

Synthesis and Chemiluminescent Property of the Novel 1,2-Dioxetanes Containing an Acridane-10-acetate Moiety as the Luminophore and Trigger Unit

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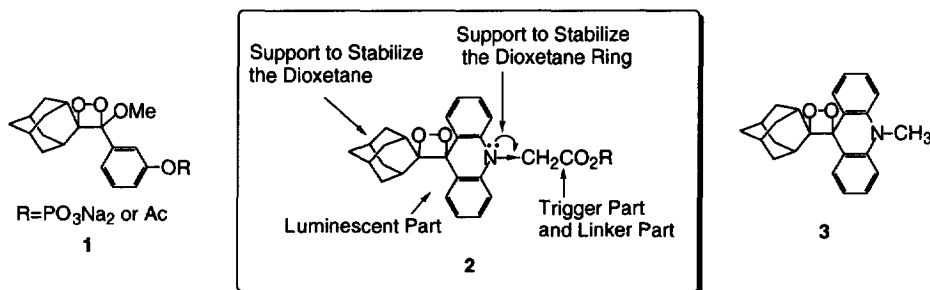
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Abstract: Novel dioxetane derivatives **2** with an acridane-10-acetate moiety were prepared and tested for a potential chemiluminescent probe. The 10-acetate was found to play an important role both in stabilization and in base-mediated smooth degradation of the dioxetane ring.

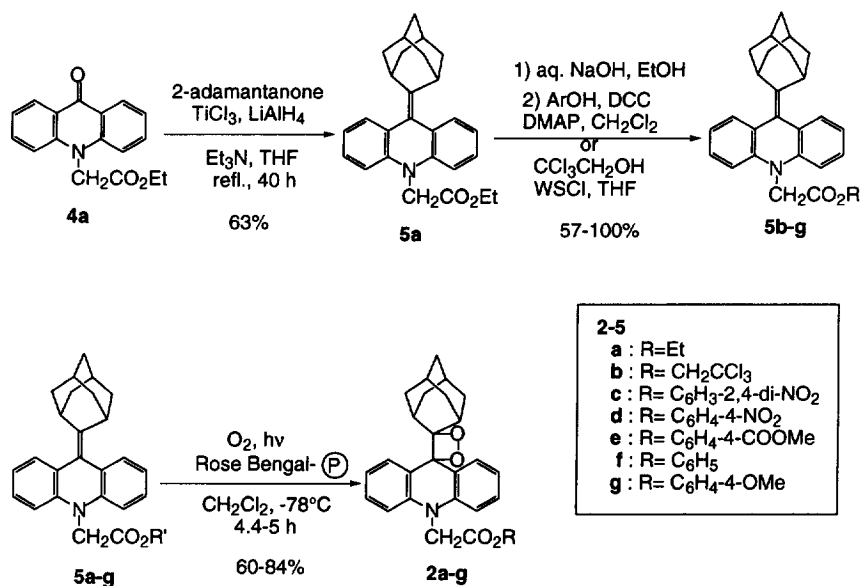
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Today, in the biological field, the most useful and versatile method to detect a trace quantity of biomolecules with large molecular weights still depends on the use of radioactive isotopes despite the serious disadvantages inherent in the use. Development of a non-radioactive and highly sensitive method to replace the radioisotope labeling has, therefore, been urgently sought. Chemiluminescent labeling is expected to be one of the most promising alternative methods.¹ In bioluminescent reactions, 1,2-dioxetanes are well known to be involved as intermediates for efficient emission of light, due to the potentially large energy of a high torsional strain of the four-membered ring system.² Accordingly, a number of dioxetane compounds such as **1** have been prepared and tested for chemiluminescent ability, but there still remain some problems in the sensitivity and/or stability of these compounds.^{3,4}

In the course of our continuous chemical studies on bio-related compounds,⁵ we have designed a novel 1,2-dioxetane **2** as potential candidate for a useful chemiluminescent probe, from the following considerations. McCapra reported that thermal decomposition of the dioxetane **3** produced 10-methylacridone with light emission, but compound **3** was too unstable to be practically handled.⁶ On the other hand, stability of 1,2-dioxetanes is reported to depend on electronic factors to a large extent. Namely, degradation of 1,2-dioxetanes having an electron donating group, such as the nitrogen in **3**, seems to be facilitated by the chemically initiated electron exchange luminescence (CIEEL) process.⁷ Our designed 1,2-dioxetane compound **2** has the acridane structure as an excellent luminophore moiety, the adamantyl and acetate groups as stabilizer of the dioxetane ring, and the acetate as a trigger for smooth decomposition of the dioxetane by rapid generation of the acetate anion on alkaline treatment. In this communication, we describe the synthesis, thermal stability, and chemiluminescent property of **2** having various substituents (R).



Synthesis of **2** was achieved as follows. Ethyl acridone-10-acetate⁸ (**4a**) was reacted with adamantanone under the typical McMurry reaction conditions to give olefin **5a**, an alkaline hydrolysis of which followed by esterification with phenols or alcohol afforded the corresponding ester congeners **5b-5g**. Photooxygenation of **5a-5g** using a 1000-w high pressure Na lamp and polymer-supported rose bengal⁹ at $-78\text{ }^{\circ}\text{C}$ smoothly took place to give the desired dioxetanes **2a-2g** in 60-84% yields.¹⁰ Although 1,2-dioxetanes are known to be very labile and sometimes to decompose quite easily on contact with silica gel,^{6a} it was found that compounds **2** were stable enough to be purified by silica gel column chromatography.



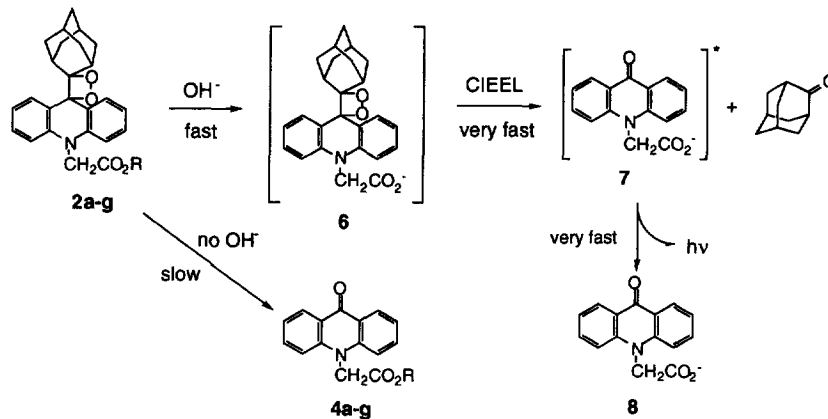
The thermal stabilities of dioxetanes **2** were measured by monitoring their UV spectral change.¹¹ First order rates of the decomposition were observed in *o*-xylene solution at 70-110 $^{\circ}\text{C}$. The half-lives ($t_{1/2}$) at 25 $^{\circ}\text{C}$ were calculated from Arrhenius plots and the results are summarized in Table 1, which shows that all of them are sufficiently stable at least in the non-polar and aprotic solvent, probably owing to the electron-withdrawing character of the acetate moiety in **2** as we expected.

Table 1. The Half-lives^a ($t_{1/2}$) of Acridine-dioxetanes **2** in *o*-Xylene.

Compound	k (25 $^{\circ}\text{C}$) (10^{-8} /sec)	$t_{1/2}$ (25 $^{\circ}\text{C}$) (month)	Compound	k (25 $^{\circ}\text{C}$) (10^{-8} /sec)	$t_{1/2}$ (25 $^{\circ}\text{C}$) (month)
2a	1.03	27.0	2e	1.08	24.7
2b	1.00	26.7	2f	1.25	21.4
2c	0.89	29.9	2g	1.12	23.8
2d	1.43	18.7			

^a See, Note 11.

The luminescent property of **2** was next examined. On treatment of a DMSO solution of **2** with a large excess of NaOH aqueous solution at room temperature, fast degradation of the dioxetane ring in **2** and strong light emission were observed.¹² A typical light-emission pattern in the case of **2d** with time function is shown in the Figure. On the other hand, the degradation was found to proceed only very slowly upon no alkaline treatment. These phenomena appear to be explained as follows. The rapid alkaline hydrolysis of the ester part in **2** affords the carboxylate anion **6**, which could induce fast decomposition of the oxetane probably *via* electron transfer from the carboxylate to the oxetane oxygen, producing the excited acridone **7**, and strong light emission is observed as a result of fluorescence on the way from the excited state to the ground state (**7**→**8**).¹³ Under neutral conditions, however, decomposition of **2** proceeds quite slowly *via* a thermal process, gradually yielding the acridone **4**.



The other dioxetanes **2a-2c**, **2e-2g** gave similar results as **2d** on treatment with the base. The rate of luminescent reaction was affected by the ester substituent and obeyed a pseudo-first order rate, suggesting that the basic hydrolysis of the ester group is the crucial and rate-limiting step in these chemiluminescent processes. The chemiluminescent quantum yields (Φ_{CL}) of **2** were calculated on the basis of luminol ($\Phi_{\text{CL}}=0.011$)¹⁴ and are summarized in Table 2. The compound **2d** ($\text{R}=\text{C}_6\text{H}_4\text{-4-NO}_2$) showed the strongest light emission, the quantum yield of which was estimated to be about 1/5 of that of luminol.

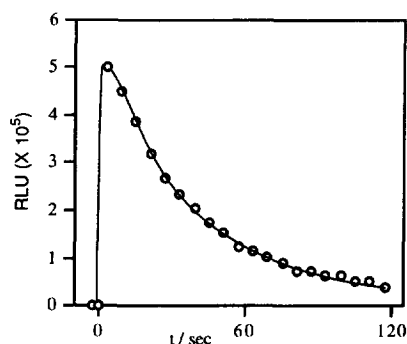


Figure. Chemiluminescent Pattern of Dioxetane **2**. A 1×10^{-7} M solution of **2d** in DMSO (0.1 ml) was added to 0.1 M NaOH (0.3 ml) at 0 second.

Table 2. Chemiluminescent Quantum Yields of **2a-g**.

Compound	R	$\Phi_{\text{CL}} \times 10^{-4}$ a)
2a	Et	4.1
2b	CH_2CCl_3	8.0
2c	$\text{C}_6\text{H}_3\text{-2,4-di-NO}_2$	11
2d	$\text{C}_6\text{H}_4\text{-4-NO}_2$	22
2e	$\text{C}_6\text{H}_4\text{-4-COOMe}$	8.9
2f	C_6H_5	17
2g	$\text{C}_6\text{H}_4\text{-4-OMe}$	7.4

a) Chemiluminescent quantum yields were calculated on the basis of luminol (ca. 0.01).

In conclusion, our designed acridine-dioxetane derivatives **2**, bearing an acetate moiety which played an important role both in stabilization and in decomposition of the dioxetane ring, showed an excellent chemiluminescent property. Study on the application to analytical use for biopolymers is now in progress.

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- 4 Compound **1** [R=Si(*t*-Bu)Me₂] was reported to show a high luminescent quantum yield ($\Phi_{CL} = 0.25$) in a dry organic solvent, but in an aqueous system a similar dioxetane **1** (R=PO₃Na₂) gave only a lower quantum yield ($\Phi_{CL} = 1.3 \times 10^{-5}$). See, ref. 3b) and 7c).
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- 10 Satisfactory spectral data for the dioxetane compounds **2a-g** were obtained. Representative data are as follows. **2d**: mp 253-255°C (from CH₂Cl₂-ether). IR ν (KBr) cm⁻¹: 2932, 1779, 1596, 1525, 1473, 1347, 1206, 1136, 757. UV λ (MeCN) nm (ϵ): 320 (5500), 270 (23000). ¹H-NMR (270 MHz, CDCl₃) δ : 0.66 (2H, br d, *J* = 13 Hz), 1.10-1.90 (10H, m), 2.30 (2H, br s), 4.96 (2H, m), 6.94 (2H, d, *J* = 8 Hz), 7.22-7.31 (4H, m), 7.44 (2H, ddd, *J* = 8, 7, 2 Hz), 8.23-8.29 (2H, m). ¹³C-NMR (68 MHz, CDCl₃) δ : 25.4, 25.6, 31.6, 32.9, 36.0, 48.2, 86.6, 97.8, 111.4, 121.5, 122.2, 125.3, 128.8, 129.3, 138.9, 145.7, 154.7, 167.2. FAB-MS *m/z* (%): 525 (M⁺+1, 67), 374 (M⁺-adamantanone, 100). *Anal. Calcd* for C₃₁H₂₈N₂O₆·3/4H₂O: C, 64.83; H, 5.05; N, 4.76. *Found*: C, 65.08; H, 5.13; N, 4.76.
- 11 Thermal stabilities of the 1,2-dioxetanes were measured in *o*-xylene at several temperatures (70, 80, 90, 100, and 110 °C) on a UV spectroscopy (Shimadzu UV 2100PC), on the basis of UV data of the authentic samples (**2a-g** and **4a-g**). The thermal parameters were obtained from Arrhenius plots.
- 12 General procedure for measurement of chemiluminescent activity: A solution of the dioxetane **2** in DMSO (1 x 10⁻⁷ M, 0.1 ml) was treated with 0.3 ml of 0.1 M NaOH aq. solution and immediately the light emission was monitored for 120 s on a photon counter (Berthold Lumat LB 9501).
- 13 The chemiluminescent spectrum of **2b** showed λ_{max} at 465 nm and closely approximates the fluorescent spectrum of **8** (λ_{max} = 457 nm). A slight difference in λ_{max} values between the chemiluminescent and fluorescent spectra was also observed in [for example : Zaklika, K.A.; Burns, P.A.; Schaap, A.P. *J. Am. Chem. Soc.* **1978**, *100*, 318-320].
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