorescence quantum yield of the emitter affects the intensity of the chemiluminescence, and, therefore, a fluorescent probe having higher

---

* To whom correspondence should be addressed.

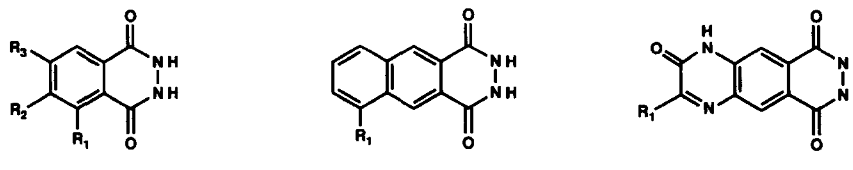
quantum yield should be used as an emitter in order to increase the sensitivity of the chemiluminescent compound.

Compounds containing naphthalene and quinoxalinone moieties are known to be highly fluorescent compounds.[6] Some new luminol related compounds containing the naphthalene system, 6-isothiocyanatobenzo[g]-phthalazine-1,4(2H,3H)-dione (IPO) (**IIa**) and quinoxalinone, 3-propyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione (PDIQ) (**IIIa**) and 3-benzyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione (BDIQ) (**IIIb**), were therefore evaluated in this investigation.

Also studied were the optimum chemiluminescence reaction conditions and the time dependence of the chemiluminescence of the compounds.

2 RESULTS AND DISCUSSION

The chemiluminescent properties of IPO, PDIQ and BDIQ were examined in order to evaluate the use of the compounds and conventional luminol analogs (Fig. 1) for highly sensitive analysis. These compounds produce chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate (III) in an alkaline medium (Fig. 2). The concentrations of hydrogen peroxide, potassium hexacyanoferrate (III) and sodium hydroxide influenced the chemiluminescence intensity (Fig. 3); 10–20 mM hydrogen peroxide, 5–10 mM potassium hexacyanoferrate (III) and 1·5–2·0 M sodium hydroxide gave almost the maximum intensity for all the compounds. Under optimum reaction conditions, the chemiluminescence intensities of eight compounds were determined (Table 1). The three new compounds (IPO, PDIQ and BDIQ) were found to produce chemiluminescence 1·7–5·5 times as large as that for luminol. On the other hand, the other luminol analogs were only about 1/30–1/1000 as bright as luminol. Figure 4 shows the time dependance of the chemilumi-



	R_1	R_2	R_3
Ia	NH_2	H	H
Ib	H	NH_2	NH_2
Ic	H	H	H

	R_1
IIa	NCS
IIb	NH_2
IIc	H

	R_1
IIIa	$-(CH_2)_2CH_3$
IIIb	$-CH_2-Ph$

Fig. 1. Structures of chemiluminescent compounds tested.

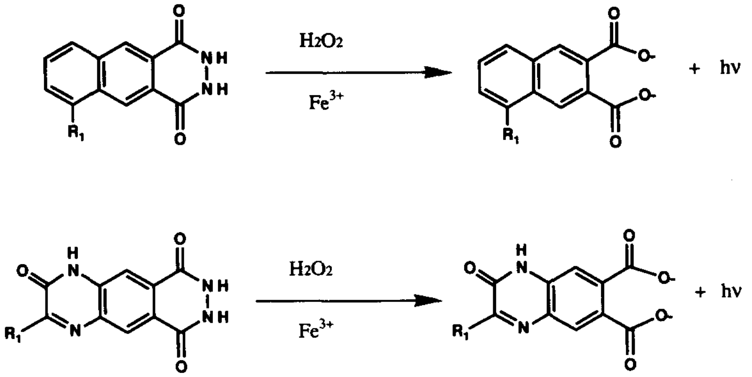


Fig. 2. Chemiluminescent reaction of luminol related compounds.

nescence reaction of the compounds. The chemiluminescence production was initiated by addition of hydrogen peroxide and potassium hexacyanoferrate (III) solutions to the compound solution. The chemiluminescence intensity was at its maximum at ≤1·5 s after the addition, and then decreased rapidly; about 1/10 of the initial intensities remained after 20 s.

In the chemiluminescence of luminol, the 3-aminophthalate ion produced during the reaction has been shown to be the light emitter. Therefore, in the case of the three new compounds, the corresponding phthalate ions (Fig. 2) could be expected to be light-emitting species. The fluorescent properties (fluorescence excitation and emission maxima and the relative intensity) of the species, after the chemiluminescence reaction was complete, were measured (Table 2), and compared with conventional

TABLE 1

Relative Chemiluminescence Intensities (RCI) of Compounds **I–III**

Compound no.	*RCI*[a]
Ia	100·0
Ib	0·9
Ic	0·1
IIa	545·0
IIb	3·6
IIc	1·8
IIIa	206·0
IIIb	169·0

[a] Integrated chemiluminescence intensity (a run time of 20·5 s) of compound **Ia** was taken as 100·0.

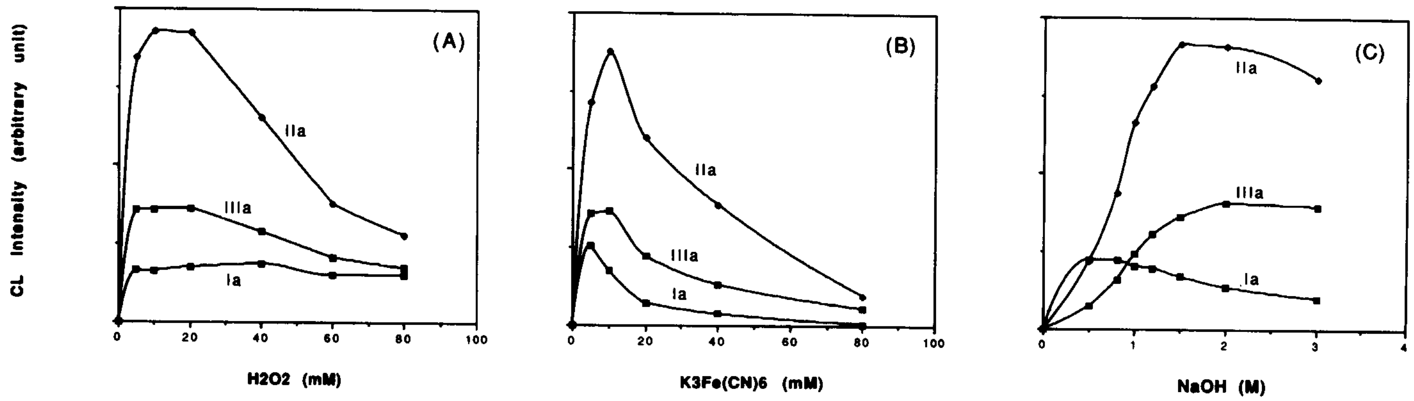


Fig. 3. Effects of (A) hydrogen peroxide, (B) potassium hexacyanoferrate (III) and (C) sodium hydroxide concentrations on integrated chemiluminescence intensities (a run time of 20·5 s).

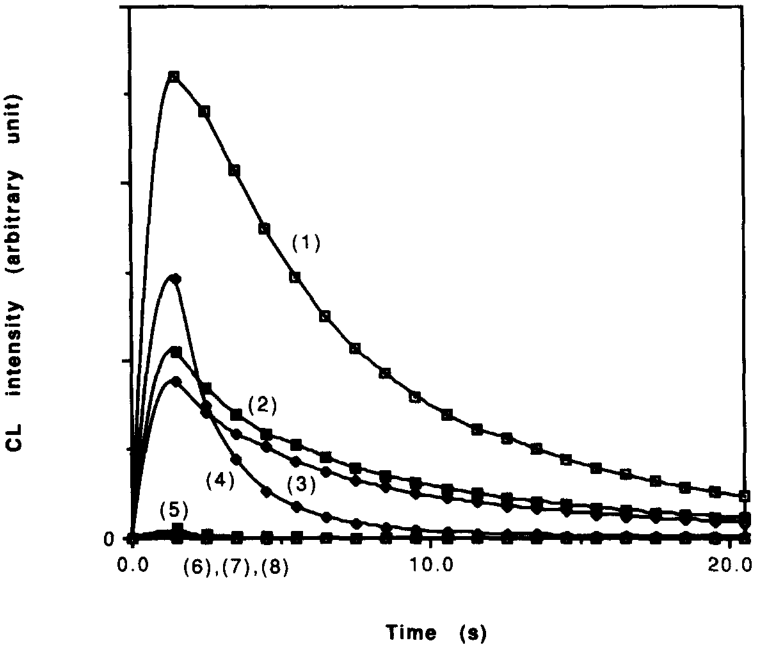


Fig. 4. Time dependence of the chemiluminescence reaction of the compounds. Curves: (1), **IIa**; (2), **IIIa**; (3), **IIIb**; (4), **Ia**; (5), **IIb**; (6), **Ib**; (7), **Ic**; (8), **IIc**.

luminol analogs. The fluorescence intensities of IPO, PDIQ and BDIQ after the reaction were 9–42 times larger than that of luminol. It is suggested that the efficiency of the chemiluminescent compound is partly dependant on the fluorescence intensities of the light-emitting species produced during the reaction.

TABLE 2
Fluorescence Excitation (Ex) and Emission (Em) Maxima and Relative Intensities (RFI) of the Compounds after the Chemiluminescence Reaction

Compound no.	*Ex (nm)*	*Em (nm)*	*RFI*[a]
Ia	314	448	100·0
Ib	322	425	76·4
Ic	353	406	14·6
IIa	360	484	949·6
IIb	361	417	42·3
IIc	294	370	269·0
IIIa	367	425	3 747·9
IIIb	371	430	4 234·9

[a] Fluorescence intensity of compound **Ia** was taken as 100·0.

3 CONCLUSION

The new chemiluminescent compounds (IPO, PDIQ and BDIQ) were found to have excellent chemiluminescent properties, and are potentially useful in sensitive analysis as chemiluminescent probes.

4 EXPERIMENTAL

4.1 General

Chemiluminescence intensities and reaction time dependencies were measured using a Microluminoreader MLR-100 (Corona Electric Co.) equipped with a Corona DP-50 autopipetter. Uncorrected fluorescence spectra and intensities were measured with a Hitachi 650-60 spectrofluorimeter in 10 × 10 mm-quartz cells; a spectral bandwidth of 5 nm was used in both the excitation and emission monochromators. Infrared spectra were recorded on a JASCO IR-810 spectrophotometer, and mass spectra on a JEOL DX-300. Luminol was purchased from Nacalai Tesque (Kyoto, Japan).

4.2 Examination of the chemiluminescence property

Solutions of compounds **I–III** (1×10^{-6} M) were prepared in dimethyl-sulfoxide. Hydrogen peroxide (5–80 mM) and potassium hexacyanoferrate (III) (5–80 mM) solutions were prepared in water and sodium hydroxide (0·5–3·0 M) solutions, respectively. A 50 μl portion of the solution was placed into the well of a black microplate. The chemilumin-escence reaction was initiated by automatic injections of 50 μl of the hydrogen peroxide solution and 100 μl of the potassium hexacyanoferrate (III) solution.

Chemiluminescence intensities were monitored immediately after the injection.

4.3 Synthesis of chemiluminescent compounds

Compounds **Ib**, **Ic**, **IIb** and **IIc** were prepared according to the literature.[7–9]

The syntheses of **IIIa** and **IIIb** have been reported previously;[10] briefly, **Ib** and α-ketovaleric acid (or phenylpyruvic acid) were dissolved in hydrochloric acid—ethanol containing β-mercaptoethanol and the mixture heated for 2 h at 100°C. The resulting precipitate was washed with ethanol and dried to give compounds **IIIa** or **IIIb**.

Compound **IIa** was synthesized from **IIb** by the following method. To a stirred solution of compound **IIb** (1·0 g) in 20 ml of water was added thiophosgene (1 ml) dropwise at room temperature. After stirring for 1 h, the precipitate which formed was filtered and washed with acetonitrile, and then dried to give **IIa** (0·9 g) as a pale brownish-yellow powder, m.p. 360°C (decomp.). Mass spectrum: 269 (M^+, base peak), ν_{max} (IR): 2100 (NCS), 3020 (lactum NH) cm^{-1}.

5 ACKNOWLEDGEMENTS

We thank Professor Masaru Nakamura (Fukuoka University) for helpful discussion. The financial support of the Grant-in-Aid for Scientific Research (No. 05671800) from the Ministry of Education, Science and Culture of Japan is gratefully acknowledged.

REFERENCES

1. Steiger, O. M., Lingeman, H., Brinkman, U. A. A., Holthuis, J. J. M., Smide, A. K. & Doornbos, D. A., *J. Chromatogr.*, **615** (1993) 97.
2. Kawasaki, T., Maeda, M. & Tsuji, A., *J. Chromatogr.*, **328** (1985) 121.
3. Townshend, A., *Analyst*, **115** (1990) 495.
4. White, E. E., Zafiriou, O., Kagi, H. H. & Hill, J. H. M., *J. Am. Chem. Soc.*, **86** (1964) 940.
5. White, E. H. & Bursey, M. M., *J. Am. Chem. Soc.*, **86** (1964) 941.
6. Ishida, J., Yamaguchi, M., Iwata, T. & Nakamura, M., *Anal. Chim. Acta*, **223** (1989) 319.
7. Williams, R. L. & Shalaby, S. W., *J. Heterocycl. Chem.*, **10** (1973) 891.
8. Drew, H. K. D. & Hatt, H. H., *J. Org. Soc.*, (1936) 16.
9. Drew, H. K. D. & Garwood, R. F., *J. Org. Soc.*, (1939) 836.
10. Arakawa, H., Ishida, J., Yamaguchi, M. & Nakamura, M., *Chem. Pharm. Bull.*, **38**(12) (1990) 3491.