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Synthesis and Chemiluminescent Properties of the Peroxy Acid Compound as an Intermediate of Coelenterate Luciferin Luminescence

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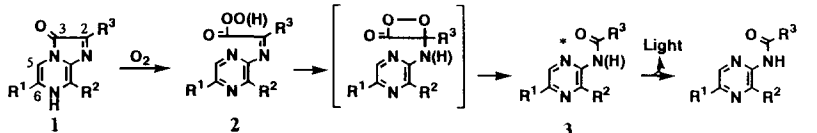
Abstract : The labile peroxy acid compound has been synthesized and is one of the postulated intermediates in chemiluminescent reactions of coelenterate luciferin, *Cypridina* luciferin, and their analogues. The peroxy acid generated amide was accompanied by light in several solvent systems. In the non-polar solvent, the peroxy acid showed luminescence with one peak at 395 nm emitted from a neutral amide, whereas in polar solvents the peroxy acid generated light with 456-470 nm emitted from an anionic amide. The variations in luminescence are caused by the acidity of the peroxy acid group. © 1997 Elsevier Science Ltd.

The mechanistic chemistry of bioluminescence and chemiluminescence on imidazo[1,2-*a*]pyrazin-3(7*H*)-ones (**1**), as shown in Scheme 1, has been intensively studied since they were first found nearly 30 years ago.[1] 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.[2] 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.*[3] Synthesized analogues of *Cypridina* luciferin generate light by oxidation with triplet oxygen in buffer solutions and with superoxide anion in aqueous solvents.[4,5] The chemiluminescence of coelenterate luciferin and its analogues has been observed in *N,N*-dimethylformamide (DMF) and hexamethylphosphoramide in the presence of oxygen.[6] In spite of the attention paid to these chemiluminescent reactions, the details of the reactions remain subject to debate, because of the extreme lability of the intermediates. While the mechanism of the oxidative chemiluminescence with molecular oxygen is still not clear, it is probable that the light emitters in the reactions are singlet excited amides **3**, as in Scheme 1. It has been suggested that the intermediate(s) in the chemiluminescent reaction is(are) 2-, 3-, or(and) 5-peroxy imidazopyrazinone compounds.[7] In order to establish the mechanism of the chemiluminescent reactions, we have investigated intermediates 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. Recently it was established that 2-hydroperoxide generates quantitatively an amide compound accompanied by light[8] and that 5-hydroperoxide is not an important intermediate[1]. Our aim of the present study was to investigate the chemiluminescent ability and properties of peroxy acid **2** as shown in Scheme 1. Herein we report synthesis and chemiluminescent properties of the peroxy acid of coelenterate luciferin.

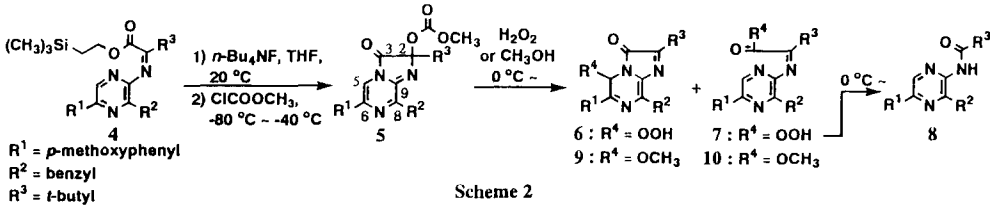
8-Benzyl-2-*tert*-butyl-2-methoxycarbonyloxy-6-(*p*-methoxyphenyl)-2,3-dihydroimidazo[1,2-*a*]pyrazin-3-one (**5**), which was too labile to be isolated, was exclusively prepared in treatment of previously reported



1 **2** **3**

Scheme 1

compound **4**[1] with *n*-Bu4NF in deuteriotetrahydrofuran followed by cyclization with methyl chloroformate as shown in Scheme 2.[9] Direct variable-temperature ^{1}H NMR analysis of the reaction of the reaction mixture including exclusively compound **5** with anhydrous hydrogen peroxide (2.5 equiv.)[10] showed that 5-hydroperoxide **6** and peroxy acid **7** were produced and that the decomposition of **7** occurred above 0 °C at the same time to give amide compound **8** quantitatively. The compound **6** was identified by comparison with reported ^{1}H NMR spectral data[1] and the structure of **8** was confirmed by ^{1}H NMR spectral data. **6** and **8** were given with 45 and 43% yields, respectively, as determined by high performance liquid chromatography (HPLC) analysis. The structure of peroxy acid **7**[11] was unequivocally characterized by comparison with ^{1}H NMR spectral data of methyl ester **10**[12], which was prepared from 2-amino-3-benzyl-5-(*p*-methoxyphenyl)pyrazine[13] and 3,3-dimethyl-α-ketobutyric acid methyl ester in the presence of 10-camphorsulfonic acid (CSA) with a 5% yield. In the case of using methyl alcohol instead of anhydrous hydrogen peroxide, 5-methoxy compound **9**[14] and methyl ester **10** were generated similar to that with treatment with hydrogen peroxide, supporting the generation and structure of the peroxy acid **7** in the reaction described above. ^{1}H NMR spectroscopy and HPLC analysis of the reactions established that compound **5** was treated with an excessive amount of anhydrous hydrogen peroxide or methyl alcohol (above 100 equiv.) to give mainly **7** or methyl ester **10**, respectively, with a slight amount of **6** or **9**, whereas it had been reported that using a restricted amount of anhydrous hydrogen peroxide (1.5 equiv.) affords mainly **6**.[1]



Scheme 2

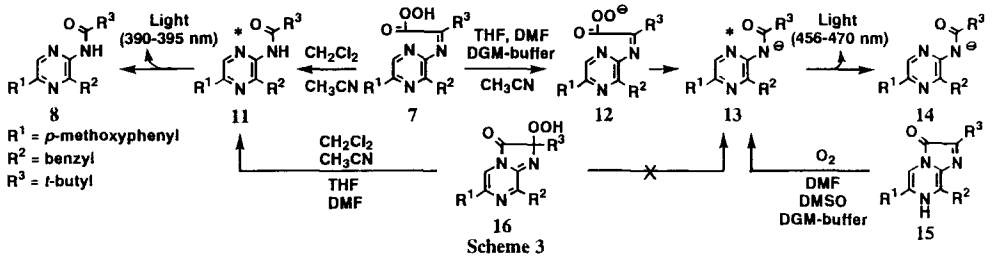
The results of the direct chemiluminescence of compound **5** (10 μM) with anhydrous hydrogen peroxide in several solvent systems are shown in Table 1. Amide compound **8** was given with 54-68% yields, passing through **7**. The chemiluminescent reactions showed three spectral variations. Among the variations, in the non-polar solvent dichloromethane (CH_2Cl_2), luminescence was shown with only one peak at 395 nm. In acetonitrile (CH_3CN) luminescence with peaks at 390 nm and 460 nm was emitted at a 1.6 : 1.0 ratio. In the polar solvent systems, tetrahydrofuran (THF), DMF, and DGM containing 0.70 volume % of 0.10 M acetate buffer at pH 5.6 (DGM-buffer), luminescence with only one peak at 456-470 nm was produced. The luminescent peaks at 456-470 nm in CH_3CN, THF, DMF, and DGM-buffer nearly coincided with the fluorescent spectra of the amide **8** in the same solvents with *t*-BuOK (Table 1), whereas the peaks at 390-395 nm in CH_2Cl_2 and CH_3CN coincided with that of **8** in the same solvents without *t*-BuOK. Our previous study showed that chemiluminescence of imidazo[1,2-*a*]pyrazin-3(7*H*)-one **15** in DMF and DGM-buffer is generated from anionic excited amide labeled **13**, shown in Scheme 3, with one peak at 465-470 nm (Table 1).[1] In an investigation of a model compound of coelenterazine, McCapra reported that the neutral excited amide emits a 380-400 nm peak.[15] Thus, it is reasonable to consider that the 456-470 nm peaks in CH_3CN, THF, DMF, and DGM-buffer are emitted from the anionic excited amide **13** and the 390-395 nm peaks are emitted from the neutral excited amide **11**.

Our previous study showed that in the chemiluminescence of the 2-hydroperoxide **16** the neutral excited amide **11** emits a 390-400 nm luminescent peak in several neutral solvents, such as CH_2Cl_2, CH_3CN, THF,

Table 1. Chemiluminescent properties of compounds **7** and **15**, and fluorescent properties of amide **8**

Solvent	Yield (%) of **8** [a]	CL_{max} (nm) of **7** [b]	CL_{max} (nm) of **15** [d]	FL_{max} (nm) of **8** [e]	FL_{max} (nm) of **8** with *t*-BuOK [f]
CH_2Cl_2	54	395	-	397	455
CH_3CN	62	390 and 460 [c]	-	395	468
THF	65	470	-	384	460
DMF	58	468	470	390	465
DGM-buffer [g]	68	456	465	390	453 [h]

[a] Compound **5** (10 μM) was treated with anhydrous H_2O_2 (0.10 M) from 0 °C to 20 °C in each solvent. Yields were evaluated by HPLC analysis. [b] The wavelength maxima of chemiluminescent spectra of compound **7** from 0 °C to 20 °C. [c] The ratio of the luminescent intensity of 390 nm to that at 460 nm was 1.6. [d] Compound **15** was treated with O_2 in each solvent. The concentration of compound **15** was 10 μM. In CH_2Cl_2, CH_3CN, and THF, **15** could not emit. [e] The wavelength maxima of fluorescent spectra of 20 ml of 10 μM solution of amide **8** in the presence of 1.1 ml of 1.8 M anhydrous H_2O_2 / Et_2O at 20 °C. [f] The wavelength maxima of fluorescent spectra of 20 ml of 10 μM solution of amide **8** in the presence of 0.1 ml of 1.0 M *t*-BuOK / THF at 20 °C. [g] DGM containing 0.70 vol% of 0.10 M acetate buffer (pH 5.6). [h] In this case, acetate buffer was not contained in the solvent.



Scheme 3

and DMF, indicating that there is no influence from solvents.[16] In comparison with the chemiluminescence of **16**, the chemiluminescence of **7** was markedly influenced by solvents, i.e. non-polar and polar solvents as described above. This phenomenon was explained by the fact that the acidity of the peroxy acid group is stronger than that of the 2-hydroperoxy group. In the non-polar solvent, CH_2Cl_2, deprotonation of the peroxy acid group did not occur, giving the neutral excited amide **11** as the result of formation of certain neutral intermediate(s), as 1,2-dioxetanone[17], whereas in polar solvents, THF, DMF, and DGM-buffer, possessing a high ability to generate hydrogen bonds, the deprotonation of the peroxy acid group can take place easily, affording anionic peroxide **12** followed by generation of an anionic intermediate, then the anionic excited amide **13**. In CH_3CN, possessing an intermediate ability to generate a hydrogen bond, **7** showed luminescence from the neutral and anionic excited amides **11** and **13**. In the case of **16**, which has the weaker acidity of the 2-hydroperoxy group than that of the peroxy acid group, the deprotonation of the 2-hydroperoxy group cannot occur even in THF or DMF, giving the neutral 1,2-dioxetanone and the subsequent neutral excited amide. Goto et al. reported that chemiluminescence of 2-methyl-6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one in DGM-buffer showed the possibility of protonation to an anionic excited amide.[4a] However, as regarding luminescence of the peroxy acid **7** in CH_2Cl_2, the generation of anionic excited amide **13** followed by the protonation to this amide cannot occur. If the anionic excited amid from **7** was produced in CH_2Cl_2, 2-hydroperoxide **16**, possessing lower acidity than **7**, should generate some luminescence from anionic excited amide in THF and in DMF.

In conclusion, this study showed synthesis of the peroxy acid **7** and its chemiluminescent properties. The results showed that the variation of the excited amide species can be caused by the acidity of the peroxy acid group.

References and Notes

1. For some of leading references, see Teranishi, K.; Hisamatsu, M.; Yamada, T.*Tetrahedron Lett.*, **1996**,*37*, 8425-8428.
2. Johnson, F. H.; Stachel, H. -D.; Taylor, E. C.; Shimomura, O. *Chemiluminescence and Fluorescence of Cypridina Luciferin and of Some New Indole Compounds in Dimethylsulfoxide*. In *Bioluminescence in Progress* ; Johnson, F. H.; Haneda, Y. Princeton University Press, 1966; pp. 67-82.
3. Goto, T.; Inoue, S.; Sugiura, S. *Tetrahedron Lett.*, **1968**, 3873- 3876.
4. (a) Goto, T.; Inoue, S.; Sugiura, S.; Nishikawa, K.; Isobe, M.; Abe, Y. *Tetrahedron Lett.*, **1968**, 4035-4038. (b) Goto, T.; Fukatsu, H. *Tetrahedron Lett.*, **1969**, 4299-4302. (c) Sawada, H.; Masuyama, K.; Nakayama, M. *Aburakagaku*, **1990**, *39*, 47-49. (d) Toya, Y.; Kayano, T.; Sato, K.; Goto, T. *Bull. Chem. Soc. Jpn.*, **1992**, *65*, 2475-2479.
5. (a) Goto, T.; Takagi, T. *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 833-834. (b) Minakami, H.; Arai, H.; Nakano, M.; Sugioka, K.; Suzuki, S.; Sotomatsu, A. *Biochem. Biophys. Res. Commun.*, **1988**, *153*, 973-978.
6. (a) Hori, K. J.; Wampler, E.; Cormier, M. J. *J. Chem. Soc., Chem. Commun.*, **1973**, 492-493. (b) Teranishi, K.; Goto, T. *Chem. Lett.*, **1989**, 1423-1426. (c) Teranishi, K.; Goto, T. *Bull. Chem. Soc. Jpn.*, **1990**, *63*, 3132-3140.
7. (a) Goto, T. *Pure and Applied Chemistry*, **1968**,*17*, 421-441. (b) Teranishi, K.; Isobe, M.; Yamada, M. *Tetrahedron Lett.*, **1994**,*35*, 2565-2568. (c) Isobe, M.; Takahashi, H.; Usami, K.; Hattori M.; Nishigohri, Y. *Pure and Appl. Chem.*, **1994**, *66*, 765-772. (d) Mager, H. I. X.; S.-C. Tu, *Photochem. Photobiol.*, **1995**, *62*, 607-614.
8. Teranishi, K.; Ueda, K.; Nakao, H.; Hisamatsu, M.;Yamada,T.*Tetrahedron Lett.*, **1994**,*35*, 8181-8184.
9. The structure of compound **5** was confirmed by comparison with ^{1}H NMR and ^{13}C NMR data of isolated 8-benzyl-2-*tert*-butyl-2-*tert*-butylcarbonyloxy-6-(*p*-methoxyphenyl)-2,3-dihydroimidazo[1,2-*a*]pyrazin-3-one (**17**), which was synthesized by treatment of **4** with *n*-Bu_4NF and then trimethylacetic acid instead of methyl chloroformate. The important NMR chemical shift data of **5** and **17** (in THF-d_8 at -10 °C), **5**: δ_H 7.66 (H–5), 3.68 ($COCH_3$), 0.93 ($C(CH_3)_3$), δ_C 100.60 (C-2), 177.76 (C-3), 108.76 (C-5), 133.38 (C-6), 158.57 (C-8), 152.80 (C-9), 38.61 ($C(CH_3)_3$), 23.56 ($C(CH_3)_3$), 145.56 (OC(O)O), 55.62 (OCH_3), **17**: δ_H 7.64 (H–5), δ_C 98.66 (C-2), 177.85 (C-3), 108.87 (C-5), 133.19 (C-6), 158.57 (C-8), 152.59 (C-9), 38.69 ($C(CH_3)_3$), 27.10 ($C(CH_3)_3$), 176.79 (OC(O)), 39.38 ($C(O)C(CH_3)_3$), 23.66 ($C(O)C(CH_3)_3$). ^{13}C chemical shifts were assigned on the basis of ^{1}H-^{13}C COSY and COLOC analyses. The detail results of this structural study is submitting in *J. Chem. Soc., Perkin Trans. 2.*
10. Saito, I.; Nagata, R.; Yuba, K.; Matsuura, T. *Tetrahedron Lett.*, **1983**, *24*, 1737-1740.
11. The ^{1}H NMR chemical shift data (in THF-d_8 at 20 °C) : δ 3.81 (3H, s, $ArOCH_3$), 4.27 (2H, s, $PhCH_2$), 6.94 (2H, d, *J* = 9.0 Hz, ArH), 7.1 - 7.31 (3H, not assigned, ArH), 7.37 (2H, d, *J* = 10 Hz, ArH), 8.02 (2H, d, *J* = 9.0 Hz, ArH), 8.60 (1H, s, CH), and 9H of *t*-butyl group was not assigned. Authors acknowledge the referee's comment leading to following conclusion. The structure of **7** on the basis of ^{1}H NMR analysis is not 1,2-dioxetanone, but peroxy acid, because the 1,2-dioxetanone decomposes at -40 °C to -10 °C accompanied by a blue light and cannot detected above 0 °C according to Usami's report. Usami, K.; Isobe, M.*Tetrahedron Lett.*, **1995**,*36*, 8613-8616.
12. The ^{1}H NMR chemical shift data (in THF-d_8 at 20 °C) : δ 1.30 (9H, s, *t*-butyl), 3.52 (3H, s, $COOCH_3$), 3.82 (3H, s, $ArOCH_3$), 4.30 (2H, s, $PhCH_2$), 6.99 (2H, d, *J* = 8.5 Hz, ArH), 7.10 (1H, t, *J* = 8.0 Hz, ArH), 7.19 (2H, t, *J* = 8.0 Hz, ArH), 7.30 (2H, d, *J* = 8.0 Hz, ArH), 8.05 (2H, d, *J* = 8.5 Hz, ArH), and 8.64 (1H, s, CH).
13. Kishi, Y.; Tanino, H.; Goto, T. *Tetrahedron Lett.*, **1972**, 2747-2748.
14. The ^{1}H NMR chemical shift data (in THF-d_8 at 20 °C) : δ 1.42 (9H, s, $C(CH_3)_3$), 3.31 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.08 (2H, s, $PhCH_2$), 6.41 (1H, s, CH), 6.95 (2H, d, *J* 9.0, ArH), 7.12 (1H, t, *J* 7.3, ArH), 7.22 (2H, dd, *J* 7.3 and 7.9, ArH), 7.35 (2H, d, *J* 7.9, ArH) and 8.05 (2H, d, *J* 9.0, ArH); *m/z* (SIMS) 418 (M+1, 14%), 417 (10) and 386 (4).
15. McCapra, F.; Manning, M. J. *J. Chem. Soc., Chem. Commun.*, **1973**, 467-468.
16. The properties of the chemiluminescence in CH_2Cl_2, CH_3CN and CH_3CN with NaOH were shown in reference 8. The data in all the other conditions are unpublished.
17. Usami and Isobe reported that a neutral 1,2-dioxetanone generated luminescence with 400 nm peak. Usami, K.; Isobe, M. *Chem.Lett.*, **1996**, 215-216.

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