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CHEMILUMINESCENCE OF N-METHYL-9-(DICARBOALKOXYMETHYL)ACRIDANES: SUCCESSIVE FORMATION OF TWO 1,2-DIOXETANONE INTERMEDIATES

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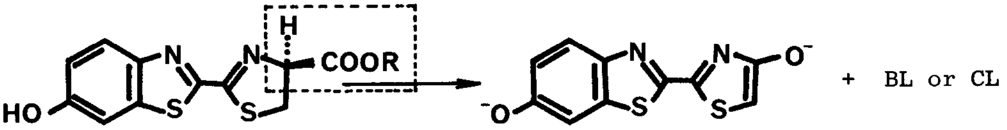
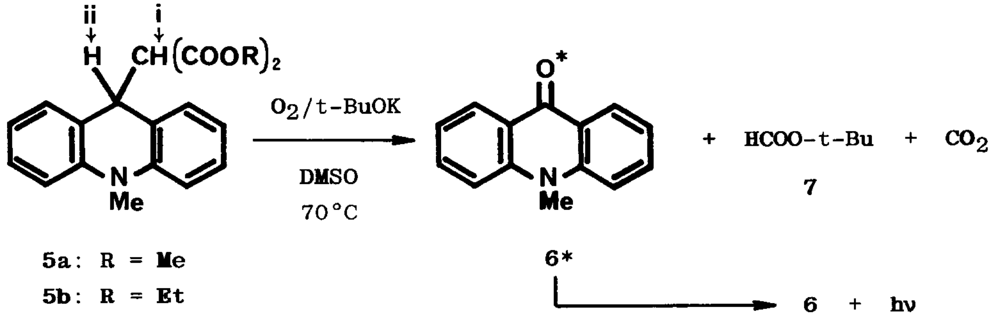
Summary: N-Methyl-9-(dicarboalkoxymethyl)acridanes (**5a-c**) which have a similar structural moiety to that of firefly luciferin and give an excellent fluorescent product were found to be chemiluminescent when being oxidized by molecular oxygen in DMSO in the presence of t-BuOK at 70°C.

Firefly luciferin (**1**), the most efficient bioluminescent system ($\Phi \sim 0.88 \pm 0.12$),[1] has a dihydroaryl -$\underset{|}{C}$HC(O)OR moiety in the activated form (**2**) and yields an efficient fluorescent final product, a dianion of firefly oxyluciferin (**4**).[2] Excellent high quantum yields of chemiluminescence (CL) of 3 ($\Phi \sim 0.5$) were also obtained in dimethyl sulfoxide (DMSO) or tetrahydrofuran (THF) by White et al.[3] The CL proceeds as similar as that of the bioluminescence (BL). The structural features seem to be essential to construction of the effective CL or BL systems.



1: R = H
2: R = AMP
3: R = C(=CH_2)OEt

firefly oxyluciferin
dianion **4**



5a: R = Me
5b: R = Et
5c: R = Ph

We describe here a new chemiluminescent system (**5**) which has such a system and yields successively two 1,2-dioxetanones similar to the firefly BL and CL, resulting in a very effective fluorescent product, N-methylacridone (**6**). One molecule of **5** would be oxidized twice (first at i and then at ii). Hence, it could give two photons a mole.

Into a solution of 5 4) (final concentration at mixing: 5.0 x 10^{-3} M) in DMSO (2 ml; dried over and distilled from CaH_2 and then saturated by O_2 before use) being flushed with O_2 gas, was added t-BuOK (1.0 x 10^{-1} M) in DMSO (2 ml) at 70 ± 1°C. Long-lasting light emission was coming out ($\tau_{1/2} \sim 40$ h); the CL and fluorescence (FL) spectra were measured on a Hitachi Fluorescence/ Phosphorescence Photometer Model MPF-2A. The final product was isolated and identified to be N-methylacridone (**6**), which was proved to be the emitting species (emitter) from its FL spectrum after a first strong flash. FL spectra of the starting materials **5** in argon gas proved that **5**'s were not the emitters. Quantum yields of CL were measured by using a photomultiplier tube exposed to the CL reacting solution (Toshiba MS-9S) and are relative to the Hasting's standard. 8) The values obtained were corrected for the phototube spectral response. The results are shown in Table 1.

Table 1. Chemiluminescence of **5a-c** and Fluorescence of **6** at 70°C in DMSO.

Substrate a)	Temp. (°C)	CL b) λ_{max}(nm)	FL c) of 5 $\lambda_{max}(O_2)$(nm)	λ_{max}(Ar)(nm)	Φ_{CL} x 10^5 d) (einstein/mol)	Product (%) e) (6)
5a	70	430	435	411	8.20	50.4
5b	70	430	435	359	7.26	18.0
5c	70	430	435	465	1.27	5.3
	43	--- f)	---	---	0.33	---
6	70	—	430 b) 435 c)	---	—	—

a) Initial concentrations: [5] = 5.0 x 10^{-3} M; [t-BuOK] = 1.0 x 10^{-1} M. b) Slit-width: 45 nm. c) Slit-width: 24 nm. d) Relative to the Hasting's standard (ref. 8). e) Isolated yields. f) ---: no data.

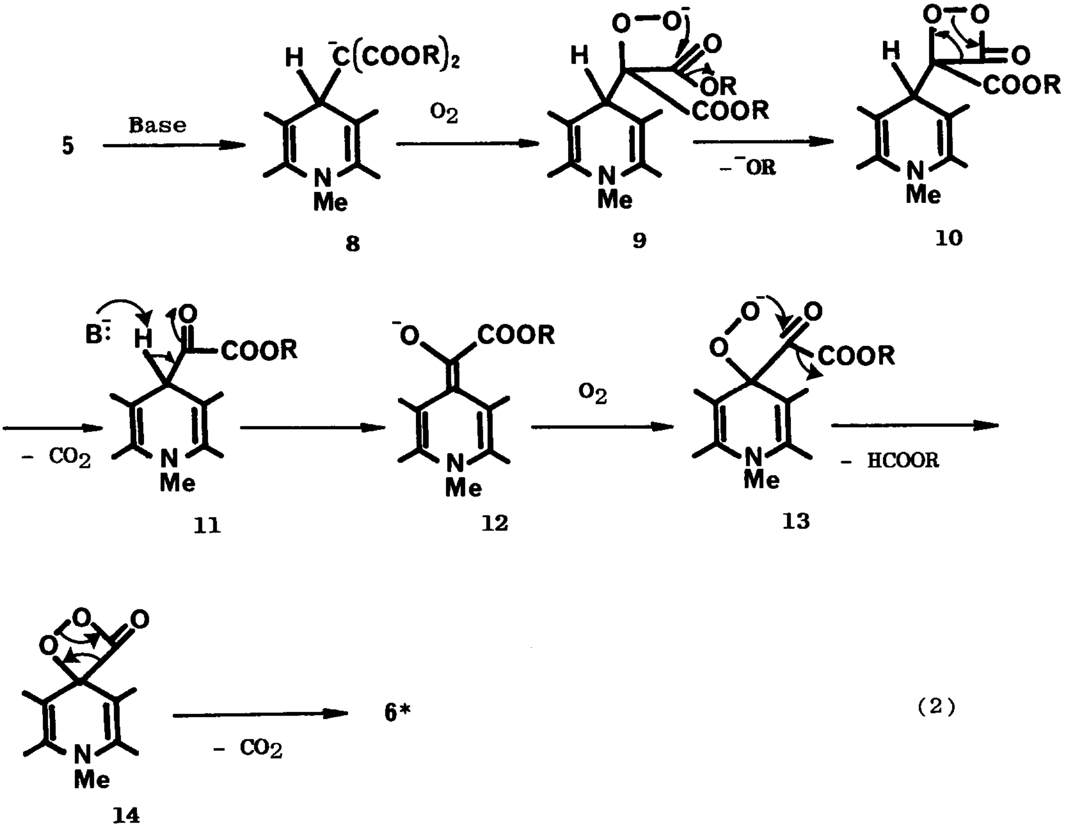
The oxygen streams bubbled through the CL reaction mixture were introduced into toluene cooled by ice bath and the resulted solution was analysed by GLC. 9) t-Butyl formate (**7**) was detected and identified instead of the methyl, ethyl, or phenyl esters by comparing with those of the authentic samples. The latter esters were proved to give the t-butyl ester **7** by transesterification under the similar conditions (Scheme 1).

$$\begin{matrix} \textbf{5a} \sim \textbf{c} \\ \text{or} \\ \textbf{HCOOR} \end{matrix} \xrightarrow[\text{DMSO} \atop 70^\circ\text{C}]{O_2/\text{t-BuOK}} \underset{\textbf{7}}{\textbf{HCOO-t-Bu}} \qquad (1)$$

(R = Me, Et, or Ph)

Introduction of the oxygen streams after acidification of the CL solution into aqueous Ca(OH)$_2$ solution proved the generation of CO$_2$ from the CL reactions.

The reaction path is best described as Scheme 2: namely the acridane **5** gave an anion **8** under the basic conditions, and then, **8** took a molecular oxygen to give a 1,2-dioxetanone **10**, through **9**. Spontaneous extrusion of CO$_2$ molecule from **10** gave an excited state of **11**, which gave light emission when being deactivated to the ground state. α-Hydrogen to the α-keto ester **11**, was abstracted by the base, again, to give the enolate anion **12**, which was oxygenated by molecular oxygen and gave the second 1,2-dioxetanone **14** and formate **7** through **13**. With elimination of CO$_2$ the dioxetanone **14** decomposed to the excited state of N-methylacridone 6*, which gave CL.

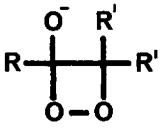


(2)

The emitter of the initial stage of the CL (probably the keto ester **11** or the anion **12**) was not proved experimentally owing to the instability of the CL in its intensity. Anyway, the similar oxygenation reaction gave no additional amounts of CL, when **6** was added beforehand (5.0 x 10^{-3} M).

A DMSO solution of **6** gave weak CL (less than 1% of the CL from **5**), when t-BuOK and O$_2$ was added at 70°C. This shows that oxygenation of DMSO to dimethyl sulfone10) is also chemiluminescent when some fluorescent compound exists.

Kamiya and Sugimoto$^{11)}$ have described similar CL reactions of ketones ($\mathrm{R\text{-}\overset{O}{\overset{\|}{C}}\text{-}CHR'_2}$: R = aryl or alkyl; R' = alkyl) in DMSO, N,N-dimethylformamide or aqueous media to give 1,2-dioxetane oxide anions **15**.



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2) N. Suzuki and T. Goto, *Tetrahedron*, **28**, 4075 (1972).

3) E. H. White, M. G. Steinmetz, J. D. Miano, P. D. Wildes, and R. Morland, *J. Am. Chem. Soc.*, 102, 3199 (1980).

4) **5a**:$^{5,6)}$ Colorless needles (petr. ether), mp 77.5-78.0°C [lit.$^{5)}$ mp 73-74°C].
5b:$^{6,7)}$ Colorless granules (ether/petr. ether), mp 140.5-141.5°C; m/z 325 (M^+).
5c:$^{6,7)}$ Colorless granules (ether), mp 144.5-146.5°C; m/z 449 (M^+).

5) F. Kröhnke and H. L. Honig, *Chem. Ber.*, **90**, 2215 (1957).

6) This substance gave satisfactory spectroscopic data.

7) This new substance gave satisfactory elemental analysis.

8) J. W. Hasting and G. Weber, *J. Opt. Soc. Am.*, **53**, 1410 (1963).

9) GLC Conditions: Yanagimoto Yanaco G-1800F (FID), 5% PEG 20 M on Gasport B in a stainless steel column (ϕ 3 mm x 2 m); column temp. 62°C, inj. temp. 75°C, carrier gas (N_2), 5.4 ml/min.

10) see N. Suzuki, M. Kato, K. Sano, and Y. Izawa, *Heterocycles*, **19**, 1415 (1982).

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