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Effects of Conformational Rigidity and Hydrogen Bonding in the Emitter on the Chemiluminescence Efficiency of Coelenterazine (Oplophorus Luciferin)

Katsunori TERANISHI and Toshio GOTO*

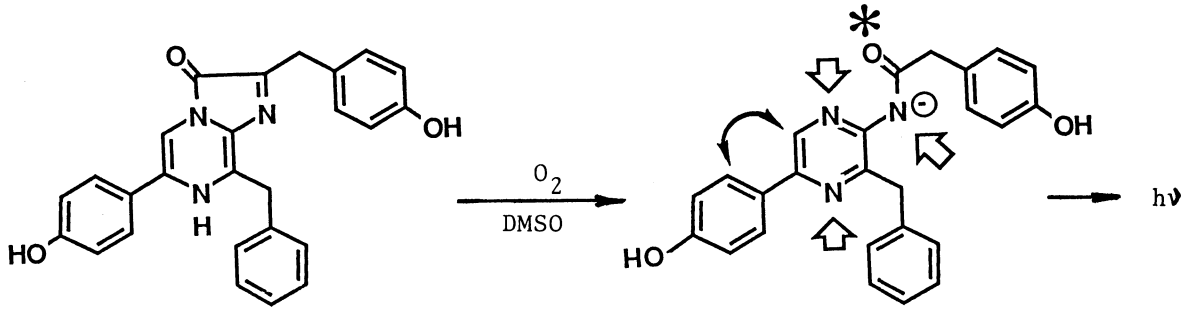
Laboratory of Organic Chemistry, Faculty of Agriculture,
Nagoya University, Chikusa, Nagoya 464

In the case of chemiluminescence of coelenterazine, effects of conformational rigidity of and hydrogen-bonding with the emitter, coelenteramide, on the chemiluminescence efficiency have been examined. 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. For example, quantum yield of Cypridina bioluminescence (a luciferin-luciferase reaction) is about 30%,[1] whereas at most only 3% has been obtained in the case of chemiluminescence of Cypridina luciferin under the best chemiluminescence condition known (diglyme containing a trace of acetate buffer, pH 5.6).[2] Similar phenomena have been observed in the bioluminescence of aequorin (quantum yield is ca 30%),[3] which is a photoprotein containing coelenterazine (Oplophorus luciferin) (1) as a light producing chromophore[3,4] similar to Cypridina luciferin.

Cypridina luciferase and apo-aequorin (protein part of aequorin) are known to be a protein of high hydrophobicity,[5] which may contribute to the high efficiency of bioluminescence.[5,6] 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). To see whether these terms are effective or not, we have prepared several derivatives of coelenterazine[7] which have a bridge for fasten between the phenyl and pyrazine rings (compds. 4-6)[8] or which have an alcoholic side chain that can make a hydrogen-bond to one of the

nitrogen atoms in the emitter (compds. 7-9). Chemiluminescence efficiency was measured in dimethyl sulfoxide (DMSO)[9-11] and the results are shown in Table 1.



Coelenterazine (1) Coelenteramide (2)
(excited state anion)

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 membered ring derivatives, 4 and 6. Shimomura et al.[8] reported that bioluminescence efficiency of the ring derivative 4 is about one half of coelenterazine (1). It may suggest that in contrast with the chemiluminescence fitting of 4 with apoaequorin (a protein) may not be perfect so that the light yield of bioluminescence is decreased.

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.

As the phenolic hydroxyl on the lower left side chain was expected to have a large light-enhancing effect by analogy with the firefly luciferin case,[12] we have synthesized the coelenterazine analogues, 10 and 11, having p-methoxyl or p-dimethylamino substituent in place of the hydroxyl group. Chemiluminescence efficiency of these analogues, however, did not differ much from that of coelenterazine (Table 1). A similar observation has been reported in the previous paper.[13]

Coelenterazine derivatives (3 - 11) showed satisfactory spectral data as well as elemental analysis. Synthesis of these compounds will be reported elsewhere.

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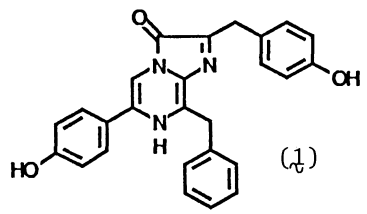
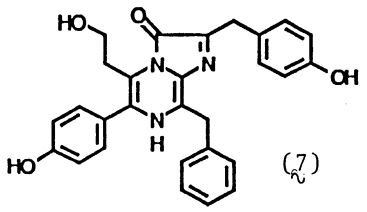
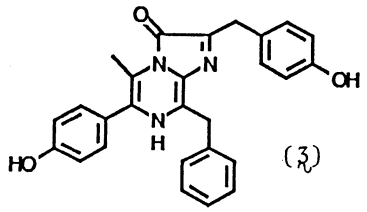
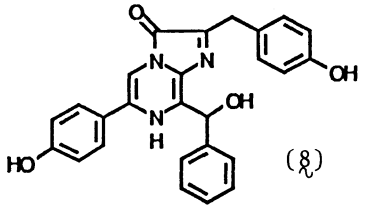
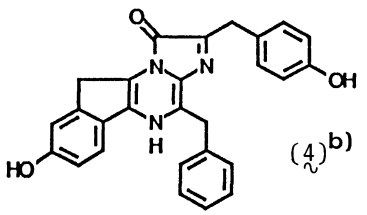
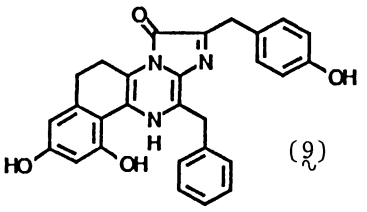
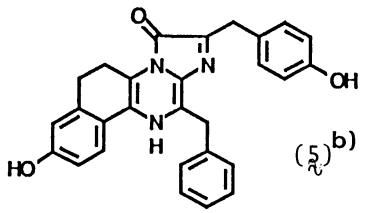
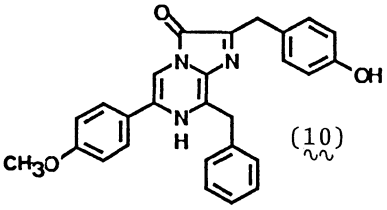
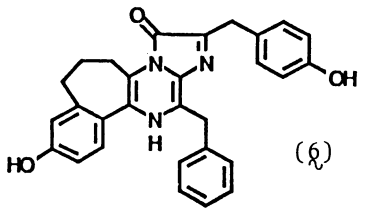
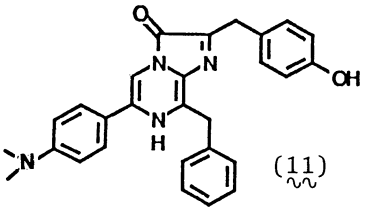
Table 1. Relative light yields of chemiluminescence

Compound	Rel. L.Y.[a]	Compound	Rel. L.Y.[a]
 (1)	1.0	 (7)	0.46
 (3)	1.1	 (8)	0.35
 (4)[b]	1.5	 (9)	0.01
 (5)[b]	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/l; temp, 25 °C.

b) These compounds were reported by Shimomura et al.[8]

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9) Solvent effect on the light yield was examined using coelenterazine (1). The results are shown in Table 2, which indicates that DMSO without additives is one of the best solvents for chemiluminescence of coelenterazine.

Table 2. Solvent effects on the relative light yield of chemiluminescence

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/l t-BuOK/t-BuOH 0.5 ml	0.06
DGM	0.1 mol/l acetate buffer pH 5.6 0.1 ml	0.006
DMSO	1.0 mol/l t-BuOK/t-BuOH 0.5 ml	0.09
DMSO	0.1 mol/l acetate buffer pH 5.6 0.1 ml	0.9

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

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