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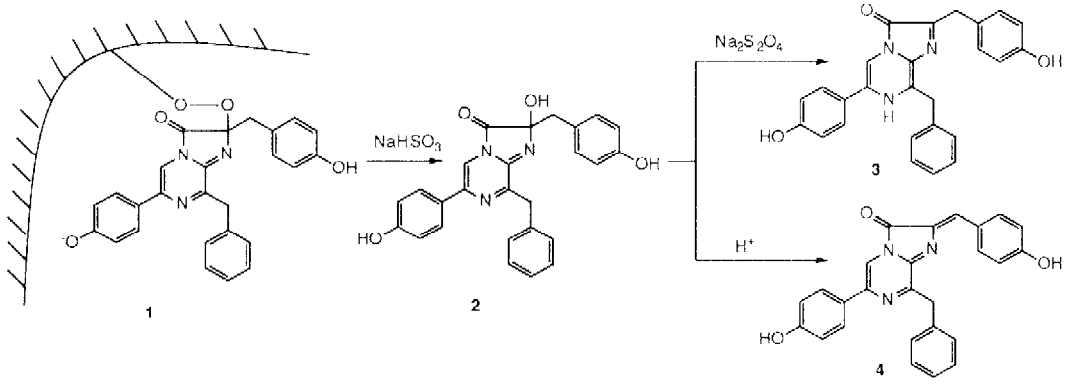
Revision of Structure of Yellow Compound, a Reduction Product from Aequorin, Photoprotein in Jellyfish, *Aequorea aequorea*

Katsunori Teranishi#, Minoru Isobe, Tetsuya Yamada# and Toshio Goto*
School of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan
#School of Bioresources, Mie University,Uehama-chou, Tsu 514, Japan

Abstract *The structure of yellow compound from aequorin was revised by means of synthesis of stable analogs having tert-butyl group at the 2-position of the imidazopyrazinone chromophore. Comparing their absorption spectra concluded that the yellow compound should have the 5-oxo structure instead of having hydroxy group at 2 position as reported previously.*

Aequorin is a photoprotein found in jellyfish to emit blue light by the action of calcium ions to this luminous system.[1] Molecular mechanism of the luminescence has recently progressed on the apoprotein by means of site specific mutagenesis.[2] Aequorin is known to have a chromophore, coelenterazine (*Oplophorus* luciferin) as the light emitting species linking to this protein through a peroxidic bond as illustrated in **1**. In 1978, Shimomura and Johnson reported that they obtained a yellow compound by reduction of aequorin with $NaHSO_3$ as shown in Scheme 1, and that they have proposed the structure **2**.[3] Kishi and Shimomura *et al.* supported this result by measuring ^{13}C-NMR spectrum of **1** and concluded its structure through incorporation experiment of $^{18}O_2$.[4] The straight forward correlation of these chromophoric structures looked consistent.

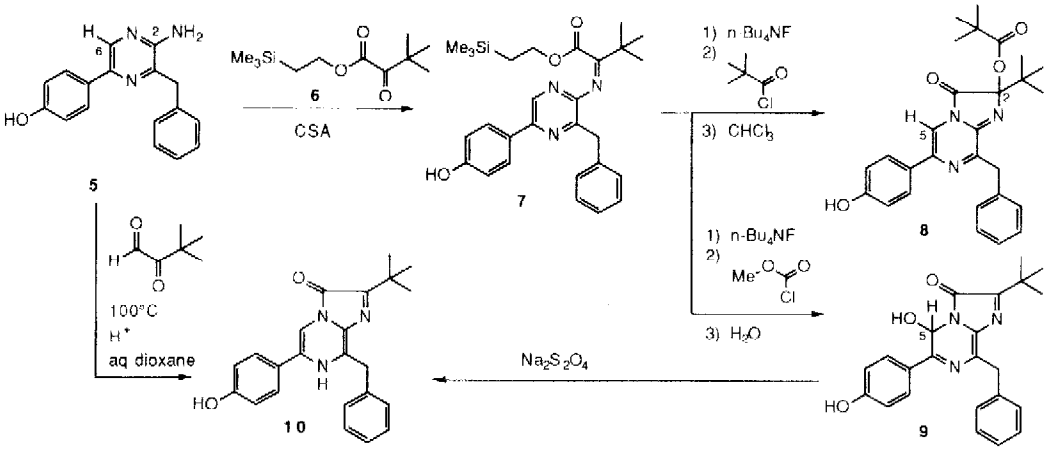


Scheme 1
Proposed structures of Aequorin chromophore (**1**) and Yellow Compound (**2**), as well as the elucidated structures of stable derivatives (**3** and **4**) by Shimomura and Johnson.[3]

Since the structure of yellow compound is directly connected to the original structural information of the aequorin chromophore, its elucidation study is indispensable. Shimomura *et al.* reported its structure as **2** leaving only UV spectrum to be comparable because of the unstability of yellow compound. Transformation of **2** under reductive or acidic conditions afforded stable products, **3** and **4**, respectively, both of which were characterized to have those structures in Scheme 1.[3]

The difficulty in collecting further information on the yellow compound lied in its unstability, which may be overcome by designing possible stable analogs related to **1** or **2** and by comparing the properties among them. In this paper, we report two syntheses of the designed molecules **8** and **9** from a common precursor **7**, which was prepared by coupling between the aminopyrazine **5**[5] and the ketoacid ester **6** (illustrated in Scheme 2).

A solution of **5** and **6** in DGM (diethylene glycol dimethyl ether) in the presence of CSA (10-camphorsulfonic acid) and molecular sieves 4A was heated at 120°C for 15 hr to afford the condensation product **7** [^{1}H-NMR of the H6 at δ 8.47 ppm (s)] [6] in 67% yield. Treatment of this trimethylsilylethyl ester with *n*-Bu_4NF in THF solvent provided the corresponding carboxylate as the ammonium salt, which was further treated with trimethylacetyl (pyvaloyl) chloride at -25°C. Further conversion of this mixed anhydride was facilitated to stand at 4°C for 12 hr in chloroform solvent[7] to obtain the pyvaloate **8**,[8] an analog of the proposed structure **2**. The structure of **8** was confirmed through full analytical data, including ^{1}H-NMR of the heteroaromatic H5 at δ 7.30 ppm (s).

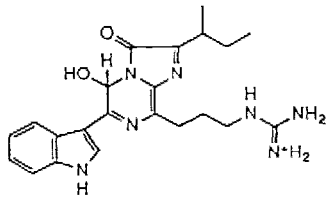


Scheme 2

Synthesis of possible analogs of Yellow Compound

Alternatively, the alcohol **9** was another candidate as a possible oxygen-regioisomer of **2**, since a structure had been known as luciferinol **11**, an analog of *Cypridina* lucferin. Luciferinol **11** had been obtained by oxidation of the *Cypridina* luciferin with lead(IV) oxide by Goto *et al.*[9] Compound **9** was synthethized here from the carboxylic acid intermediate obtained by treatment of **7** with the fluoride. The

acid was activated with methyl chloroformate[7] and the corresponding mixed anhydride was treated in water as shown to give 9 [^{1}H-NMR of the H5 at δ 6.60 ppm (d, J= 5.0Hz)][10] in 22% yield in Scheme 2. Reduction of the 5-oxy derivative 9 with $Na_2S_2O_4$, applying the reductive condition of 2 into 3, provided a coelenterazine analog 10 [quantitative yield; m/z 374(M+1)$^+$], which was identified with authentic sample.[11]



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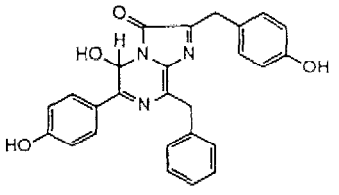

12

The absorption spectra of the 2-oxy and 5-oxy analogs, 8 and 9, respectively, was compared with that of yellow compound reported by Shimomura.[12] And the results are illustrated in Fig 1. All of the three spectra similarly showed two maxima at around 430-450 nm and 290-310 nm, respectively. The relative intensities, however, at those two maxima with the three compounds were quite different to each other. 2-Oxy compound 8 showed weak absorption at 430 nm (ε = 5,240) relative to that at 290 nm (ε = 20,300), the ratio $\varepsilon_{430/290}$ being 0.26 ; while 5-oxy compound 9 exhibited opposit relative intensities at 434 nm (ε = 23,700) and 303 nm (ε = 7,730) the ratio $\varepsilon_{434/303}$ being 3.1. The yellow compound showed the relative value of 1.4, but the value can be corrected by compensating an end absorbing base line (due to the difficult *purification of the naturally drived sample) to be ca.* 3.

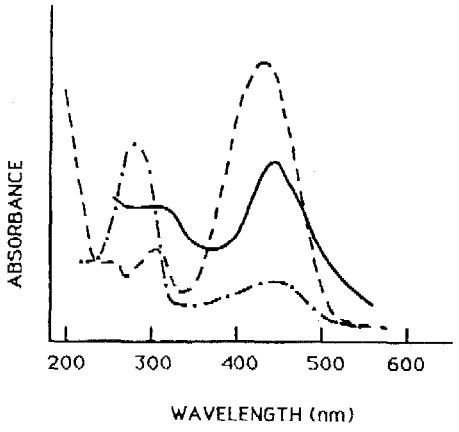

Fig 1 Absorption spectra (EtOH)

———— Yellow Compound

— — — — 2-oxy analog **8**

---------- 5-oxy analog **9**

The current study concluded that the structure of yellow compound obtained by reduction from aequorin was **12**, which is closely related to luciferinol derived by oxidation from *Cypridina* luciferin. The results would provide further molecular mechanism on the bioluminescence in aequorin.

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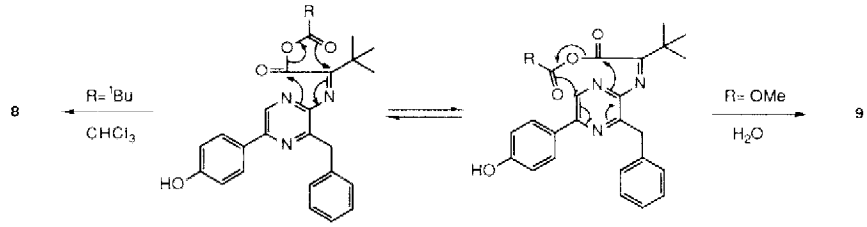
References and Notes

† Deceased on August 29, 1990.

1. (a) O. Shimomura, F. H. Johnson and Y. Saiga, *J. Cell Comp. Physiol.*, **59**, 223 (1962). (b) *idem, ibid*, **62**, 1 (1963). (c) Review article, T. Goto, "Marine Natural Products", ed by P. Scheure, *Academic Press Inc.*, New York pp 179-222 (1980).
2. J. Nakajima-Shimada, H. Iida, F. I. Tsuji and Y. Anraku, *Biochem. Biophys. Res. Commun.*, **174**, 115 (1991).
3. O. Shimomura and F. H. Johnson, *Proc. Natl. Acad. Sci. USA*, **75**, 2611 (1978).
4. B. Musicki, Y. Kishi and O. Shimomura, *J. Chem. Soc., Chem. Commun.*, 1566 (1986).
5. Y. Kishi, H. Tanino and T. Goto, *Tetrahedron Lett.*, 2747 (1972).
6. **7** ^{1}H-NMR ($CDCl_3$ 500MHz), δ -0.07(9H, s), 0.64(2H, m), 1.33(9H, s), 4.04(2H, m), 4.29(2H, s), 5.23(1H, br s), 6.89(2H, d, J= 9.3 Hz), 7.17(1H, t, J= 7.4), 7.25(2H, t, J= 7.4). 7.35(2H, d, J= 7.4), 7.89(2H, d, J= 9.3), 8.47(1H, s).
7. The mechanism of the mixed anhydride to convert different product may be depending upon the following equilibrium and possible hydrolysis in water solvent.



8. **8** ^{1}H-NMR ($CDCl_3$ 500MHz), δ 0.98(9H, s), 1.24(9H, s), 4.08(1H, d, J= 13.4 Hz), 4.30(1H, d, J= 13.4), 5.09(1H, br s), 6.82(2H, d, J= 8.9), 7.21(2H, t, J= 7.1), 7.28(2H, t, J= 7.1), 7.30(1H, s), 7.45(2H, d, J= 7.1), 7.61(2H, d, J= 8.9). UV λ_{max}. (EtOH) 438 nm(ε = 5,240) 290 nm(ε = 20,300).
9. Y. Toya, S. Nakatsuka and T. Goto, *Tetrahedron Lett.*, 5753 (1983).
10. **9** ^{1}H-NMR ($CDCl_3$ 500MHz), δ 1.44(9H, s), 3.84(1H, d, J= 5.0 Hz; disappear with D_2O), 4.08(1H, d, J= 13.3), 4.14(1H, d, J= 13.3), 5.30(1H, br s), 6.60($1H_5$, d, J= 5.0; changed to singlet by D_2O addition), 6.87(2H, d, J= 9.2), 7.21(1H, t, J= 7.5), 7.30(2H, t, J= 7.5), 7.42(2H, d, J= 7.5), 8.01(2H, d, J= 9.2). UV λ_{max}. (EtOH) 434 nm(ε = 23,700), 303 nm(ε = 7,730), 249 nm(ε = 7,140).
11. Authentic sample of **10** was prepared in 33% yield as shown in Scheme 2.
12. The value of ε of the yellow compound was not reported in the ref. #3.

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