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Studies on the Mechanism of Chemiluminescence: Synthesis and Chemiluminescent Properties of the 5-Hydroperoxide Analogue of Coelenterate Luciferin

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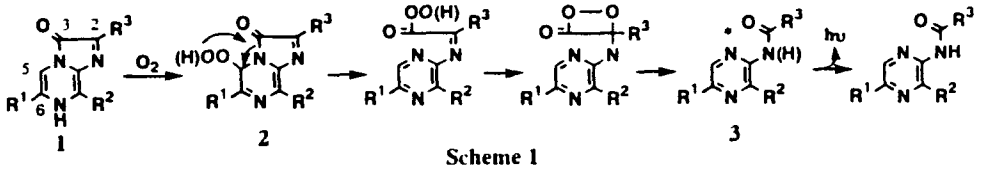
Abstract : The 5-hydroperoxide analogue of coelenterate luciferin has been synthesized and is one of the postulated intermediates in chemiluminescent reactions of coelenterate luciferin, *Cypridina* luciferin, and its analogues. The 5-hydroperoxide analogue emitted weak light in chemiluminescent reactions in several solvent systems. However, it did not generate any amide. This research leads to the conclusion that 5-hydroperoxide is not an important intermediate in chemiluminescent reactions.

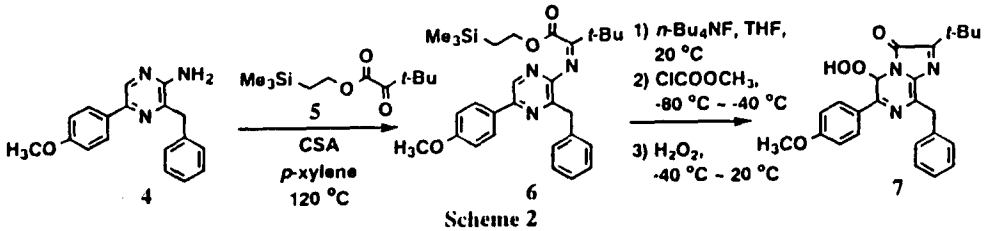
The chemiluminescent reaction of *Cypridina* luciferin without enzyme in dimethyl sulfoxide (DMSO) and in the presence of oxygen was first reported in 1966 by Johnson and coworkers.[1] Diethylene glycol dimethyl ether (DGM), containing a trace amount of acetate buffer (pH 5.6), was later reported as an efficient solvent by Goto et al.[2] Many synthesized analogues of *Cypridina* luciferin, such as 2-methyl-6-phenylimidazo[1,2-a]pyrazin-3(7*H*)-one (CLA) and 2-methyl-6-(*p*-methoxyphenyl)imidazo[1,2-a]pyrazin-3(7*H*)-one (MCLA) generate light by oxidation with triplet oxygen in basic buffer solutions or with superoxide anion in aqueous solvents.[3,4] The chemiluminescence of coelenterate luciferin (*Oplophorus* luciferin), 2-(*p*-hydroxybenzyl)-6-(*p*-hydroxyphenyl)-8-benzylimidazo[1,2-a]pyrazin-3(7*H*)-one, and its analogues have been observed in *N*, *N*-dimethylformamide (DMF), hexamethylphosphoramide (HMPA), or DMSO in the presence of oxygen.[5]

Since the discovery of the chemiluminescent properties of the compounds labeled **1**, shown in Scheme 1, the mechanisms of the reactions have been studied extensively.[6] In spite of the attention paid to the chemiluminescent reactions, the details of the reactions remain subject to debate. While the mechanism of the oxidation reactions with molecular oxygen is still uncertain, it is probable that light emitters in the reactions are singlet excited coelenteramide analogues **3**, in Scheme 1. In order to clarify the mechanism of the chemiluminescent reactions, we have investigated a key intermediate in the chemiluminescent reaction of the coelenterate luciferin analogue, 2-*tert*-butyl-6-(*p*-methoxyphenyl)-8-benzylimidazo[1,2-a]pyrazin-3(7*H*)-one, compound **8**, in Scheme 3.[7] Recently we suggested that 5-hydroperoxide, compound **2**, is involved in the chemiluminescent reactions of compound **1**, as illustrated in Scheme 1,[6h,8] and reported on synthesis and chemiluminescence of 5-silyl peroxide of the coelenterate luciferin analogue. However, we were unable to confirm, isolate or characterize the 5-hydroperoxide or 5-peroxide anion because of their unstable properties under the conditions of silyl group deprotection. To determine whether the 5-hydroperoxide or its anion is an important intermediate, we tried to synthesize the 5-hydroperoxide. In this paper, we describe the synthesis and chemiluminescence properties of the 5-hydroperoxide analogue of coelenterate luciferin.



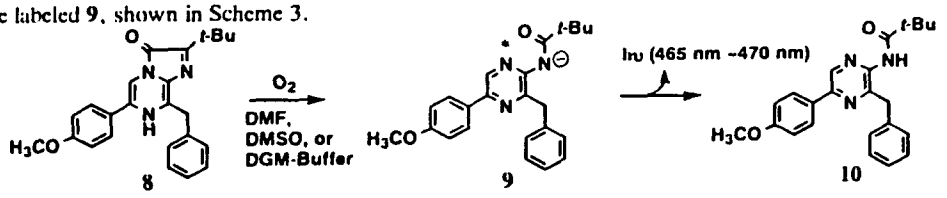
Scheme 1

Compound **6**, the precursor of 5-hydroperoxide (**7**) have been synthesized by modification of published procedures as shown in Scheme 2.[6d] Condensation of aminopyrazine (**4**) [9] and 3,3-dimethyl-α-ketobutyric acid trimethylsilylethyl ester (**5**) in *p*-xylene, containing a trace amount of 10-camphorsulfonic acid (CSA), at 120 °C for 5 hr gave compound **6** with a 24% yield. After deprotection of the silyl group of compound **6** with tetrabutylammonium fluoride in tetrahydrofuran (THF), compound **6** was subjected to treatment with methyl chloroformate at -80 °C and was then kept at -40 °C for 10 min. Then, dried diethyl ether solution of anhydrous hydrogen peroxide (1.5 equiv.)[10] at -40 °C was added, followed by treatment at 20 °C for 20 min. to give compound **7** with a 65% yield by high performance liquid chromatography (HPLC) analysis. Compound **7** was isolated and purified by HPLC, with the following elution conditions: Fuji silysia Chromatorex-ODS column, CH_3OH-H_2O as elution solvent, at 4 °C). Purified compound **7** was obtained at a 4% yield.[11] Although the half-life of compound **7** (6.1×10^{-4} M) in CH_3OH and in THF at 20 °C were 1.3 hr and 63 hr respectively, in the reaction solution at 20 °C its half-life was less than 1 hr. A portion of compound **7** decomposed during reaction and isolation due to its instability.



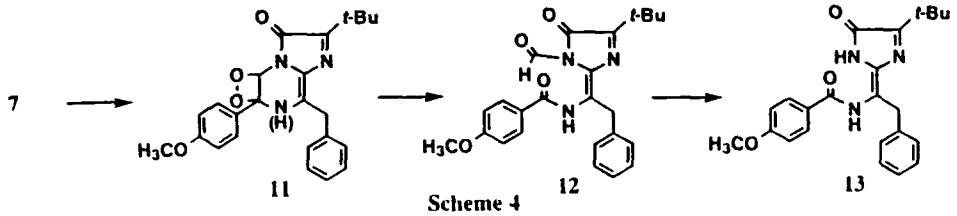
Scheme 2

Cormier et al. observed chemiluminescence of 2-methyl-6-(*p*-methoxyphenyl)-8-benzylimidazo[1,2-a]pyrazin-3(7*H*)-one, which is structurally similar to that of analogue **8**, in DMF in the presence of oxygen.[5a] The products were CO_2 and the corresponding coelenteramide analogue, which was obtained with an 80% yield.[5a] We measured chemiluminescent reactions of analogue **8** in a few organic solvents as shown in Scheme 3 and Table 1.[12] The wavelength maxima of the chemiluminescence spectra of analog **8** in DMF, DMSO, and DGM containing 0.70 volume % of 0.10 M acetate buffer at pH 5.6 were 470nm, 470nm, and 465nm, respectively. These luminescent spectra did not coincide with the fluorescent spectra of compound **10** in DMF, DMSO or DGM solvents, but nearly coincided with fluorescence spectra of compound **10** in the same solvents with potassium *tert*-butoxide, added.[13] These observations correspond with the observations by Cormier et al., who suggested that the light emitter is an amide anion and show that the light emitter is anionic amide labeled **9**, shown in Scheme 3.



Scheme 3

Decomposition of compound **7** (6.1×10^{-4} M) in CH_2Cl_2 and in CH_3OH at 25 °C yielded product **13**, which was assigned on the basis of spectroscopic data, in 95% and 60% yields, respectively.[14] After the decomposition of compound **7** in CD_3OD, HCO_2CD_3 and compound **13** were present in a 1:1 ratio, as shown by ^{1}H-NMR analysis. Compound **13** is probably produced by the mechanism shown in Scheme 4. The hydroperoxide adds to the C-6 position to give a dioxetane, compound **11** (not detected), which can decompose to compound **12**. Finally, compound **13** is produced by methanolysis of **12**.



Scheme 4

The results of chemiluminescent reactions of compound **7** (1.0×10^{-5} M), measured in several conditions, are shown in Table 1. The light intensity of compound **7** in DMF and in DMF containing NaOH are about 1% of that of compound **8**. The chemiluminescent light of compound **7** was not generated in DMSO, because compound **8** was reduced to 2-*tert*-butyl-5-hydroxy-6-(*p*-methoxyphenyl)-8-benzyl-3,5-dihydroimidazol[1,2-a]pyrazin-3-one with a 94% yield. These chemiluminescent reactions of compound **7** did not yield any amide **10**, which is the neutral species of emitter **9** in the chemiluminescence of compound **8**, and many decomposition compounds were produced. Compound **13** was formed as one of the decomposition products. Chemiluminescent spectra of compound **7** in solvents listed above were broad (λmax are between 480 nm to 550 nm), and not similar to that of chemiluminescence of compound **8**. These spectra show that the light emitter(s) is (are) different from anionic amide **9** or neutral amide **10**.[15] These results, therefore, show neither chemiluminescence of compound **8** is formed from compound **7**, nor **7** is a key intermediate in the chemiluminescent reactions of **8**.

Table 1. Chemiluminescent properties of compounds **8** and **7**.[a]

Solvent	Compound **8**		Compound **7**		
	Relative light intensity	Yield of **10** (%)[b]	Relative light intensity	Yield of **10** (%)[b]	Yield of **13** (%)[b]
DMF	1.0	88	0.0082	0	9
DMF (1×10^{-5}M NaOH)[c]	-	-	0.0082	0	30
DMF (1×10^{-4}M NaOH)[d]	-	-	0.012	0	55
DMSO	0.63	99	0	0	0.5
DGM-Acetate buffer[e]	1.6	73	0.025	0	20
DGM (1×10^{-4}M NaOH)[f]	-	-	0.0053	0	47

[a] All chemiluminescent reactions were run at 25 °C. [b] Concentrations of compounds **8** and **7** were 1.0×10^{-5} M respectively. Yields were evaluated by HPLC. [c] DMF containing 1.0 vol% of 0.0010 N NaOH/H_2O. [d] DMF containing 1.0 vol% of 0.010 N NaOH/H_2O. [e] DGM containing 0.70 vol% of 0.10 M acetate buffer of pH 5.6. [f] DGM containing 1.0 vol% of 0.010 N NaOH/H_2O.

We conclude that the generation of the 5-hydroperoxide analogue **7** is not the major reaction pathway in the chemiluminescent reaction of compound **8**, which has a *tert*-butyl group at the C-2 position. We therefore suggest that 5-hydroperoxide, compound **2**, could not be an important intermediate in chemiluminescent

reactions of coelenterate luciferin, *Cypridina* luciferin, and its analogues, which do not have a *tert*-butyl group at the C-2 position.

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11. **7** : amorphous powder from CH_3CN-H_2O, UV λmax (CH_3CN) 428 (ε = 17000), 296 (ε = 7700). ^{1}H-NMR (THF-d_8): δ 1.43 (9H, s), 3.83 (3H, s), 4.06 (1H, d, J = 14.0 Hz), 4.10 (1H, d, J = 14.0 Hz), 6.81 (1H, s), 6.94 (2H, d, J = 9.2 Hz), 7.10 (1H, m), 7.20 (2H, d,d, J = 7.3, 7.9 Hz), 7.34 (2H, d, J = 7.3 Hz), 8.11 (2H, d, J = 9.2 Hz), 11.22 (1H, s; disappear with D_2O). SIMS m/z 420 $[M+1]^+$.
12. Usami et. al. reported about chemiluminescence of **8** in acidic, neutral, or basic DGM.[6k]
13. Usami et. al. showed that amide **10** was protonated in basic DGM after luminescence of **8**.[6k]
14. **13** : amorphous powder, UV λmax (CH_3CN) 347 (ε = 22000), 267 (ε = 9600). ^{1}H-NMR (THF-d_8): δ 1.39 (9H, s), 3.81 (3H, s), 4.43 (2H, s), 6.91 (2H, d, J = 9.2 Hz), 7.10 (1H, m), 7.17 (2H, m), 7.22 (2H, d, J = 7.3 Hz), 7.78 (2H, d, J = 9.2 Hz), 8.61 (1H, s; disappear with D_2O), 10.26 (1H, s; disappear with D_2O). ^{13}C-NMR (THF-d_8): 27.75, 34.35, 35.34, 55.65, 114.16, 122.46, 126.92, 127.60, 128.94, 129.92, 130.33, 138.98, 140.39, 163.62, 164.50, 165.93, 170.94. SIMS m/z 392 $[M+1]^+$.
15. Emitter(s) in these chemiluminescent reactions is (are) not decided yet.

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