Synthesis and Chemiluminescence of Coelenterazine (*Oplophorus* Luciferin) Analogues[1)]

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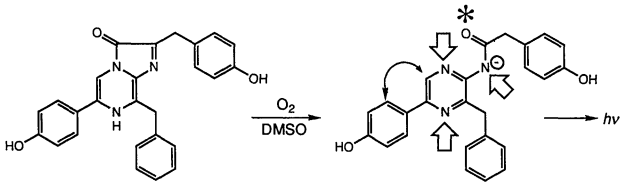
In the case of chemiluminescence of coelenterazine, effects of conformation rigidity of and hydrogen bonding with the emitter, coelenteramide, on the chemiluminescence efficiency have been examined with several coelenterazine analogues synthesized. Conformational rigidity has a light enhancing effect, whereas decreasing light yield was observed by hydrogen-bond formation with the emitter.

In general, bioluminescence efficiency is far better than chemiluminescence efficiency.[2)] For example, quantum yield of *Cypridina* bioluminescence (a luciferin-luciferase reaction) is about 28%,[3)] whereas at most only 3% has been obtained in the case of chemiluminescence of *Cypridina* luciferin under the bset chemiluminescence conditions known (diethylene glycol dimethyl ether containing a trace of acetate buffer, pH 5.6).[4)] Similar phenomena have been observed in the bioluminescence of aequorin (qauntum yield is ca. 23%),[5)] which is a photoprotein containing coelenterazine (*Oplophorus* luciferin) [2-(*p*-hydroxybenzyl)-6-(*p*-hydroxyphenyl)-8-benzyl-imidazo[1,2-*a*]pyrazin-3-(7*H*)-one] (**1**) as a light producing chromophore[5,6)] similar to *Cypridina* luciferin.

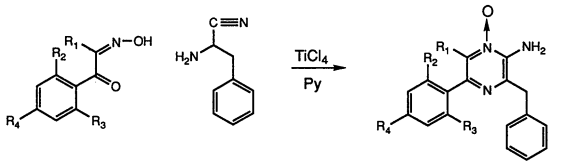
Cypridina luciferase and apo-aequorin (protein part of aequorin) are known to be a protein of high hydrophobicity,[7)] which may contribute to the high efficiency of bioluminescence.[7,8)] We assumed that the high light-emitting efficiency observed in the hydrophobic proteins may come from (1) a conformational rigidity of the emitter in the protein and/or (2) inhibition of hydrogen bonding between water and the emitter, *C.* oxyluciferin or coelenteramide [2-(*p*-hydroxyphenylacetylamino)-3-benzyl-5-(*p*-hydroxyphenyl)pyrazine] (**2**). To see whether these terms are effective or not, we have synthesized several analogues of coelenterazine (1) which have a bridge for fasten the dihedral angle between the phenyl and pyrazine rings (compds **4**—**6**)[9)] or which have a hydroxyl group that can make a hydrogen bonding to one of the nitrogen atoms in the emitter (compds **7**—**9**), and measured their chemiluminescence efficiency.



Synthesis of Coelenterazine Analogues (3—11) and Coelenteramide Analogues (12—19). The key intermediates, coelenteramine analogues (**27**, **32**—**39**), have been synthesized by application of the procedure of Kishi et al.[10)] Condensation of the coelenteramine analogues with 3-(*p*-acetoxyphenyl)-2-oxopropanal was carried out by a modified method of Inoue et al.[6)]

5-Methylcoelenterazine (**3**) was synthesized from 1-(*p*-methoxyphenyl)-1,2-propanedione 2-oxime (**20**). The oxime **20** was condensed in pyridine with 2-amino-3-phenylpropionitrile in the presence of $TiCl_4$ to give the 2-aminopyrazine 1-oxide **26**, which was reduced with Raney Ni to the *O*-methylcoelenteramine **32**. De-*O*-methylation of the coelenteramine **32** was carried out by heating with pyridine hydrochloride giving the coelenteramine **37**. Condensation of **37** with 3-(*p*-acetoxyphenyl)-2-oxopropanal under acidic conditions gave the corresponding coelenteramino acid **40**, which was characterized as its methyl ester. Cyclization of **40** was effected by treatment with dicyclohexylcarbodiimide (DCC) in pyridine at room temp giving 5-methylcoelenterazine (**3**).

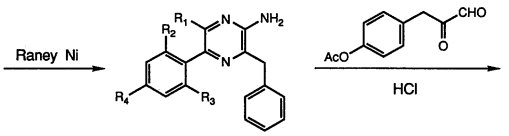
The bridged coelenterazines **4**—**6** having five-,[9)] six-,[9)] and seven-membered ring were synthesized similarly by starting from 5-methoxy-1,2-indanedione 2-oxime (**21**), 6-benzyloxy-3,4-dihydro-1,2-naphthalenedione 2-oxime (**22**), and 2-methoxy-8,9-dihydro-5*H*-benzocycloheptene-5,6(7*H*)-dione-6-oxime (**23**), respectively. Condensation of the five-membered ring derivative **38** with the 2-oxopropanal afforded the coelenterazine **4** directly, whereas in other cases were isolated the coelenteramino acid intermediates, which were then cyclized with DCC to the coelenterazine analogues. In the synthesis of the six-membered ring derivative **5**, the benzyl protecting group was removed at the final stage by reducing with Pd–C/H_2. 5-(2-Hydroxyethyl)coelenterazine (**7**) was also synthesized similarly by starting from 1-(*p*-isopropoxyphenyl)-4-(triphenylmethyloxy)-1,2-butanedione 2-oxime (**24**). Introduction of a hydroxyl group on a side chain of the *O*-benzylcoelenteramine (**46**) was effected by air oxidation in the presence of *t*-BuOK (giving the ketone **47**) followed by reduction with $NaBH_4$ to the desired alcohol **48**. The benzyl protecting group was removed by hydrogenolysis with Pd–C/H_2 to give the hydroxycoelenteramine **49**, which was converted to the



2-Aminopyrazine-1-oxides

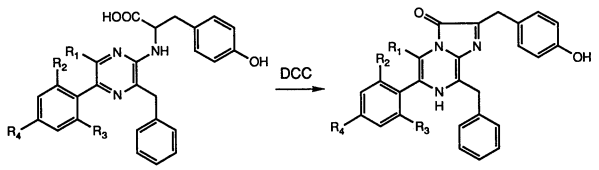
	R_1	R_2	R_3	R_4
(20)	CH_3	H	H	CH_3O
(21)	CH_2		H	CH_3O
(22)	CH_2CH_2		H	BnO
(23)	$CH_2CH_2CH_2$		H	CH_3O
(24)	CH_2CH_2OTr	H	H	$(CH_3)_2CHO$
(25)	CH_2CH_2		BnO	BnO
(26)	CH_3	H	H	CH_3O
(28)	CH_2CH_2		H	BnO
(29)	$CH_2CH_2CH_2$		H	CH_3O
(30)	CH_2CH_2OTr	H	H	$(CH_3)_2CHO$
(31)	CH_2CH_2		BnO	BnO

Bn=benzyl Tr=triphenylmethyl



Coelenteramines

	R_1	R_2	R_3	R_4
(27)	CH_2		H	CH_3O
(32)	CH_3	H	H	CH_3O
(33)	CH_2CH_2		H	BnO
(34)	$CH_2CH_2CH_2$		H	CH_3O
(35)	CH_2CH_2OH	H	H	OH
(36)	CH_2CH_2		BnO	BnO
(37)	CH_3	H	H	OH
(38)	CH_2		H	OH
(39)	$CH_2CH_2CH_2$		H	OH

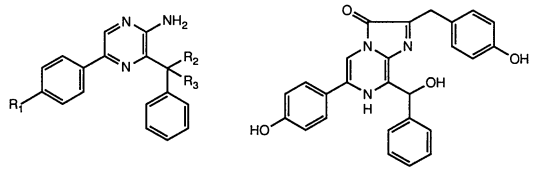


Coelenteramino acids

	R_1	R_2	R_3	R_4
(40)	CH_3	H	H	OH
(41)	CH_2CH_2		H	BnO
(42)	$CH_2CH_2CH_2$		H	OH
(43)	CH_2CH_2OH	H	H	OH

Coelenterazines

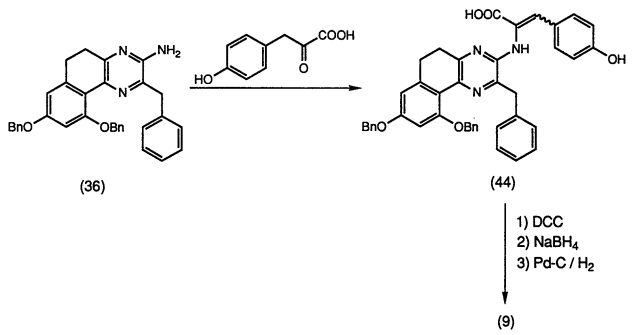
	R_1	R_2	R_3	R_4
(3)	CH_3	H	H	OH
(4)	CH_2		H	OH
(5)	CH_2CH_2		H	OH
(6)	$CH_2CH_2CH_2$		H	OH
(7)	CH_2CH_2OH	H	H	OH
(9)	CH_2CH_2		OH	OH
(10)	H	H	H	CH_3O
(11)	H	H	H	$(CH_3)_2N$



(8)

	R_1	R_2	R_3
(46)	BnO	H	H
(47)	BnO	O	
(48)	BnO	OH	H
(49)	OH	OH	H

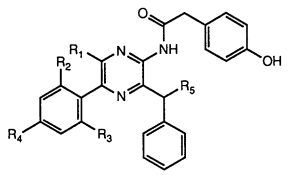
hydroxycoelenterazine **8** by condensation with 3-(*p*-acetoxyphenyl)-2-oxopropanal.



(36) (44)

Condensation of 6,8-bis(benzyloxy)-3,4-dihydro-1,2-naphthalenedione 2-oxime (**25**) with the aminonitrile afforded the pyrazine 1-oxide **31**, which was reduced as usual to the coelenteramine **36**. In this case, *p*-hydroxyphenylpyruvic acid was used for condensation with **36**, giving the unsaturated coelenteramino acid **44**. The acid **44** was cyclized with DCC to give the corresponding dehydrocoelenterazine,[11] which was reduced with $NaBH_4$ followed by deprotection with Pd–C/H_2 to the hydroxycoelenterazine **9**. The dimethylaminocoelenterazine (**11**) were synthesized similarly.

Coelenteramide analogues (**12—19**) are main prod-



Coelenteramides

	R_1	R_2	R_3	R_4	R_5
(12)	CH_3	H	H	OH	H
(13)	CH_2		H	OH	H
(14)	CH_2CH_2		H	OH	H
(15)	$CH_2CH_2CH_2$		H	OH	H
(16)	CH_2CH_2OH	H	H	OH	H
(17)	H	H	H	OH	OH
(18)	CH_2CH_2	H	OH	OH	H
(19)	H	H	H	$N(CH_3)_2$	H

ucts of the chemiluminescence of coelenterazine analogues (**3—9**, **11**) and considered to be the light emitter. They were synthesized from coelenteramine analogues (**33**, **35—39**, **45**, **49**) by treatment with *p*-acetoxyphenylacetyl chloride in pyridine followed by hydrolysis with aq NaOH.

Electronic Spectra of Coelenterazines[12] and Coelenteramines. Electronic spectra of coelenterazines are shown in Figs. 1 and 2. The dihedral angle between the imidazopyrazine ring and the *p*-hydroxyphenyl ring seems to influence the spectra as shown in Fig. 1. The spectra of the five-membered **4** and the hydroxy derivative **8** in Fig. 2 are similar in shape to that of

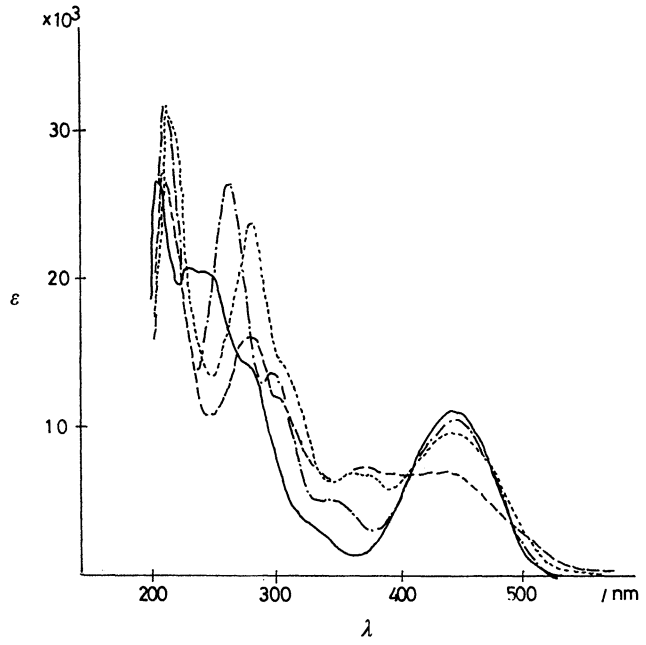


Fig. 1. Electronic spectra of coelenterazine analogues **3**—**6** in methanol. —— (**3**); ——— (**4**); ------ (**5**); ——— (**6**).

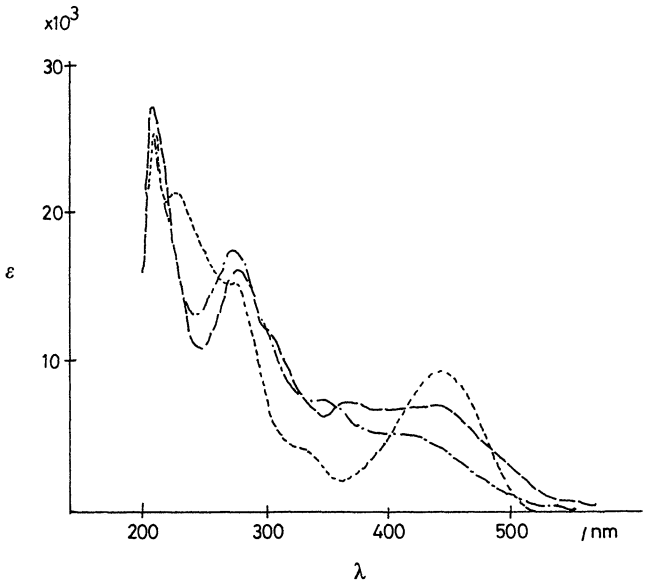


Fig. 2. Electronic spectra of coelenterazine analogues **4**, **7**, and **8** in methanol. ——— (**4**); ------ (**7**); ——— (**8**).

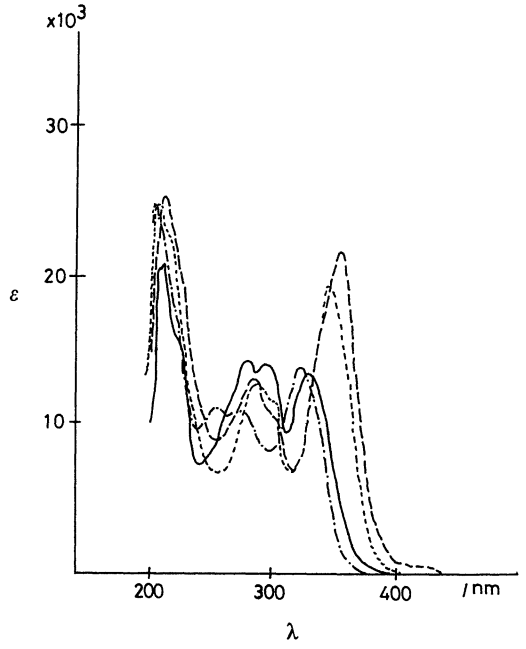


Fig. 3. Electronic spectra of coelenteramides **2** and **13**—**15** in methanol. —— (**2**); ——— (**13**); ------ (**14**); ——— (**15**).

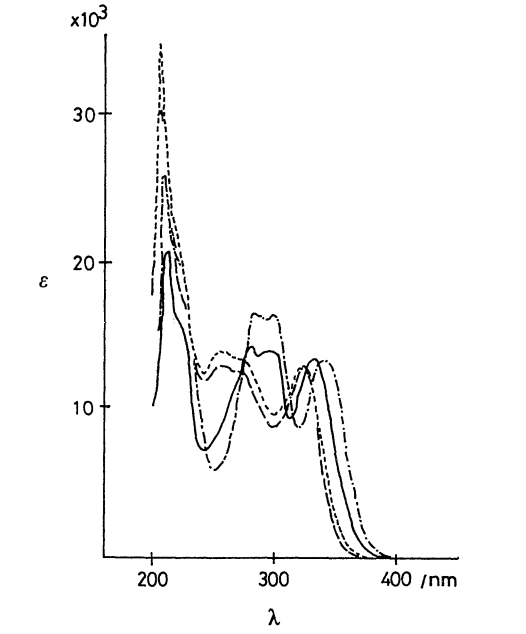


Fig. 4. Electronic spectra of coelenteramides **2**, **12**, **16**, and **17** in methanol. —— (**2**); ——— (**12**); ------ (**16**); ——— (**17**).

coelenterazine (**1**) in acidic methanol.[13] The hydroxy derivative **8** has a hydrogen bonding between the hydroxyl group and the nitrogen atom at 1-position, resulting the electronic spectrum similar to that of **1** in the acidic methanol. The reason why the electronic spectrum of the five-membered analogue **4** is similar to that of the hydroxy derivative **8** is not known.

The electronic spectra of coelenteramides (Figs. 3 and 4) are strongly influenced from the conformation of the chromophore; the larger the dihedral angle between the pyrazine and the *p*-hydroxyphenyl ring is, the shorter the wavelength of the absorption maximum seems to be. Thus, the coelenteramide analogue **13** having 5-membered ring absorbs at the longest wavelength, whereas coelenteramide (**2**) absorbs at similar wavelength with that of the 7-membered analogue **15**. The 6-methyl and 6-(2-hydroxyethyl)

derivatives, **12** and **16**, absorb at shorter wavelengths than that of coelenteramide (**2**), because of larger steric hindrance of the 6-substituents than hydrogen.

Since the coelenteramides are the emitter of chemiluminescence, change of the electronic spectra influences of the chemiluminescence spectra as expected (see Fig. 6).

Chemiluminescence of Coelenterazine in Organic Solvents. Cormier et al.[14] observed chemiluminescence of a coelenterazine analogue in *N,N*-dimethylformamide (DMF) in the presence of oxygen. The products are CO_2 and the corresponding coelenteramide analogue, which was obtained in 80% yield. We have measured chemiluminescence efficiency in several organic solvents as shown in Table 1, which indicates that dimethyl sulfoxide (DMSO) without additives is one of the best solvents for chemiluminescence of coelenterazine.

Figure 5 shows chemiluminescence spectra of coelenterazine (**1**) in a few organic solvents. Nearly identical spectra suggest that the emitters are same. The luminescence spectrum in DMSO, however, does not coincide with the fluorescence spectrum of coelenteramide (**2**) in neutral DMSO nor in DMSO containing *t*-BuOK. The spent solution of chemiluminescence of **1** gave its fluorescence similar to that of **2**, but different from chemiluminescence emission. These observations coincide with the observations by Cormier et al.,[14] who suggested that the light emitter is the monoanion having a minus charge on the amide moiety.

Effects of Conformational Rigidity on the Chemiluminescence Efficiency. Chemiluminescence efficiency of the coelenterazine analogues having a rigid dihedral angle between the pyrazine and the *p*-hydroxyphenyl ring was measured in DMSO[1,14,15] and the results are shown in Table 2.

Conformational rigidity of the *p*-hydroxyphenyl group in coelenteramide (**2**) has some enhancement effects on the light yield; the six-membered ring derivative **5** is superior to the five- and seven-

Table 1. Solvent Effects on the Relative Light Yield of Chemiluminescence of Coelenterazine (**1**)

Solvent	Additives	Rel. L. Y.
DMSO	None	1.0[a]
DMF	None	1.3
HMPA	None	2.4
DGM	None	0
DGM	1.0 mol dm^{-3} *t*-BuOK/*t*-BuOH 0.5 ml	0.06
DGM	0.1 mol dm^{-3} Acetate buffer pH 5.6 0.1 ml	0.006
DMSO	1.0 mol dm^{-3} *t*-BuOK/*t*-BuOH 0.5 ml	0.09
DMSO	0.1 mol dm^{-3} Acetate buffer pH 5.6 0.1 ml	0.9

DMSO: dimethyl sulfoxide; DMF: dimethylformamide; HMPA: hexamethylphosphoric triamide; DGM: diethyleneglycol dimethyl ether. Condition: concn, ca. 1×10^{-5} mol dm^{-3}; volume, 3 ml; temp, 25 °C.
a) Chemiluminescence quantum yield=0.21%.

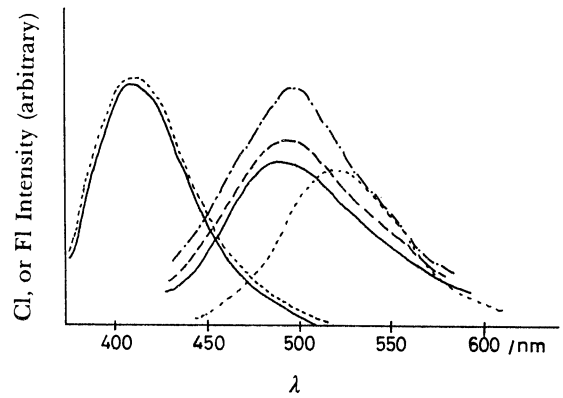
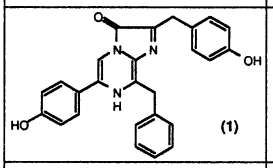
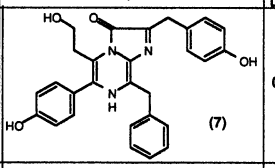
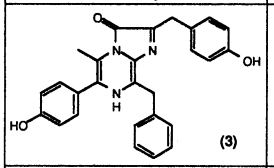
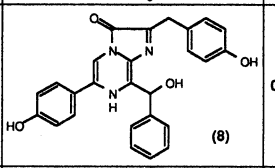
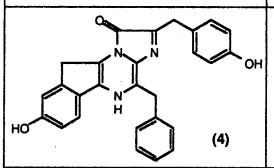
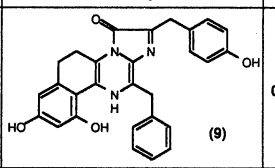
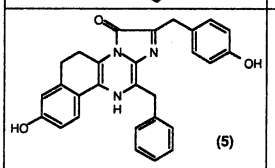
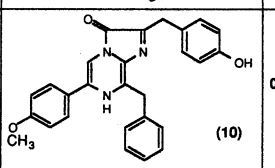
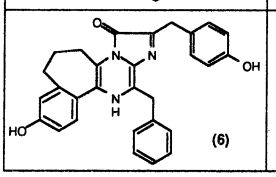
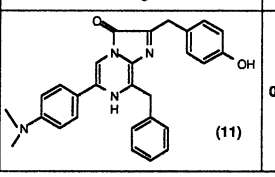


Fig. 5. Chemiluminescence spectra of coelenterazine (**1**) and fluorescence spectra of coelenteramide (**2**).
Chemiluminescence: —— (**1**) in DMSO; ——— (1) in DMF; --- (**1**) in HMPA.
Fluorescence: —— (right) (**2**) in DMSO; ----- (right) (**2**) in DMSO+*t*-BuOK; ----- (left) spent DMSO solution of chemiluminescence.

Table 2. Relative Light Yields of Chemiluminescence

Compound	Rel.a) L. Y.	Compound	Rel.a) L. Y.
 (1)	1.0	 (7)	0.46
 (3)	1.1	 (8)	0.35
 (4)	1.5	 (9)	0.01
 (5)	2.3	 (10)	0.92
 (6)	1.5	 (11)	0.33

a) Rel. L. Y.=relative light yield. Condition: solvent, DMSO; additive, none; concn, ca. 1×10^{-5} mol dm^{-3}; temp, 25 °C.

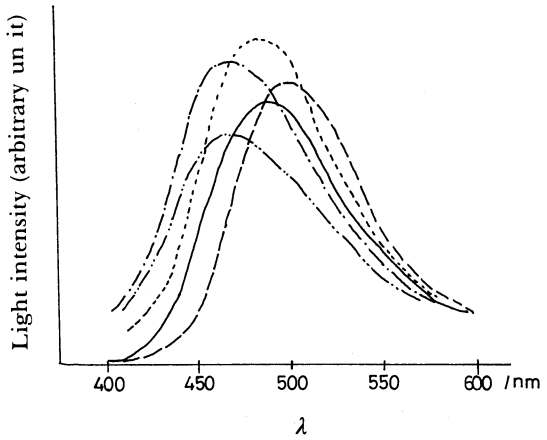
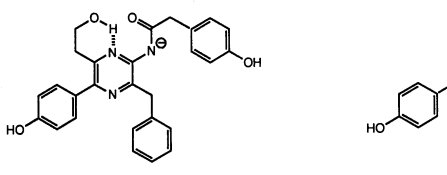


Fig. 6. Chemiluminescence spectra of coelenterazine (**1**) and its analogues **3**—**6** in DMSO. —— (**1**); ---- (**3**); ——— (**4**); ------ (**5**); ——— (**6**).

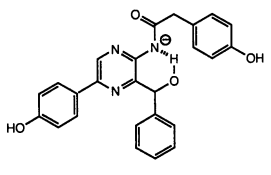
membered ring derivatives, **4** and **6**. Shimomura et al.[9] reported that bioluminescence efficiency of the ring derivative **5** is about one half of coelenterazine (**1**). It may suggest that in contrast with the chemiluminescence fitting of **5** with apoaequorin (a protein) may not be perfect so that the light yield of bioluminescence is decreased.

Fixation of the conformation of coelenterazine chromophore has some effect on the chemiluminescence spectra as shown in Fig. 6. The luminescence maximum of the six-membered ring compound **5** is almost identical with that of coelenterazine (**1**). Bathochromic shift is observed with the five-membered ring compound **4**, whereas the seven-membered ring (**6**) has an opposite effect. That a methyl substituent in **3** has larger hypsochromic effect than that of seven-membered ring in **6** may suggest that the dihedral angle between the pyrazine and *p*-hydroxyphenyl ring is larger in **3** than in **6** as discussed in the section of electronic spectra.

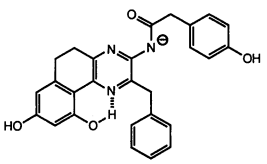
Effects of Hydrogen-Bonding in the Emitter on the Chemiluminescence Efficiency. An intramolecular hydrogen-bond formation on the excited state of coelenteramide (**2**, light emitter) at the nitrogen atom indicated with an arrow always decreases light emitting efficiency remarkably as expected from the hydrophobic nature of the luciferase and apoaequorin. Thus, the hydrogen-bonded emitters, **A**, **B**, and **C**, may be produced from the coelenterazines, **7**, **8**, and **9**, respectively. In the case of **9**, the strongly decreasing effect may come not only from the hydrogen-bonding, but also from conjugation of the phenolic hydroxyl group with the chromophore.



(A)



(B)



(C)

Effects of an Electron-Releasing Group on the Chemilimunescence Efficiency. In the case of the firefly bioluminescence, ionization of the phenolic hydroxyl group of firefly luciferin is important for high efficiency of light production as expected from the CIEEL (chemically initiated electron exchange luminescence) mechanism suggested by Schuster et al.[16] and by McCapra.[17] Thus, strongly electron-donating ability of phenoxide anion accelerates the rate of decomposition as well as the formation of a singlet excited state molecule.

As the phenolic hydroxyl group on the lower left side chain of coelenterazine (**1**) might have such a light-enhancing effect, we have synthesized the coelenterazine analogues, **10** and **11**, having *p*-methoxyl or *p*-dimethylamino substituent, which has no ionizable proton but electron-donating ability, in place of the hydroxyl group.[18] Chemiluminescence efficiency of these analogues, however, did not differ much from that of coelenterazine (Table 2). Different from the firefly luminescence case, the negative charge produced on the nitrogen during formation of the intermediate dioxetane donates enough electrons to the dioxetane moiety to produce singlet excited state molecule so that no other electron-donating moiety such as phenoxide ion would be necessary. A similar observation and some discussions on the mechanism have been reported in the previous paper.[19]

Experimental

All melting points were measured on a Mitamura Riken mp apparatus and uncorrected. ^{1}H NMR spectra were recorded on a JNM-FX200 spectrometer. Chemical shifts (δ) are given in ppm from internal TMS and coupling constants (J) 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 Otuka 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.

Synthesis of the 2-Aminopyrazine 1-Oxides. The 2-Aminopyrazine 1-Oxide 26: A solution of 1-(*p*-methoxyphenyl)-1,2-propanedione 2-oxime (**20**) (3.5 g) and 2-amino-3-phenylpropionitrile (3.2 g) in pyridine (60 ml) was deoxygenated by passing argon gas. To this solution was added $TiCl_4$ (2.4 ml) dropwise at −20 °C. After being stirred at

room temp for 15 min, the mixture was cooled to 0 °C and neutralized with 10% $NaHCO_3$. The mixture was filtered through Celite and the filtrate was extracted with ethyl acetate three times. The organic extracts were washed with water and sat. NaCl solution, dried over anhydrous Na_2SO_4, and evaporated to dryness. The product was crystallized from methanol and ether to give the *N*-oxide **26** (3.4 g) as white plate. The second crop (0.6 g) was obtained from the mother liquor by silica gel column chromatography. Total yield was 4.0 g (63%); mp 159—160 °C; MS *m/z* 321 (M^+); IR (KBr) 3390, 3262, 1613 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 347 (7400), 260(sh) (16800), 245 (19700); ^1H NMR ($CDCl_3$–CD_3OD) δ (*J*)=2.55 (3H, s), 3.87 (3H, s), 4.21 (2H, s), 7.01 (2H, d, 9.0), 7.2—7.4 (5H, m), 7.46 (2H, d, 9.0). Calcd for $C_{19}H_{19}O_2N_3$: C, 71.01; H, 5.96; N, 13.08%. Found: C, 71.00; H, 6.01; N, 13.18%.

The 2-Aminopyrazine 27: Slightly yellowish plates, mp 168—169 °C; MS *m/z* 303 (M^+); UV (MeOH) λ_{max} nm (ε) 379 (16700), 288 (18900); ^1H NMR ($CDCl_3$) δ (*J*)=3.78 (2H, s), 3.88 (3H, s), 4.22 (2H, s), 4.30 (2H, br. s), 6.98 (1H, dd, 2.5 & 8.5), 7.09 (1H, d, 2.5), 7.2 (5H, m), 7.85 (1H, d, 8.5). Calcd for $C_{19}H_{17}ON_3$: C, 75.22; H, 5.65; N, 13.85%. Found: C, 75.20; H, 5.68; N, 13.93%.

The 2-Aminopyrazine 1-Oxide 28: Slightly yellowish plates, mp 183—186 °C; MS *m/z* (409) (M^+); UV (MeOH) λ_{max} nm (ε) 372 (10400), 298 (23300), 284 (sh) (21100); ^1H NMR ($CDCl_3$) δ (*J*)=3.00 (2H, t, 7.5), 3.30 (2H, t, 7.5), 4.21(2H, s), 5.11 (2H, s), 5.29 (2H, br. S), 6.84 (1H, d, 2.5), 6.96 (1H, dd, 2.5 & 8.5), 7.2—7.5 (10H, m), 8.13 (1H, d, 8.5). Calcd for $C_{26}H_{23}O_2N_3$: C, 76,26; H, 5.66; N, 10.24%. Found: C, 76.17; H, 5.74; N, 10.42%.

The 2-Aminopyrazine 1-Oxide 29: White plates (MeOH), mp 186—188 °C; MS *m/z* 347 (M^+); IR (KBr) 3394, 3240, 1610 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 354 (9400), 280 (20900), 257 (21800), 223 (16800); ^1H NMR ($CDCl_3$) δ (*J*)=2.36 (2H, tt, 7.0 & 7.2), 2.58 (2H, t, 7.0), 3.03 (2H, t, 7.2), 3.86 (3H, s), 4.24 (2H, s), 5.36 (2H, br. s), 6.28 (1H, 2.5), 6.93 (1H, dd, 2.5 & 8.5), 7.20—7.40 (5H, m), 7.68 (1H, d, 8.5). Calcd for $C_{21}H_{21}O_2N_3$: C, 72.60; H, 6.09; N, 12.10%. Found: C, 72.62; H, 6.18; N, 12.19%.

The 2-Aminopyrazine 1-Oxide 30: White needles (MeOH), mp 90—91 °C; UV (MeOH) λ_{max} nm (ε) 348 (10400), 252 (25000); ^1H NMR ($CDCl_3$) δ (*J*)=1.40 (6H, d, 6.0), 3.30 (2H, t, 6.5), 3.54 (2H, t, 6.5), 4.20 (2H, s), 4.63 (1H, septet, 6.0), 5.24 (2H, br. s), 6.94 (2H, d, 8.5), 7.1—7.4 (20H, m), 7.50 (2H, d, 8.5). Calcd for $C_{41}H_{39}O_3N_3$: C, 79.20; H, 6.32; N, 6.76%. Found: C, 79.12; H, 6.74; N, 6.56%.

The 2-Aminopyrazine 1-Oxide 31: Slightly yellow needles (MeOH), mp 160—162 °C; MS *m/z* 515 (M^+); IR (KBr) 3458, 3320, 1604 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 367 (10200), 308 (17000), 289 (19400), 280 (19400); ^1H NMR ($CDCl_3$) δ (*J*)=2.90 (2H, t, 7.0), 3.24 (2H, t, 7.0), 4.20 (2H, s), 5.10 (2H, s), 5.14 (2H, s), 5.28 (2H, br. s), 6.54 (1H, d, 2.5), 6.65 (1H, d, 2.5), 7.1—7.6 (15H, m). Calcd for $C_{33}H_{29}O_3N_3$: C, 76.87; H, 5.67; N, 8.15%. Found: C, 76.86; H, 5.79; N, 8.38%.

Synthesis of the Coelenteramines. The Coelenteramine 32: Raney nickel (W2) was added to a solution of the *N*-oxide **26** (4.0 g) in dichloromethane (40 ml) and the mixture was stirred under hydrogen atmosphere for 96 h. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was crystallized from methanol to give white plates (3.5 g, 92%); mp 143—145 °C; MS *m/z* 305 (M^+); IR (KBr) 3430, 3332, 1618 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 337 (12800), 263 (19300); ^1H NMR ($CDCl_3$) δ (*J*)=2.44 (3H, s), 3.84 (3H, s), 4.14 (2H, s), 4.28 (2H, br. s), 6.99 (2H, d, 9.0), 7.2—7.4 (5H, m), 7.50 (2H, d, 9.0). Calcd for $C_{19}H_{19}ON_3$: C, 74.73; H, 6.27; N, 13.76%. Found: C, 74.76; H, 6.34; N, 13.86%.

The Coelenteramine 33: Slightly yellow rocks (MeOH), mp 138—139 °C; MS *m/z* 393 (M^+), UV λ_{max} nm (ε) 364 (16200), 291 (23500), 249 (7400); ^1H NMR ($CDCl_3$) δ (*J*)=2.96 (4H, br. s), 4.15 (2H, s), 4.32 (2H, br. s), 5.10 (2H, s), 6.84 (1H, d, 2.5), 6.94 (1H, dd, 2.5 & 8.5), 7.2—7.5 (10H, m), 8.10 (1H, d, 8.5). Calcd for $C_{26}H_{23}ON_3$: C, 79.36; H, 5.90; N, 10.68%. Found: C, 79.36; H, 5.99; N, 10.88%.

The Coelenteramine 34: Whites rocks (MeOH), mp 144—145 °C; MS *m/z* 331 (M^+); IR (KBr) 3170, 1611 cm^{-1}; UV λ_{max} nm (ε) 343 (12100), 274 (17800); ^1H NMR ($CDCl_3$) δ (*J*)=2.32 (2H, tt, 7.0 & 6.5), 2.58 (2H, t, 7.0), 2.60 (2H, t, 6.5), 3.86 (3H, s), 4.18 (2H, s), 4.30 (2H, br. s), 6.82 (1H, d, 2.5), 6.93 (1H, dd, 2.5 & 8.5), 7.2—7.4 (5H, m), 7.66 (1H, d, 8.5). Calcd for $C_{21}H_{21}ON_3$: C, 76.10; H, 6.39; N, 12.68%. Found: C, 76.12; H, 6.43; N, 12.79%.

The Coelenteramine 36: Slightly yellowish needles (MeOH–CH_2ClCH_2Cl), mp 149—152 °C; MS *m/z* 499 (M^+); IR (KBr) 3476, 3270, 3150, 1601 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 358 (15800), 300 (sh), (12500), 288 (18800); ^1H NMR ($CDCl_3$) δ (*J*)=2.86 (4H, s), 4.15 (2H, s), 4.25 (2H, br. s), 5.08 (2H, s), 5.14 (2H, s), 6.54 (1H, d, 2.5), 6.64 (1H, d, 2.5), 7.1—7.6 (15H, m). Calcd for $C_{33}H_{29}O_2N_3$: C, 79.33; H, 5.85; N, 8.41%. Found: C, 79.34; H, 5.91; N, 8.40%.

The Coelenteramine 35: The *N*-oxide **30** (420 mg) was reduced with Raney Ni to give a product (365 mg), which was dissolved in CH_2Cl_2 (11 ml) and treated with BCl_3 in CH_2Cl_2 (1M, 2.5 ml, M=mol dm^{-1}) at room temp under argon atmosphere for 10 min. Usual work-up gave a product, which was chromatographed on a silica-gel column to give **35** (196 mg, 85%). White needles (MeOH–CH_2Cl_2), mp 99—100 °C; MS *m/z* 332 ($M+H^+$); IR (KBr) 3374, 1613 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 336 (9500), 260 (14300); ^1H NMR ($CDCl_3$) δ (*J*)=2.96 (2H, t, 5.5), 3.94 (2H, t, 5.5), 4.16 (2H, s), 4.36 (2H, br. s), 6.86 (2H, d, 9.0), 7.2—7.3 (5H, m), 7.36 (2H, d, 9.0). Calcd for $C_{19}H_{19}O_2N_3$: C, 71.01; H, 5.96; N, 13.08%. Found: C, 70.99; H, 6.05; N, 13.16%.

Demethylation of *p*-Methoxyphenyl Group in the Coelenteramines. The Coelenteramine 37: A mixture of the 2-amino-5-(*p*-methoxyphenyl)pyrazine (**32**) (0.80 g) and pyridine hydrochloride (3.0 g) was heated at 210 °C for 30 min. After cooling, the mixture was dissolved in ethyl acetate, neutralized with 10% $NaHCO_3$, and extracted with ethyl acetate three times. The extracts were washed with sat. NaCl, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed on a silica-gel column (ethyl acetate–hexane) to give the aminopyrazine **37** (0.63 g, 83%) as white plates(CH_2Cl_2), mp 162—163 °C; MS *m/z* 291 (M^+); IR (KBr) 3424, 3300, 3190, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 338 (9700), 262 (14900); ^1H NMR ($CDCl_3$–CD_3OD) δ (*J*)=2.42 (3H, s), 4.12 (2H, s), 6.90 (2H, d, 9.0), 7.2—7.3 (5H, m), 7.39 (2H, d, 9.0). Calcd for $C_{18}H_{17}ON_3$: C, 74.20; H, 5.88; N, 14.42%. Found: C, 74.17; H, 5.88; N, 14.44%.

The Coelenteramine 38: Slightly yellowish needles, mp 232—234 °C; MS *m/z* 289 (M^+); UV (MeOH) λ_{max} nm (ε) 381 (15900), 288 (18500); ^1H NMR ($CDCl_3$–CD_3OD) δ (*J*)=3.72

(2H, s), 4.18 (2H, s), 6.91 (1H, dd, 2.5 & 8.5), 7.01 (1H, d, 2.5), 7.24 (5H, m), 7.89 (1H, d, 8.5). Calcd for $C_{18}H_{15}ON_3$: C, 74.72; H, 5.23; N, 14.53%. Found: C, 74.88; H, 5.26; N, 14.57%.

The Coelenteramine 39: White powder (ether), mp 167—168 °C, MS *m/z* 317 (M^+); IR (KBr) 3450, 3400, 3300, 3200, 1617 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 345 (18200), 273 (27200); ^1H NMR ($CDCl_3$–CD_3OD) δ (*J*)=2.20 (2H, tt, 6.5 & 7.0), 2.45 (2H, t, 6.5), 2.47 (2H, t, 7.0), 4.08 (2H, s), 6.66 (1H, d, 2.5), 6.76 (1H, dd, 2.5 & 8.3), 7.1—7.3 (5H, m), 7.40 (1H, d, 8.3). Calcd for $C_{20}H_{19}ON_3$: C, 75.68; H, 6.03; N, 13.24%. Found: C, 75.70; H, 6.11; N, 13.29%.

Synthesis of the Coelenteramino Acids. The Coelenteramine Acid 40(Characterized as its methyl ester): A mixture of the coelenteramine **37** (280 mg), 3-(*p*-acetoxyphenyl)-2-oxopropanal (280 mg), 10% HCl (1.0 ml), water (2 ml) and dioxane (4.0 ml) was heated at 100 °C for 13 h. The reaction mixture was extracted with ethyl acetate five times and the combined organic layers were washed with brine and concentrated. The residue was chromatographed on silica gel plates to give the coelenteramino acid **40** (287 mg, 66%).

The amino acid **40** was methylated with diazomethane in methanol to give **40** methyl ester as white rock from methanol, mp 192—195 °C; MS *m/z* 469 (M^+); IR (KBr) 3430, 3220, 1735 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 339 (10500), 268 (19400), 220 (16500); ^1H NMR ($CDCl_3$) δ (*J*)=2.35 (3H, s), 2.86 (1H, dd, 7.0 & 1.4), 3.00 (1H, dd, 5.0 & 14), 3.64 (3H, s), 4.02 (2H, br. s), 4.66 (1H, d, 7.0), 4.86 (1H, dt, 5.0 & 7.0), 6.55 (2H, d, 8.5), 6.66 (2H, d, 8.5), 6.70 (2H, d, 8.5), 7.0—7.25 (5H, m), 7.28 (2H, d, 8.5). Calcd for $C_{28}H_{27}O_4N_3$: C, 71.62; H, 5.80; N, 8.95%. Found: C, 71.74; H, 5.83; N, 9.03%.

The Coelenteramino Acid 41 Methyl Ester: Slightly yellow oil; MS *m/z* 571 (M^+); UV (MeOH) λ_{max} nm (ε) 368 (16100), 294 (23000), 252 (8000); IR (KBr) 3422, 1735, 1611; ^1H NMR ($CDCl_3$) δ (*J*)=2.9—3.1 (6H, m), 3.64 (3H, s), 4.06 (2H, s), 4.70 (1H, d, 7.5), 4.92 (1H, m), 5.10 (2H, s), 5.36 (1H, br. s), 6.62 (2H, d, 8.5), 6.76 (2H, d, 8.5), 6.82 (1H, d, 2.5), 6.92 (1H, dd, 2.5 & 8.5), 7.0—7.5 (10H, m), 8.05 (1H, d, 8.5). Calcd for $C_{36}H_{33}O_4N_3$: C, 75.63; H, 5.82; N, 7.35%. Found: C, 75.58; H, 5.99; N, 7.22%.

The Coelenteramino Acid 42: Slightly yellowish microcrystalline powder (ethyl acetate), mp 134—136 °C; MS (FAB) *m/z* 482 ($M+H^+$); IR (KBr) 3410, 1720 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 350 (13100), 282 (23900); ^1H NMR ($CDCl_3$) δ (*J*)=2.20 (2H, tt, 6.5 & 7.5), 2.46 (2H, t, 7.5), 2.50 (2H, t, 6.5), 2.92 (1H, dd, 7.0 & 14), 3.10 (1H, dd, 5.0 & 14), 4.06 (2H, s), 4.86 (1H, dd, 5.0 & 7.0), 6.58 (2H, d, 8.5), 6.70 (1H, d, 2.0), 6.78 (1H, dd, 2.0 & 8.0), 6.82 (2H, d, 8.5), 7.0—7.3 (5H, m), 7.38 (1H, d, 8.0). Calcd for $C_{29}H_{27}O_4N_3$: C, 72.23; H, 5.65; N, 8.73%. Found: C, 72.25; H, 5.67; N, 8.64%.

The Coelenteramino Acid 43: Slightly yellowish plate (AcOEt), mp 176—178 °C; MS (FAB) *m/z* ($M+H^+$); IR (KBr) 3414, 1708, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 342 (9690), 269 (18800); ^1H NMR ($CDCl_3$) δ (*J*)=2.89 (2H, t, 7.0), 2.96 (1H, dd, 8.0 & 14), 3.12 (1H, dd, 5.0 & 14), 3.86 (1H, dd, 5.0 & 7.0), 3.93 (1H, dd, 5.0 & 7.0), 3.98 (1H, d, 15), 4.10 (1H, d, 15), 4.73 (1H, dd, 5.0 & 8.0), 6.62 (2H, d, 8.5), 6.87 (2H, d, 8.5), 6.87 (2H, d, 8.5), 7.1—7.3 (5H, m), 7.32 (2H, d, 8.5). Calcd for $C_{28}H_{27}O_5N_3$: C, 69.26; H, 5.61; N, 8.86%. Found: C, 69.24; H, 5.73; N, 8.57%.

The Unsaturated Coelenteramino Acid 44: Brown powder (MeOH–AcOEt), mp 160—163 °C; MS (FAB) *m/z* 663 ($M+H^+$); IR (KBr) 3394, 1604 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 374 (19300), 308 (sh) (21300), 290 (24100); ^1H NMR (DMSO-d_6) δ (*J*)=2.34 (2H, br.), 2.58 (2H, br. s), 2.84 (1H, br. s), 4.20 (2H, br. s), 5.08 (2H, s), 5.24 (2H, s), 6.42 (2H, d, 8.0), 6.52 (1H, br. s), 6.70 (1H, br. s), 6.90 (2H, d, 8.0), 7.0—7.7 (16H, m). Calcd for $C_{42}H_{35}O_5N_3$: C, 76.23; H, 5.33; N, 6.35%. Found: C, 76.14; H, 5.55; N, 6.41%.

Synthesis of the Hydroxycoelenteramine 49. The Keto Coelenteramine 47: To a solution of *O*-benzyl-coelenteramine (**46**) (1.2 g) in THF (200 ml) was added *t*-BuOK (3.7 g) at room temp and the mixture stirred vigorously under air. After 40 min the solution was diluted with 1M HCl at 0 °C and concentrated to a small volume. The residue was extracted with dichloromethane three times. The combined extract was dried over sodium sulfate and evaporated in vacuo. The residue was chromatographed on a silica-gel column to give **47** (0.30 g, 24%), which was crystallized from methanol to give yellow needles, mp 156—157 °C; MS *m/z* 381 (M^+); IR (KBr) 3426, 3138, 1648, 1617 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 417 (8000), 298 (27700), 272 (sh) (22700); ^1H NMR ($CDCl_3$) δ (*J*)=5.12 (2H, s), 6.82 (2H, br. s), 7.04 (2H, d, 9.0), 7.3—7.6 (8H, m), 7.82 (2H, d, 9.0), 8.06 (2H, dd, 1.6 & 8.5), 8.68 (1H, s). Calcd for $C_{24}H_{19}O_2N_3$: C, 75.57; H, 5.02; N, 11.02%. Found: C, 75.57; H, 5.06; N, 10.93%.

The Hydroxycoelenteramine 48: A solution of the coelenteramine **47** (250 mg) in methanol (10 ml) was treated with $NaBH_4$ (30 mg) at 0 °C. After 15 min the mixture was acidified with 1M HCl and extracted four times with dichloromethane. The combined extract was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on a silica-gel column to obtain **48** (230 mg, 92%), which was crystallized from methanol as white needles, mp 157—158 °C, MS *m/z* 383 (M^+); IR (KBr) 3366, 3280, 1608 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 352 (10500), 284 (26000); ^1H NMR ($CDCl_3$) δ (*J*)=2.10 (1H, br. s), 4.66 (2H, br. s), 5.16 (2H, s), 5.66 (1H, s), 7.10 (2H, d, 9.0), 7.4—7.6 (10H, m), 7.90 (2H, d, 9.0), 8.39 (1H, s). Calcd for $C_{24}H_{21}O_2N_3$: C, 75.17; H, 5.32; N, 10.96%. Found: C, 75.18; H, 5.57; N, 11.01%.

The Hydroxycoelenteramine 49: A mixture of **48** (100 mg), 10% Pd-C (20 mg), MeOH (3.0 ml) and dichloromethane (2.0 ml) was stirred under hydrogen atmosphere at room temp for 3 days and then filtered. The filtrate was evaporated to dryness and the residue was separated by silica-gel TLC to give **49** (60 mg, 78%), which was crystallized from methanol as white crystalline powder, mp 178—179 °C; MS *m/z* 293 (M^+); IR (KBr) 3500, 3378, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 353 (9700), 282 (24600); ^1H NMR (CD_3OD) δ (*J*)=5.94 (1H, s), 6.86 (2H, d, 9.0), 7.2—7.5 (5H, m), 7.76 (2H, d, 9.0), 8.24 (1H, br. s). Calcd for $C_{17}H_{15}O_2N_3$: C, 69.61; H, 5.15; N, 14.33%. Found: C, 69.60; H, 5.23; N, 14.17%.

Synthesis of Coelenterazine Analogues. Methylcoelenterazine 3: The coelenteramino acid **40** (170 mg) and dicyclohexylcarbodiimide (230 mg) were dissolved in pyridine (3.0 ml) under argon atmosphere and the solution was allowed to stand at room temp for 1 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on a silica-gel column to give **3** (120 mg, 75%), yellow crystalline powder (MeOH), mp 175 °C (decomp); FABMS *m/z* 438 ($M+H$); IR (KBr) 3166, 1630, 1511; UV (MeOH) λ_{max} nm (ε) 445 (11400), 320 (sh) (4400), 280 (sh) (14500), 244 (20200), 228 (20600); ^1H NMR (CD_3OD) δ (*J*)=2.64 (3H, s),

4.00 (2H, br. s), 4.26 (2H, br. s), 6.70 (2H, d, 8.0), 6.88 (2H, d, 8.0), 7.1—7.4 (9H, m). Calcd for $C_{27}H_{23}O_3N_5$: C, 74.12; H, 5.30+; N, 9.61%. Found: C, 74.10; H, 5.37; N, 9.62%.

The Coelenterazine Analogue 4:[9] Yellow powder (MeOH), mp 150 °C (decomp); MS (FAB) *m/z* 436 (M^++H^+); UV (MeOH) λ_{max} nm (ε) 435 (7000), 368 (7100), 300 (sh) (12000), 276 (16300); ^{1}H NMR (CD_3OD) δ (*J*)=4.08 (2H, s), 4.31 (2H, br. s), 4.44 (2H, s), 6.70 (2H, d, 8.5), 6.80 (1H, dd, 2.5 & 8.5), 7.00 (1H, d, 2.5), 7.1—7.4 (7H, m), 7.56 (1H, d, 8.5). Calcd for $C_{27}H_{21}O_3N_3$: C, 74.47; H, 4.86; N, 9.65%. Found: C, 74.48; H, 4.91; N, 9.50%.

The Coelenterazine Analogue 5:[9] Yellow powder mp 160 °C (decomp); MS (FAB) *m/z* 450 (M+H$^+$); UV (MeOH) λ_{max} nm (ε) 442 (9600), 376 (6900), 360 (6900), 304 (sh), (14400), 280 (24000); ^{1}H NMR (CD_3OD) δ (*J*)=2.90 (2H, t, 9.0), 3.74 (2H, t, 9.0), 3.99 (2H, s), 4.39 (2H, s), 6.64—6.76 (4H, m), 7.14 (2H, d, 8.5), 7.20—7.40 (5H, m), 7.48 (1H, br. d, 8.5). Calcd for $C_{28}H_{23}O_3N_3$: C, 74.81; H, 5.16; N, 9.35%. Found: C, 74.77; H, 5.28; N, 9.35%.

The Coelenterazine Analogue 6: Yellow crystalline powder (MeOH), mp 170 °C (decomp); MS (FAB) *m/z* 464 (M+H); IR (KBr) 3178, 1612, 1553, 1510 °cm^{-1}; UV (MeOH) λ_{max} nm (ε) 445 (10800), 348 (5300), 297 (13900), 261 (26300); ^{1}H NMR (CD_3OD) δ (*J*)=2.34 (2H, br. m), 2.56 (2H, br. t, 7.0), 3.20 (2H, br. s), 4.04 (2H, s), 4.36 (2H, s), 6.6—6.8, 7.1—7.4 (12H, m). Calcd for $C_{29}H_{25}O_3N_3$: C, 75.14; H, 5.44; N, 9.07%. Found: C, 75.12; H, 5.50; N, 9.05%.

The Coelenterazine Analogue 7: Yellow powder (MeOH–Et_2O–hexane), mp 150 °C (decomp); MS (FAB) *m/z* 468 (M+H$^+$); IR (KBr) 3398, 3200, 1620, 1550, 1512 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 443 (9300), 324 (sh) (4400), 274 (15300), 226 (21400); ^{1}H NMR (CD_3OD) δ (*J*)=3.2 (2H, t, 6.0), 3.80 (2H, t, 6.0), 4.04 (2H, s), 4.30 (2H, s), 6.70 (2H, d, 9.0), 6.90 (2H, d, 9.0), 7.16 (2H, d, 9.0), 7.26 (2H, d, 9.0), 7.2—7.4 (5H, m). Calcd for $C_{28}H_{25}O_4N_3$; C, 71.93; H, 5.39; N, 8.99%. Found: C, 71.82; H, 5.66; N, 9.05%.

The Coelenterazine Analogue 8: Yellow powder (MeOH), mp 100 °C (decomp); FABMS *m/z* 440 (M+H); IR (KBr) 3376, 1611, 1513 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 420 (5100), 348 (7200), 277 (17800); ^{1}H NMR (CD_3OD) δ (*J*)=4.04 (2H, br. s), 6.40 (1H, s), 6.68 (2H, d, 8.0), 6.88 (2H, d, 8.0), 7.12 (2H, d, 8.0), 7.2—7.7 (7H, m), 7.80 (1H, br. s). Calcd for $C_{26}H_{21}O_4N_3$: C, 71.06; H, 4.82; N, 9.56%. Found: C, 71.01; H, 4.97; N, 9.41%.

The Coelenterazine Analogue 9: Brown powder (acetone–CH_2Cl_2), mp 180 °C (decomp); MS (FAB) *m/z* 466 (M+H$^+$); IR (KBr) 3222, 1630, 1600, 1551 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 450 (8700), 371 (6500), 324 (14600), 312 (sh) (13800), 284 (17600), 224 (26400); ^{1}H NMR (acetone-d_6) δ (*J*)=2.84 (2H, t, 7.0), 3.66 (2H, t, 7.0), 4.02 (2H, s), 4.42 (2H, s), 6.14 (1H, d, 2.5), 6.18 (1H, d, 2.5), 6.68 (2H, d, 9.0), 7.1—7.5 (7H, m). Calcd for $C_{28}H_{23}O_4N_3$: C, 72.24; H, 4.98; N, 9.03%. Found: C, 72.19; H, 5.14; N, 9.11%.

The Coelenterazine Analogue 11: Synthesized from the coelenteramine **45**.[16] Yellow powder (EtOAc), mp 140 °C (decomp); MS (FAB) *m/z* 451 (M+H$^+$); UV (MeOH) λ_{max} nm (ε) 437 (12600), 303 (24000), 216 (sh) (25400), 206 (35300); ^{1}H NMR (CD_3OD–CD_2Cl_2) δ (*J*)=2.96 (6H, s), 4.07 (2H, br. s), 4.40 (2H, br. s), 6.72 (2H, d, 8.0), 6.76 (2H, d, 8.0), 7.10—7.45 (9H, m), 7.51 (1H, br. s). Calcd for $C_{28}H_{26}O_2N_4$: C, 74.64; H, 5.82; N, 12.44%. Found: C, 74.69; H, 6.08; N, 12.46%.

Synthesis of the Coelenteramides. Methylcoelenteramide 12: A solution of methylcoelenteramine **37** (80 mg) and *p*-acetoxyphenylacetyl chloride (87 mg) in pyridine (0.3 ml) and CH_2Cl_2 (1.0 ml) was stirred at room temp for 1.5 h. The reaction mixture was treated with 10% $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in methanol (3.0 ml) and 1M NaOH aq (0.25 ml) and the solution stirred at room temp for 20 min. The mixture was acidified to pH 1 and extracted with a mixture of CH_2Cl_2–AcOEt–MeOH. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was subjected to silica-gel TLC to give methylcoelenteramide **12** (75 mg, 71%), white needles (AcOEt–Et_2O), mp 152—165 °C; MS *m/z* 425 (M^+); IR (KBr) 3400, 3256, 1672, 1611 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 322 (12300), 272 (sh) (11800), 253 (12300); UV (DMSO) λ_{max} nm (ε) 325 (13700), 270 (15500); ^{1}H NMR (CD_3OD) δ (*J*)=2.52 (3H, s), 4.07 (2H, s), 6.76 (2H, d, 8.5), 6.87 (2H, d, 8.5), 6.97 (2H, dd, 2.5 & 7.0), 7.16 (2H, d, 8.5), 7.10—7.2 (3H, m), 7.44 (2H, d, 8.5). Calcd for $C_{26}H_{23}O_3N_3$; C, 73.39; H, 5.45; N, 9.88%. Found; C, 73.31; H, 5.70; N, 9.57%.

The Coelenteramide 13: White powder (EtOH), mp 236—237 °C; MS *m/z* 423 (M^+); IR (KBr) 3372, 3250, 1666, 1615 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 353 (22100), 300 (sh) (10700), 282 (13200); UV (DMSO) λ_{max} nm (ε) 355 (21400), 300 (sh) (10300), 282 (13200); ^{1}H NMR (CD_3OD) δ (*J*)=3.54 (2H, s), 3.86 (2H, s), 4.12 (2H, s), 6.74 (2H, d, 8.5), 6.90 (1H, dd, 2.0 & 8.5), 7.00 (2H, dd, 2.0 & 8.0), 7.04 (1H, d, 2.0), 7.14 (2H, d, 8.5), 7.1—7.3 (3H, m), 7.88 (1H, d, 8.5). Calcd for $C_{26}H_{21}O_3N_3$; C, 74.48; H, 5.58; N, 9.31%. Found: C, 74.42; H, 5.81; N, 9.23%.

The Coelenteramide 14: White powder (AcOEt), mp 209—210 °C; MS *m/z* 437 (M^+); IR (KBr) 3510, 3400, 3260, 1658, 1611 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 347 (19500), 300 (sh) (11500), 284 (13000); UV (DMSO) λ_{max} nm (ε) 351 (23000), 300 (sh) (14200), 286 (16300); ^{1}H NMR ($CDCl_3$–CD_3OD) δ (*J*)=3.00 (4H, m), 3.60 (2H, s), 4.09 (2H, s), 6.68 (1H, d, 2.5), 6.79 (1H, dd, 2.5 & 8.5), 6.80 (2H, d, 8.5), 7.00 (2H, dd, 2.0 & 7.0), 7.08 (2H, d, 8.5), 7.10 (3H, m), 8.08 (1H, d, 8.5). Calcd for $C_{27}H_{23}O_3N_3$; C, 74.12; H, 5.30; N, 9.61%. Found; C, 74.04; H, 5.41; N, 9.56%.

The Coelenteramide 15: White needles (AcOEt), mp 179—180 °C; MS *m/z* 451 (M^+); IR (KBr) 3400, 3246, 1665, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 328 (14200), 277 (11000), 256 (11400); UV (DMSO) λ_{max} nm (ε) 330 (16200), 280 (13500); ^{1}H NMR ($CDCl_3$–CD_3OD) δ (*J*)=2.30 (2H, tt, 6.5 & 7.0), 2.52 (2H, t, 6.5), 2.65 (2H, t, 7.0), 3.60 (2H, s), 4.16 (2H, s), 6.74 (1H, d, 2.5), 6.79 (2H, d, 8.5), 6.85 (1H, dd, 2.5 & 8.5), 6.99 (2H, dd, 2.0 & 7.0), 7.07 (2H, d, 8.5), 7.18 (3H, m), 7.52 (1H, d, 8.5). Calcd for $C_{28}H_{25}O_3N_3$: C, 73.74; H, 5.00; N, 9.92%. Found; C, 73.60; H, 5.20; N, 9.52%.

The Coelenteramide 16: White needles (EtOH), mp 223—224 °C; MS (FAB) *m/z* 456 (M+H$^+$); IR (KBr) 3400, 3272, 1671, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 321 (12800), 268 (13600), 254 (13800); UV (DMSO) λ_{max} nm (ε) 324 (13500), 272 (15900); ^{1}H NMR (CD_3OD) δ (*J*)=3.04 (2H, t, 6.5), 3.56 (2H, s), 3.88 (2H, t, 6.5), 4.06 (2H, s), 6.74 (2H, d, 8.5), 6.88 (2H, d, 9.0), 6.96 (2H, dd, 2.0 & 8.0), 7.1—7.2 (3H, m), 7.14 (2H, d, 9.0), 7.42 (2H, d, 8.5). Calcd for $C_{27}H_{25}O_4N_3$: C, 71.19; H, 5.53; N, 9.23%. Found: C, 71.15; H, 5.53; N, 9.17%.

The Coelenteramide 17: White powder (AcOEt), mp 207—208 °C; MS *m/z* 427 (M^+); IR (KBr) 3400, 3296, 1675, 1611 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 339 (14800), 296 (17000), 282 (17300), 224 (sh) (20100); UV (DMSO) λ_{max} nm (ε) 340 (13100), 302 (16200), 282 (16700); ^1H NMR (CD_3OD) δ (*J*)=3.58 (2H, br. s), 5.92 (1H, s), 6.80 (2H, d, 9.0), 6.90 (2H, d, 9.0), 7.14 (2H, d, 9.0), 7.1—7.3 (5H, m), 7.95 (2H, d, 9.0), 8.68 (1H, s). Calcd for $C_{25}H_{21}O_4N_3$: C, 70.24; H, 4.95; N, 9.83%. Found: C, 70.20; H, 5.20; N, 9.38%.

The Coelenteramide 18: Slightly green powder (AcOEt–CH_2Cl_2), mp 212—213 °C; MS *m/z* 453 (M^+); IR (KBr) 3380, 3250, 1629, 1597 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 362 (17000), 289 (12000); UV (DMSO) λ_{max} nm (ε) 368 (22000), 290 (15800); ^1H NMR (CD_3OD) δ (*J*)=3.00 (4H, m), 3.64 (2H, s), 3.98 (2H, s), 6.11 (1H, d, 2.5), 6.22 (1H, d, 2.5), 6.76 (2H, d, 9.0), 7.06 (2H, dd, 2.0 & 7.5), 7.20 (2H, d, 9.0), 7.1—7.3 (3H, m). Calcd for $C_{27}H_{23}O_4N_3$: C, 71.51; H, 5.11; N, 9.27%. Found: C, 71.42; H, 5.29; N, 9.13%.

The Coelenteramide 19: Slightly yellow needles (EtOH–CH_2Cl_2), mp 222—223 °C; MS *m/z* 438 (M^+); IR (KBr) 3400, 3254, 1670, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 367 (17900), 332 (16700), 270 (9800), 224 (17900); UV (DMSO) λ_{max} nm (ε) 370 (24100), 336 (21300), 274 (11400); ^1H NMR (CD_3OD–$CDCl_3$) δ (*J*)=3.40 (6H, s), 3.58 (2H, s), 4.12 (2H, s), 6.80 (2H, d, 8.5), 6.82 (2H, d, 9.0), 7.03 (2H, dd, 2.0 & 8.0), 7.16 (2H, d, 8.5), 7.1—7.25 (3H, m), 7.82 (2H, d, 9.0), 8.63 (1H, s). Calcd for $C_{27}H_{26}O_2N_4$; C, 73.95; H, 5.98; N, 12.78%. Found: C, 73.76; H, 6.07; N, 12.79%.

Chemiluminescence Measurements: To a solution (10 μl) of a coelenterazine analogue (concn ca. 3×10^{-3} mol^{-3}) was added dimethyl sulfoxide (3 ml) at room temp. The resulting light emission was recorded with a luminometer as decribed previously.[20]

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References

1) A part of this paper was preliminarily reported in *Chem. Lett.*, **1989**, 1423.
2) T. Goto, "Marine Natural Products," ed by P. Scheuer Academic Press, New York (1980), pp. 179—222.
3) F. H. Johnson, O. Shimomura, Y. Saiga, L. C. Gershman, G. T. Reynolds, and J. R. Waters, *Cell. Comp. Physiol.*, **60**, 85 (1962).
4) T. Goto, S. Inoue, and S. Sugiura, *Tetrahedron Lett.*, **1968**, 3873.
5) O. Shimomura and F. H. Johnson, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2611 (1978).
6) S. Inoue, S. Sugiura, H. Kakoi, T. Hashizume, T. Goto, and H. Iio, *Chem. Lett.*, **1975**, 141.
7) F. I. Tsuji, S. Inouye, T. Goto, and Y. Sakaki, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8107 (1986); M. DeLuca, *Biochemistry*, **8**, 160 (1969).
8) T. Goto and H. Fukatsu, *Tetrahedron Lett.*, **1969**, 4299.
9) O. Shimomura, B. Musicki, and Y. Kishi, *Biochem. J.*, **251**, 405 (1988).
10) Y. Kishi, H. Tanino, and T. Goto, *Tetrahedron Lett.*, **1972**, 2747.
11) S. Inoue, H. Taguchi, M. Murata, H. Kakoi, and T. Goto, *Chem. Lett.*, **1977**, 259.
12) T. Goto, M. Isobe, Y. Kishi, S. Inoue, and S. Sugiura, *Tetrahedron Lett.*, **31**, 939 (1975).
13) S. Inoue, S. Sugiura, H. Kakoi, K. Hashizume, T. Goto, and H. Iio, *Chem. Lett.*, **1975**, 141.
14) K. Hori, J. E. Wampler, and M. J. Cormier, *J. Chem. Soc., Chem. Commun.*, **1973**, 492.
15) T. Goto, I. Kubota, N. Suzuki, and Y. Kishi, "Chemiluminescence and Bioluminescence," ed by M. J. Cormier et al., Plenum Press, New York (1973), pp. 325—335.
16) J. -Y. Koo, S. P. Schmidt, and G. B. Schuster, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 30 (1978); G. B. Schuster, B. Dixon, J. -Y. Koo, S. P. Schmidt, and J. P. Smith, *Photochem. Photobiol.*, **30**, 17 (1979).
17) F. McCapra, *J. Chem. Soc., Chem. Commun.*, **1977**, 946.
18) E. H. White, H. Worther, H. H. Seliger, and W. D. McElroy, *J. Am. Chem. Soc.*, **88**, 2015 (1966).
19) K. Teranishi and T. Goto, *Bull. Chem. Soc. Jpn.*, **62**, 2009 (1989).
20) H. Nakamura and T. Goto, *Photochem. Photobiol.*, **30**, 27 (1979).