

Synthesis of photosensitive luminol releasing compound, luminol-*O*-2-nitrobenzylate

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Abstract

Luminol-*O*-2-nitrobenzylate (**1**), which releases luminol upon light irradiation at 366 nm and can measure light power, was developed. **1** has a 2-nitrobenzyl group at the carbonyl oxygen of luminol. The released luminol emitted light in the presence of NaOH and H₂O₂. In the concentration range of 0.01–1 μM **1**, CL intensities of released luminol increased in proportion to increase of the concentration of **1** with a light irradiation time of 5 min. The CL intensity of **1** increased in proportion to the light power in the range of 450–650 mW/cm². © 2004 Elsevier B.V. All rights reserved.

Keywords: Luminol-*O*-2-nitrobenzylate; Photosensitive compound; Luminol derivative; Chemiluminescence

1. Introduction

3-Aminophthalhydrazide (luminol) emits light in the presence of peroxide in an alkaline medium. The chemiluminescence (CL) species is 3-aminophthalic acid. Luminol has a high CL quantum yield ($\phi = 0.036$) among the CL compounds [1]. The derivatives of luminol are used for simple and highly sensitive CL assays of DNA and enzyme activity [2–4].

Several compounds, which release fluorescent or bioactive compounds with light irradiation, have been reported. Photosensitive compounds, which have a 2-nitrobenzyl group in the structure, have been utilized for developing novel caged compounds [5]. For example, 5- or 6-(carboxymethyl-carbamoyl)-3', 6'-*O,O'*-bis(2-nitrobenzyl)fluorescein and *P*-[1-(2-nitrophenyl)ethyl]adenosine-3',5'-cyclic monophosphate contain the 2-nitrobenzyl group in the structure and release carboxyfluorescein and cyclic adenosine monophosphate with light irradiation at 365 nm and xenon arc flash lamp, respectively. They were developed for investigating the microtubule flux in the mitotic spindle and second messengers in *Aplysia* sensory neurons [6,7]. Aequorin was added to 1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane-*N,N,N',N'*-tetraacetic acid loaded with CaCl₂. When the solution was photoirradiated at 347 nm, Ca was released and the photoemission of aequorin was observed [8].

The luminol derivative, which has a methyl group at the carbonyl oxygen of luminol, was nonchemiluminescence [9]. This means that the nonchemiluminescence and photosensitive luminol derivative which releases the luminol with light irradiation can be utilized for novel CL assays. A luminol derivative, which releases luminol upon light irradiation and can measure light power, has not been reported. Thus we synthesized the luminol derivative (luminol-*O*-2-nitrobenzylate, **1**) which has a 2-nitrobenzyl group at the carbonyl oxygen of luminol and estimated the CL characteristics with the light irradiation using an ultrahigh-pressure mercury lamp (Fig. 1). Furthermore, **1** was applied to measure light power.

2. Experimental

2.1. Reagents and solutions

Deionized and distilled water purified by a Milli-Gradient (Japan Millipore, Tokyo, Japan) was used. 2-Nitrobenzyl chloride, sodium hydride and dimethylformamide (DMF) were purchased from Wako Chemicals (Osaka, Japan). All other chemicals and solvents were of analytical reagent grade.

2.2. Apparatus and its operations

Preparative thin layer chromatography was performed with a silica gel 60 F₂₅₄ plate (Merck Japan Limited, Tokyo,

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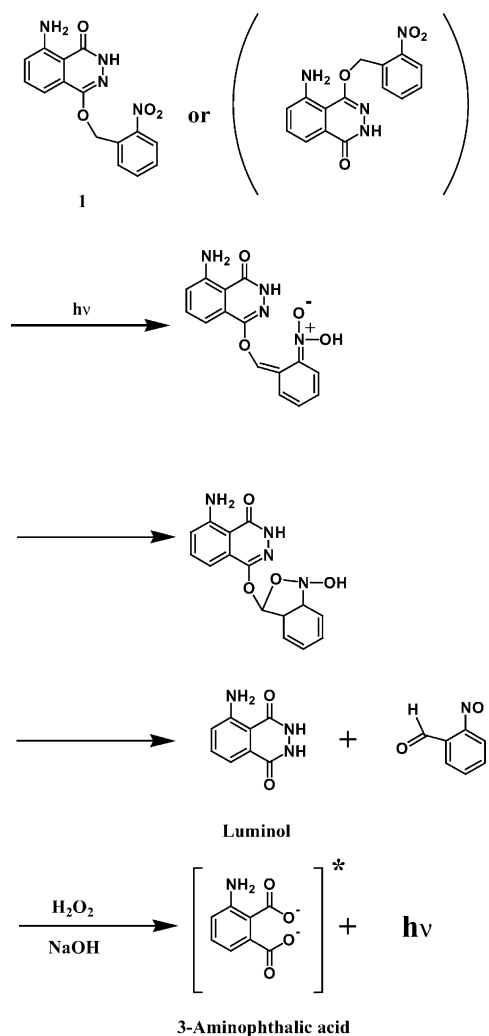


Fig. 1. Possible photosensitive luminol release mechanism.

Japan) for purification. The $^1\text{H-NMR}$ spectra were taken with a Varian UNITY plus (USA) spectrometer at 500 MHz. The FAB MS spectra of **1** and luminol were obtained using a JEOL JMS 600 (Tokyo, Japan). The irradiation system constructed with Optical Module X (model SX-UI500 MQQ) and a power supply unit (model BA-H500) (360 nm cutoff, 450 mW/cm^2 and the distance between the collimator lens and the quartz cuvette was 40 cm) was obtained from USIO Electronics (Tokyo, Japan). The light power was changed by adjusting the lamp current. Cutoff filters of 360, 420 and 500 nm were purchased from Stream (Saitama, Japan). A light power meter was purchased from Advantest (Tokyo, Japan). Lumat LB 9501 (Berthold, Wildbad, Germany) was used to measure the CL with a round-bottom glass tube (75 mm \times 12 mm i.d.).

2.3. Synthesis

2.3.1. Luminol-O-2-nitrobenzylate **1**

To stirred DMF (100 ml) was added luminol (0.89 g, 5 mmol) and sodium hydride (60% in oil, 0.2 g, 5 mmol), and

the solution was stirred at ambient temperature for 10 min. 2-Nitrobenzyl chloride (1.28 g, 7.5 mmol) was added to the solution. The mixture was stirred at ambient temperature for 16 h. H_2O (250 ml) was added to the solution and the organic layer was extracted with ethyl acetate (600 ml). The organic layer was dried with magnesium sulfate and the filtrate was concentrated. $\text{CHCl}_3\text{-MeOH}$ (2:1, v/v, 180 ml) solution was added to the solution and the precipitate was recrystallized from MeOH to give **1** as a yellow powder (0.23 g, 14.6% yield, mp 240°C). $^1\text{H-NMR}$ ($(\text{CD}_3)_2\text{S=O}$): 5.6 (s, 2H, benzyl H), 6.92 (m, 1H, ArH), 6.97 (m, 1H, ArH), 7.48 (m, 1H, ArH), 7.61 (s, 1H, ArH), 7.79 (m, 2H, ArH), 8.12 (m, 1H, ArH), 11.5 (s, 1H, $-\text{CO-NH-}$). FAB MS: 313 $[\text{M} + \text{H}]^+$, Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{O}_4\text{N}_4$: C, 57.7; H, 3.87; N, 17.9. Found: C, 57.31; H, 3.99; N, 17.50.

2.4. CL measurement of **1** after light irradiation

Three milliliters of the $1\ \mu\text{M}$ **1** in DMF was added to the quartz cuvette. The solution was photoirradiated for 0.5–10 min with a 360 nm cutoff using the ultrahigh-pressure mercury lamp. The $200\ \mu\text{l}$ of the photoirradiated solution was added to $100\ \mu\text{l}$ of 5–50 mM NaOH. After standing for 25 s, to the mixture was added $100\ \mu\text{l}$ of 100–750 mM H_2O_2 using the automatic injection system in the luminometer. The CL emission was measured for 5 min, and the integral photon counts were used for estimating the CL intensities.

2.5. Production of luminol after light irradiation of **1**

Three milliliters of 5 mM **1** in DMF was added to the quartz cuvette. The solution was photoirradiated for 2 h with a 360 nm cutoff using the ultrahigh-pressure mercury lamp. The solution was purified by preparative thin layer chromatography using $\text{CHCl}_3\text{-MeOH}$ (10:1, v/v) as the eluting solvent to give luminol as a yellow powder (0.41 mg, 15.4% yield, mp $> 300^\circ\text{C}$). $^1\text{H-NMR}$ ($(\text{CD}_3)_2\text{S=O}$): 6.8 (d, $J = 7.5\ \text{Hz}$, 1H, ArH), 6.9 (d, $J = 7.5\ \text{Hz}$, 1H, ArH), 7.4 (t, $J = 7.5\ \text{Hz}$, 1H, ArH). FAB MS: 177 $[\text{M}]^+$.

2.6. Light power measurement using **1**

Three milliliters of the $1\ \mu\text{M}$ **1** in DMF was added to the quartz cuvette. The solution was photoirradiated for 10 s with a 360 nm cutoff using the ultrahigh-pressure mercury lamp ($450\text{--}650\text{ mW/cm}^2$). To $200\ \mu\text{l}$ of the photoirradiated solution was added $100\ \mu\text{l}$ of 10 mM NaOH. After standing for 25 s, to the mixture was added $100\ \mu\text{l}$ of 500 mM H_2O_2 using the automatic injection system in the luminometer. The CL emission was measured for 5 min, and the integral photon counts were used for estimating the CL intensities.

3. Results and discussion

1 was synthesized as described in Section 2. However, the structure of **1** could not be identified. The

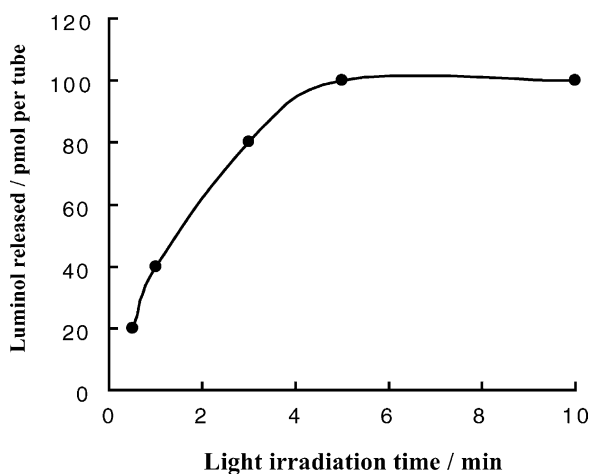


Fig. 2. Effect of light irradiation time of **1**. $[I] = 1 \mu\text{M}$, $[\text{NaOH}] = 10 \text{ mM}$, $[\text{H}_2\text{O}_2] = 500 \text{ mM}$.

photosensitive compound which has 2-nitrobenzyl group in the structure produces the precursor and 2-nitrosobenyl derivative with light irradiation via the photorearrangement [10,11]. It is thought that the **1** produces luminol and 2-nitrosobenzaldehyde with a light irradiation. Thus, the intramolecular hydrogen abstraction of benzylic H in 2-nitrobenzyl group and nucleophilic reaction of oxygen anion in 2-nitrobenzyl group occur (Fig. 1). However, production of 2-nitrosobenzaldehyde was not identified. When cutoff filters at 420 and 500 nm were used for the light irradiation, **1** could not release luminol. This indicated that **1** cannot release luminol with visible light irradiation. Thus, **1** released luminol with the light irradiation at 366 nm from the standpoint of line spectra of the ultrahigh-pressure mercury lamp. When the light irradiating times of **1** were 0.5, 1, 3, and 5 min, 20, 40, 80 and 100 pmol luminol per tube in this CL assay were released, respectively (Fig. 2). The total photon count reached a maximum with the light irradiation time of 5–10 min. Therefore, the light irradiation time of 5 min was used for evaluating the maximum CL conditions of the released luminol.

The luminol derivative, which has a methyl group at the carbonyl oxygen of luminol, was nonchemiluminescence [11]. The CL intensity of $1 \mu\text{M}$ **1** in DMF without light irradiation was eight-fold compared to that of DMF. This indicated that **1** emitted light in the presence of H_2O_2 under alkaline conditions (Fig. 3). Thus, **1** was not nonchemiluminescence. The CL intensities of **1** obtained with and without light irradiation were apparently different (Fig. 3). The maximum CL intensity of photoirradiated **1** was obtained when 10 mM NaOH and 500 mM H_2O_2 were used as the reagent concentrations. The CL intensities increased in proportion to the light power in the range of 450–650 mW/cm^2 (Fig. 4). This indicated that **1** could measure light power. When the CL intensities of **1** with and without light irradiation were the sample and blank, respectively, the CL ratio of the sample to blank with the light irradiation of **1** increased

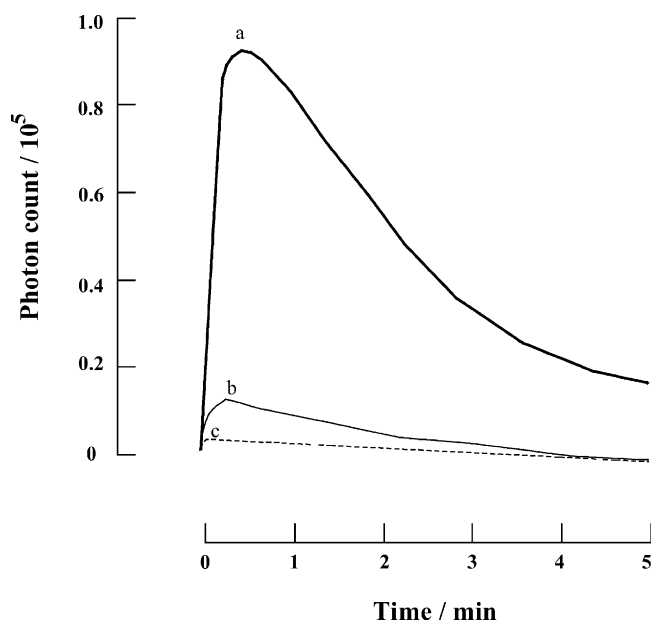


Fig. 3. Time course of CL of **1** with and without light irradiation. Curves: (a) irradiation: $[I] = 1 \mu\text{M}$; (b) irradiation: $[I] = 0.1 \mu\text{M}$; (c) nonirradiation: $[I] = 1 \mu\text{M}$, $[\text{NaOH}]$ and $[\text{H}_2\text{O}_2]$ were 10 and 500 mM under each CL reaction condition.

with increasing concentration of **1** (Table 1). The CL ratio of the sample to blank of $1 \mu\text{M}$ photoirradiated **1** was 175. In the concentration range of 0.01– $1 \mu\text{M}$ **1**, CL intensities of released luminol increased in proportion to increase of the concentration of **1** with a light irradiation time of 5 min (Table 1). In the section of *production of luminol by after light irradiation of 1*, it was confirmed that **1** released luminol with light irradiation. Compared to luminol, it is postulated that CL compounds, which have a relatively strong CL intensity such as the acridinium ester, lucigenin and lophine, have difficulty in synthesizing the CL derivative which

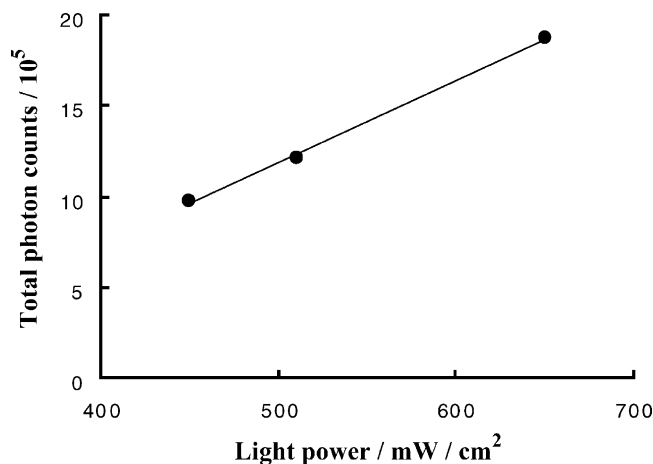


Fig. 4. Measurement of light power. $[I] = 1 \mu\text{M}$, $[\text{NaOH}] = 10 \text{ mM}$, $[\text{H}_2\text{O}_2] = 500 \text{ mM}$.

Table 1

Effect of concentration of **1** and CL ratio of sample to blank with and without the light irradiation of **1**

1 (μM)	NaOH (mM)	H ₂ O ₂ (mM)	Integral photon count ($\times 10^5$)	S/B ^a
0.01	10	500	1.8	5.5
0.1	10	500	15.3	51
1	10	500	156	175

^a Sample and blank show CL intensities of **1** with and without light irradiation, respectively. Each light irradiation time is 5 min.

releases the CL compound with light irradiation by substituting the 2-nitrobenzyl group from the standpoint of these CL mechanisms. Therefore, **1** is a novel photosensitive CL compound.

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