FLUORESCENCE AND PHOSPHORESCENCE SPECTRA OF FIREFLY AND CYPRIDINA OXYLUCIFERINS: A QUESTION FOR THE MULTIPLICITY OF THE EXCITED STATES PRODUCED IN THE BIOLUMINESCENT SYSTEMS

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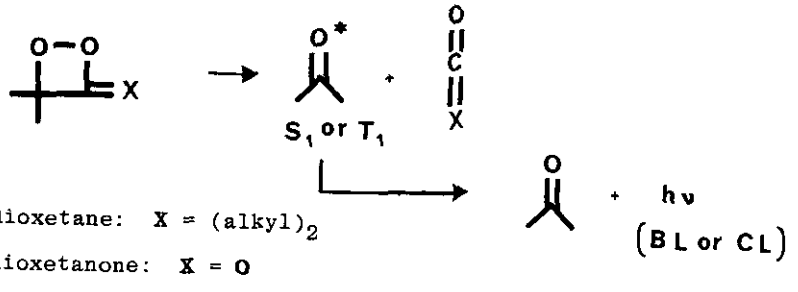
Abstract —— Firefly oxyluciferin (1), the emitter of firefly bio- and chemiluminescence, gives identical FL and PL spectra under a certain condition. Hence, we showed that it would be necessary to examine the fluorescence and phosphorescence spectra before the identification of the multiplicity of the emitting species in the every bio- and chemiluminescence.

Bioluminescent systems have been considered to give carbonyl products, one of which is produced predominantly in a singlet excited state (S_1)[1)] and gives light emisson. On the contrary, most chemiluminescent, synthetic 1,2-dioxetanes[1)] and dioxetanones[1c)] give directly a triplet excited state (T_1) carbonyl compound in major parts. This contrast has been a serious puzzle. The chemically initiated electron-exchange luminescence (CIEEL) mechanism was proposed for the explanation.[1b,d,f)]

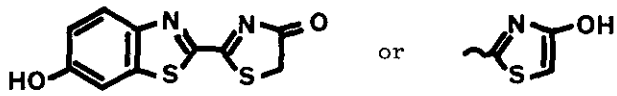


1,2-dioxetane: X = $(alkyl)_2$

1,2-dioxetanone: X = O

They seem to provide a priori that fluorescence (FL) and phosphorescence (PL) spectra of the carbonyl compounds, which are produced in the bioluminescence (BL) and/or chemiluminescence (CL), are different from each other. However, no report was found on the correlation between S_1 and T_1 states of the emitting species, the carbonyl compounds produced on the BL or CL. We now wish to describe that firefly oxyluciferin (1), the emitter in the firefly bio- and chemiluminescence, and its related compounds gave identical "PL" spectra with the FL

spectra under the conditions used in the CL reaction except the temperature (77 K).



Firefly Oxyluciferin 1[2)]

A solution (200 µl) of 1[2)] (final concentration: 1.0×10^{-5} M) in dimethyl sulfoxide (DMSO) was evacuated (10^{-4} mmHg x 4 times/77 K) in a quartz tube (ϕ 2 mm). To this solution, a separately evacuated solution (300 µl) of t-BuOK (final concentration: 5.0×10^{-3} M) in DMSO was added at room temperature.[3,4)] Its FL[6)] and PL[6)] spectra at 77 K were identical (λ_{max} 490 ± 6 nm; exc. at 400 nm). The FL spectrum at room temperature was identical with CL spectra reported in literature (λ_{max} 560 ± 6 nm; exc. at 400 nm) (Fig. 1). PL spectrum of 1 could not be detected at room temperature, however.

Generally to say, this coincidence of the FL and the "PL" spectra is not so a strange phenomenon. Several examples are found in the literature,[8)] e.g., anthracene in EtOH ($10^{-4} \sim 10^{-5}$ M) at 20°C and 77 K shows spectra which are identical to the ordinary FL spectrum both in the λ_{max} and the shapes under the measuring conditions of PL. The intersity is, however, 0.28% of the ordinary one. The true PL from the T_1 state is not detectable (too weak). The energy diagram at 77 K in EPA was reported.[9,10)] The "PL" spectrum identical to its FL spectrum is known to be delayed FL and could be explained either by thermal popping up of T_1 to S_1 after a fast intersystem crossing ($S_1 \rightarrow T_1$) (sequence 1) or by T - T annihilation (sequence 2).[8,9)]

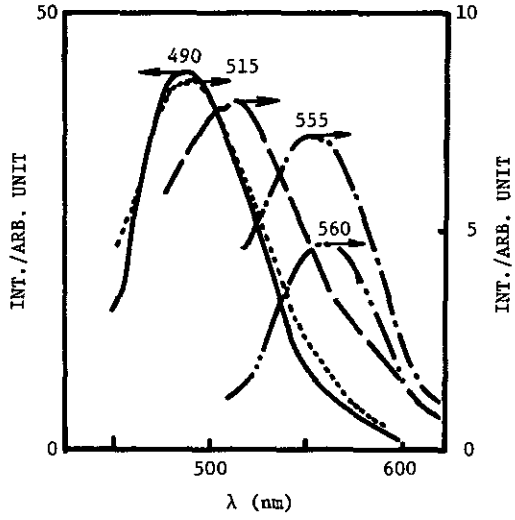


Fig. 1. FL and "PL" Spectra of 1. FL at 77K, (———); at -78°C, (— — —); at 0°C, (— · —); and at room temp., (— ·· —). PL at 77K, (·····).

$$S_0 + h\nu \rightarrow S_1 \rightarrow T_1 \overset{\Delta}{\rightarrow} S_1 \rightarrow S_0 + h\nu' \qquad (1)$$

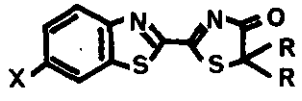
$$T_1 + T_1 \rightarrow S_0 + S_1 \rightarrow S_0 + h\nu' \qquad (2)$$

The present data suggest a possibility that the BL produces directly the T_1 excited state instead of the S_1 excited state to yield light emission from it and/or the S_1 state produced from the T_1 state, as most of the synthetic dioxetane systems do. As the CL reaction in DMSO and BL reaction in an aqueous medium do not proceed at

the temperature below the melting points (18.5°C and ca. 0°C, respectively), the coincidence of the FL and "PL" spectra at 77 K, however, would not indicate directly the facts that the BL or CL reaction of **1** produces the T_1 excited state, predominantly. At least, the present results suggest that it would be worthy to examine the FL and PL spectra of every bioluminescent emitter for the assignment of the electronic multiplicity of the excited states produced.

For that purpose we examined FL and PL spectra of some other firefly oxyluciferins[11] (**2** and **3**) and Cypridina oxyluciferin[12] (**4**) under the similar conditions. The results are summarized in Table 1.

Table 1 shows that every firefly oxyluciferin (**2** and **3**) behaved similarly to **1** under the conditions examined. Cypridina oxyluciferin (**4**), however, showed a different PL from FL. They showed FL spectra whose λ_{max} are identical at room temperature with the CL spectra of the corresponding luciferins. These results confirmed the necessity of the examination mentioned above.



2: R=H, X=MeO
3: R=Me, X=OH

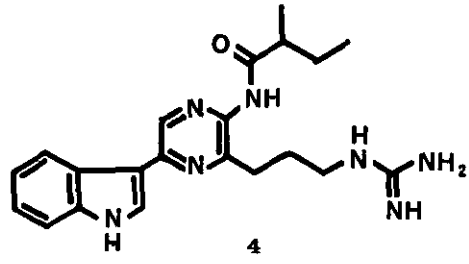


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Table 1. FL and "PL" of Firefly and Cypridina Oxyluciferins (**1** ∿ **4**) in DMSO

Oxyluciferin[a]	Temp.(°C)	λ_{max} (nm) FL(exc)[b]	"PL"(exc)[b]	CL(ref)[c]
1	r.t.	560±6(470)	——	556±6 (7)
	0	555±6(470)	——	——
	-78	515±6(380)	——	——
	-196	490±6(400)	490(320)	
2	r.t.	580±6(470)	——	592±6 (7)
	0	580±6(480)	——	——
	-78	522±6(440)	——	——
	-196	500±6(420)	500(320)	——
3	r.t.	620±6(520)	——	630±5 (7)
	0	605±6(580)	——	——
	-78	583±6(470)	——	——
	-196	555±6(470)	555(320)	——
4	r.t.	425±6(360)	——	417 (12)
	0	417±6(330)	——	——
	-78	400±6(300)	——	——
	-196	400±6(290)	492(320)	——

a) Final concentration: [Oxyluciferin] = 10^{-5} M; [t-BuOK] = 5 x 10^{-3} M.
b) exc: Wave length of excitation (FL: ±6 nm; "PL": ±20 nm).
c) reported in the literatures cited.

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