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Chemiexcitation efficiency for the charge-transfer-induced chemiluminescent decomposition of 3-hydroxyphenyl-substituted dioxetanes in an aqueous system

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ABSTRACT

The decomposition of 3-oxyphenyl-3-methoxy-4-(2′-spiroadamantane)-1,2-dioxetane (**A**) and 5-*tert*-butyl-4,4-dimethyl-1-(3-oxyphenyl)bicyclo[3.2.0]heptane (**B**) in NaOH/H_2O gives light in poor yield, which is several orders of magnitude lower than that in aprotic solvents. To understand the poor chemiluminescence efficiency in NaOH/H_2O, we investigated the behaviors of the authentic emitters, methyl 3-oxidobenzoate (**C**) and 2,2,4,4-tetramethyl-3-oxopentyl 3-oxidobenzoate (**D**). We found that **D** was weakly fluorescent though hydrolyzed in NaOH/H_2O, and estimated that the singlet-chemiexcitation efficiency Φ_S was 6.1×10^{-3} for the decomposition of **B** in NaOH/H_2O. On the other hand, Φ_S for **A** could not be estimated, since **C** was hydrolyzed too rapidly to observe its fluorescence.

The intramolecular charge-transfer-induced decomposition (CTID) of oxidophenyl-substituted dioxetanes has received considerable attention due to interest in the mechanisms of bioluminescence and chemiluminescence and because of possible applications in modern biological and clinical analyses using chemiluminescence.[1–4] Typical examples are adamantylidene-substituted dioxetanes **1** and bicyclic dioxetanes **2**, which undergo chemiluminescent CTID through unstable oxidophenyl-substituted dioxetane **3** or **4** produced by deprotonation or deprotection (Scheme 1).[2,5,6] Although dioxetanes **1** and **2** both effectively emit light in an aprotic polar medium, they give light in quite poor yield in an aqueous medium: the chemiluminescence efficiency Φ^{CL} in H_2O versus CH_3CN was ca. 1/16,000 for **1**, and ca. 1/10,000 for **2**.

This significant defect has been considerably improved through the addition of a fluorescer and/or a surfactant for practical use in an aqueous system.[7,8] However, it is still unclear whether the markedly low Φ^{CL} is mainly due to poor singlet-chemiexcitation efficiency Φ_S and/or to poor fluorescence efficiency Φ^{fl} of the emitter produced for CTID of **1** or **2** in H_2O. Since Φ^{CL} is given as $\Phi_S \times \Phi^{fl}$ for dioxetane-based chemiluminescence, it is important for the estimation of Φ_S to characterize the emitter and to understand its fluorescence properties. Thus, we can first reliably estimate Φ_S as well as Φ^{fl}, when the fluorescence spectrum of the authentic emitter coincides with the chemiluminescence spectrum.

For the CTID of **3** and **4**, the emitters produced are methyl 3-oxidobenzoate **5** and its 2,2,4,4-tetramethyl-3-oxopentyl analog **6**, respectively. However, both the fluorescence spectrum of the authentic emitter **5** and the chemiluminescence spectrum of **3** have been reported to be considerably different from each other in NaOH/H_2O, while they are similar in an aprotic polar solvent such as DMSO or acetonitrile.[9] A similar discrepancy has also been reported between CTID emission from **4** and fluorescence of **6**.[10] It has very recently been reported that **5** undergoes rapid hydrolysis to give a dianion **15** (vide infra) of 3-hydroxybenzoic acid **9**, which shows a strong fluorescence with λ^{fl}_{max} = 412 nm in a basic aqueous solution.[11] This work prompted us to report our findings that may lead to a better understanding of the markedly low Φ^{CL} for CTID of **3** and **4** in an aqueous system.

Bicyclic dioxetane **10** bearing a 4-hydroxy-2-methylbenzoxazol-6-yl group has been reported to show Φ^{CL} that is considerably higher than that for **2** in a NaOH/H_2O system.[12] As in the case of **1** and **2**, the fluorescence spectrum of the spent reaction mixture does not coincide with the chemiluminescence spectrum of **10** in NaOH/H_2O (Scheme 2). The authentic emitter **11** prepared by dissolving 2,2,4,4-tetramethyl-3-oxopentyl 4-hydroxy-2-methylbenzoxazole-6-carboxylate (**12**) in NaOH/H_2O showed fluorescence (λ^{fl}_{max} = 413 nm), the spectrum of which resembled that of the spent

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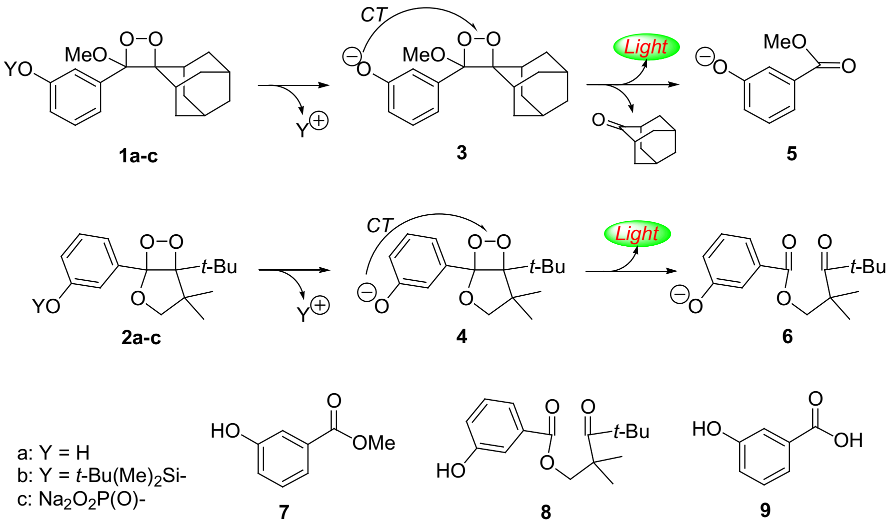
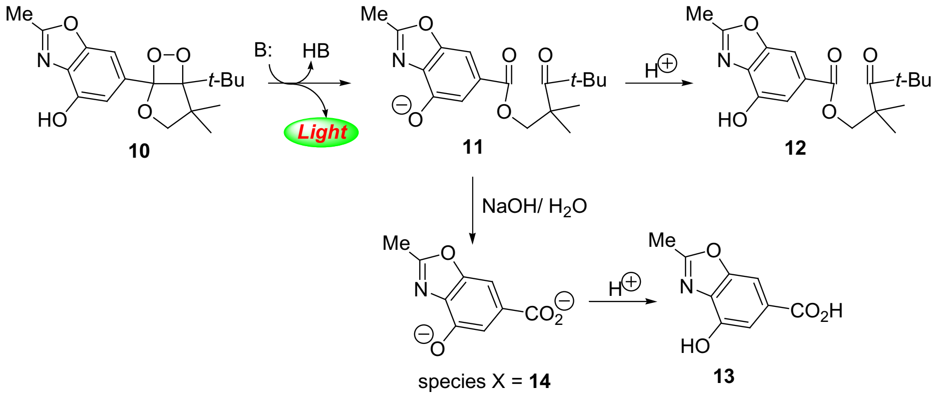


Scheme 1. Base-induced chemiluminescent decomposition of 3-oxyphenyl-substituted 1,2-dioxetanes.



Scheme 2. Base-induced chemiluminescent decomposition of bicyclic dioxetane **10** bearing a 4-hydroxy-2-methylbenzoxazol-6-yl group.

reaction mixture when it was irradiated with light of λ_{ex} = 313 nm. Furthermore, fluorescence with λ^{fl}_{max} = 413 nm increased gradually as time passed. On the other hand, when the solution of **11** was irradiated with light of λ_{ex} = 370 nm, it showed weak fluorescence with λ^{fl}_{max} = 469 nm, the spectrum of which coincided with that of chemiluminescence from **10** (Fig. 1).

Thus, we first attempted to carefully investigate the time-course of absorption and the fluorescence spectra for freshly prepared **11** in NaOH/H_2O at 25 °C.[13] Figure 2 shows that the absorption at λ^{abs} = 337 nm decreased while the absorption at λ^{abs} = 320 nm increased over time. On the other hand, Figure 3 shows fluorescence spectra (λ_{ex} = 370 nm) in which a peak at λ^{fl}_{max} = 469 nm decreased while a peak at λ^{fl}_{max} = 413 nm increased over time. Irradiation of the same sample with light of λ_{ex} = 313 nm gave only fluorescence with λ^{fl}_{max} at 413 nm that increased over time, as shown in Figure 4.

These results strongly suggested that **11** with an absorption maximum λ^{abs}_{max} at 337 nm and a fluorescence maximum λ^{fl}_{max} at 469 nm changed gradually into a species **X** with λ^{abs}_{max} at 320 nm and λ^{fl}_{max} at 413 nm in NaOH/H_2O: species **X** was presumed to be a dianion **14** of 4-hydroxy-2-methylbenzoxazole-6-carboxylic acid **13** based on the findings in a previous report (Scheme 2).[11] Thus, we subjected keto ester **12** to hydrolysis with NaOH in aqueous EtOH to effectively give authentic **13**: for the hydrolysis of **12** in NaOH/H_2O as a control experiment, the isolation of pure **12** was quite hazardous. As expected, the authentic **13** showed strong fluorescence with λ^{fl}_{max} at 413 nm when it was dissolved in NaOH/H_2O.

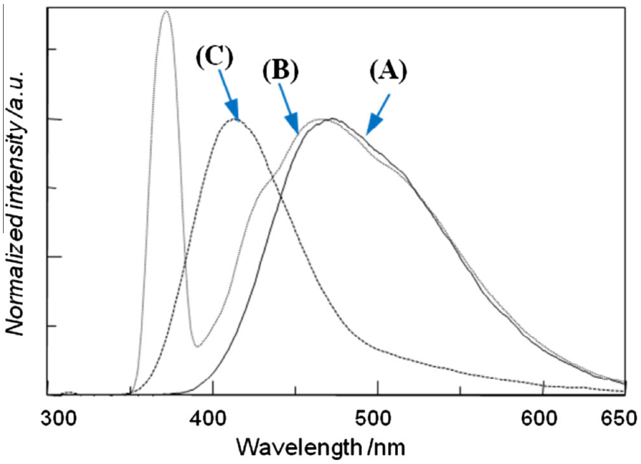


Figure 1. (A) Chemiluminescence spectrum of **10**, (B) fluorescence spectrum of authentic keto ester **11** (λ_{ex} = 370 nm), and (C) fluorescence spectrum of **11** (λ_{ex} = 313 nm) in NaOH/H_2O.

The above results prompted us to reinvestigate the absorption and fluorescence spectra of **6**, as the authentic emitter for CTID of **2**, prepared from ester **8** in NaOH/H_2O. When a freshly prepared solution of **6** was irradiated with light of λ_{ex} = 370 nm, it showed

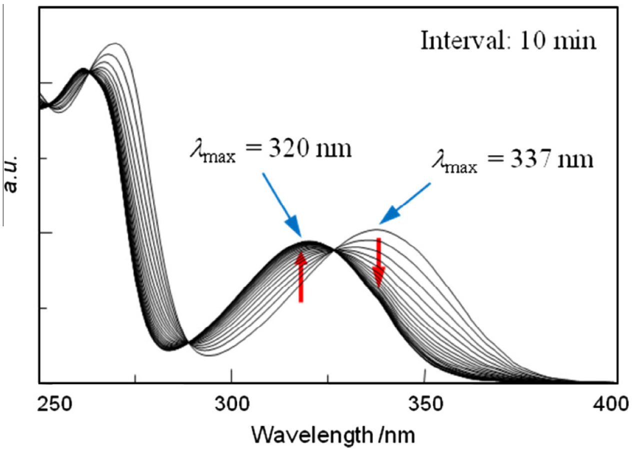


Figure 2. Time-course of absorption spectra for keto ester **11** in NaOH/H_2O.

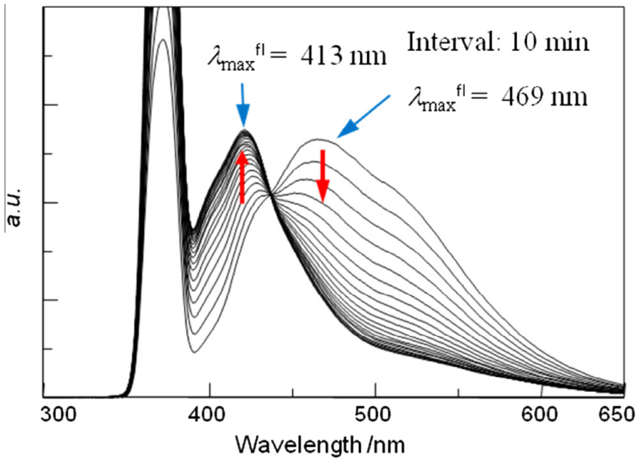


Figure 3. Time-course of fluorescence spectra for keto ester **11** (λ_{ex} = 370 nm) in NaOH/H_2O.

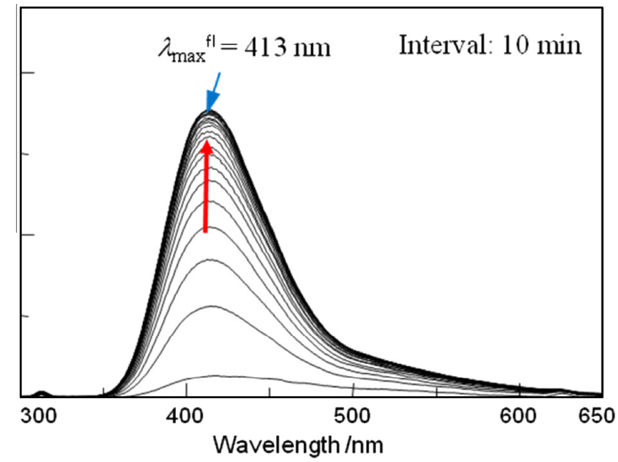


Figure 4. Time-course of fluorescence spectra for keto ester **11** (λ_{ex} = 313 nm) in NaOH/H_2O.

weak fluorescence with λ_{max}^{fl} = 466 nm, which rapidly disappeared. On the other hand, when the solution of **6** was irradiated with light of λ_{ex} = 313 nm, it showed strong fluorescence with λ_{max}^{fl} = 413 nm, the peak of which increased with time (Fig. 5). In the absorption

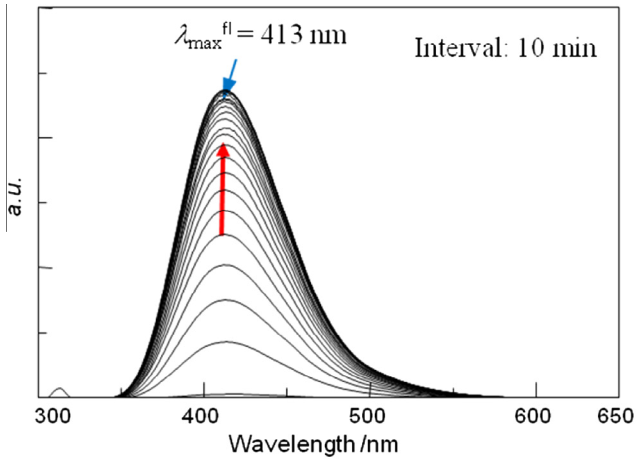


Figure 5. Time-course of fluorescence spectra for ketoester **6** (λ_{ex} = 313 nm) in NaOH/H_2O.

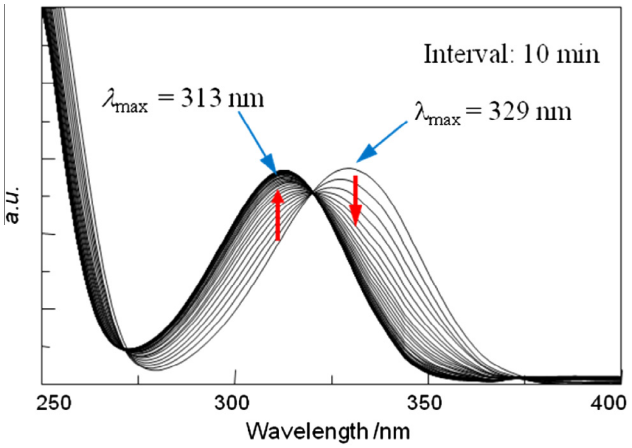
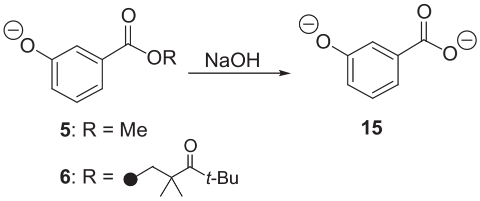


Figure 6. Time-course of absorption spectra for ketoester **6** in NaOH/H_2O.



Scheme 3. Rapid hydrolysis of an emitter, 3-oxidobenzoate, in NaOH/H_2O.

spectra of the solution of **6**, a peak initially observed at λ_{max}^{abs} = 329 nm decreased and finally disappeared, while a peak at λ_{max}^{abs} = 313 nm appeared and increased with time (Fig. 6). These results showed that the time-courses of absorption and fluorescence spectra for the solution of **6** resembled those for **11** in a NaOH/H_2O system. Thus, we can see that the authentic emitter **6** showed very weak fluorescence with λ_{max}^{fl} = 466 nm, which coincided with λ_{max}^{CL} of **2**, though **6** rapidly underwent hydrolysis to give dianion **15** of *m*-hydroxybenzoic acid (Scheme 3).

As has very recently been reported,[11] a solution of authentic emitter **5** prepared from methyl *m*-hydroxybenzoate **7** in NaOH/H_2O showed time-dependent changes in fluorescence and absorption which resembled the case of **6**, as illustrated in Figures 7 and 8. However, fluorescence with λ_{max}^{fl} = 465-470 nm, which corresponded to the λ_{max}^{CL} of **2**, was not observed. These results showed

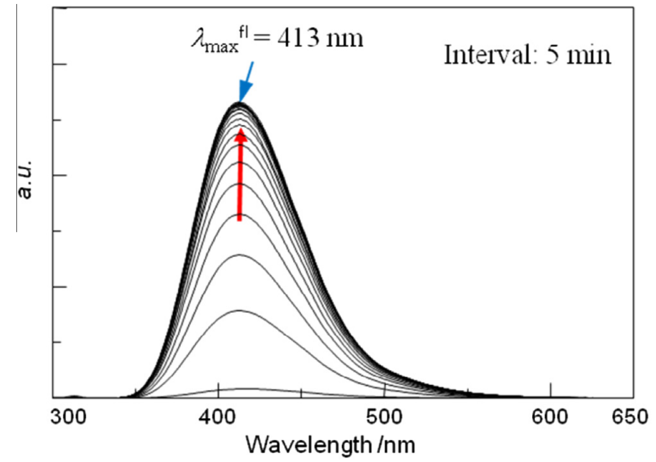


Figure 7. Time-course of fluorescence spectra for methyl ester **5** (λ_{ex} = 313 nm) in NaOH/H_2O.

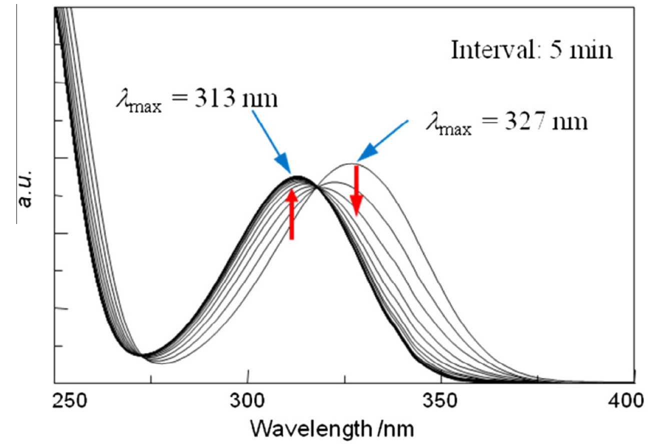


Figure 8. Time-course of absorption spectra for methyl ester **5** in NaOH/H_2O.

that ester **5** was hydrolyzed far more rapidly than **6** so that fluorescence of **5** could hardly be observed.

Next, we attempted to estimate the rates of hydrolysis for **5**, **6** and **11**, by monitoring the time-course of fluorescence intensity at λ^{fl}_{max} = 413 nm due to dianion **15** or **14**. The hydrolysis proceeded according to pseudo-first order kinetics in the present investigation where a large excess of NaOH (0.1 M vs 2×10^{-4} M of a substrate) in H_2O was used. Thus, we found that methyl ester **5** was hydrolyzed 30–40 times faster than **6** and **11**: the rate constants estimated k/s^{-1} were 1.1×10^{-3} for **5**, 3.1×10^{-4} for **6**, and 4.3×10^{-4} for **11**.

Finally, we estimated the values of Φ^{fl} for mono-anions of 3-oxidobenzoates to be 1.8×10^{-3} for **6** and 3.9×10^{-3} for **11** in NaOH/H_2O after analyses of the absorption and fluorescence spectra of freshly prepared authentic emitters. On the other hand, Φ^{fl} for dianion of 3-hydroxybenzoic acid **15** was estimated to be 0.33, which was two orders of magnitude higher than those for anions of esters **6** and **11**. Furthermore, based on Φ^{fl} for **6** and for **11** shown above, we estimated the singlet chemiexcitation efficiency $\Phi_S = 6.1 \times 10^{-3}$ for CTID of **2a** (**4**) and 1.9×10^{-2} for **10** in NaOH/H_2O. Thus, we can understand that the markedly low Φ^{CL} is attributed to poor Φ^{fl} of the emitter **6** as well as poor singlet-chemiexcitation efficiency Φ_S for CTID of **4** in an aqueous system.[14,15] This conclusion is presumably applicable to the case of **1**, though the Φ^{fl} of **3** could not be estimated.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.01.089.

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13. All electronic spectral data shown here were obtained by using a 2×10^{-4} M solution of a substrate at 25 °C.
14. Both Φ^{fl} of the emitter **6** and Φ_S for the CTID of **4** have been reported far higher in a TBAF/acetonitrile system than in an aqueous system:[10,15] Φ^{fl} of **6** = 0.24 and Φ_S for the CTID of **4** = 0.46.
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