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Effect of some aminoanthraquinone derivatives as red fluorescers on chemiluminescence systems originating from bis-(2,4,6-trichlorophenyl) oxalate and lucigenin

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Abstract

Peroxyoxalates such as bis-(2,4,6-trichlorophenyl) oxalate (TCPO) can transfer energy to fluorescer molecules via formation of dioxetanedione intermediate. The aminoanthraquinone derivatives used in this study found to produce a red light in the chemiluminescence systems containing TCPO or lucigenin. Both the fluorescence and chemiluminescence spectra of each of aminoanthraquinone fluorescer system were obtained and the influence of anthraquinone derivatives on the λ_{max} of fluorescer was investigated. The blue light of chemiluminescenct system containing lucigenin is of direct chemilumiescence type, while the red chemiluminescence produced in the presence of anthraquinones is indirect in nature.

Keywords: Alkylaminoanthraquinone; Chemiluminescence; TCPO; Lucigenin; Red fluorescer

1. Introduction

The essential requirements for chemiluminescence (CL) include (1) a chemical reaction enable to generate a high energy intermediate, (2) decomposition of the intermediate with concurrent transfer of excess chemical energy to a receptor molecule which becomes electronically excited, and (3) light emission as the excited receptor molecule relaxes to the ground state.

Two classical mechanisms have been proposed for explanation of the chemiexcitation phenomenon. The first is one in which a high energy molecule is converted through a thermal reaction to an electronically excited state. This electronically excited state may then emit a photon of light either as fluorescence or phosphorescence (direct chemiluminescence) or undergo a chemical reaction. Alternatively, this electronically excited state may transfer energy to a suitable acceptor (fluorescer) and the thus formed excited acceptor may then go on to emit a photon of light (indirect chemiluminescence) or undergo a chemical reaction [1–4]. Indirect CL has been reported in a bioluminescent system of coelentrates [4,5]. In several of the luminescent coelentrates, the color of the light emission of the living organism is green due to green fluorescent protein (indirect CL), whereas the emission of the isolated in vitro reaction is blue (direct CL) [4,5]. Aromatic fluorescent compounds can accept energy and change the bacterial bioluminescent spectra [6]. This indicates that there is an energy transfer from the excited molecule to a second species followed by emission from its excited state.

In this paper, we studied the influence of four recently synthesized aminoanthraquinone derivatives 1-amino-2-methyl-

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9,10-anthraquinone (A1), 1-amino-2-ethyl-9,10-anthraquinone (A2), 1-amino-2,3-dimethyl-9,10-anthraquinone (A3) and 1-amino-2,4-dimethyl-9,10-anthraquinone (A4) on the chemiluminescence systems containing bis-(2,4,6-trichlorophenyl) oxalate (TCPO) and lucigenin.

The aminoanthraquinones used found to produce a red light in both chemiluminescence systems employed, while, TCPO and lucigenin produced a blue-green light. In fact, the aminoanthraquinone derivatives acted as fluorescers and accepted energy from TCPO or lucigenin and emitted red light. The proposed mechanisms for the direct and indirect CL are summarized as follows:

Direct chemiluminescence:

$$A + B \rightarrow C^* + D$$

$$C^* \rightarrow C + \text{light (blue-green)}$$

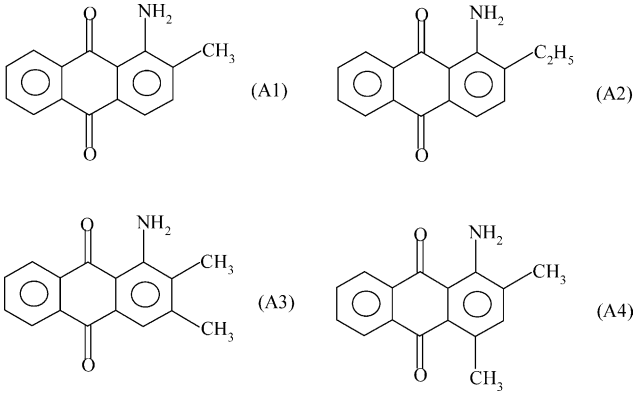
Indirect chemiluminescence:

$$A + B \rightarrow C^* + D$$

$$C^* + F \rightarrow F^* + C$$

$$F^* \rightarrow F + \text{light (red)}$$

Some derivatives of polycyclic aromatic hydrocarbons have already been introduced as red fluorescers [7,8].



2. Experimental

2.1. Apparatus

All fluorescence and chemiluminescence spectra were recorded on a Model LS-50B Perkin-Elmer instrument. The xenon lamp of spectrofluorometer was off when the CL spectra were obtained in the visible region. The fluorescence spectra for aminoanthraquinones in the presence and absence of TCPO and lucigenin (i.e., before and after CL reaction) were recorded at excitation wavelengths of 254 and 366 nm, respectively.

2.2. Reagents

The aminoanthraquinone derivatives A1–A4 were synthesized [9] and used after recrystallization from reagent grade benzene and vacuum-drying. The purities (>99.99%) of aminoanthraquinone derivatives were confirmed by spectroscopic data and elemental analysis. All other reagents and solvents were of reagent grade and purchased from Fluka (CH-9470 Buchs, Switzerland). Bis-(2,4,6-trichlorophenyl) oxalate (TCPO) was prepared by the method of Mohan and Turro [10] from reaction of 2,4,6-trichlorophenol and oxalyl chloride in the presence of triethylamine and recrystallised from ethyl acetate. Hydrogen peroxide (30%) was concentrated via freeze drying (using a model FD-1 Eyela freeze dryer, Tokyo, Japan) up to 60% and mixed with dimethyl phthalate in a 1:1 v/v portions and shook well on an electrical shaker. After about 10 h, the organic phase was separated, dried on anhydrous Na_2SO_4, and the H_2O_2 concentration was determined by a standard potassium permangenate solution. Standard solutions of hydrogen peroxide (1.5 M in 80:20 dimethyl phthalate:*tert*-butyl alcohol containing 0.005 M sodium salycilate) were prepared from this solution.

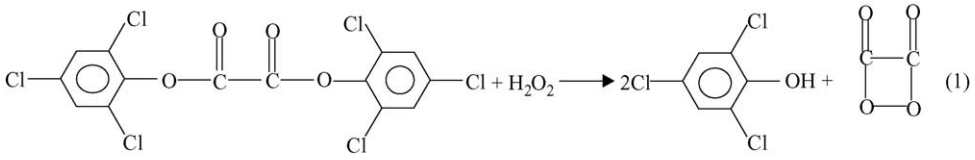
2.3. Procedures

To a fluorometric cell containing 1 ml TCPO (0.01 M in ethyl acetate) and 1 ml of aminoanthraquinone (0.01 M in ethyl acetate) was added 0.2 ml hydrogen peroxide (1.5 M in 80:20 dimethylphthalate:*tert*-butyl alcohol containing 0.005 M sodium salicylate) and after vigorous stirring both the fluorescence and chemiluminescence spectra were recorded.

To a fluorometric cell containing 1 ml lucigenin (0.002 M in methanol) and 1 ml of aminoanthraquinone (0.01 M in ethyl acetate) and 0.2 ml hydrogen peroxide (10–20% in water) was added 0.2 ml of a NaOH solution (0.1 M in water) and after vigorous stirring both the fluorescence or chemiluminescence spectra were recorded.

3. Results and discussion

The initial example of peroxyoxalate CL was reported in 1963 by Chandross [11] who found that oxalyl chloride, when treated with hydrogen peroxide in the presence of a fluorescent compound such as 9,10-diphenylantracene, produces a bright, short-lived blue emission corresponding to the fluorescence of the hydrocarbon. The energy produced in the chemical reaction was transferred to the fluorescer, producing the excited singlet state of the fluorescer, which emits in a typical fluorescence process. Rauhut and his co-workers at Cyanamid subsequently developed the chemiluminescent reactions of aryl oxalate esters and hydrogen peroxide with a variety of fluorescers in quest of a long-lived light source for commercial and military used [12–17].



$$[C_2O_4] + F \rightarrow \quad F^* + 2\ CO_2 \qquad (2)$$

$$F^* \longrightarrow F + h\nu \qquad (3)$$

Scheme 1. The mechanism of PO–CL reaction.

Peroxyoxalate–chemiluminescence (PO–CL) is well-known as one of the most efficient non-biological light producing systems. Like many chemiluminescence reactions, the PO–CL reaction can be schematized in three basic steps [4,15,16,18–20] (Scheme 1). In the first step, an aryl oxalate ester like TCPO reacts with H_2O_2 to produce a key chemical intermediate, C_2O_4, containing the necessary excitation energy. The second step involves the chemiexcitation of a fluorophore, like aminoanthraquinone, to electronically excited states by the reactive intermediate via conversion of the chemical energy into electronic excitation energy. The final step is the emission of light energy by returning the excited fluorophore molecule to the ground state.

In preliminary experiments, it was found that the addition of a few drops of the stock solution of H_2O_2 to an ethyl acetate solution containing TCPO and aminoanthraquinone results in a relatively intense red light. The sensitized PO–CL spectrum of aminoanthraquinone A1 is shown in Fig. 1. Similar PO–CL spectra were obtained for compounds A2–A4. The wavelengths of maximum intensity for the resulting fluorescence and CL spectra are tabulated in Table 1. As it is seen, since the light emission steps for both chemiluminescence and fluorescence are essentially analogous, the emission wavelength maxima in both processes are similar [13,21]. Hence, the color of the CL emission can be manipulated readily by introducing fluorescent additives with known emission characteristics. For example, a purple light can be achieved with 9,10-diphenylanthracene (λ_{max} = 430 nm), blue with perylene (λ_{max} = 471 nm), green with 9,10-diphenylanthracene (λ_{max} = 513 nm) and orange with rubrene (λ_{max} = 556 nm) [4,7,8,16,22].

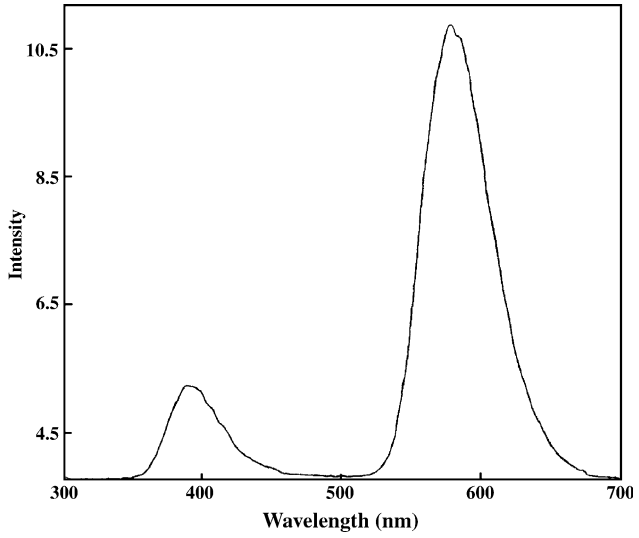


Fig. 1. PO–CL in the presence of A1.

As expected, the intensity of the PO–CL emission peak was found to be proportional to the initial concentration of the reactants. Sample of the corresponding plots for H_2O_2–TCPO–L1 system are shown in Fig. 2. In the presence of sodium salicylate (0.0023 M), the light intensity is much higher than that in the absence of the base. The observed behavior is clearly indicative of the catalytic effect of sodium salicylate on the PO–CL system studied [15,16,23]. However, further addition of sodium salicylate revealed a gradual decrease of the CL intensity. This is most probably due to the quenching effect of the base at higher concentrations, which begins to decompose the reactive intermediate, dioxetanedione, and hence reduces the CL light. As is obvious from Fig. 2, there was also a nice linear correlation between the CL intensity and the hydrogen peroxide and TCPO concentrations. The basis for such linear correlation has already been discussed in detail in the literature [24]. Meanwhile, as it has been clearly shown before [25], there is an exponential increase in chemiluminescence of the H_2O_2-TCPO–L1 system with increasing concentration of the fluorescer.

Lucigenin (*N*,*N*′-dimethyl-9,9′-diacridinium nitrate) undergoes a CL reaction with H_2O_2 in alkaline solution to yield *N*-methylacridone (NMA) and light. Several side prod-

Table 1
Wavelengths of maximum intensity (λ_{max}) for the fluorescence and chemiluminescence spectra of alkylaminoanthraquinones in the peroxyoxalate chemiluminescence system

Compound	λ_{max} (nm)			
	FL	FL in reaction mixture	FL after reaction	CL
A1	558.3	552.2	579.4	576.1
A2	569.4	567.3	582.5	582.1
A3	583.5	574.4	590.5	586.1
A4	572.3	570.3	577.4	575.1

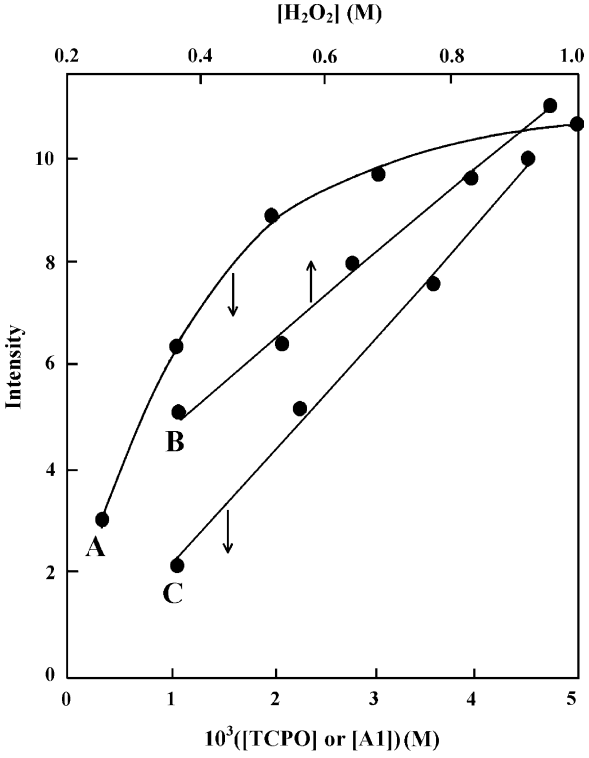
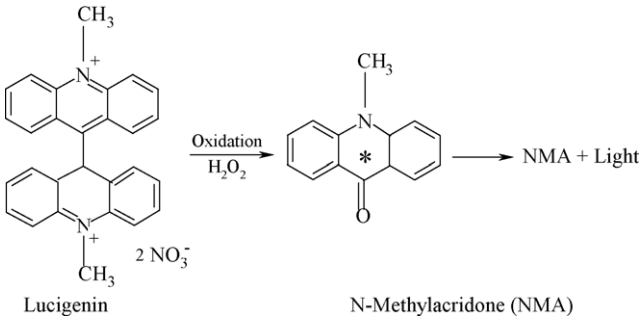


Fig. 2. Effect of concentrations of A1 (A), H_2O_2 (B) and TCPO on the PO–CL of TCPO–H_2O_2–A1 system.

ucts can also be formed. Due to the formation of the excited state *N*-methylacridone (NMA), which emits from about 420–550 nm with a maximum at about 440 nm, the emission is green. In the presence of fluorescers, energy can be transfered and emitted in other spectral region [4,16]. Lucigenin CL reaction is shown in Scheme 2.

When hydrogen peroxide was added to a solution containing TCPO and an aminoanthraquinone derivative, there appeared an intense reddish indirect CL. In the lucigenin CL system, a blue-violet light was observed. The intensity of the emission peaks was found to be proportional to the initial concentration of the reactants. The fluorescence spectra of aminoanthraquinones before and after CL reaction in the TCPO and lucigenin systems revealed that these compounds act as fluorescers, which are stable in the solution and can be commercially used as suitable red emitter fluorescers.



Scheme 2. The mechanism of lucigenin CL reaction.

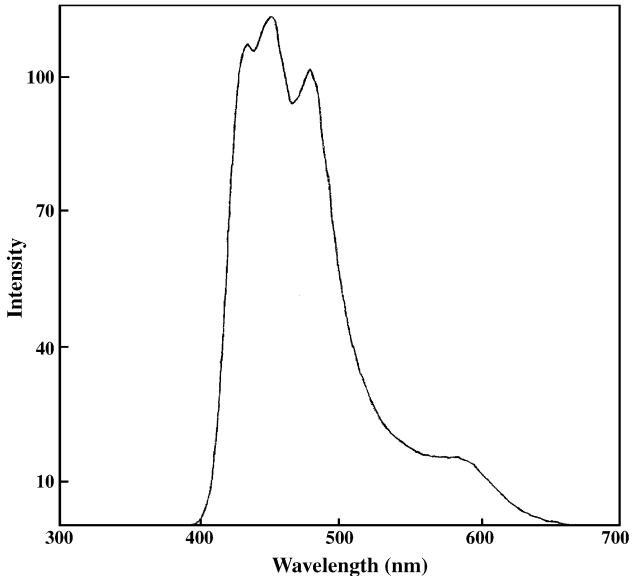


Fig. 3. Lucigenin–CL in the presence of A3.

As the light emission steps for both fluorescence and CL are essentially analogous, the emission wavelength maxima in both processes are similar for the same emitter and the same concentration range (Table 1). The fluorescers' stability in reaction medium is dependent on their resistance to hydrogen peroxide, which might result in some small shifts in the resulting FL spectra taken in the reaction mixture, as they are compared with the corresponding FL spectra recorded after the CL reaction. The low CL quantum yield of lucigenin and a broad peak of direct CL for this compound was affected the energy transfer process. As we can observe from the CL of lucigenin in the presence of 1-amino-2,3-dimethylantraquinone, the shoulder observed at about 582.1 nm belongs to the resulting indirect CL (Fig. 3). This low intensity peak was also observed in the flurescence spectrum of the reaction mixture and has about the same wavelength (585.1 nm) with respect to the CL spectrum.

As it is obvious from Table 1, the wavelengths of maximum intensity for both the FL and PO–CL spectra varied with the position and number of substituted alkyl groups on the frame of 1-amino-9,10-anthraquinone. The λ_{max} of both the CL and FL spectra increases in the order A1 < A2 < A4 < A3. Because the ethyl group is a more electron donor than the methyl group, the CL and FL of A2 shows some bathochromic shift with respect to A1. The influence of the substitution of two donating methyl groups on A3 and A4, as it compared to A1 containing only one methyl substitution, is quite obvious from Table 1. As expected, they show maximum intensity at longer wavelengths. The most bathochromic shift belongs to A3, which has two methyl substituents on 2- and 3-positions. The longer emission wavelengths for A3 is due to the facilitated hyperconjugation effect of the two substituted methyl groups on 2- and 3-positions. While, in the case of compound A4 with two methyl groups in 2- and 4-postions, there is only one methyl group located in the para

position in relation to one of the carbonyl groups suitable for hyperconjucation. It is also interesting to note that the λ_{max} values of the CL and FL emissions of A3 in the lucigenin are very close to those of the TCPO system.

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