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Base-induced chemiluminescence of 5-*tert*-butyl-1-(4-hydroxybenz[*d*]oxazol-6-yl)-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptanes: chemiluminescence–chemiexcitation profile in aqueous medium

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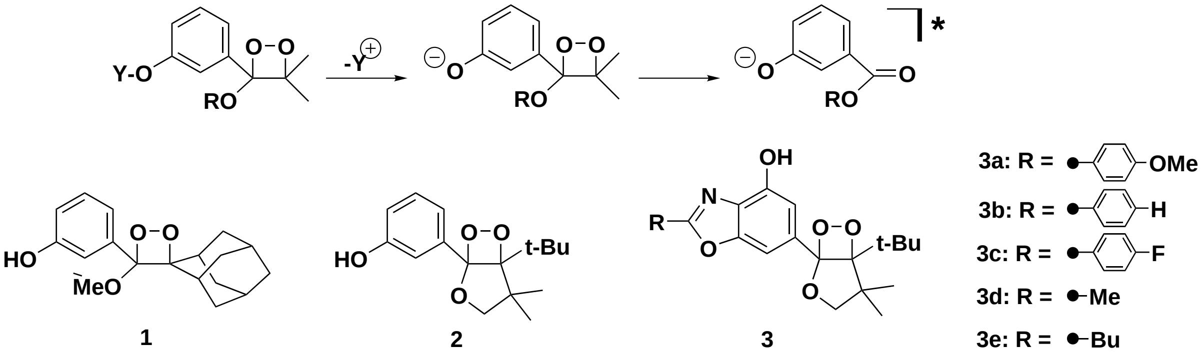
Abstract—Bicyclic dioxetanes bearing a 2-aryl-4-hydroxybenz[d]oxazol-6-yl moiety (**3a**–**3c**) and their 2-alkyl-analogs (**3d**, **3e**) were synthesized. All these dioxetanes (**3a**–**3e**) afforded light with high efficiency on treatment with tetrabutylammonium fluoride in acetonitrile. In the NaOH–H_2O system, chemiluminescence efficiency (Φ^{CIEEL}) for alkyl-analogs (**3d**, **3e**) decreased markedly, while Φ^{CIEEL} for **3a**–**3c** was still considerably high. Fluorescence study revealed that the marked decrease of Φ^{CIEEL} for alkyl-analogs would be attributed to a synergetic effect of decreased chemiexcitation yield and fluorescence yield of the emitter in NaOH–H_2O system. © 2001 Elsevier Science Ltd. All rights reserved.

The intramolecular CIEEL (chemically initiated electron exchange luminescence) of a dioxetane bearing a phenolic anion is a promising entry to highly efficient chemiluminescent substrates.[1–3] An adamantylidenedioxetane (**1**) is a typical example for such CIEEL-active dioxetanes and its phosphate-protected form is now used in modern chemiluminescence bioassays.[3–5] The CIEEL from **1** occurs to emit light effectively in aprotic solvents such as DMSO and acetonitrile while its chemiluminescence efficiency (Φ^{CIEEL}) decreases markedly in aqueous medium (Φ^{CIEEL} in H_2O/Φ^{CIEEL} in DMSO$\,=1/39000$).[4–6] Another noteworthy phenomenon observed for the CIEEL of **1** is that the fluorescence maximum wavelength ($\lambda_{max}{}^{fl}$) of the emitter does not agree with the maximum wavelength ($\lambda_{max}{}^{CIEEL}$) of the CIEEL emission and is blue-shifted by ca. 50 nm in aqueous medium, while the CIEEL spectra and the fluorescence spectra of the emitter coincide in aprotic solvents.[7] Similar phenomena have been observed for a bicyclic dioxetane (**2**).[8,9] Thus, there still remained an important question, namely how is the marked decrease of chemiluminescence efficiency of **1** and **2** in aqueous medium attributed to the singlet-chemiexcitation yield (Φ_S) and/or fluorescence yield



Scheme 1.

Keywords: singlet oxygenation; 1,2-dioxetane; CIEEL; aqueous medium.

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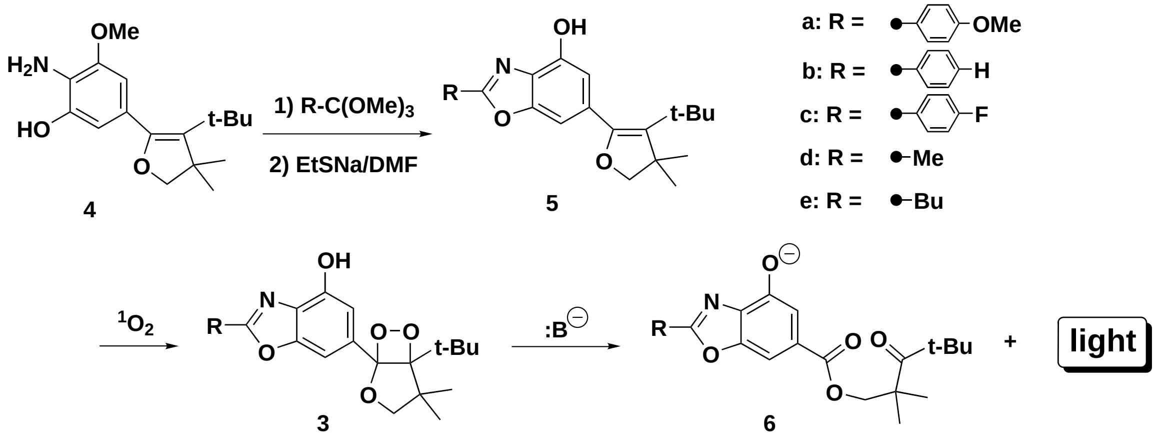
(Φ^{fl}) of the emitter, though chemiexcitation yields have been estimated for CIEEL-decay of various dioxetanes in aprotic solvents (Scheme 1).

In the course of our investigation on the design of new chemiluminescent substrates with high efficiency, we synthesized a variety of bicyclic dioxetanes, 5-*tert*-butyl-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptanes (**3**) bearing a 2-substituted 4-hydroxybenz[*d*]oxazol-6-yl moiety, and our findings can provide an answer to the above question regarding the marked decrease of Φ^{CIEEL} in H_2O.

Benzoxazole-substituted dioxetanes (**3a**–**3e**) were synthesized in>82% yield by singlet oxygenation of the corresponding dihydrofurans (**5a**–**5e**), which were prepared from 2-amino-3-methoxyphenol (**4**) by condensation with an orthoester and by successive demethylation of the methoxy to a hydroxy group (Scheme 2). Typical singlet oxygenation was as follows: a solution of dihydrofuran (**5a**) (100 mg) and tetraphenylporphin (TPP) (1 mg) in CH_2Cl_2 (10 mL) was irradiated with a 940 W Na lamp under an oxygen atmosphere at 0°C for 40 min to give a dioxetane (**3a**), which was obtained in 93.2% yield after chromatographic purification (SiO_2/hexane–AcOEt 3:1).[10] All dioxetanes (**3a**–**3e**) synthesized here were stable enough to permit handling at room temperature.

When dioxetane (**3a**) was treated with a large excess of tetrabutylammonium fluoride (TBAF) in acetonitrile,[11] decomposition occurred rapidly to follow pseudo-first-order kinetics independent of the TBAF concentration and to emit yellow light (maximum wavelength: $\lambda_{max}^{CIEEL} = 512$ nm, chemiluminescence efficiency: $\Phi^{CIEEL} = 0.15$,[12] half-life of CIEEL-decay: $t_{1/2} = 9.0$ s). Similar treatment of the other dioxetanes (**3b**–**3e**) with TBAF/acetonitrile caused also the CIEEL-decay to afford light whose chemiluminescent properties are summarized in Table 1. It should be noted here that λ_{max}^{CIEEL} for **3a**–**3e** coincided with λ_{max}^{fl} of the corresponding spent reaction mixture. Table 1 shows that



Scheme 2.

Table 1. Base-induced chemiluminescent decomposition of dioxetanes bearing a 2-substituted 4-hydroxybenz[*d*]oxazol-6-yl group (**3**)

Dioxetane	TBAF–Acetonitrile[a]				NaOH–H_2O[b]				
	λ_{max}^{CIEEL} (nm)	λ_{max}^{fl} (nm)	Φ^{CIEEL}	$t_{1/2}$ (s)	λ_{max}^{CIEEL} (nm)	λ_{max}^{fl} (nm)	Φ^{CIEEL}	Relative Φ^{CIEEL}	$t_{1/2}$ (s)
3a	512	512	0.15	9.0	505	508	4.3×10^{-3}	58	29
3b	543	545	0.08	23	534	536	1.2×10^{-3}	16	66
3c	533	534	0.12	26	520	526	1.9×10^{-3}	26	73
3d	466	466	0.12	12	482	408 (476)[c]	7.4×10^{-5}	1	85
3e	463	463	0.19	7.8	474	411 (470)[c]	4.1×10^{-5}	0.55	75
1	466[d]	466[d]	0.12[e]	13[e]	466[d]	415[d]	7.5×10^{-6}[f]	0.10	180
2	467[g]	467[g]	0.11	25	467[g]	416[g]	1.1×10^{-5}	0.15	810

[a] A solution of a dioxetane in acetonitrile (1.0×10^{-6} mol dm^{-3}, 1 mL) was added to a TBAF solution in acetonitrile (1.0×10^{-3} M, 2 mL) at 25°C.

[b] A solution of a dioxetane in acetonitrile (1.0×10^{-3} mol dm^{-3}, 0.1 mL) was added to a NaOH solution in water (0.1 M, 2.9 mL) at 25°C.

[c] A figure in parenthesis means wavelength of a shoulder.

[d] Ref. 7.

[e] Ref. 5.

[f] Ref. 4.

[g] Ref. 9.

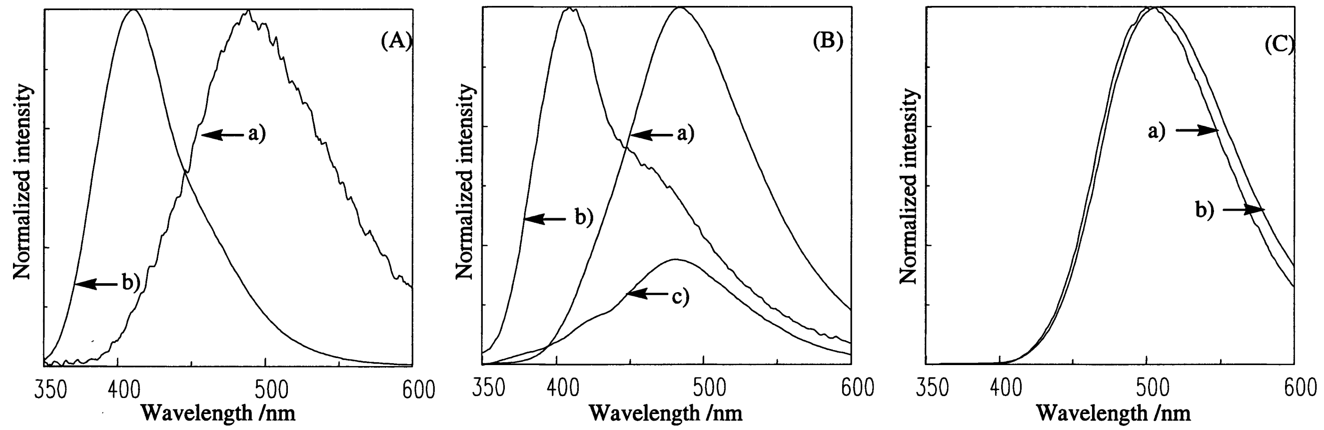


Figure 1. Normalized spectra in NaOH–H_2O: (A) (a) CIEEL of **2** and (b) fluorescence of 2,4,4-trimethyl-3-oxo-1-pentyl 3-hydroxybenzoate, (B) (a) CIEEL of **3d**, (b) fluorescence of **6d** excited with light of $\lambda_{ex}=320$ nm and (c) fluorescence (not normalized) of **6d** excited with light of $\lambda_{ex}=370$ nm, and (C) (a) CIEEL of **3a** and (b) fluorescence of **6a**.

both the chemiluminescence efficiency (Φ^{CIEEL}) and half-life of the CIEEL-decay ($t_{1/2}$) were not substantially different between 2-aryl- (**3a**–**3c**) and 2-alkyl-analogs (**3d**, **3e**) in acetonitrile, though considerable difference in the color tone of luminescence was observed: aryl-analogs (**3a**–**3c**) emit yellow light ($\lambda_{max}{}^{CIEEL}=512$–$543$ nm), while alkyl-analogs (**3d**, **3e**) emit blue light ($\lambda_{max}{}^{CIEEL}=463$–$466$ nm).

Next, we carried out NaOH-induced decomposition of the present dioxetanes (**3a**–**3e**). Treatment of **3a** (1×10^{-3} M in acetonitrile, 0.1 mL) with NaOH (0.1 M in H_2O, 2.9 mL) gave intense light with $\lambda_{max}{}^{CIEEL}=505$ nm, $\Phi^{CIEEL}=4.3\times10^{-3}$, and $t_{1/2}=29$ s at 25°C. The chemiluminescence of **3a** was intense and visible to the naked eye even in NaOH–H_2O.[13] The other aryl-analogs (**3b** and **3c**) gave similarly intense light in NaOH–H_2O (see Table 1). On the other hand, the alkyl-analogs (**3d**, **3e**) gave light far less effectively than **3a**–**3c** but a little more than **1** and **2** in NaOH–H_2O (see relative Φ^{CIEEL} in Table 1). The relation between the maximum wavelength of the CIEEL emission ($\lambda_{max}{}^{CIEEL}$) and the fluorescence maximum ($\lambda_{max}{}^{fl}$) of the spent reaction mixture for aryl-analogs (**3a**–**3c**) was markedly different from that for alkyl-analogs (**3d**, **3e**) in NaOH–H_2O. Thus, $\lambda_{max}{}^{CIEEL}$ and $\lambda_{max}{}^{fl}$ of the spent reaction mixture coincided for **3a**–**3c**, while the spent reaction mixture of alkyl-analogs (**3d**, **3e**) showed a fluorescence peak at $\lambda_{max}{}^{fl}=408$–411 nm accompanied with a shoulder peak near the region where the CIEEL peak ($\lambda_{max}{}^{CIEEL}=474$–$482$ nm) appeared. The observed relations between $\lambda_{max}{}^{CIEEL}$ and $\lambda_{max}{}^{fl}$ for alkyl-analogs (**3d**, **3e**) and for aryl-analogs (**3a**–**3c**) were both different from that reported for **1** and **2**, where $\lambda_{max}{}^{fl}$ of the emitter is blue-shifted by ca. 50 nm from $\lambda_{max}{}^{CIEEL}$ in NaOH–H_2O.[7,9]

To confirm the results described above, we measured the fluorescence spectra of ketoesters (**6a**) and (**6d**) as a representative of an authentic emitter for 2-aryl- (**3a**–**3c**) and 2-alkyl-analogs (**3d**, **3e**), respectively. The fluorescence maximum ($\lambda_{max}{}^{fl}$) of the authentic emitter (**6a**) agreed with the CIEEL maximum ($\lambda_{max}{}^{CIEEL}$) of **3a** as well as the fluorescence maximum of its spent reaction mixture irrespective of the solvent system used: $\lambda_{max}{}^{fl}=512$ nm, and $\Phi^{fl}=0.30$ in TBAF–acetonitrile, while $\lambda_{max}{}^{fl}=508$ nm, and $\Phi^{fl}=0.12$ in NaOH–H_2O [Fig. 1, (C)].[15] Using these fluorescence quantum yields (Φ^{fl}) and chemiluminescence yields (Φ^{CIEEL}) summarized in Table 1, singlet-chemiexcitation yields ($\Phi_S=\Phi^{CIEEL}/\Phi^{fl}$) are estimated to be 0.50 in TBAF–acetonitrile and 0.036 in NaOH–H_2O. Consequently, decrease of Φ^{CIEEL} for an aryl-analog (**3a**) should be attributed mainly to low efficiency of the singlet-chemiexcitation process in NaOH/H_2O: (Φ^{fl} in H_2O)/(Φ^{fl} in acetonitrile) = 0.4, while (Φ_S in H_2O)/(Φ_S in acetonitrile) = 0.07.

As in the case of a pair (**3a** and **6a**), the fluorescence spectrum of the authentic emitter (**6d**) agreed both with the CIEEL emission spectrum of **3d** and with the fluorescence spectrum of its spent reaction mixture in TBAF–acetonitrile. Fluorescence yield (Φ^{fl}) was estimated to be 0.20 for **6d** in TBAF–acetonitrile, so that singlet-chemiexcitation for an alkyl-analog (**3d**) should be as effective as for an aryl-analog (**3a**) in TBAF–acetonitrile. On the other hand, the fluorescence spectrum of **6d** in NaOH–H_2O showed a peak at $\lambda_{max}{}^{fl}=408$ nm with a shoulder at 470–480 nm (wavelength of excitation: $\lambda_{ex}=320$ nm) [Fig. 1, (B), b): compared with (A), b)]. This spectrum coincided with that for the spent reaction mixture after the CIEEL-decay of **3d** in NaOH–H_2O. When light with wavelength at $\lambda_{ex}=370$ nm was used for excitation, the fluorescence spectrum of **3d** in NaOH–H_2O showed a peak at $\lambda_{max}{}^{fl}=482$ nm but little peak at 408 nm [Fig. 1, (B), (c)]. Fluorescence of the authentic emitter (**6d**) at $\lambda_{max}{}^{fl}=482$ nm, whose Φ^{fl} was estimated to be 0.023, should correspond to the CIEEL emission for **3d** in NaOH–H_2O, though its intensity is far lower than that of fluorescence at $\lambda_{max}{}^{fl}=408$ nm.

These results suggest that the authentic emitter (**6d**) exists at least as two different species in NaOH–H_2O, of which one emitting fluorescence at 482 nm corresponds to the CIEEL emitter produced from **3d**, and the other species emitting fluorescence at 408 nm should be an oxyanion of **6d** effected by strong hydrogen bonding as mentioned for the case of **1** and **2** by Adam.[7,9,16] According to this suggestion, chemiexcitation yield (Φ_s) is estimated to be 0.0032 for **3d** in NaOH–H_2O. Therefore, the very low chemiluminescence efficiency of **3d** in NaOH–water would be attributed to a synergetic effect of the low fluorescence yield of the emitter and low chemiexcitation yield. Comparing the chemiluminescent decomposition of an alkyl-analog (**3d**) with an aryl-analog (**3a**), which emits light ca 60 times more effectively than **3d** in NaOH–H_2O, one realizes that the fluorescence efficiency (Φ^{fl}) of the emitter and the chemiexcitation yield (Φ_s) are both higher for **3a** than for **3d**.

The present results provide the first instance that decreased chemiexcitation yield and decreased fluorescence yield of the emitter effect synergetically a marked drop of chemiluminescence efficiency for the CIEEL-decay of a dioxetane bearing a phenolic moiety in NaOH–H_2O. By an analogy, marked decrease of chemiluminescence efficiency for **1** and **2** may be attributed both to low chemiexcitation efficiency and fluorescence efficiency of the emitter so low that a peak could not be observed at a region where the CIEEL peak appears in NaOH–H_2O.

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10. Selected data for **3a**: colorless granules (from hexane–CH_2Cl_2), mp 150.5–151.5°C. ^1H NMR (400 MHz, $CDCl_3$): δ_H 1.03 (s, 9H), 1.17 (s, 3H), 1.41 (s, 3H), 3.84 (d, $J=8.2$ Hz, 1H), 3.90 (s, 3H), 4.60 (d, $J=8.2$ Hz, 1H), 7.02 (d, $J=8.9$ Hz, 2H), 7.14 (d, $J=1.0$ Hz, 1H), 7.49 (d, $J=1.0$ Hz, 1H), 8.15 (d, $J=8.9$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 18.7, 25.2, 27.1, 36.9, 45.7, 55.5, 80.2, 103.3, 105.0, 111.5, 114.4, 116.5, 118.9, 129.5, 131.5, 134.0, 146.8, 151.0, 162.4, 163.2; mass (m/z, %) 425 (M^+, 16), 369 (26), 285 (26), 268 (100).
11. A solution of **3a** in acetonitrile (1.0×10^{-6} mol dm^{-3}, 1 mL) was added to a TBAF solution in acetonitrile (1.0×10^{-3} M, 2 mL) at 25°C.
12. Chemiluminescence efficiency (Φ^{CIEEL}) was based on the reported value for *tert*-butyldimethylsilyl ether of **1**: $\Phi_{CL}=0.29$ in DMSO (Ref. 5).
13. Improved chemiluminescence yield as high as that of **3a** has been attained for the CIEEL of dioxetanes bearing a 4-acetyl-3-hydroxyphenyl or a 3-hydroxy-4-iminophenyl moiety, though their light intensity is weak because of long CIEEL-decay rate.[6,14]
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15. Quinine bisulfate was used as the fluorescence standard.
16. Fluorescence of ketoester (**6d**) was observed at $\lambda_{max}=341$ nm in acetonitrile without base, while fluorescence of **6d** in aqueous acetonitrile (1:1) without base exhibited a broad spectrum with two peaks at $\lambda_{max}=358$ and 470 nm. Although further experiments should be required to discuss in detail, fluorescence at $\lambda_{max}=341$–358 nm is presumably attributed to undissociated phenolic form of **6d**.