Synthesis and Chemiluminescence of 5-[(2-Pyridyl)-, (2-Pyrazinyl)-, and (Substituted 2-pyrazinyl)amino]-1,2,4-trioxanes

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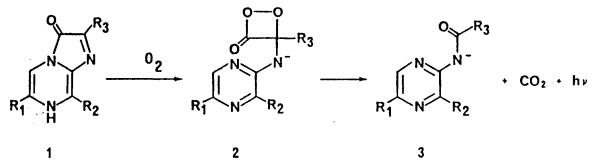
Synopsis. From pyridyl- and pyradinylamines and isobutylaldehyde were prepared the corresponding 5-arylamino-1,2,4-trioxanes, whose chemiluminescence was studied by comparing with those of *Cypridina* luciferin analogs.

Cypridina luciferin (**1a**) is one of the most efficient chemiluminescent substances in addition to its bioluminescent activity.[1] Accumulated results of studies on both of the bio- and chemiluminescence suggest the light-producing mechanism (Scheme 1) involving an intermediate, 1,2-dioxetane **2a**, which decomposes rapidly to yield predominantly a singlet excited state molecule. The efficient chemiluminescence and instability of the dioxetane **2a** would be explained in terms of conjugation of the electron-donating and highly fluorescent chromophore with the dioxetane ring. Akutagawa et al. treated 9-aminoanthracene **4a** with isobutyraldehyde in the presence of atmospheric oxygen and obtained a stable peroxide,[2] the 5-(9-anthrylamino)-1,2,4-trioxane **6a**[2-8] that caused efficient chemiluminescence when it was treated with a base in a polar aprotic solvent. Aminodioxetane **7a** is assumed to be an intermediate in the light producing process.[4,8] If the anthracene moiety is replaced with a pyrazine derivative, the expected aminodioxetane intermediate would have the structural similarity to the aminodioxetane **2a** in the *Cypridina* bioluminescence. In this paper, we describe the synthesis and chemiluminescence of the trioxanes (**6b—i**) which have the chromophore similar to *Cypridina* luciferin and *Watasenia* preluciferin (coelenterazine).[1]

Arylamines (**4b—i**) were oxidized with atmospheric oxygen in the presence of a large excess of isobutyraldehyde to afford the corresponding trioxanes (**6a—i**) in 6.5—50% yields (Table 1). The trioxanes may be formed via a Shiff-base as shown in Scheme 2. These compounds were fairly stable and showed a molecular ion peak in the EI mass spectra except **6b** and **6c**. The



1 2 3

a: R_1= 3-indolyl, R_2= $CH_2CH_2CH_2$-NHC(=NH)NH_2, R_3= $CH(CH_3)CH_2CH_3$
b: R_1= phenyl, R_2=H, R_3= CH_3
c: R_1= 3-indolyl, R_2= H, R_3= CH_3

Scheme 1.

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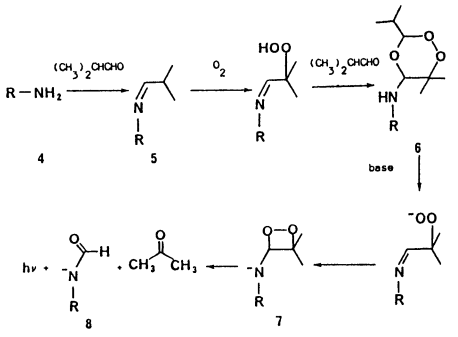
^{1}H NMR spectrum of **6b** showed signals assignable to an isobutyl group at δ 0.98 (6H), 1.84 (1H), and 4.80(1H), and to a methine proton at δ 5.28 (1H), which was coupled with an NH proton signal at δ 4.82. In the ^{13}C NMR spectrum of **6b**, signals for an acetal methine carbon, a methine carbon bearing oxygen and nitrogen atoms, and a quaternary carbon attached to an oxygen atom were observed at δ 107.1, 84.0, and 79.9, respectively. The ^{1}H and ^{13}C NMR spectra of the trioxanes (**6c—g** and **6i**) resemble very closely those of **6b**. In the ^{1}H NMR spectra of **6a** and **6h**

Table 1. Synthetic Yield and Chemiluminescence Properties of the Trioxanes and Luciferin Analogs in Dimethyl Sulfoxide Containing *t*-BuOK

Compound	Yield/%	Φ_{CL}[a]$\times 10^2$	Φ_F[b]$\times 10^2$	Φ_S[c]$\times 10^2$
6a	12	4.6	37	12
6b	18	0.0086	2.7	0.32
6c	29	—	—	—
6d	19	0.045	8.4	0.54
6e	6.5	0.10	15	0.67
6f	26	0.049	8.1	0.60
6g	50	0.14	21	0.67
6h	45	0.13	20	0.65
6i	47	0.22	52	0.42
1b	—	0.12	11	1.1
1c	—	0.024	43	0.056

a) Quantum efficiency of chemiluminescence. b) Quantum efficiency of fluorescence of the corresponding amide (**8**). c) Quantum efficiency of excited singlet state formation.



4 5 6 7 8

a: R = 9-anthryl
b: R = 2-pyridyl
c: R = 2-pyrazinyl
d: R = 5-methyl-2-pyrazinyl
e: R = 5-phenyl-2-pyrazinyl
f: R = 5-(p-bromophenyl)-2-pyrazinyl
g: R = 5-(p-methoxyphenyl)-2-pyrazinyl
h: R = 3-benzyl-5-(p-methoxyphenyl)-2-pyrazinyl
i: R = 5-(3-indolyl)-2-pyrazinyl

Scheme 2.

having substituents at ortho position to the amino group, an anisotropic effect on a methyl signal was observed.[4]

The trioxanes (**6b, d—i**) emitted light under basic conditions in dimethyl sulfoxide as well as **6a** (Table 1), but efficiencies of chemiluminescence and singlet excited state formation were much lower than those of **6a**. The luciferin analog **lb** also produced light under the same conditions and the efficiency of singlet excited state formation was about two times larger than those of the analogous trioxane **6e**, suggesting a slight difference between the dioxetanone and the dioxetane moiety for light production. On the other hand, the luciferin analog **1c** having an indole side chain showed only very weak chemiluminescence under the condition while it gave light efficiently in diethylene glycol dimethyl ether (diglyme) containing a trace of acetate buffer (pH 5.6);[1] the efficiency of singlet excited state formation being 0.16. Efficiency of singlet excited state formation of the analogous trioxane **6i** was about 8 times larger than that of **1c** under the basic condition, indicating the presence of some side reactions or quenching processes in the chemiluminescent reaction of **1c**.

Experimental

All melting points were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-MH100 (100 MHz) and a JNM-FX100 (25 MHz) spectrometer, respectively. Chemical shifts (δ) are given in ppm from internal TMS and coupling constants (J) in Hz. IR spectra were taken on a JASCO IR-G spectrometer. UV spectra were obtained on a HITACHI EPS-3T spectrometer. Mass spectra were measured on a JEOL JMS-D100 and a JMS-0lSG-2 instrument. Fluorescence spectra were recorded on a Hitachi MPF-3 spectrofluorometer. Dimethyl sulfoxide and diglyme were distilled from KOH and sodium metal, respectively, under reduced pressure. The other solvents were of reagent grade (Wako Chemical Co., Ltd.). 2-Aminopyrazines (**4d—i**) were prepared by the methods described in our previous reports.[9]

Preparation of 5-(9-Anthrylamino)-3-isopropyl-6,6-dimethyl-1,2,4-trioxane (6a). This compound was prepared according to the method of Akutagawa et al.[2] Pale yellow prisms from CH_2Cl_2-hexane, mp 122—124 °C (lit,[2] 113 °C).

Preparation of the Trioxanes (6b—h). A solution of arylamine (**4b—h**) (2 mmol) and isobutyraldehyde (60 mmol) in diethyl ether (20 ml) was kept in a dark with stirring under atmospheric oxygen at room temperature. After about 10 days, the solution was diluted with diethyl ether, washed with aq. $NaHCO_3$ and saturated aq. NaCl, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on TLC plates to give the corresponding trioxane (**6**).

6b: Needles (from hexane), mp 85—86 °C; ^{1}H NMR ($CDCl_3$) δ=0.98 (6H, d, J=6.5, 10 and 11-Me), 1.20 and 1.52 (each 3H, s, 7 and 8-Me), 1.84 (1H, m, 9-H), 4.82 (1H, d, J=11, NH), 5.16 (1H, d, J=5.2, 3-H), 5.28 (1H, d, J=11, 5-H), 6.60 (1H, d, J=8, 3-H), 6.72 (1H, dd, J=8 and 4), 7.48 (1H, td, J=8 and 1), 8.18 (1H, br d, J=4); ^{13}C NMR ($CDCl_3$) δ= 16.6 and 16.8 (each q, 10 and 11-Me); 17.5 and 21.7 (each q, 7 and 8-Me); 31.0 (d, C-9), 79.9 (s, C-6), 84.0 (d, C-5), 107.1 (d, C-3), 107.9 (d), 115.2 (d), 137.8 (d), 148.0 (d), 156.8 (s). Found: C, 61.65; H, 7.86; N, 10.96%. Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88: H, 7.86; N, 10.96%.

6c: Colorless oil; ^{1}H NMR ($CDCl_3$) δ= 0.99 (6H, d, J=6.9), 1.20 (3H, s), 1.52 (3H, s), 1.83 (1H, m), 5.10 (1H, d, J=5.5), 5.17 (1H, d, J=10, NH), 5.42 (1H, d, J=10), 7.8—8.4 (3H, m); ^{13}C NMR ($CDCl_3$) δ=16.6 (q), 16.8 (q), 17.4 (q), 21.6 (q), 31.0 (d), 79.8 (s), 83.0 (d), 107.2 (d), 132.5 (d), 135.2 (d), 141.8 (d), 153.1 (s).

6d: Needles (from hexane), mp 104—105 °C; ^{1}H NMR ($CDCl_3$) δ= 0.95 (6H, d, J=6.8), 1.19 (3H, s), 1.52 (3H, s), 1.85 (1H, m), 2.43 (3H, s), 4.88 (1H, d, J=10, NH), 5.17 (1H, d, J=4.9), 5.38 (1H, d, J=10), 7.98 (2H, s); ^{13}C NMR ($CDCl_3$) δ=16.7 (q), 16.8 (q), 17.5 (q), 21.7 (q), 20.2 (q, ArMe), 31.0 (d), 79.9 (s), 83.4 (d), 107.3 (d), 131.2 (d), 141.0 (d), 143.8 (s), 151.0 (s). Found: C, 58.20; H, 7.81; N, 16.00%. Calcd for $C_{13}H_{21}N_3O_3$: C, 58.41; H, 7.92; N, 15.72%.

6e: Amorphous solid (from hexane), mp 100—110 °C; MS m/z 329 (M^+); ^{1}H NMR ($CDCl_3$) δ=0.98 (6H, d, J=6.4), 1.22 (3H, s), 1.56 (3H, s), 1.85 (1H, m), 4.90 (1H, d, J=10, NH), 5.18 (1H, d, J= 4.5), 5.42 (1H, d, J=12), 7.3—8.5 (7H, m); ^{13}C NMR ($CDCl_3$) δ= 16.7 (q), 16.8 (q), 17.5 (q), 21.7 (q), 31.0 (d), 79.8 (s), 83.2 (d), 107.3 (d), 125.8 (d), 128.4 (d), 128.8 (d), 131.4 (d), 136.7 (s), 139.1 (d), 144.2 (s), 151.6 (s). Found: C, 65.33; H, 7.03; N, 12.57%. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76%.

6f: Pale yellow amorphous solid (from hexane), mp 157—159 °C; MS m/z 407, 409 (M^+); ^{1}H NMR ($CDCl_3$) δ= 0.98 (6H, d, J=6.8), 1.23 (s), 1.56 (s), 1.88 (1H, m), 5.14 (1H, d, J=11, NH), 5.20 (1H, d, J=5.0), 5.48 (1H, d, J=11), 7.66 (4H, AB q), 8.12 (1H, d, J=1), 8.50 (1H, d, J=1); ^{13}C NMR ($CDCl_3$) δ=16.7 (q), 16.8 (q), 17.5 (q), 21.7 (q), 31.1 (d), 79.7 (s), 83.2 (d), 107.4 (d), 122.7 (s), 127.2 (d), 131.6 (d), 132.0 (d), 135.7 (s), 138.7 (d), 142.9 (s), 151.9 (s). Found: C, 51.87; H, 5.31; N, 10.04%. Calcd for $C_{18}H_{22}N_3O_3Br \cdot 1/2H_2O$: C, 51.81; H, 5.55; N, 10.06%.

6g: Amorphous solid (from hexane), mp 129.5—130.5 °C; MS m/z 359 (M^+); ^{1}H NMR ($CDCl_3$) δ= 0.96 (6H, d, J=6.8), 1.19 (3H, s), 1.53 (3H, s), 1.85 (1H, m), 3.80 (3H, s, OMe), 5.19 (1H, d, J=11, NH), 5.21 (1H, d, J=5.0), 5.47 (1H, d, J=11), 6.99 (2H, d, J=9), 7.87 (2H, d, J=9), 8.13 (1H, d, J=1), 8.51 (1H, d, J=1); ^{13}C NMR ($CDCl_3$) δ= 16.7 (q), 16.9 (q), 17.5 (s), 21.7 (s), 31.0 (d), 55.2 (q, OMe), 79.9 (s), 83.4 (d), 107.3 (d), 114.3 (d), 127.0 (d), 129.5 (s), 131.3 (d), 138.3 (d), 144.0 (s), 151.3 (s), 160.0 (s). Found: C, 63.44; H, 6.90; N, 11.76%. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69%.

6h: Plates (from hexane), mp 75.5—82 °C; MS m/z 449 (M^+); ^{1}H NMR ($CDCl_3$) δ=0.84 (6H, d, J=5.6), 1.13 (3H, s), 1.79 (3H, s), 1.79 (1H, m), 3.86 (3H, s, OMe), 4.24 (2H, AB q), 4.49 (1H, d, J=11, NH), 5.12 (1H, d, J=5.5), 5.59 (1H, d, J=11), 7.00 (2H, d, J=9), 7.2—7.4 (5H, m), 7.92 (2H, d, J=9), 8.42 (1H, br s); ^{13}C NMR ($CDCl_3$) δ=16.4 (q), 16.9 (q), 17.3 (q), 21.7 (q), 31.1 (d), 41.5 (t), 55.3 (q, OMe), 79.9 (s), 82.0 (d), 107.1 (d), 114.2, 127.1, 127.3, 128.4, 129.1, 129.4, 136.5, 136.6, 140.8, 142.6, 149.3, 159.8. Found: C, 69.23; H, 6.87; N, 9.24%. Calcd for $C_{26}H_{31}N_3O_4$: C, 69.46; H, 6.95; N, 9.35%.

Preparation of Trioxane 6i. A solution of aminopyrazine **4i** (200 mg, 0.91 mmol) and isobutyraldehyde (10.4 ml, 114 mmol) in diethyl ether (21 ml) was treated as described for the preparation of **6b**. After 5 days, pale yellow precipitates of **6i** were collected by filtration. The filtrate was treated as described in the case of **6b** to give additional amount of **6i**. **6i**: pale yellow amorphous solid, mp 204.5—205.5 °C; MS m/z 368 (M^+); ^{1}H NMR ($CDCl_3$) δ=0.98 (6H, d, J=6.8), 1.24 (3H, s), 1.57 (3H, s), 1.94 (1H, m), 4.91 (1H, d, J=12, NH), 5.20 (1H, d, J=5.0), 5.40 (1H, d, J=12), 7.71 (1H, d, J=2.5), 8.16 (1H, d, J=1), 8.40 (1H, br s, NH), 8.55 (1H, d, J=1), 7.1—8.2 (4H, m); ^{13}C NMR (DMSO-d_6) δ=16.5 (q), 16.6 (q), 17.7 (q), 21.4 (q), 30.5 (d), 80.1 (s), 82.8 (d), 106.5 (d), 111.6 (d), 112.8 (s), 119.5 (d), 120.9 (d), 121.4 (d), 123.5 (d), 124.9 (s), 131.9 (d), 136.7 (s), 137.5 (d), 141.3 (s), 150.6 (s). Found: C, 65.13; H, 6.54; N, 15.21%. Calcd for $C_{20}H_{24}N_4O_3$: C, 65.20; H, 6.57; N, 15.21%.

Preparation of the Formamides (8a, b, d—i). To a solution of each of the arylamines (**4**) (0.4 mmol) in pyridine (1 ml) was added acetic anhydride (0.1 ml) under cooling in an ice-water bath. The solution was warmed to room temperature and stirred for 2 h. The solvent was evaporated under reduced pressure to give the corresponding crude formamide (**8**), which was recrystallized from the solvent given.

8a: Pale yellow needles (from MeOH), mp 189—191 °C; MS *m/z* 221 (M^+). Found: C, 81.18; H, 5.21; N, 6.39%. Calcd for $C_{15}H_{11}NO$: C, 81.43; H, 5.01; N, 6.33%.

8b: Rods (from MeOH), mp 75—76 °C; MS *m/z* 122 (M^+). Found: C, 59.17; H, 4.95; N, 22.94%. Calcd for $C_6H_6N_2O$: C, 59.01; H, 4.95; N, 22.94%.

8d: Slightly yellowish plates (from benzene), mp 121—124 °C; MS *m/z* 137 (M^+). Found: C, 52.55; H, 5.17; N, 30.84%. Calcd for $C_6H_7N_3O$: C, 52.54; H, 5.15; N, 30.64%.

8e: Leaflets (from MeOH), mp 162—164 °C; MS *m/z* 199 (M^+). Found: C, 66.27; H, 4.70; N, 20.96%. Calcd for $C_{11}H_9N_3O$: C, 66.32; H, 4.55; N, 21.10%.

8f: Plates (from MeOH), mp 212—214 °C; MS *m/z* 277, 279 (M^+). Found: C, 47.49; H, 2.75; N, 15.11%. Calcd for $C_{11}H_8N_3OBr$: C, 47.50; H, 2.90; N, 15.11%.

8g: Plates (from MeOH), mp 205—206 °C; MS *m/z* 229 (M^+). Found: C, 62.60; H, 4.70; N, 18.43%. Calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33%.

8h: Pale yellow needles (from MeOH), mp 190.5—191 °C; MS *m/z* 319 (M^+). Found: C, 71.39; H, 5.30; N, 13.07%. Calcd for $C_{19}H_{17}N_3O_2$: C, 71.45; H, 5.37; N, 13.16%.

8i: Slightly yellowish amorphous solid (from MeOH), mp 262—263 °C; MS *m/z* 238 (M^+). Found: C, 65.53; H, 4.04; N, 23.75%. Calcd for $C_{13}H_{10}N_4O$: C, 65.53; H, 4.23; N, 23.52%.

Chemiluminescence and Fluorescence Measurements. A solution of dimethyl sulfoxide or diglyme (1 ml) containing 0.85 mol l^{-1} *t*-BuOK (*t*-BuOH solution, 5 μl) or acetate buffer pH 5.6 (50 μl) was added to a solution of a trioxane or a luciferin analog in dimethyl sulfoxide or diglyme (1 ml). The resulting light emission was recorded with a luminometer. Quantum yields of chemiluminescence and fluorescence were determined as previously described.[10]

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