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SYNTHESIS OF SILYL PEROXIDE OF COELENTERAZINE (*OPLOPHORUS* LUCIFERIN) ANALOGUE FOR PRECURSOR OF LUMINESCENCE

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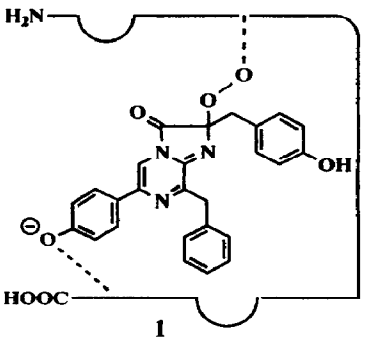
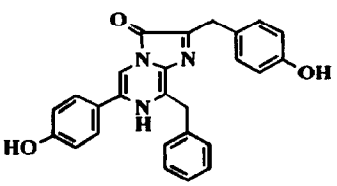
Abstract : Unstable tert-butyldimethylsilyl peroxide of coelenterazine (Oplophorus luciferin) analogue has been synthesized by radical reaction of tert-butyldimethylsilyl hydroperoxide. This compound may be a key intermediate model in the bioluminescence and chemiluminescence of coelenterazine .

Aequorin (**1**) is a calcium-binding protein found in jellyfish, *Aequorea vicutoria*, to emit blue light (460nm) by the action of calcium ions to this luminescence system.[1] Molecular mechanism studies 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 and Shimomura *et al.* supported this result by measuring ^{13}C-NMR spectrum of **1** and concluded its structure through incorporation experiment of $^{18}O_2$.[5] Recently we reported that the structure of yellow compound, which is directly connected to the original structural information of the aequorin chromophore, is the 5-oxy structure **3**.[6]



1



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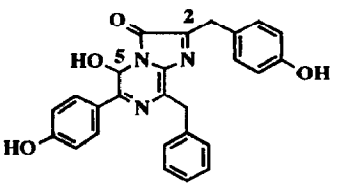
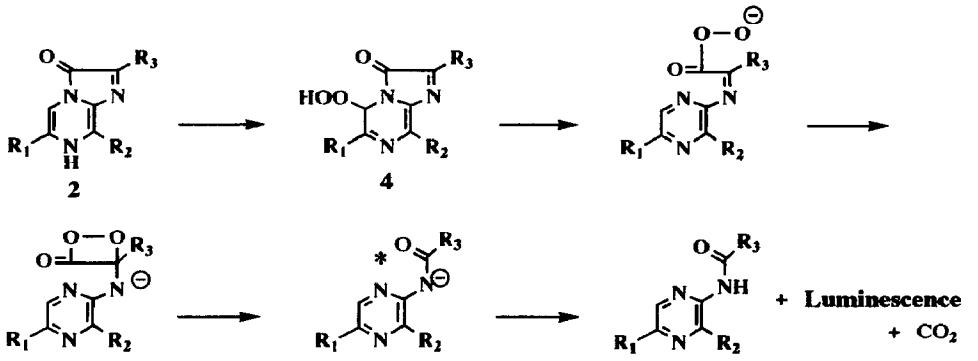


3

Coelenterazine and its analogues emit blue light in organic solvents , for example dimethyl sulfoxide or dimethylformamide (DMF), under aerobic condition without apoprotein.[7] While molecular mechanism of this chemiluminescence has been studied for many years, it is still uncertain.[8]

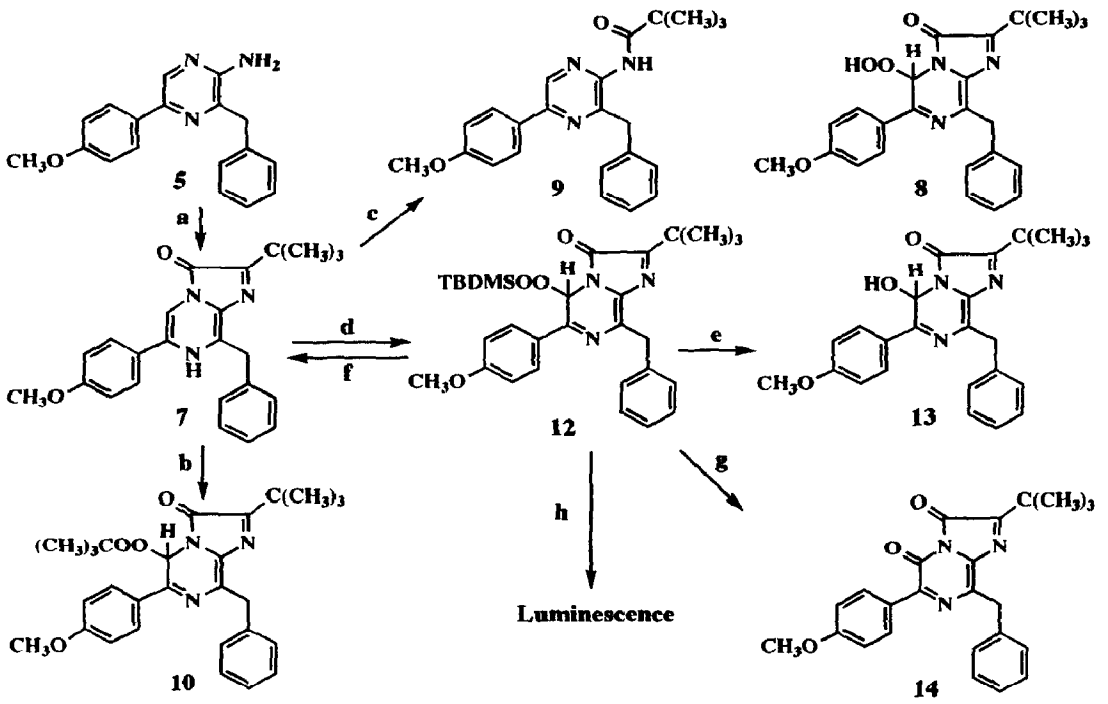
We has suggested that hydroperoxide **4** of coelenterazine is involved in these luminescence reactions as illustrated in Scheme 1. Our interest in the chemistry of coelenterazine peroxide has prompted us to synthesize peroxides related to **4** in an effort to evaluate our proposal.



Scheme 1. Posturated mechanism of luminescence reaction.

A number of synthetic approaches to peroxides related to **4** have been examined (illustrated in Scheme 2). These approaches all began with the coelenterazine analogue **7**, having *tert* -butyl group at 2-position of the imidazopyrazinone, which was readily prepared by coupling [9] between the 2-aminopyrazine **5** [10] and the *tert*-butyl glyoxal **6** [11] in 90% yield as shown in Scheme 2. In the initial approach, hydrogen peroxide - cuprous chloride system and photooxygenation system were employed as the oxidizing system, however not the hydroperoxide **8** but 2-amidpyrazine **9** was formed under these conditions. Subsequently it was found that *tert*-butyl peroxide compound **10** [12] could be particularly easy obtained in 30 ~ 90% yield[13] using anhydrous *tert*-butyl hydroperoxide - cuprous chloride system [14]. Similarly, *tert*-butyldimethylsilyl hydroperoxide (TBDMSOOH) **11** [15] - cuprous chloride system gave *tert* -butyldimethylsilyl peroxide **12.**

The experimental procedure is as follows; to a distilled methylene chloride solution of coelenterazine analogue **7** under an argon atmosphere was added TBDMSOOH (2.0 equiv) at -20°C followed by addition of a catalytic amount of cuprous chloride. The resulting mixture was stirred for 3 ~ 30 hr at -15°C, then poured into a mixture of ice-cooled, 0.01N $Na_2S_2O_3$ aqueous solution and methylene chloride, and extracted with methylene chloride. The combined methylene chloride extracts were thoroughly washed with distilled water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at 5 ~ 10°C. The residue was chromatographed thrice on silica gel column by using 1 : 30 mixture of ethyl acetate and *n-hexane*, methylene chloride and 1 : 20 mixture of ethyl acetate and *n*-hexane at -50°C ~ -40°C respectively to give *tert*-butyldimethylsilyl peroxide **12** in 2% yield. About 30% loss of **12** occurred in each chromatography due to instability of this compound. Product structure was assigned on the basis of spectroscopic data [16] and confirmed by chemical transformations. For example, **12** was reduced to **13** with dimethyl sulfide, which was identified with authentic sample [17], reduced to **7** with $Na_2S_2O_4$ and converted directly to **14** [18] by treatment of **12** for a few minutes at 0°C with silica gel. While other silyl hydroperoxides, for example, triphenylsilyl hydroperoxide, and other radical sources were employed, never desired silyl peroxide compound was obtained.



Scheme 2. Synthesis of peroxides of coelenterazine analogue. Reagents; (a) $HCOCOC(CH_3)_3$ (**6**), HCl, H_2O, 1,4-Dioxane, 100°C ; (b) $(CH_3)_3COOH$, CuCl, CH_2Cl_2, -15°C ; (c) [O] ; (d) TBDMSOOH (**11**), CuCl, CH_2Cl_2, -15°C ; (e) $(CH_3)_2S$, THF, 0°C ; (f) $Na_2S_2O_4$, CH_3OH, H_2O, 0°C ; (g) silica gel, 0°C ; (h) DMF, 20°C or *n*-Bu_4NF, AcOH, THF, -78°C~-20°C.

Purified **12** is very unstable. The stability in solution decreases in the order : chloroform, methanol, DMF. **12** can be kept for a day at 20°C in chloroform, in which **12** is most stable, but it rapidly decomposes in methanol to give compound **14** and a few compounds. In DMF under either aerobic or anaerobic condition, **12** emitted weak light for 2 days.[19] Desilylation of **12** in tetrahydrofuran at -78°C with *n*-Bu_4NF in the presence of acetic acid should lead to the hydroperoxide or the hydroperoxide anion and these species fragmented to emit blue light (490 nm) at -30°C~-20°C under anaerobic condition.[19] Unfortunately, the hydroperoxide **8** or the hydroperoxide anion could not be detected as an intermediate. Thus, **8** or the anion would appear to be very labile in this desilylation condition. Further work to detect or isolate the hydroperoxyde **8** is now in progress.

In summary, this work has first demonstrated that peroxy coelenterazine analogue for the luminous precursor is formed by using TBDMSOOH - cuprous chloride system. Further, it has been shown that silyl peroxide of coelentelazine leads to precursor of luminous species. This work would provide further mechanism on the bioluminescence and chemiluminescence of coelenterazine.

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12. **10** ^{1}H-NMR ($CDCl_3$, 20°C, 270MHz), δ 0.92(9H, s), 1.44(9H, s), 3.87(3H, s), 4.09(1H, d, J= 14.2 Hz), 4.21(1H, d, J= 14.2 Hz), 6.67(1H, s), 6.94(2H, d, J= 8.9 Hz), 7.15~7.30(3H, m), 7.40(2H, d, J= 6.9 Hz), 8.06(2H, d, J= 8.9 Hz). UV λmax. (MeOH) 432nm(ε= 19700), 303nm(ε= 6670), 248nm(ε= 6410). MS m/z 476 $[M+1]^+$, mp 134~135°C (decompose).
13. The yield of this reaction is ruled by lots of anhydrous *tert*-butyl hydroperoxide solution.
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15. To a dried ether solution of anhydrous H_2O_2 (5.0 equiv)[20] was added *tert*-butyldimethylsilyl chloride (1.0 equiv) and imidazole (1.0 equiv) at 0°C followed by extractive isolation with *n*-hexane and evaporation under reduced pressure at 15°C. The residue was chromatographed on silica gel at 0°C by using methylene chloride to give *tert*-butyldimethylsilyl hydroperoxide (88% yield, mp 44~45°C).
16. **12** ^{1}H-NMR ($CDCl_3$, 20°C, 270MHz), δ -0.12(3H, s), -0.11(3H, s), 0.75(9H, s), 1.44(9H, s), 3.86(3H, s), 4.20(1H, d, J= 13.9Hz), 4.26(1H, d, J= 13.9Hz), 6.70(1H, s), 6.90(2H, d, J= 8.9Hz), 7.2~7.4(3H, m), 7.45(2H, d, J= 6.9Hz), 8.03(2H, d, J= 8.9Hz). UV λmax. (MeOH) 431nm(ε= 23100), 303nm(ε= 7950), 247nm(ε= 8180). MS m/z 534 $[M+1]^+$. mp 107~108°C (decompose).
17. Authentic sample of **13** was prepared as shown in the previous report [6]. **13** ^{1}H-NMR ($CDCl_3$, 20°C, 270MHz), δ 1.44(9H, s), 3.86(3H, s), 4.07(1H, d, J= 15Hz), 4.17(1H, d, J= 15Hz), 6.62(1H, s), 6.94(2H, d, J= 9.0Hz), 7.1~7.2(3H, m), 7.22(2H, d, J= 6.0Hz), 8.06(2H, d, J= 9.0Hz).
18. **14** ^{1}H-NMR($CDCl_3$, 20°C, 270MHz), δ 1.46(9H, s), 3.87(3H, s), 4.21(2H, s), 6.94(2H, d, J= 9.2Hz), 7.15~7.40(3H, m), 7.43(2H, d, J= 7.0Hz), 8.38(2H, d, J= 9.2Hz). UV λmax. (MeOH) 472nm(ε= 1800), 362nm(ε= 21000), 253nm(ε= 15900). MS m/z 402 $[M+1]^+$. mp 156~157°C.
19. The chemiluminescence of **12** will be described in a subsequent paper.
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