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Chemiluminescent properties of some luminol-related compounds — Part 3

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Abstract

Novel luminol-related compounds containing a 2-arylbenzothiazole moiety were synthesized by the reaction of a series of aromatic aldehydes with 7-amino-6-sulfanylphthalazine-1,4(2*H*,3*H*)-dione (ASPH). The chemiluminescent properties of the ASPH derivatives were examined and compared with luminol and ASPH. All compounds produced chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in sodium hydroxide solution. The chemiluminescence intensities of the chemiluminophores were influenced by the concentrations of hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide. The ASPH derivatives of the aromatic aldehydes examined were found to produce chemiluminescence 1.1–40 times more intensely than luminol.

Keywords: Chemiluminescence; Luminol; Chemiluminophores; 7-Amino-6-sulfanylphthalazine-1,4(2*H*,3*H*)-dione

1. Introduction

Luminol and isoluminol are the most investigated chemiluminescent compounds [1,2], and their wide use in various sensitive analyses has been reviewed [3–6]. The 3-aminophthalate anion of luminol is a light emitter [7]. In the case of some luminol-type chemiluminophores, the fluorescence intensity of the corresponding emitters affects the chemiluminescence intensity [8,9], and therefore, a fluorophore having high emitter fluorescence quantum yield will tend also to have high chemiluminescence intensity.

On that basis, we have already developed some luminol-type chemiluminophores; i.e. 3-propyl-7,8-dihydropyridazino[4,5-*g*]quinoxaline-2,6,9(1*H*)-trione [1,10] and 6-isothiocyanatobenzo[*g*]phthalazine-1,4(2*H*,3*H*)-dione [2,11,12]. These compounds chemiluminesce more intensely than luminol, because they and their corresponding emitters afford high fluorescence intensities owing to their quinoxalinone and naphthalene skeletons, respectively. 2-Arylbenzothiazoles are also known to generate intense fluorescence [13–15]. Therefore, we have developed 6-(5′,6′-dimethoxybenzothiazolyl)phthalazine-1,4(2*H*,3*H*)-dione (DBPH) [16] as an intense chemiluminophore having a 2-arylbenzothiazole moiety. The chemiluminogenic benzothiazoles, 6-(5′-hydroxybenzothiazolyl)-phthalazine-1,4(2*H*,3*H*)-dione [17] and 5-(5′-azo-

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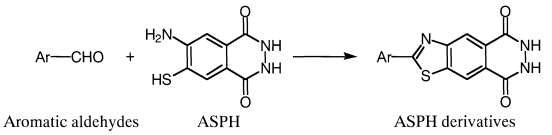
luciferinyl)phthalazine-1,4(2*H*,3*H*)-dione [18], which also generate chemiluminescence more intensely than luminol, have been reported by other workers.

In this work, some luminol-type compounds having a 2-arylbenzothiazole moiety, viz. 2-(4′-methylphenyl)phthalazino[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (TA-ASPH), 2-(4′-carboxyphenyl)-phthalazino[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (FB-ASPH), 2-(4′-aminophenyl)phthalazino[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (AB-ASPH), 2-[4′-(*N*,*N*-dimethylamino)phenyl]phthalazino[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (DA-ASPH), 2-{4′-[3′-hydroxy-5′-(hydroxymethyl)-2′-methylpyridyl]}phthalazino-[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (PD-ASPH) and 2-(1′-pyrenyl)phthalazino[6,7-*d*]thiazole-5,8(6*H*,7*H*)-dione (PY-ASPH), have been synthesized as the condensation reaction products of 7-amino-6-sulfanylphthalazine-1,4(2*H*,3*H*)-dione (ASPH) and the corresponding aromatic aldehydes (Fig. 1). These ASPH derivatives not only have the 2-arylbenzothiazole structure as a highly fluorescent site, but also a cyclic phthalhydrazide structure as a chemiluminescence emitting site, as is the case with DBPH. We examined the chemiluminescent properties (relative intensity, optimum reaction conditions and time dependence) of the ASPH derivatives of some aromatic aldehydes, and compared them with those of luminol.



Aromatic aldehyde	Ar	Abbreviation of ASPH derivative
4-Tolualdehyde	—CH_3	TA-ASPH
4-Formylbenzoic acid	—COOH	FB-ASPH
4-Aminobenzaldehyde	—NH_2	AB-ASPH
4-(*N*,*N*-Dimethylamino)benzaldehyde	—$N(CH_3)_2$	DA-ASPH
Pyridoxal	HO, CH_3, N, HOH_2C	PD-ASPH
1-Pyrenecarboxaldehyde		PY-ASPH

Fig. 1. Synthesis of ASPH derivatives with aromatic aldehydes.

2. Experimental

2.1. Apparatus

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured on a Jeol (Tokyo, Japan) JNM-GX-400 spectrometer at 400 MHz in dimethylsulfoxide-d_6 with tetramethylsilane as an internal standard. Splitting patterns were designated as follows: s, singlet; br, broad. Fast atom bombardment mass spectra (FAB-MS) were taken with a Jeol DX-300 spectrometer. High resolution FAB-MS measurements were made on a Jeol JMS-HX110 instrument. Uncorrected melting points were obtained with a Gallenkamp (Loughborough, UK) melting point apparatus.

Chemiluminescence intensities and their reaction time dependences were measured using a Corona (Ibaragi, Japan) MLR-100 microluminoreader equipped with a Corona DP-50 autopipetter. Uncorrected fluorescence excitation and emission spectra, and their intensities were measured with a Hitachi (Tokyo, Japan) F-3010 fluorescence spectrophotometer in 10×10 mm-quartz cells; a spectral bandwidth of 5 nm was used in both the excitation and emission monochromators.

2.2. Chemicals and solutions

All chemicals and solvents were of the highest purity available unless noted otherwise, and were used as received. Distilled water, purified with a Milli-QII system (Millipore, Milford, MA, USA), was used for all aqueous solutions. Hydrogen peroxide (31%, w/v) was purchased from Mitsubishi Gas Kagaku (Tokyo, Japan). Luminol was obtained from Nacalai Tesque (Kyoto, Japan), and used without further purification.

Stock solutions (1×10^{-3} M) of each chemiluminophore were prepared in *N*,*N*-dimethylformamide (DMF), stored at −20°C, and diluted further with various solvents to the required concentrations prior to use. These stock solutions were stable for at least 2 weeks. The solutions of hydrogen peroxide (1.0–20 mM) and potassium hexacyanoferrate(III) (1.0–20 mM) were prepared in water and sodium hydroxide (0.1–3.0 M) solution, respectively, and were used within 12 h.

2.3. Synthesis of chemiluminescent compounds

The evaluated ASPH derivatives (TA-AMPH, FB-AMPH, AB-AMPH, DA-AMPH, PD-AMPH and PY-AMPH) were synthesized by the cyclization reaction of ASPH and the corresponding aromatic aldehydes as illustrated in Fig. 1.

2.3.1. 6-Amino-7-sulfanylphthalazine-1,4(2H,3H)-dione (ASPH)

ASPH was synthesized *via* phthalimido[4,5-*d*]-thiazol-2-ylamine (compound I) from 4-aminophthalimide by the following steps.

4-Aminophthalimide (6.49 g, 40 mmol) and ammonium thiocyanate (6.09 g, 80 mmol) were suspended in methanol (100 mL). To the suspension, methanolic solution of bromine (16.0 g, 100 mmol/50 ml) was added dropwise at 0°C. The solution was stirred for 2 h at 0°C and for 8 h at room temperature. The resulting precipitates were collected, dried under reduced pressure and recrystallized from DMF–benzene (1:2, v/v) to give compound I as a yellow crystalline powder (2.69 g, 30.8%). FAB-MS, $m/z = 220$ ($[M+H]^+$, base peak).

Compound I (2.19 g, 10 mmol) was dissolved in ethanol (100 ml) at 60°C. To the solution, hydrazine monohydrate (100 ml) was added, and the mixture was refluxed for 72 h. The solvent was evaporated under reduced pressure and the residue was recrystallized from ethanol to afford ASPH as a pale yellow crystalline material (1.40 g, 6.7 mmol, 67.0%), m.p. 119–121°C. ^{1}H-NMR, δ 3.31 (br, 2H + 1H, hydrazino moiety and SH), δ 6.19 (br, 2H, NH_2), δ 7.03 (s, 1H, C8 aromatic proton), δ 7.67 (s, 1H, C5 aromatic proton). FAB-MS, $m/z = 210$ ($[M+H]^+$, base peak). High-resolution FAB-MS, $m/z = 210.1257$ (as calcd for $C_8H_8N_3O_2S$).

The total yield of the ASPH synthesis was *ca.* 20%.

2.3.2. 2-(4′-Methylphenyl)phthalazino[6,7-d]-thiazole-5,8(6H,7H)-dione (TA-ASPH)

ASPH (0.21 g, 1 mmol) and 4-tolualdehyde (0.12 ml, 1 mmol) were dissolved in DMF (20 ml), and a solution of sodium sulfite (0.06 g) and disodium hydrogenphoshite (0.63 g) in 1.5 M sulfuric acid (20 ml) was added. The mixture was heated at 80°C for 1 h with stirring and was then stirred for 3 h at room temperature. The resulting precipitates formed by cooling in ice-water, were collected and recrystallized from methanol to give TA-ASPH as a pale yellow crystalline material (132 mg, 42.7%), m.p. > 300°C. FAB-MS, $m/z = 310$ ($[M+H]^+$, base peak).

2.3.3. 2-(4′-Carboxyphenyl)phthalazino[6,7-d]-thiazole-5,8(6H,7H)-dione (FB-ASPH)

4-Formylbenzoic acid (0.15 g, 1 mmol) was reacted with ASPH in the same way as was 4-tolualdehyde (2.3.2). Pale yellow needles [(118 mg, 34.8%), m.p. > 300°C] were obtained. FAB-MS, $m/z = 340$ ($[M+H]^+$, base peak).

2.3.4. 2-(4′-Aminophenyl)phthalazino[6,7-d]-thiazole-5,8(6H,7H)-dione (AB-ASPH)

4-Acetamidobenzaldehyde (0.16 g, 1 mmol) was reacted with ASPH in the same way as was 4-tolualdehyde (2.3.2). The acetyl group of 4-acetamidobenzaldehyde was hydrolytically cleaved in the derivatization reaction in acidic medium. Yellow needles [(114 mg, 36.8%), m.p. > 300°C] were obtained. FAB-MS, $m/z = 311$ ($[M+H]^+$, base peak).

2.3.5. 2-[4′-(N,N-Dimethylamino)phenyl]-phthalazino[6,7-d]thiazole-5,8(6H,7H)-dione (DA-ASPH)

4-(*N*,*N*-Dimethylamino)benzaldehyde (0.15 g, 1 mmol) was reacted with ASPH in the same way as was 4-tolualdehyde (2.3.2). Light brown needles [(98 mg, 29.0%), m.p. > 300°C] were obtained. FAB-MS, m/z = 339 ($[M+H]^+$, base peak).

2.3.6. 2-{4′-[3′-Hydroxy-5′-(hydroxymethyl)-2′-methylpyridyl]}phthalazino[6,7-d]thiazole-5,8(6H,7H)-dione (PD-ASPH)

Pyridoxal hydrochloride (0.20 g, 1 mmol) was reacted with ASPH in the same way as was 4-tolualdehyde (2.3.2). Brown needles [(120 mg, 33.7%), m.p. > 300°C] were obtained. FAB-MS, $m/z = 357$ ($[M+H]^+$, base peak).

2.3.7. 2-(1′-Pyrenyl)phthalazino[6,7-d]thiazole-5,8(6H,7H)-dione (PY-ASPH)

1-Pyrenecarboxaldehyde (0.23 g, 1 mmol) was reacted with ASPH in the same way as was 4-tolualdehyde (2.3.2) except that DMF-water (3:1, v/v) was used as the recrystallization solvent. A light brown crystalline powder [(206 mg, 49.2%), m.p. $>300^{\circ}$C] was obtained. FAB-MS, $m/z=420$ ($[M+H]^+$, base peak).

ASPH and its derivatives (TA-ASPH, FB-ASPH, AB-ASPH, DA-ASPH, PD-ASPH and PY-ASPH) were stable in the crystalline state at room temperature in the dark for at least 6 month in a desiccator containing silica gel.

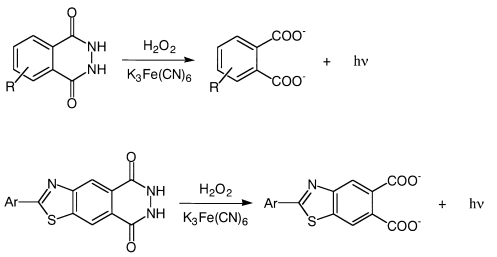


Fig. 2. Chemiluminescence reactions of luminol-related compounds.

2.4. Examination of the chemiluminescent properties

2.4.1. Standard procedure

A 50 μl portion of a 1×10^{-7} M solution of each compound was placed into the well of a Greiner (Frickenhausen, Germany) 96 hole black FIA-plate and the chemiluminescence reaction was initiated by the simultaneous automatic injections of 50 μl of hydrogen peroxide (10 mM for TA-ASPH, 2.5 mM for PD-ASPH, 5.0 mM for the others) solution and 100 μl of potassium hexacyanoferrate(III) (2.5 mM for ASPH, 10 mM for PD-ASPH, 5.0 mM for the others) solution dissolved in sodium hydroxide (0.1 M for luminol, 1.5 M for the others).

The chemiluminescence intensities were monitored immediately after the injection of the

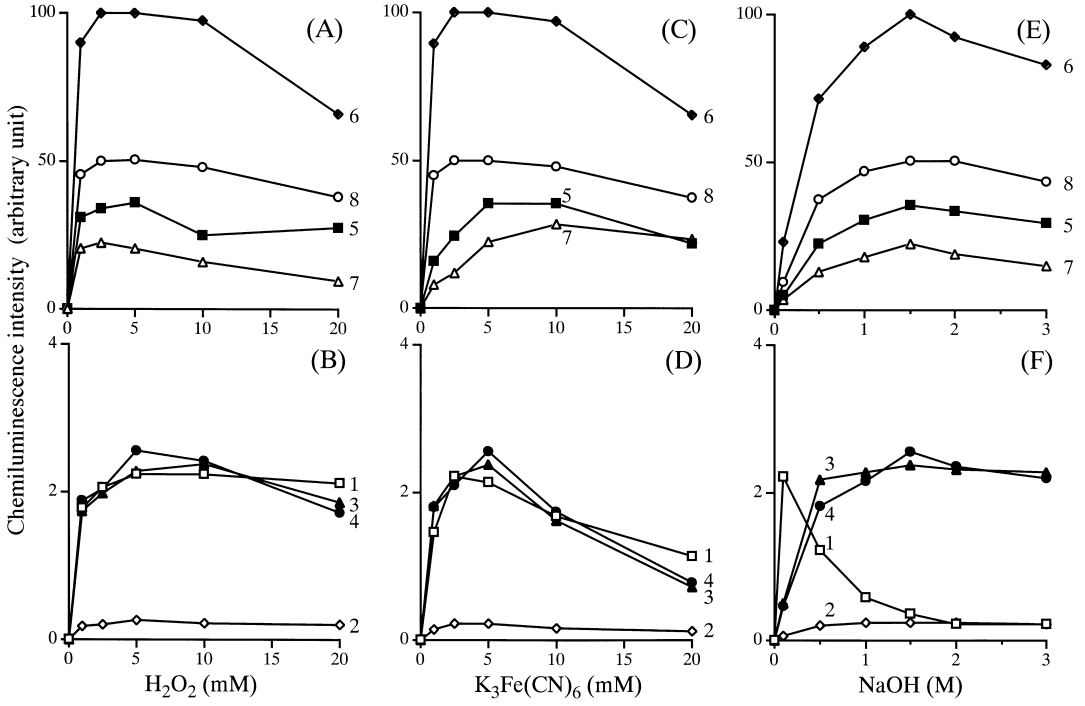


Fig. 3. Effects of concentrations of hydrogen peroxide (A, B), potassium hexacyanoferrate(III) (C, D) and sodium hydroxide (E, F) on integrated chemiluminescence intensities (a run time of 60 s). Curves: 1, luminol; 2, ASPH; 3, TA-ASPH; 4, FB-ASPH; 5, AB-ASPH; 6, DA-ASPH; 7, PD-ASPH; 8, PY-ASPH. Chemiluminescence conditions: see Experimental (2.4.2).

oxidizing eagent solutions. Integrated photon counts for 60 s after the injections were defined as the chemiluminescence intensities.

2.4.2. Optimization of the chemiluminescence conditions

The kind of solvent of chemiluminophores and the concentrations of the oxidizing reagents (hydrogen peroxide, potassium hexacyanoferrate(III) and sodium hydroxide) were varied one at a time to establish the optimum chemiluminescence conditions. The provisionally settled conditions were DMF solution of chemiluminophores, 5.0 mM hydrogen peroxide and 5.0 mM potassium hexacyanoferrate(III) prepared in 1.5 M sodium hydroxide.

Table 1
Relative chemiluminescence intensities (R.C.I.) of luminol-related compounds examined in various solvents

	R.C.I.[a]					
Compound	H_2O	CH_3OH	CH_3CN	DMSO	DMF	Dioxane
Luminol	41	42	49	92	100	34
ASPH	< 1	< 1	< 1	1	2	< 1
TA-ASPH	54	55	69	83	110	71
FB-ASPH	62	71	68	110	100	69
AB-ASPH	690	710	960	1400	1300	690
DA-ASPH	1200	2600	3300	3800	4100	3600
PD-ASPH	300	310	660	910	770	480
PY-ASPH	1300	1400	1400	2000	1700	1600

[a] Integrated chemiluminescence intensity (a run time of 60 s) of luminol in DMF was taken as 100.

3. Results and discussion

The chemiluminescent properties of ASPH and its derivatives (TA-ASPH, FB-ASPH, AB-ASPH, DA-ASPH, PD-ASPH and PY-ASPH) were examined in order to evaluate the use of these compounds as highly chemiluminescent probes. These seven compounds, like luminol, produce chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in an alkaline medium (Fig. 2). The concentrations of the three components of the oxidizing reagent system had a major influence on chemiluminescence intensity (Fig. 3); 1.0–10 mM hydrogen peroxide, 2.5–10 mM potassium hexacyanoferrate(III) and 1.0–2.0 M sodium hydroxide

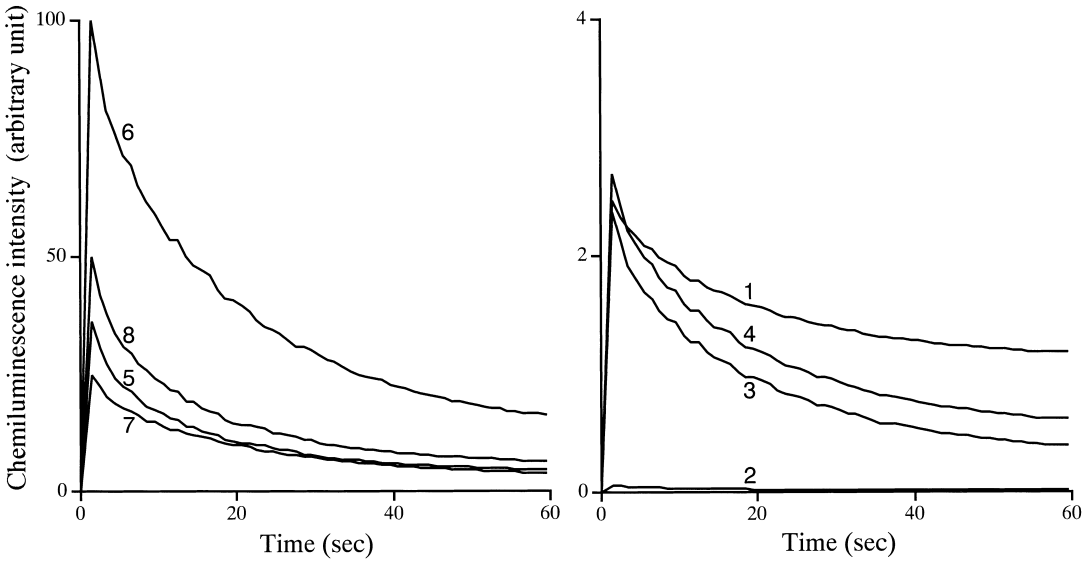


Fig. 4. Time dependence of the chemiluminescence reaction of the compounds. Curves: 1, luminol; 2, ASPH; 3, TA-ASPH; 4, FB-ASPH; 5, AB-ASPH; 6, DA-ASPH; 7, PD-ASPH; 8, PY-ASPH. Chemiluminescence conditions: see Experimental (2.4.1).

gave the maximum intensity. The optimum chemiluminescence conditions employed were described in section 2.4.1.

Under these optimum reaction conditions, the chemiluminescence intensities of eight compounds in various solvents were examined (Table 1). The ASPH derivatives in dimethylsulfoxide (DMSO) and/or DMF were found to produce chemiluminescence 1.1–40 times larger than that of luminol. In turn, the chemiluminescence of luminol was 50 times greater than ASPH itself.

Fig. 4 illustrates the time dependence of the chemiluminescence intensity of the new luminol-type compounds and luminol. For all the compounds, chemiluminescence was immediately initiated by the addition of hydrogen peroxide and alkaline potassium hexacyanoferrate(III). Chemiluminescence intensities reached their maxima within 1.5 s and then decreased rapidly.

The chemically excited 3-aminophthalate anion, which is produced in the oxidization reaction, has been shown to be the light emitter in the chemiluminescence of luminol [5]. Therefore, in the case of these new luminol-type chemiluminophores, the corresponding dicarboxylate ions (Fig. 2) could be expected to be the light-emitting species. The fluorescent properties (excitation and emission maxima of the fluorescence and their relative intensities) of the species in the chemiluminescence reaction mixture were measured and compared with those of luminol (Table 2). The fluorescence intensities of the ASPH-derivative's dicarboxylate ions were 39–3100 times larger than that of luminol's 3-aminophthalate anion. These results suggest that the chemiluminescence intensities of the luminol-type chemiluminophores are highly dependent on the fluorescence intensities of the light-emitting species, the corresponding dicarboxylate ions, that are produced in the chemiluminescence reaction. Otherwise, it is known that the chemiluminescence spectra of luminol-type compounds are almost identical to the fluorescence emission spectra of the corresponding dicarboxylate ions [1,2,7,16–18]. Thus, AB-ASPH, DA-ASPH, PD-ASPH and PY-ASPH were expected to afford chemiluminescence of longer wavelengths than luminol, which are preferable for the sensitive assays of hydrogen peroxide and peroxidase, and easily observed with the naked eyes. This work is now in progress.

Table 2
Fluorescence excitation (Ex) and emission (Em) maxima and relative intensities (R.F.I.) of the dicarboxylate anions corresponded to the chemiluminophores

Chemiluminophore	Ex (nm)	Em (nm)	R.F.I.[a]
Luminol	316	425	100
ASPH	313	432	75
TA-ASPH	314	387	4600
FB-ASPH	318	400	3900
AB-ASPH	362	459	88,000
DA-ASPH	382	454	310,000
PD-ASPH	404	486	36,000
PY-ASPH	376	457	110,000

[a] Fluorescence intensity for luminol was taken as 100.

4. Conclusions

Novel luminol-type compounds containing a 2-arylbenzothiazole moiety, TA-ASPH, FB-ASPH, AB-ASPH, DA-ASPH, PD-ASPH and PY-ASPH, were found to produce intense chemiluminescence by reaction with hydrogen peroxide in the presence of potassium hexacyanoferrate(III) in an alkaline medium. DA-ASPH and PY-ASPH particularly are potentially useful as highly chemiluminescent probes.

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