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# CYPRIDINA BIOLUMINESCENCE IV. SYNTHESIS AND CHEMILUMINESCENCE OF 3,7-DIHYDROIMIDAZO[1,2-a]PYRAZIN-3-ONE AND ITS 2-METHYL DERIVATIVE

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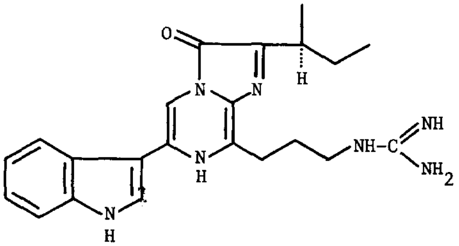
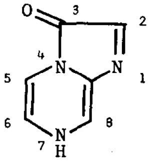
CYPRIDINA luciferin, a bioluminescent substance isolated from Cypridina hilgendorfii, has been shown to have structure I (1). Johnson et al. (2) reported that the luciferin luminesces very weakely in dimethyl sulfoxide (DMSO) without enzyme. We found that when diethylene glycol dimethyl ether (diglyme) (3) containing acetate buffer (pH 5.6) is used as a solvent, light yield of the chemiluminescence is improved nearly 100 times more than that in DMSO; it being over 10% of the quantum yield of the Cypridina bioluminescence, which was reported as ca. 0.28 (4). This high chemiluminescence efficiency suggests that both chemi- and bio-luminescence involve similar mechanisms. Thus, study of mechanism of the chemiluminescence would contribute for elucidation of the bioluminescence mechanism.



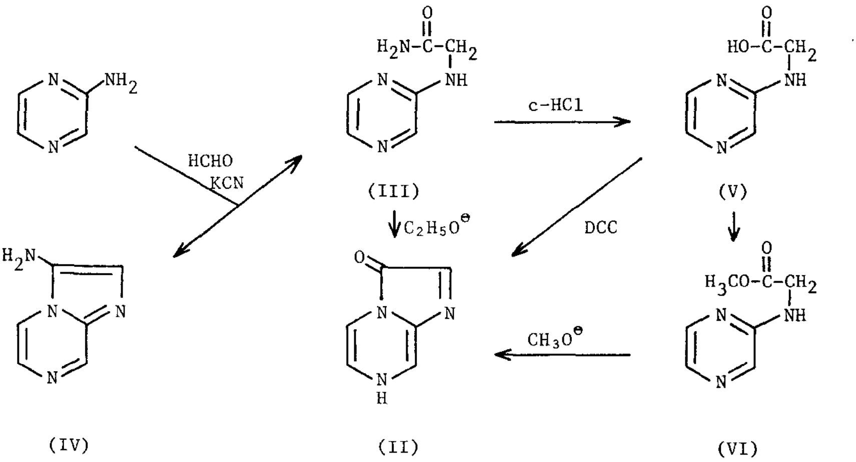
(I)



(II)

Since Cypridina luciferin (I) produces during bioluminescence oxyluciferin and etioluciferin (1), the structure essential for the luminescent reaction is considered to be 3,7-dihydroimidazo[1,2-a]pyrazin-3-one (II). Synthesis of derivatives of II was first achieved in the course of the total synthesis of Cypridina luciferin (5), although over-all yield was very low. Recently, McCapra and Chang (6) synthesized 2,8-dimethyl-6-phenyl derivative, but this method is difficult to be applied for the synthesis of the parent compound (II). We succeeded in obtaining II by the following route.

2-Aminopyrazine was condensed with formaldehyde (as bisulfite adduct) and sodium cyanide to give 2-pyrazylaminoacetamide* (III), m.p. 159° (7), and 3-aminoimidazo[1,2-a]pyrazine* (IV), m.p. 208-209° (dec.) (8). Treatment of the amide (III) with sodium ethoxide in ethanol gave compound II. Alternatively, hydrolysis of the amide (III) with conc. hydrochloric acid afforded in a low yield the corresponding acid (V) as its hydrochloride, which was converted without purification to its methyl ester* (VI), m.p. 81-83°, by heating with methanol. Reaction of the ester (VI) with sodium methoxide in methanol resulted in formation of II. Treatment of the acid (V) with dicyclohexylcarbodiimide in ethanol also gave II. Although II could not be obtained in a sufficiently pure state for elementary analysis, but the following physical data confirmed the proposed structure: m.p. above 300°; mass spectr. $M^+=135$; NMR (in DMSO-$d_6$, ppm from int. TMS) 8.25, 7.60, 7.32, and 7.13 (1H each); $\lambda_{max.}^{MeOH}$ mμ (log ε ) 430 (3.73), 311 (3.48), 257 (4.09); $\lambda_{max.}^{MeOH-HCl}$ 382 (3.64), 280sh (3.53), 252 (3.97); $\lambda_{max.}^{MeOH-KOH}$



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* Satisfactory analyses were obtained with the compounds indicated by asterisks.

399(3.71), 303 (3.47), 256 (4.19). 2-Methyl derivative[*] (VII) was also synthesized from acetaldehyde, instead of formaldehyde, by the method essentially same as that described above. Physical properties of VII: m.p. 239-240° (dec.); mass spectr. $M^{+}$=149; NMR 7.73, 7.22, and 6.79 (1H each), 2.32 (3H); $\lambda_{max.}^{MeOH}$ 427 (3.96), 318 (3.56), 258 (4.11); $\lambda_{max.}^{MeOH-HCl}$ 382 (3.86), 289sh (3.51), 257 (4.00); $\lambda_{max.}^{MeOH-KOH}$ 405 (3.87), 313 (3.56), 260 (4.17).

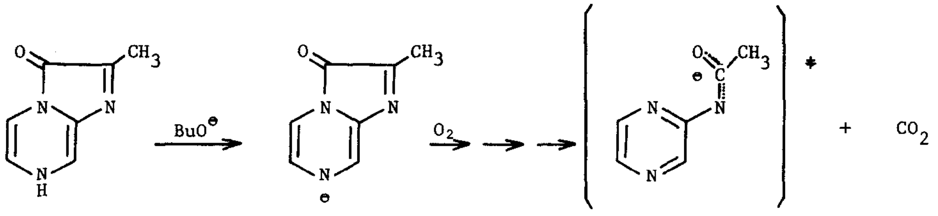
As being expected, the compound II and VII do emit light in aprotic solvents, such as diglyme, DMSO, tetrahydrofuran, etc. in the absence or presence of small amounts of base. The following data were obtained from the study of chemiluminescence of the methyl derivative (VII) (standard condition for chemiluminescence: to a solution of 5 μg of VII in 5 μl n-BuOH was added 2 ml of diglyme containing 10 μl of 0.8 M t-BuOK in t-BuOH).

First-order dependence of light production on VII is observed under a constant pressure of oxygen and the period of half-life is independent to the concentration of VII. The luminescence rate is in direct proportion to the oxygen pressure, but is independent to the concentration of t-butoxide when a large excess of the reagent is used. VII consumes one mole of oxygen during the reaction. Total light yield is proportional to the amount of VII and approximate quantum yield under this condition is 0.0005. These results led to the following equation.

$$\frac{dI}{dt} = k\ [\text{Anion of VII}]\ [O_2] \qquad k = 0.017\ \text{sec}^{-1}\ (20°,\ 1\ \text{atm. air})$$

I = Integrated light yield

McCapra and Chang (6) reported that 6-phenyl-2,8-dimethyl derivative of II is chemiluminescent in DMSO in the presence of base and produces 2-acetamino-3-methyl-5-phenylpyrazine; the chemiluminescence spectrum being identical with the fluorescence spectrum of the acetaminopyrazine in DMSO containing t-butoxide. They proposed that the anion of the pyrazine is the emitter. Similar results were obtained in the case of VII. Thus, the product of the reaction is mainly 2-acetaminopyrazine (VIII), whose fluorescence spectrum in diglyme containing potassium t-butoxide is in agreement with the luminescence spectrum ($\lambda_{max.}$ 450 mμ ). In DMSO, VII gives light ($\lambda_{max.}$ 455 mμ ) without added base, but its emission spectrum is identical with the fluorescence spectrum of the pyrazine in DMSO containing -butoxide, and not that in a neutral solution.



(VII) (Anion of VII) (Excited state of VIII anion)

→ (Anion of VIII) + hν

## REFERENCES AND FOOTNOTES

1. Y. Kishi, T. Goto, Y. Hirata, O. Shimomura and F. H. Johnson, Bioluminescence in Progress, p. 89. Princeton Univ. Press, U.S.A. (1966); Tetrahedron Letters 3427 (1966).
2. F. H. Johnson, H.-D. Stachel, E. C. Taylor and O. Shimomura, Bioluminescence in Progress, p. 67 (1966).
3. Diglyme was purified by warming with sodium at 120° for 5 hrs. and then by distillation under reduced pressure. It was stored in a deep freezer (-20°). Small amounts of peroxide in the solvent, if present, do not affect chemiluminescence.
4. F. H. Johnson, O. Shimomura, Y. Saiga, L. C. Gershman, G. T. Reynolds and J. R. Waters, Cell. and Comp. Physiol. **60**, 85 (1962).
5. Y. Kishi, T. Goto, S. Inoue, S. Sugiura and H. Kishimoto, Tetrahedron Letters 3445 (1966).
6. F. McCapra and Y. C. Chang, Chem. Commun. 1011 (1967).
7. The structure of III was confirmed by analysis of its NMR spectrum. Thus, a $CH_2$ signal at $\delta$ 3.85 is coupled with an NH signal at $\delta$ 7.19 (J = 6 cps, in DMSO-$d_6$); this coupling being disappeared by addition of $D_2O$.
8. The amino form, instead of the corresponding imino form, was assigned by analysis of NMR and UV spectra of this and related compounds. This compound is weakly chemiluminescent.