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3-(4-Acyl-3-hydroxyphenyl)-1,2-dioxetanes as a Chemiluminescent Substrate with High Efficiency in an Aqueous System

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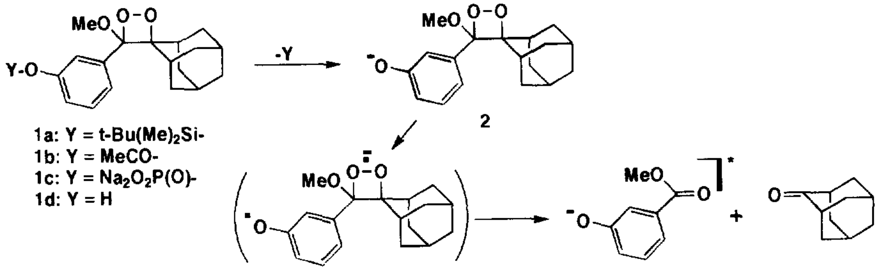
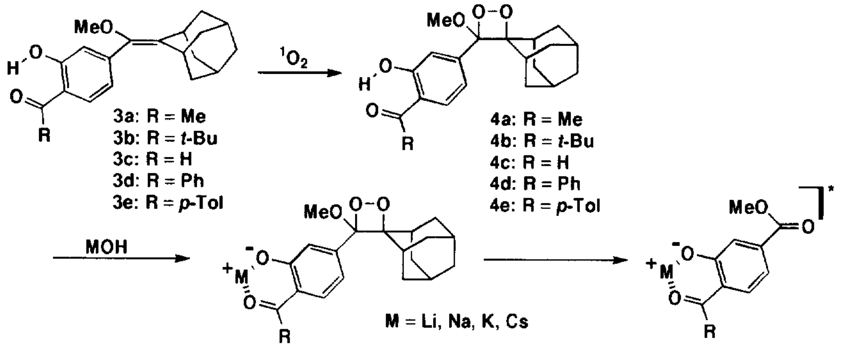
Abstract: A 3-(4-acetyl-3-hydroxyphenyl)-1,2-dioxetane (**4a**) emits light in a high yield (λmax: 486 nm, Φ_{CL}: 0.13, *t1/2*: 2.3 x 10^4 s) on treatment with TBAF in DMSO. Even in an aqueous solution, base-induced chemiluminescence of **4a** occurs effectively (Φ_{CL}: 1/20 of the DMSO system). On the other hand, **1d** bearing a 3-hydroxyphenyl affords in the aqueous system only 1/31000 light of the DMSO system.

Dioxetanes bearing a fluorescent electron donor display chemically initiated electron exchange luminescence (CIEEL).[1,2] The phenomenon has recently been extended to thermally stable dioxetanes (**1**) which release a CIEEL-active phenolate-substituted dioxetane (**2**) on treatment with a base or enzyme.[3,4] The dioxetane (**2**) emits light in a high yield in an aprotic solvent such as DMSO,[3] whereas it affords very weak light in an aqueous solution (for **1c**, Φ_{CL}: < 1/10000 of **1a** / DMSO system),[4] which is needed for an enzymatic triggering system useful in an immunoassay system and DNA probes.[5] Such a low light yield in the aqueous system has been improved to some extent (<400 times enhancement) by adding a surfactant, which serves as pockets keeping **2** in a hydrophobic environment, or by tethering a fluorescer to a dioxetane.[4,6] There has been, however, little study on designing a fluorescent electron donor attached to a dioxetane ring to attain efficient chemiluminescence in an aqueous medium. We report here that dioxetanes bearing a 4-acyl-3-hydroxyphenyl moiety emit light in high yield even in an aqueous solution.



A solution of a (4-acetyl-3-hydroxyphenyl)ethylene (**3a**) (136 mg) and tetraphenylporphin (TPP, 3 mg) in CH_2Cl_2 (8 ml) was irradiated with 940 W Na-lamp under an oxygen atmosphere at -78 °C for 90 min. The photolysate was concentrated and the residue was crystallized from methanol-ethyl acetate to give a dioxetane bearing a 4-acetyl-3-hydroxyphenyl group (**4a**) as white crystals (m. p. 104 - 106 °C) in a 83.4 % yield.[7] When a solution of **4a** in DMSO (0.98×10^{-3} mol dm^{-3}, 1 ml) was added into a tetrabutylammonium fluoride (TBAF) solution in DMSO (0.1 mol dm^{-3}, 2 ml) at 25 °C, **4a** emitted light with extremely long half-life (λ_{max} = 486 nm, Φ_{CL} = 0.13, $t_{1/2} = 2.3 \times 10^4$ s). On the other hand, both a phenolic dioxetane (**1d**) and its silyl-protected form (**1a**) afforded light with short half-life in the TBAF / DMSO system (**1a**; λ_{max} = 465 (lit.[3]; 470, lit.[8]; 463) nm, Φ_{CL} = 0.25,[3] $t_{1/2}$ = 5.0 (lit.[3]; 5, lit.[8]; 4.7) s, **1d**; λ_{max} = 465 nm, Φ_{CL} = 0.22,[9] $t_{1/2}$ = 5.1 s). These results suggest that an acetyl as an electron-withdrawing group at the 4-position of the phenolic substituent of **4a** suppresses significantly a charge transfer from the phenoxide to the dioxetane ring.



Next, the chemiluminescence of **4a** induced with various alkali metal hydroxides was examined in aqueous methanol (H_2O / MeOH = 2 / 1) and the results are summarized in Table 1 together with those for **1d**. Table 1 revealed a marked effect of the 4-acetyl functionality on light yield; Φ_{CL} of **4a** in an aqueous methanol system (λ_{max}: 496 - 497 nm) still retained ca 1/10 of Φ_{CL} in the TBAF/DMSO system, whereas Φ_{CL} of **1d** decreased to ca 1/10000 by changing the solvent from DMSO to aqueous MeOH (λ_{max}: 474 nm). It is noteworthy that the half-life ($t_{1/2}$) of chemiluminescence of **4a** in aqueous methanol system decreased in the order: $Li^+ > Na^+ > K^+ > Cs^+$, which corresponds to the reverse order of ion radii of alkali metals.[10]

CsOH-induced chemiluminescences of **4a** and **1d** were further examined in aqueous MeOH in various proportions (H_2O : MeOH = 0 : 100 - 97 : 3).[11] The correlation between Φ_{CL} and ratios of H_2O *vs* MeOH is illustrated in Fig 1, from which light yields in H_2O were estimated by extrapolation to be 6.4×10^{-3} for **4a** and 7.0×10^{-6} for **1d**. Experiments for **4a** in aqueous DMSO in various proportions were also carried out to give an extrapolated value of Φ_{CL} of **4a** in H_2O, which is similar to the value described above (see also Fig. 1). The pronounced decrease of Φ_{CL} for **1d** in an aqueous system is likely attributed to quenching of a singlet excited anionic methyl *m*-hydroxybenzoate formed from the dioxetane (**1d**). Such quenching is probably caused by complex interactions between an excited emitter bearing an oxyanion and H_2O such as dipole-dipole interaction, proton transfer, and hydrogen bonding in an aqueous solution. On the other hand, from such interactions, especially hydrogen bonding with H_2O molecules, the oxyanion center of 4-acetyl-3-

hydroxybenzoate as an emitter derived from **4a** is likely protected by coordination (chelation) with an alkali metal ion. The contribution of the chelation to maintain high light yield was further supported by the following experiments.

For a 3-hydroxy-4-pivaloylphenyl moiety, neither an intramolecular hydrogen bonding nor chelation with an alkali metal ion should be less favorable than a 4-acetyl-3-hydroxyphenyl because of a steric repulsion between a *tert*-butyl and the adjacent aromatic proton. Accordingly, providing that the chelation with an alkali metal ion plays an important role in chemiluminescent efficiency for **4a**, base-induced chemiluminescence of a dioxetane (**4b**), bearing a 3-hydroxy-4-pivaloylphenyl moiety instead of 4-acetyl-3-hydroxyphenyl, is expected to have a light yield lower than **4a** in an aqueous solution. Thus, a dioxetane (**4b**) was synthesized similarly to the case of **4a** (81.4 % yield).

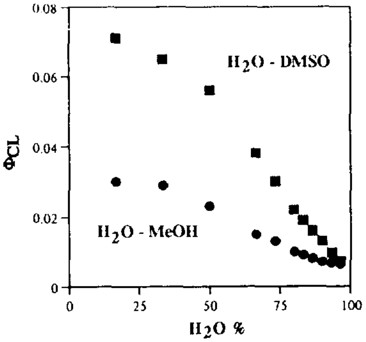


Fig. 1 Chemiluminescence of a dioxetane (**4a**) induced with CsOH in H_2O-MeOH and in H_2O-DMSO

Table 1 Base-induced chemiluminescence of dioxetanes (**1d**) and (**4**)$^{a)}$

Dioxetane	Solvent	Base	λmax / nm	$t_{1/2}$ / s	$\Phi_{CL}$$^{b)}$	Relative Φ_{CL}
(**4a**)	DMSO	TBAF	486	2.3×10^4	1.3×10^{-1}	1
	H_2O-MeOH (2 : 1)	LiOH	496	2.1×10^4	1.5×10^{-2}	
	H_2O-MeOH (2 : 1)	NaOH	496	1.9×10^4	1.4×10^{-2}	
	H_2O-MeOH (2 : 1)	KOH	496	1.8×10^4	1.4×10^{-2}	
	H_2O-MeOH (2 : 1)	CsOH	497	1.7×10^4	1.4×10^{-2}	1/9
	$H_2O$$^{c)}$	CsOH	497	8.0×10^3	6.4×10^{-3}	1/20
(**4b**)	DMSO	TBAF	488	1.2×10^2	7.9×10^{-2}	1
	H_2O-MeOH (2 : 1)	CsOH	496	4.6×10^2	5.1×10^{-5}	1/1500
	$H_2O$$^{c)}$	CsOH	497	2.5×10^2	1.6×10^{-5}	1/4900
(**1d**)	DMSO	TBAF	465	5.1	2.2×10^{-1}	1
	H_2O-MeOH (2 : 1)	CsOH	474	2.7×10^2	2.3×10^{-5}	1/9600
	$H_2O$$^{c)}$	CsOH	474	1.8×10^2	7.0×10^{-6}	1/31000

a) Measured at 25 °C. TBAF / DMSO system; for **1d**, dioxetane: 3.3×10^{-7} M, TBAF: 6.6×10^{-4} M, for **4a**, dioxetane: 3.3×10^{-4} M, TBAF: 6.6×10^{-2} M, for **4b**, dioxetane: 3.3×10^{-5} M, TBAF: 6.6×10^{-3} M. H_2O-MeOH (2 : 1) system and MeOH system; dioxetane: 10^{-3} M, alkali metal hydroxide: 10^{-1} M. b) Relative quantum yields based on the value for **1a** (ref. 3): λ_{max} = 470 nm (465 nm), Φ_{CL} = 0.25, $t_{1/2}$ = 5 (5.0) s; values in parentheses were obtained in the present work. c) Estimated values by extrapolation of experimental data obtained in aqueous MeOH in various proportions (100% MeOH - 3% MeOH); see also Fig. 1.

The dioxetane (**4b**) emits light (λ_{max} = 488 nm, Φ_{CL} = 0.08, $t_{1/2}$ = 120 s) by treatment with TBAF in DMSO, whereas **4b** afforded weak light (λ_{max} = 496 nm) in aqueous MeOH as shown in Table 1. The light yield of **4b** in H_2O was estimated to be 1.6 x 10^{-5} after experiments similar to the case of **4a**, and was, as expected, lower than the case of **4a** but still higher than the case of **1d**. The fact that $t_{1/2}$ was shorter for **4b** than for **4a** is likely attributed also to a steric repulsion of a pivaloyl which diminishes an electron-withdrawing effect of the acyl substituent on the aromatic π-system as an electron donor for CIEEL.

Dioxetanes bearing 4-formyl-3-hydroxyphenyl (**4c**), 4-benzoyl-3-hydroxyphenyl (**4d**), or 3-hydroxy-4-(4-methylbenzoyl)phenyl (**4e**) were also synthesized (**4c**: 38.6 %, **4d**: 54.2 %, **4e**: 63.2 % yield). All these dioxetanes afforded light shifted to the red region though their Φ_{CL} were significantly low on treatment with TBAF in DMSO (**4c**: λ_{max} = 510 nm, Φ_{CL} = 8.2 x 10^{-3}, $t_{1/2}$ = 2.1 x 10^5; **4d**: λ_{max} = 565 nm, Φ_{CL} = 1.9 x 10^{-5}, $t_{1/2}$ = 2.1 x 10^3 s; **4e**: λ_{max} = 560 nm, Φ_{CL} = 9.7 x 10^{-5}, $t_{1/2}$ = 1.8 x 10^3 s). These results suggest that, for (4-acyl-3-hydroxyphenyl)dioxetanes (**4**), an acyl substituent has a subtle effect on yield of the singlet excited carbonyl fragment.

References and Notes

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7. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.05 -1.89 (m, 12H), 2.18 (m, 1H), 2.68 (s, 3H), 3.03 (m, 1H), 3.23 (s, 3H), 7.16 - 7.82 (m, 3H), and 12.28 (s, 1H) ppm; IR (KBr) 3448, 2919, 2860, and 1648 cm^{-1}; Mass (m/z, %) 344 (M+, trace), 312 (24), 278 (4), 194 (60), and 179 (100).
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9. Relative quantum yield based on the value for **1a** (ref. 3).
10. Further experiments are required to discuss these tendencies in detail, though they were not observed for **1d**.
11. The CsOH system was chosen as a representative because it induced the shortest half-life emission of **4a**.

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