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SYNTHESIS OF HYDROPEROXIDE VIA PHOTOOXYGENATION FOR A MODEL AEQUORIN BIOLUMINESCENCE†

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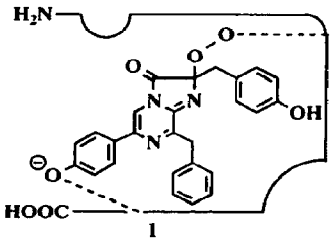
Abstract : Unstable hydroperoxide of coelenterazine (Oplophorus luciferin) analog has been synthesized by the reaction of coelenterazine analog with polymer-bound Rose Bengal via photooxygenation. This compound may be a key intermediate model in the bioluminescence of aequorin and the chemiluminescence of coelenterazine.

Aequorin (1) is a calcium-binding protein found in jellyfish, *Aequorea victoria*, which emits blue light (460 nm ~ 470 nm) by the action of calcium ions to this luminescence system.[1] A number of studies of molecular mechanism of the luminescence reaction, and applications of the apoprotein [2] and chromophore [3] have recently progressed. Aequorin is known to have a chromophore, coelenterazine (*Oplophorus* luciferin) 2, as the light emitting species linking to this protein through a peroxidic bond as illustrated in 1. In 1978, Shimomura and Johnson reported that a yellow compound obtained by reduction of aequorin with $NaHSO_3$ has a tertiary alcohol at the imidazolone carbon to which the *p*-hydroxybenzyl group is attached.[4] Kishi *et al.* supported this result by measuring ^{13}C-NMR spectra of 1 and concluded its structure through incorporation experiment of $^{18}O_2$.[5] Besides, coelenterazine and its analogs emit blue light in organic solvents, such as dimethyl sulfoxide or dimethylformamide, under aerobic condition without apoprotein.[6] While the molecular mechanism of these bio- and chemiluminescence is still uncertain, the working hypothesis outlined in Scheme 1 is consistent with the available data.[7] Our interest in the chemistry of coelenterazine peroxide has prompted us to synthesize hydroperoxide related to 3 and to study this chemistry. We provide herein the first report of synthesis of hydroperoxide of coelenterazine analog and the chemiluminescence of this hydroperoxide.



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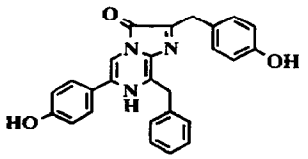

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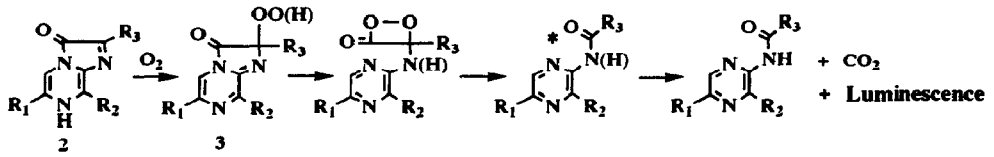
† The first author wishes to dedicate this paper to Professor Toshio Goto (Nagoya University) who deceased on August 29, 1990.



Scheme 1. Posturated mechanism of luminescence reaction.

Photooxygenation of coelenterazine analog **4** [8] (0.10 g, 0.26 mmol), having *tert*-butyl group at 2 position of the imidazopyrazinone, in CH_2Cl_2 (20 ml) at -95 °C with polymer-bound Rose Bengal [9] (0.40 g) as a sensitizer and a 220-W high pressure sodium lamp gave mixtures of products (Scheme 2). This reaction solution produced chemiluminescence (λ_{max} = 395 nm) above about -50 °C. After keeping this solution for 1 h at -20 °C followed by warming to ambient temperature, amide **7a** was observed as a major product in the crude reaction mixture by ^{1}H-NMR spectroscopy and HPLC analysis . This product is presumably derived from dioxetanone **6a** by the well-established cleavage process. **7a** was isolated in 27 % yield by silica gel column chromatography and identified by comparison of its spectral data with a sample prepared by an independent method [10].

After photooxygenation of **4** in CD_2Cl_2 by the above method, the sensitizer was removed by filtration with a tetrafluoroethylene-membrane filter below -80 °C. In this crude reaction mixture, hydroperoxide **5** was present as a major product as shown by ^{1}H-NMR analysis at -80 °C.[11] However, **7a** and dioxetanone **6a** were not detected in this solution. Subsequently ^{1}H-NMR spectrum of this reaction mixture at -50 °C showed generation of a small quantity of **7a** and that of this reaction mixture at -20 °C showed that **7a** was nearly the exclusive product. The hydroperoxide **5** , which are only stable below about -50 °C, was converted to **7a** in good yield on warming to -20 °C.

The chemiluminescence spectra (λ_{max} = 395 nm) of 10^{-4} M solutions of the photooxygenated reaction mixture in CH_2Cl_2 or CH_3CN at -20 °C match the fluorescence spectra of **7a** in the same conditions (Figure). Addition of NaOH (10 equiv. for **4**) to 10^{-4} M solution of the photooxygenated reaction mixture in CH_3CN produced blue chemiluminescence (λ_{max} = 470 nm) at -20 °C (Figure). This chemiluminescence was identical to the fluorescence spectrum of **7a** in CH_3CN with excess NaOH. These findings demonstrate that **5** is converted to **6a** or dioxetanone **6b** followed by generation of singlet excited **7a** or singlet excited amide anion **7b**.

Peroxide derivative **8** was readily available (Scheme 2). Photooxygenation of **4** at -95 °C in CH_2Cl_2 followed by addition of trimethylsilyl cyanide (100 equiv.) at -95 °C and keeping at -80 °C for 14 days smoothly afforded trimethylsilyl peroxide **8** (16% yield from **4**) and **7a** (6% yield). Isolation of **8** [12] was carried out by means of HPLC (elution condition : Fuji Silysia Chromatorex-ODS column, CH_3CN as elution solvent, at 10 °C), though **8** is labile (half-lives at 25 °C ; 5 min in CH_3OH, 16 min in CH_3CN). Compound **8** decomposed to only **7a** in CH_2Cl_2, but in CH_3CN or CH_3OH, to **7a** and **10** at the ratio 2 to 1. The column chromatography gave **7a** in 6% yield, indicating that a portion of **8** decomposed to **7a** under these conditions.

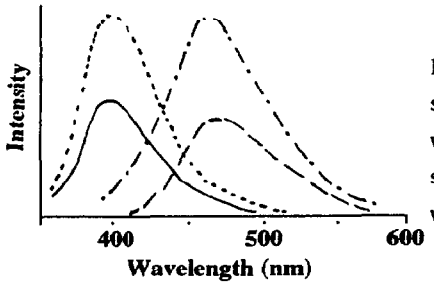
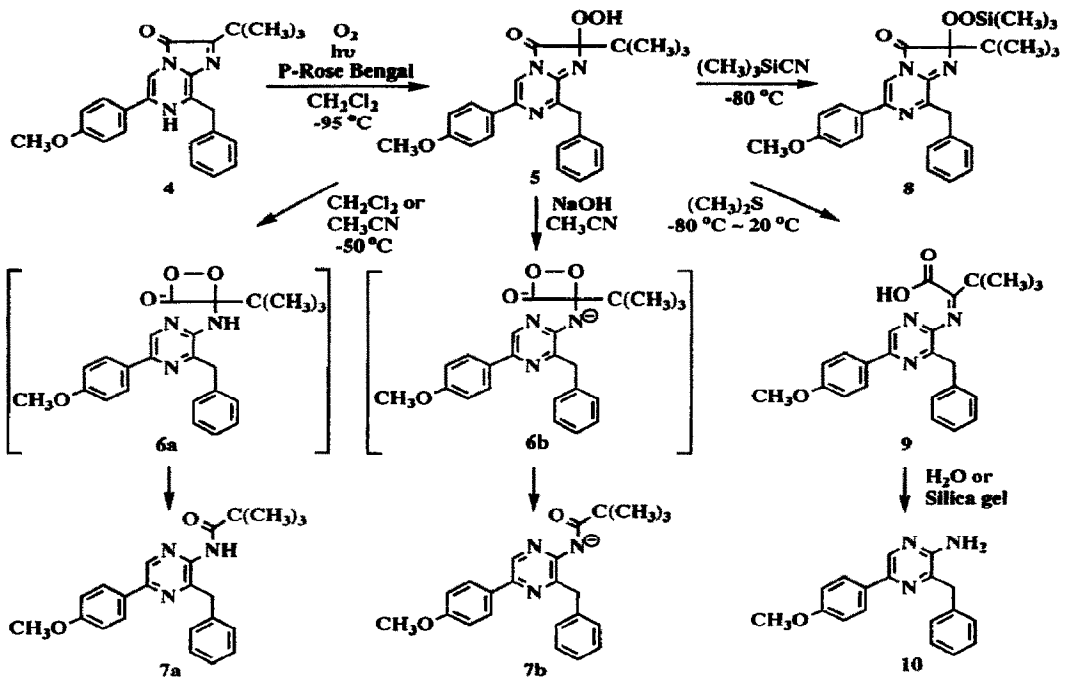


Figure. Chemiluminescence spectra of the photooxygenated solution of **4** in CH_3CN at -20 °C (——) and in CH_3CN with NaOH (10 equiv.) at -20 °C (- - -). Fluorescence spectra of **7a** in CH_3CN at -20 °C (.....) and in CH_3CN with excess NaOH at -20 °C (- . - . -).



Scheme 2

Hydroperoxide **5** was reduced to carboxylic acid **9** with dimethyl sulfide (50 equiv.) for 2 h at -80 °C.[13] **9** was identified by comparison of silica gel TLC and HPLC analysis with those of an authentic sample.[14] **9** was rapidly converted to **10**[15] by treatment with H_2O or silica gel column chromatography at -20 °C.

This study have shown for the first time that photooxygenation of coelenterazine analog **4** can afford hydroperoxide **5** and that thermal decomposition of **5** generate singlet excited **7a**, **7b**, and corresponding chemiluminescence. We will report the chemiluminescence of trimethylsilyl peroxide **8**.

Acknowledgments. This research was supported by the Sasakawa Scientific Research Grant from The Japan Science Society and the Grant In Aid for Scientific Research (No. 5760098), Ministry of Education, Science and Culture.

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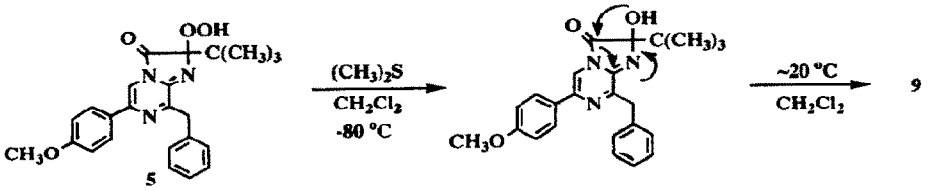
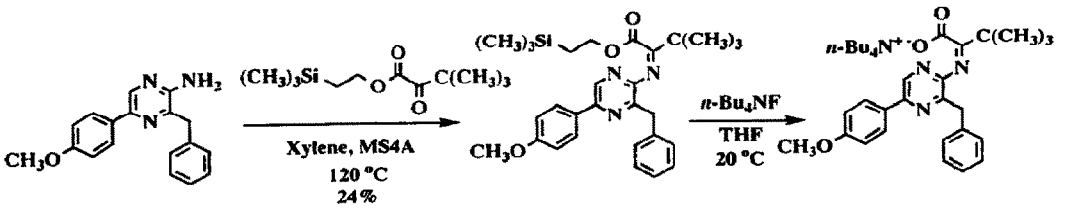
10. Authentic sample of **7a** was prepared by treatment of **10** with pivaloyl chloride and triethylamine in CH_2Cl_2 at 20 °C.
11. **5** : ^{1}H-NMR (CD_2Cl_2, -80 °C, 400 MHz), δ 1.22 (9H, s), 3.89 (3H, s), 4.17 (2H, br.), 7.03 (2H, d, J = 8.9 Hz), 7.2~7.4 (3H, m), 7.32 (1H, s), 7.69 (2H, d, J = 8.8 Hz), 7.92 (2H, d, J = 8.9 Hz).
12. **8** : amorphous powder, IR υ max (KBr) cm^{-1}: 2950, 1755, 1600, 1510, 1480, 1440, 1250. ^{1}H-NMR (CD_2Cl_2, 15 °C, 270 MHz), δ 0.18 (9H, s), 0.97 (9H, s), 3.83 (3H, s), 4.12 (1H, d, J = 13.9 Hz), 4.35 (1H, d, J = 13.9 Hz), 6.94 (2H, d, J = 9.0 Hz), 7.2 ~ 7.4 (3H, m), 7.29 (1H, s), 7.49 (2H, d, J = 7.3 Hz), 7.71 (2H, d, J = 9.0 Hz). ^{13}C-NMR ($CDCl_3$, 15 °C, 270 MHz), δ -1.09, 24.18, 38.84, 39.86, 55.33, 106.49, 107.37, 114.61, 126.31, 126.78, 128.10, 128.32, 129.82, 132.03, 136.02, 150.79, 158.80, 159.82, 177.29. SIMS m/z 492 $[M+1]^+$.
13. The mechanism of conversion of **5** into **9** may be shown in following scheme.



14. Authentic sample of **9** was prepared as shown in following scheme.



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