No Electron-Donating Substituent Effect on the Singlet Excited State Formation from the 5-(5-Aryl-2-pyrazinylamino)-1,2,4-trioxanes in Dimethyl Sulfoxide Triggered by Potassium *t*-Butoxide

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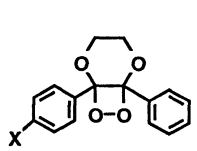
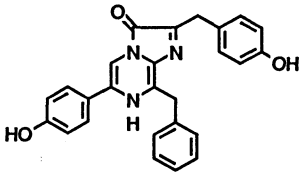
5-(5-Phenyl-2-pyrazinylamino)-1,2,4-trioxane derivatives having a substituent on the 4 position of the phenyl group gave chemiluminescence in dimethyl sulfoxide by addition of potassium *t*-butoxide. No electron-donating substituent effect was observed on the yield of singlet excited state formation. This may be explained by the negative charge formed on the nitrogen atom next to the transient dioxetane moiety, which may produce singlet excited-state molecule by the intramolecular CIEEL mechanism.

Simple, isolable dioxetanes such as tetramethyl-1,2-dioxetane are relatively stable at room temperature and afford predominantly triplet excited-state molecules on thermolysis,[1] whereas singlet excited products were efficiently produced in the case of bioluminescent systems such as firefly and *Cypridina*[2] (Scheme 1). In the latter cases, the transient 1,2-dioxetane intermediate produced must be extremely unstable[3] so as in the case of efficiently chemiluminescent compounds. The high efficiency of singlet excited state production and the instability of the intermediate dioxetanes in bioluminescence and efficient chemiluminescence may come from the conjugation of an electron-donating (easily oxidizable) and highly fluorescent chromophore with the excited-state carbonyl group to be formed.[4]

Koo et al.[5] explained these effects in terms of the chemically initiated intramolecular electron-exchange luminescence (intramolecular CIEEL) mechanism. In the firefly case, for example (Scheme 2), electron transfer from the easily oxidizable phenoxide anion to the highly energized dioxetanone moiety to cause a heterolytic O-O bond fission followed by rapid decarboxylation with electron exchange generates an excited state product (emitter). Thus, the phenoxide anion formation would accelerate strongly the dioxetane decomposition[6] and singlet state formation.

This is further supported by the observation of Schaap and Gagnon[7] in chemiluminescence of some dioxetanes. Dioxetane **7b** having a *p*-hydroxyphenyl substituent is fairly stable at room temperature in *o*-xylene (tau 1/2 at 25 °C, 57 h), but addition of *t*-BuOK/18-crown-6 dramatically accelerates the rate of decomposition (4.4×10^6 times) of **7b** with produc-


(7a) X=H
(7b) X=OH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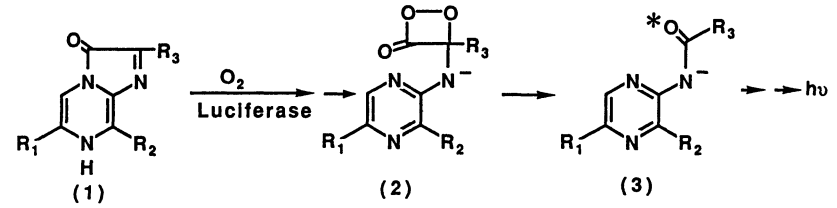
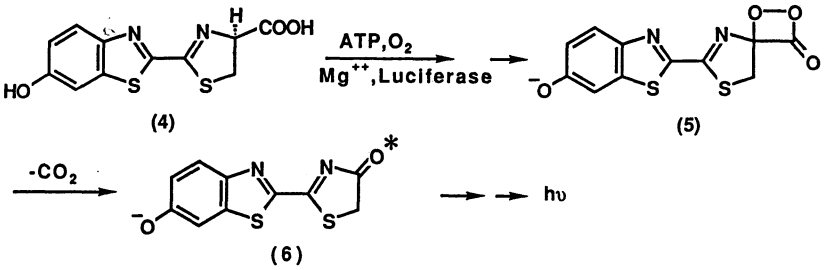
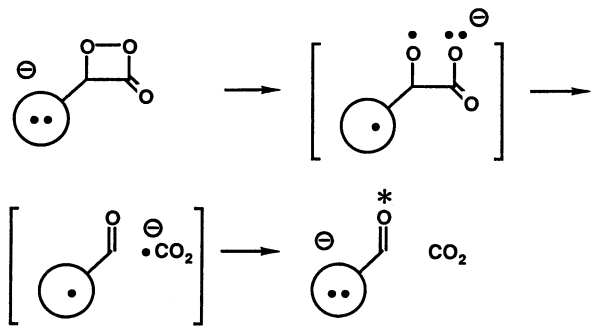




Scheme 1. Mechanism of *Cypridina* and firefly bioluminescence.



Scheme 2. Schematic presentation of intramolecular CIEEL mechanism.

tion of singlet excited molecules ca. 160 times more than that formed from the neutral molecules. An amino group also has a strongly electron-donating ability and hence is expected to have the effect similar to $-O^-$.[4,8-10]

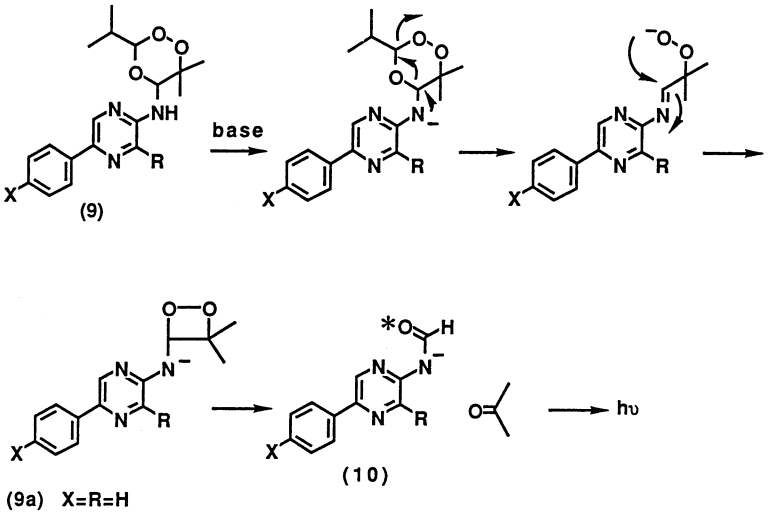
Coelenterazine (*Oplophorus* luciferin) (**8**)[2] which is a luminescent chromophore of a photoprotein, aequorin, has a *p*-hydroxyphenyl substituent on the 3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one chromophore, which is transformed to a dioxetane intermediate during bioluminescent oxidation (see Scheme 1). Shimomura et al.[11] previously suggested that the phenolic hydroxyl group must be dissociated in the photoprotein. It might indicate that the phenoxide anion, as in the firefly luciferin case, contributes to the quantum yield of singlet excited state formation, but very recently Shimomura et al.[12] also suggested that such an anion formation in aequorin would not be important. To see whether this dissociation of the phenolic hydroxyl group is necessary or not to produce a high-quantum-yield luminescence similar to the case of firefly bioluminescence, we have examined the trioxane **9e** in comparison with those having other substituents, since such the trioxanes **9** have been found to give strong chemiluminescence with base[13,14] and are regarded as a luminescent system similar to coelenterazine (**8**) and *Cypridina* luciferin (**1**) (Scheme 3).[15]

As shown in Table 1, the *p*-hydroxyphenyl-substituted trioxane **9e** gave a slightly higher chemiluminescence quantum yield than the phenyl or methoxyphenyl trioxane, **9a** or **9c**, but quantum yield of singlet excited-state molecules was rather decreased since the fluorescence quantum yield of the product **10e** was better than that of others. In this case the phenolic hydroxyl group was dissociated to form phenoxide anion, which has almost no effect on the formation of the singlet excited molecule. 4-Dimethylaminophenyl and 3-indolyl derivatives, **9f** and **9g**, behave

Table 1. Chemiluminescence Properties of the Trioxanes in DMSO Containing *t*-BuOK

Compound	$\phi_{CL}\times10^2$	$\phi_F\times10^2$	$\phi_S^*\times10^2$	λ_{max}/nm	Reference
9a	0.10	15	0.67	450	16
9b	0.049	8.1	0.60	465	16
9c	0.14	21	0.67	454	16
9d	0.13	20	0.65	454	16
9e	0.21	63	0.33	545	This work
9f	0.13	50	0.26	471	This work
9g	0.22	52	0.42	545	16

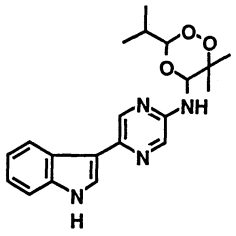
ϕ_{CL}=quantum efficiency of chemiluminescence. ϕ_F=quantum efficiency of fluorescence of the corresponding amide anion **10**. ϕ_S^*=quantum efficiency of excited singlet state formation calculated from an equation $\phi_{CL}=\phi_S^*\times\phi_F$.



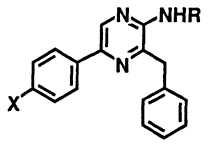
(9a) X=R=H
(9b) X=Br R=H
(9c) X=CH_3O R=H
(9d) X=CH_3O R=benzyl
(9e) X=OH R=benzyl
(9f) X=NMe_2 R=benzyl

Scheme 3. Chemiluminescence reaction of the 5-amino-1,2,4-trioxanes.

similarly. The difference in the behavior between the Schaap's compound **7** and the present case may be explained as follows. In the present case, cyclization of the intermediate hydroperoxide anion forms the dioxetane having a negative charge on the nitrogen atom next to the dioxetane moiety. The negative charge donates enough electrons to the dioxetane moiety to produce singlet excited state molecule **10** so



(9g)



(10e) R=CHO X=OH
(10f) R=CHO X=NMe_2
(11e) R=H X=OH
(11f) R=H X=NMe_2

that no other electron-donating moiety would be necessary. On the other hand, firefly luciferin (**4**) and the Schaap's compound **7a** without a phenoxide anion on the molecule have insufficient electrton-donating ability to the intermediate dioxetane moiety so that only triplet state produces predominantly. Thus, ionization of the phenolic hydroxyl group in the firefly luciferin (**4**) and **7b** dramatically changes the yield of singlet excited state molecules, whereas no such a change is observed in the present case. This explanation may also be extended to the luminescence of *Cypridina* luciferin (**1**), since the initially produced excited molecule was proven to be anion of the oxyluciferin (**3**).[16]

Experimental

All melting points were measured on a Mitamura Riken mp aparatus and uncorrected. ^{1}H NMR spectra were recorded on a JNM-FX200 spectrometer. Chemical shifts are given in ppm from internal TMS and coupling constants in Hz. IR spectra were taken on a JASCO IR-700 infrared spectrometer. UV spectra were obtained on a Hitachi 228 spectrometer. Mass spectra were measured on a JEOL JMS-DX300 instrument. Chemiluminescence and fluorescence spectra were recorded on an Otsuka Electronics MCPD-110A and JASCO FP-770 spectrometers, respectively. Dimethyl sulfoxide was distilled from calcium hydride under reduced pressure. The other solvents were of reagent grade.

5-(4-Dimethylaminophenyl)-3-benzyl-2-aminopyrazine (11f): To a solution of *p*-dimethylaminophenylglyoxal oxime[17] (3.0 g) and 2-amino-3-phenylpropionitrile (3.0 g) in pyridine (50 ml) was added $TiCl_4$ (2.0 ml) dropwise under argon at 0 °C. After being allowed to stand for 10 min, the mixture was heated at 50 °C for 30 min. To this reaction mixture was added slowly 10% aq $NaHCO_3$ at 0 °C to neutralize it and the resulting mixture was filtered through Celite bed. The filtrate was diluted with water and extracted three times with ethyl acetate. The organic layer was washed with water and sat. NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was crystallized from ethyl acetate to give the *N*-oxide of **11f** (2.8 g). The *N*-oxide was dissolved in MeOH (100 ml) and CH_2Cl_2 (20 ml) and reduced with Raney Ni (W2) at 50 °C under hydrogen atmosphere for 5 h with stirring, and the mixture was filtered. The filtrate was concentrated to a small volume and diluted with diethyl ether to give the aminopyrazine **11f** (2.5 g, 52%); slightly brownish needles (from methanol), mp 175—176 °C; IR (KBr) cm^{-1} 3478, 3294, 3134, 1610, 1463; UV (MeOH) λ_{max}/nm (ε) 304 (24800), 366 (10500); MS *m/z* 304 (M^+); ^{1}H NMR ($CDCl_3$) δ (*J*) 3.00 (6H, s), 4.30 (2H, s), 4.40 (2H, br. s), 6.80 (2H, d, 9.0), 7.30 (5H, s), 7.84 (2H, d, 9.0), 8.30 (1H, s). Calcd for $C_{19}H_{20}N_4$: C, 74.97; H, 6.62; N, 18.41%. Found: C, 75.00; H, 6.70; N, 18.63%.

5-[5-(4-Dimethylaminophenyl)-3-benzyl-2-pyrazinylamino]-3-isopropyl-6,6-dimethyl-1,2,4-trioxane (9f): A solution of the aminopyrazine **11f** (400 mg) and isobutyraldehyde (5.0 ml) in hexane (10 ml) was heated at 90 °C for 3 h and then stirred at 20 °C for 3 days with a $CaCl_2$ tube. The mixture was poured in 5% aq $NaHCO_3$ and extracted twice with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column with ethyl acetate-hexane (1 : 20) to give a mixture of two diastereomers of **9f** (350 mg, 58%), which was crystallized from hexane. Recrystallizations of the mixture from hexane gave a main isomer of **9f** as slightly brownish needles, mp 129—130 °C; IR (KBr) cm^{-1} 1611, 1499; UV (MeOH) λ_{max}/nm (ε) 306 (23900), 362 (12700); MS *m/z* 462 (M^+); ^{1}H NMR ($CDCl_3$) δ (*J*) 0.80 (3H, s), 0.90 (3H, d, 7), 0.91 (3H, s, 7), 1.12 (3H, s) 1.79 (1H, m), 3.00 (6H, s), 4.13 (1H, d, 16), 4.30 (1H, d, 16), 4.42 (1H, br. d, 10), 5.12 (1H, d, 5.0), 5.58 (1H, d, 10), 6.84 (2H, br. d, 9.0), 7.25 (5H, m), 7.86 (2H, d, 9.0), 8.40 (1H, s). Calcd for $C_{27}N_{34}O_3N_4$: C, 70.10; H, 7.41; N, 12.11%. Found: C, 70.23; H, 7.46; N, 12.39%.

5-[5-(4-Hydroxyphenyl)-3-benzyl-2-pyrazinylamino]-3-isopropyl-6,6-dimethyl-1,2,4-trioxane (9e): The trioxane **9e** was prepared from the aminopyrazine **11e**[18] (170 mg), isobutyraldehyde (5.0 ml) and hexane (20 ml) according to the procedure described in the preparation of **9f**. After chromatography was obtained **9e** (180 mg, 70%) as a 4 : 1 mixture of diastereomers; slightly yellowish amorphous solid (from ether-hexane); mp 65—67 °C; IR (KBr) cm^{-1} 3430, 1498; UV (MeOH) λ_{max}/ nm (ε) 280 (22200), 346 (10500); MS *m/z* 435 (M^+). Calcd for $C_{25}N_{29}O_4N_3$: C, 68.94; N, 6.71; N, 9.65%. Found: C, 68.89; N, 6.84; N, 9.58%. ^{1}H NMR ($CDCl_3$) δ (*J*): **major isomer** (20 °C) 0.80 (3H, s), 0.90 (6H, d, 6.8), 1.12 (3H, s), 1.75 (1H, m), 4.14 (1H, d, 16), 4.30 (1H, d, 16), 4.50 (1H, d, 10), 5.12 (1H, d, 4.5), 5.58 (1H, d, 10), 5.80 (1H, br. s), 6.90 (2H, d, 8.7), 7.20—7.40 (5H, m), 7.80 (2H, d, 8.7), 8.38 (1H, s); **minor isomer** (60 °C) : 0.77 (3H, d, 7), 0.78 (3H, d, 7), 0.90 (3H, s),1.60 (3H, s) 1.7 (1H, m), 4.14 (1H, d, 15), 4.29 (1H, d, 15), 4.40, (1H, d, 5.0) 5.42 (1H, d, 9.0), 5.58 (1H, d, 9.0), 6.90 (2H, d, 9.0), 7.4 (5H, m), 7.86 (2H, d, 9.0), 8.41 (1H, s).

5-(4-Dimethylaminophenyl)-3-benzyl-2-(formylamino)-pyrazine (10f): To a solution of the aminopyrazine **11f** (50 mg) in pyridine (0.8 ml) was added acetic formic anhydride (0.2 ml) dropwise at 0 °C. After being stirred further 2 h, the mixture was evaporated to dryness to give the amide **10f**

(45 mg) as yellow needles from methanol; mp 200—202 °C; IR (KBr) 1681 cm^{-1}; UV (MeOH) λ_{max}/nm (ε) 328 (996), 370 (11400); MS m/z 332 (M^+); ^1H NMR ($CDCl_3$-CD_3OD, 80 °C) δ (*J*) 3.00 (6H, s), 4.24 (2H, s), 6.80 (2H, d, 9.5), 7.25 (5H, s), 7.87 (2H, d, 9.5), 8.50 (1H, s), 9.04 (1H, br.s). Calcd for $C_{20}N_{20}ON_4$: C, 72.27; N, 6.07; N, 16.86%. Found: C, 72.23; N, 6.05; N, 17.03%.

5-(4-Hydroxyphenyl)-3-benzyl-2-formylaminopyrazine (10e): To a solution of the aminopyrazine **11e** (50 mg) in pyridine (0.8 ml) was added acetic formic anhydride (0.2 ml) at 0 °C. After being stirred further 2 h, the reaction mixture was evaporated to dryness and the residue purified by silica gel TLC to give **10e** (20 mg, 36%) as colorless needles from methanol; mp 208—210 °C; IR (KBr) cm^{-1} 3368, 1674; UV (MeOH) λ_{max}/nm (ε) 277 (18000), 294 (15800), 341 (15600); MS m/z 305 (M^+); ^1H NMR ($CDCl_3$-acetone-d_6) δ (*J*) 4.30 (2H, s), 6.96 (2H, d, 8.0), 7.28 (5H, s), 7.88 (2H, d, 8.0), 8.20 (1H, br.d, disappeared with D_2O), 8.30 (1H, s, diappeared with D_2O), 8.52 (1H, s), 9.3 (1H, br.s). Calcd for $C_{18}N_{15}O_2N_3$: C, 70.80; H, 4.95; N, 13.76%. Found: C, 70.83; H, 4.95; N, 13.95%.

Chemiluminescence and Fluorescence Measurements. To a solution of a trioxane (concn 1×10^{-5} mol l^{-1}) in dimethyl sulfoxide (2 ml) was added a solution of *t*-BuOK (0.25 mol l^{-1}) in *t*-BuOH (0.1 ml) at 25 °C. The resulting light emission was recorded with a luminometer. Quantum yields of chemiluminescence and fluorescence were determined as described previously.[4]

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