Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 46 (2005) 6075–6078
Tetrahedron Letters

Bicyclic dioxetanes bearing a 4-(benzoazol-2-yl)-3-hydroxyphenyl moiety: chemiluminescence profile for base-induced decomposition in aprotic medium and in aqueous medium

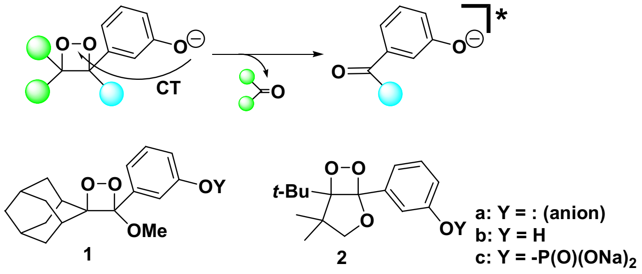
Masakatsu Matsumoto,* Taichi Akimoto, Yuki Matsumoto and Nobuko Watanabe

Department of Chemistry, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa 259-1293, Japan

Received 20 May 2005; revised 27 June 2005; accepted 1 July 2005
Available online 21 July 2005

Abstract—Four bicyclic dioxetanes bearing a 4-(benzothiazol-2-yl)-3-hydroxyphenyl or 4-(benzoxazol-2-yl)-3-hydroxyphenyl group were synthesized. These dioxetanes underwent base-induced decomposition with accompanying emission of light with high efficiency in NaOH/H_2O as well as in tetrabutyl ammonium fluoride (TBAF)/acetonitrile. Among them, benzothiazol-analogs decomposed faster in the aqueous solution than in acetonitrile.

The intramolecular charge-transfer (CT)-induced decomposition of a dioxetane bearing a phenolic anion is a promising entry to highly efficient chemiluminescent substrates.[1–3] Adamantylidenedioxetane (**1a**) and bicyclic dioxetane (**2a**) are typical examples for such dioxetanes, and their phosphate-protected forms (**1c**) and (**2c**) are now being used in modern chemiluminescence biochemical and medical analyses.[2–6] Dioxetanes (**1a**) and (**2a**) emit light in high chemiluminescent yields (Φ^{CL}) in an aprotic solvent system such as DMSO and acetonitrile,[1,7,8] whereas they afford very weak light in an aqueous system: Φ^{CL} in H_2O/Φ^{CL} in DMSO = 1/39,000 for **1a**, and 1/15,000 for **2a**. Another noteworthy phenomenon observed for chemiluminescence from **1a** and **2a** is that the rates of decomposition induced by CT (rate constant: k^{DICT}) tend to decrease in aqueous system: k^{DICT} in H_2O/k^{DICT} in DMSO = 1/40 for **1a**, 1/180 for **2a**.[4,7–9] Similar solvent effect on the rate of decomposition has been observed for various dioxetanes.[9,10] Such tendency is rather unfavorable for application to chemiluminescence analyses such as immunoassays, since the prolonged decomposition rate of dioxetane leads to decrease of light intensity per unit time. In the course of our investigation to design highly efficient chemiluminescent substrates, we found that bicyclic dioxetanes (**3a**,**c**) bearing a 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety exhibit unique chemiluminescence with high Φ^{CL} even in an aqueous system and k^{DICT} larger in an aqueous system than in an aprotic solvent such as acetonitrile (Scheme 1).



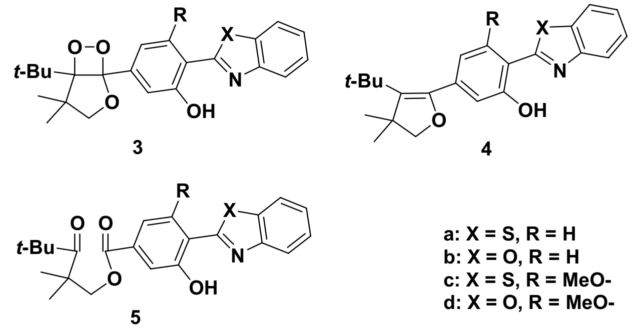
Scheme 1.

Dioxetanes (**3a**,**b**) and the related dioxetanes (**3c**,**d**) bearing a 4-(benzoazol-2-yl)-3-hydroxy-5-methoxyphenyl moiety were synthesized effectively by the reaction of the corresponding dihydrofurans (**4a**–**d**) with singlet oxygen, which was generated photochemically by the use of teptraphenylporphin as a sensitizer and a 940 W Na-lamp as a light source at 0 °C in CH_2Cl_2. The structures of these four dioxetanes were determined by ^1H NMR, ^{13}C NMR, IR, Mass, and HR Mass spectral analysis.[11] All dioxetanes (**3a**–**d**) were quite stable thermally, though they decomposed exclusively into the corresponding ketoesters (**5a**–**d**) on prolonged heating in hot xylene (Scheme 2).

---

* Corresponding author. Tel.: +44 463 59 4111; fax: +44 463 58 9684; e-mail: matsumo-chen@kanagawa-u.ac.jp

0040-4039/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2005.07.008



Scheme 2.

When a solution of dioxetane (**3a**) in acetonitrile (1.0×10^{-5} M, 1 mL) was added to a solution of tetrabutylammonium fluoride (TBAF) in acetonitrile (1.0×10^{-2} M, 2 mL) at 45 °C, dioxetane (**3a**) decomposed to emit blue light with maximum wave-length $\lambda^{CL}_{max} = 490$ nm, chemiluminescent efficiency $\Phi^{CL} = 0.39$,[12] and rate of decomposition $k^{DICT} = 4.4 \times 10^{-4}\ s^{-1}$. The chemiluminescence spectrum coincided with the fluorescence spectrum of the spent reaction mixture, from which ketoester (**5a**) was isolated in high yield after neutralization. The fluorescence spectrum of authentic **5a**, when dissolved in TBAF/acetonitrile, agreed with the chemiluminescence spectrum of **3a**, and its fluorescence efficiency (Φ^{fl}) was estimated to be 0.66, so that singlet-chemiexcitation yield ($\Phi_S = \Phi^{CL}/\Phi^{fl}$) for **3a** was estimated to be 0.59. Similarly, dioxetanes (**3b–d**) were treated with large excess of TBAF in acetonitrile to exhibit chemiluminescence with properties as summarized in Table 1, in which fluorescence efficiencies for the corresponding emitters (anionic form of **5b–d**) and the estimated singlet-chemiexcitation yields are also cited. The results in Table 1 reveal that (a) dioxetanes (**3a**) and (**3b**) exhibit chemiluminescence with markedly high Φ^{CL} and (b) their methoxy-analogs (**3c**) and (**3d**) decompose somewhat more rapidly than **3a** and **3b** in TBAF/acetonitrile, but give light with considerably low Φ^{CL}, though they are still higher than that for **2b** ($\Phi^{CL} = 0.11$ in TBAF/acetonitrile).[9]

Next, NaOH-induced decomposition of dioxetanes (**3a–d**) was examined in an aqueous system. When solutions of **3a–d** in acetonitrile (1.0×10^{-3} M, 100 μL) were added to aqueous solutions of NaOH (0.1 M, 2.9 mL) at 45 °C, **3a–d** decomposed into the corresponding ketoesters with accompanying emission of light. As shown in Table 1, both **3a** and **3b** exhibited markedly high Φ^{CL} even in the aqueous system. Φ^{CL} for **3c** and **3d** was also considerably high, though they were 1/6 of those for the corresponding analogs (**3a**) and (**3b**).

With respect to the CT-induced decomposition rate (k^{DICT}), dioxetanes bearing a benzoxazolylphenol moiety (**3b**) and (**3d**) behaved similarly to various dioxetanes known already, that is, their chemiluminescent decomposition occurred more slowly in NaOH/H_2O than in TBAF/acetonitrile. In contrast, both dioxetanes bearing a benzothiazolylphenol moiety (**3a**) and (**3c**) decomposed faster in NaOH/H_2O than in TBAF/acetonitrile.

To know the unusual behavior of **3a** (and **3c**) with respect to the decomposition rate in more detail, the NaOH-induced decomposition rates (k^{DICT}) of **3a** and **3b** were examined as representatives in H_2O–CH_3CN mixed solvent with various ratios. The results illustrated in Figure 1 reveal that the k^{DICT} for **3b** at the ratio (v/v) of H_2O–CH_3CN = 1:5 decreased into only 1/11 of k^{DICT} at the ratio of H_2O–CH_3CN = 0:1,[13] and then k^{DICT} increased again, as the content of H_2O increased, and recovered finally 82% of the value in CH_3CN at the solvent ratio = 29:1. A similar tendency of the change in k^{DICT} depending on the ratio of H_2O versus organic solvent has been observed for **1b**: as representatives, the changes in k^{DICT} for **1b** and a dioxetane bearing a 4-hydroxybenzoxazol-6-yl group were illustrated in a small dashed square in Figure 1.[14] On the other hand, the rate (k^{DICT}) for **3a** increased monotonously from CH_3CN, and reached finally 5.7 times of the value in H_2O–CH_3CN (29:1).

The significant decrease of k^{DICT} for **3b** in CH_3CN with low water-content is most likely rationalized as follows. Since the sodium ion cannot be sufficiently hydrated in a low water-content-medium, the phenoxy anion of **6b**, produced from**3b**, should coordinate strongly to the sodium ion in such medium. Hence, the negative charge of the phenoxy anion in **6b** is relieved so that the phenoxy anion becomes less oxidized, and thereby, the intramolecular CT-induced decomposition occurs less easily. This idea was supported by an experiment using tetrabutylammonium hydroxide ($Bu_4N^+ \cdot OH^-$) as a base; Bu_4N^+ is well-known to exhibit affinity also to organic

Table 1. Base-induced chemiluminescent decomposition of dioxetanes bearing a 4-(benzoazol-2-yl)-3-hydroxyphenyl moiety (**3a**–**d**)

Dioxetane	TBAF/acetonitrile[a]					NaOH/H_2O[b]				
	λ_{max}/nm	Φ^{CLc}	Φ^{fl}	Φ_S	k^{DICT}/s^{-1}	λ_{max}/nm	Φ^{CLc}	Φ^{fl}	Φ_S	k^{DICT}/s^{-1}
3a	490	0.39	0.66	0.59	4.4×10^{-4}	486	0.12	0.39	0.30	2.5×10^{-3}
3b	487	0.46	0.61	0.75	1.2×10^{-3}	482	0.18	0.44	0.41	9.8×10^{-4}
3c	486	0.14	0.36	0.38	7.7×10^{-4}	486	0.02	0.08	0.25	2.0×10^{-3}
3d	485	0.26	0.36	0.72	1.3×10^{-3}	473	0.03	0.14	0.21	5.0×10^{-4}

[a] A solution of dioxetane in acetonitrile (1.0×10^{-5} mol dm^{-3}, 1 mL) was added to a solution of TBAF in acetonitrile (1.0×10^{-2} mol dm^{-3}, 2 mL) at 45 °C.

[b] A solution of dioxetane in acetonitrile (1.0×10^{-3} mol dm^{-3}, 100 μL) was added to a solution of NaOH in H_2O (1.0×10^{-1} mol dm^{-3}, 2.9 mL) at 45 °C.

[c] Relative quantum yields based on the value for dioxetane (**1**, Y = t-BuMe$_2$Si–) in TBAF/DMSO system (Ref. 7: $\Phi^{CL} = 0.29$).

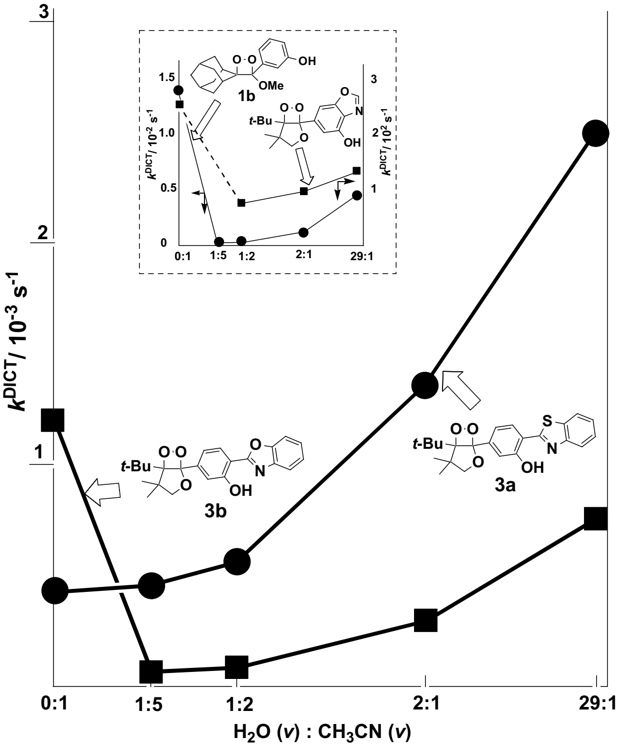
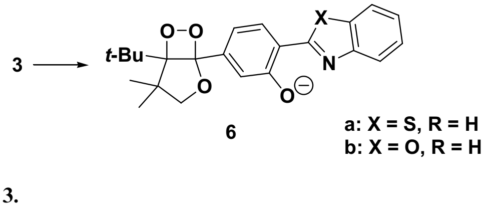


Figure 1. Change of rate constant (k^{DICT}) depending on the ratio of CH_3CN versus H_20 for NaOH-induced decomposition of dioxetanes (**3a**) and (**3b**).

solvents. When the chemiluminescent decomposition of **3b** was carried out by the use of $Bu_4N^+{\cdot}OH^-$ instead of NaOH in H_2O–CH_3CN (1:5), the rate for **3b** increased upto 1.3 times of the value for the NaOH system. The k^{DICT} for **3b** turned out to be little different between NaOH and $Bu_4N^+{\cdot}OH^-$ as a base used in H_2O–CH_3CN (29:1).

On the other hand, minimal effect of a cation, namely Na^+ or Bu_4N^+, on the decomposition rate (k^{DICT}) was observed for dioxetane (**3a**) in H_2O–CH_3CN ranging from the ratio 1:5 to 29:1. This fact suggests that the phenoxy anion of **6a** should coordinate weakly to Na^+ as does Bu_4N^+. This suggestion agrees with the facts that (a) 2-(benzothiazol-2-yl)phenol is an acid stronger than 2-(benzoxazol-2-yl)phenol,[15] so that the conjugate phenoxy anion of the former would be more stable than that of the latter and (b) **3a** decomposes more slowly than **3b** in TBAF/CH_3CN: this means that a naked phenoxy anion of **6a** is presumably more stable and thus is less oxidized than that of **6b** (Scheme 3).

The base-induced decomposition rates (k^{DICT}) of **3b** and most dioxetanes bearing a phenolic moiety reported already are slower for an aqueous medium than for a nonaqueous medium such as CH_3CN. The phenomenon is presumably rationalized by the fact that a phenoxide anion of intermediary dioxetane such as **1a**, **2a**, and **6b** is hydrated in an aqueous medium so that it becomes more stable and less oxidized. However, this idea cannot be applied to **6a**, k^{DICT} of which increased markedly in an aqueous medium. Since a phenoxide anion of **6a** would be also more or less hydrated, other factor(s) should be considered to account for the unusual behavior of **3a**. Referring to the fact that decomposition of a dioxetane is accelerated by adsorption on silica gel,[16] H_2O molecule(s) may participate to increase the CT-induced decomposition rate of dioxetanes by hydrogen bonding to O–O. Therefore, the CT-induced decomposition rate of dioxetanes may vary in the balance between the hydration of the phenoxy anion and the hydrogen bonding of H_2O molecule(s) to O–O of an intermediary dioxetane such as **1a**, **2a**, **6a**, and **6b** in an aqueous medium.



Scheme 3.

Acknowledgements

The authors gratefully acknowledge financial assistance provided by a Grant-in Aid (Nos. 14540506 and 15550043) for Scientific Research by the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References and notes

1. Schaap, A. P.; Chen, T.-S.; Handley, R. S.; DeSilva, R.; Giri, B. P. *Tetrehedron Lett.* **1987**, *28*, 1155–1158.
2. Beck, S.; Köster, H. *Anal. Chem.* **1990**, *62*, 2258–2270.
3. Matsumoto, M. *J. Photochem. Photobiol. C* **2004**, *5*, 27–53.
4. Adam, W.; Bronstein, I.; Edwards, B.; Engel, T.; Reinhardt, D.; Schneider, F. W.; Trofimov, A. V.; Vasil'ev, R. F. *J. Am. Chem. Soc.* **1996**, *118*, 10400–10407.
5. Yamada, M.; Matsumoto, M.; Watanabe, N. In *Bioluminescence and Chemiluminescence Progress and Perspectives*; Tsuji, A., Matsumoto, M., Maeda, M., Kricka, L. J., Stanley, P. E., Eds.; World Scientific: Singapore, 2005, pp 483–486.
6. Yamada, M.; Kitaoka, K.; Matsumoto, M.; Watanabe, M. In *Bioluminescence and Chemiluminescence Progress and Perspectives*; Tsuji, A., Matsumoto, M., Maeda, M., Kricka, L. J., Stanley, P. E., Eds.; World Scientific: Singapore, 2005, pp 487–490.
7. Trofimov, A. V.; Mielke, K.; Vasil'ev, R. F.; Adam, W. *Photochem. Photobiol.* **1996**, *63*, 463–467.
8. Matsumoto, M.; Watanabe, N.; Kasuga, N. C.; Hamada, F.; Tadokoro, K. *Tetrahedron Lett.* **1997**, *38*, 2863–2866.
9. Matsumoto, M.; Mizoguchi, Y.; Motoyama, T.; Watanabe, N. *Tetrahedron Lett.* **2001**, *42*, 8869–8872.
10. Matsumoto, M.; Sakuma, T.; Watanabe, N. *Luminescence* **2001**, *16*, 275–280.
11. Selected data for **3a**: colorless needles melted at 147.0–148.5 °C (from *p*-xylene); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.05 (s 9H), 1.17 (s, 3H), 1.40 (s, 3H), 3.85 (d, $J = 8.2$ Hz, 1H), 4.60 (d, $J = 8.2$ Hz, 1H), 7.23–7.25 (m, 1H), 7.38 (d, $J = 1.6$ Hz, 1H), 7.44 (dd with fine coupling, $J = 7.9$ and

7.2 Hz, 1H), 7.53 (dd with fine coupling, $J = 8.2$ and 7.2 Hz, 1H), 7.72 (d, $J = 8.2$ Hz, 1H), 7.92 (d, $J = 7.9$ Hz, 1H), 8.01 (d, $J = 8.2$ Hz, 1H), 12.6 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 18.4, 25.0, 26.9, 36.8, 45.6, 80.4, 105.3, 116.1, 117.5, 118.1, 119.4, 121.6, 122.3, 125.8, 126.8, 127.9, 132.6, 140.6, 151.7, 157.4, 168.6 ppm; IR (KBr): ν 3442, 2968, 2893, 1631, 1574 cm^{-1}; Mass (*m*/*z*, %): 411 (24) [M$^+$], 355 (22), 271 (28), 255 (18), 254 (100), 227 (17), 226 (18), 198 (24), 57 (33); HRMS: 434.1381, calcd for $C_{23}H_{25}NO_4SNa$ [M+Na$^+$] 434.1402. Selected data for **3b**: colorless plates melted at 155.0–156.0 °C (from *p*-xylene); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.04 (s 9H), 1.17 (s, 3H), 1.41 (s, 3H), 3.85 (d, $J = 8.2$ Hz, 1H), 4.61 (d, $J = 8.2$ Hz, 1H), 7.31 (dd, $J = 8.3$ and 1.6 Hz, 1H), 7.38-7.43 (m, 3H), 7.60–7.65 (m, 1H), 7.73–7.78 (m, 1H), 8.06 (d, $J = 8.3$ Hz, 1H), 11.5 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 18.4, 25.0, 26.9, 36.8, 45.6, 80.4, 105.4, 110.7, 111.3, 116.1, 117.7, 119.4, 119.5, 125.1, 125.7, 126.6, 139.9, 141.4, 149.1, 158.2, 162.3 ppm; IR (KBr): ν 3431, 2968, 2893, 1637, 1581, 1549 cm^{-1}; Mass (*m*/*z*, %) 395 (14) [M$^+$], 363 (6), 348 (11), 339 (27), 255 (32), 239 (15), 238 (100), 211 (15), 210 (20), 182 (23), 57 (38); HRMS(ESI): 418.1593, calcd for $C_{23}H_{25}NO_5Na$ [M+Na$^+$] 418.1630.

12. Relative value based on Φ^{CL} reported for **1b**.[7]
13. The rate for TBAF/CH_3CN system was used.
14. Unpublished results.
15. Roberts, E. L.; Dey, J.; Warner, I. M. *J. Phys. Chem.* **1996**, *100*, 19681–19686.
16. Schaap, A. P.; Burns, P. A.; Zaklika, K. A. *J. Am. Chem. Soc.* **1977**, *99*, 1270.