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THE STRUCTURE CONFIRMATION OF THE LIGHT-EMITTING MOIETY OF BIOLUMINESCENT JELLYFISH AEQUOREA

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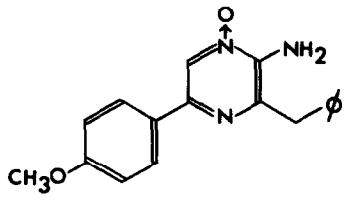
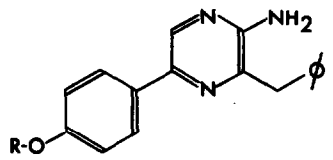
The Shimomura and Johnsons' extensive studies[1] on the bioluminescent jellyfish, Aequorea, revealed that Aequorea stands in a specific position among the numerous bioluminescent organisms. Namely, only the photoprotein, aequorin, and a trace amount of calcium or strontium ion are required for the luminescence in aqueous solution. Aequorin contains a single chromophore, which is functional in the light-emitting reaction, and has been separated from the protein and designated AF-350 from its uv absorption maximum at 350nm. Recently, Shimomura and Johnson[1] proposed the 2-aminopyrazine (5) as the structure of AF-350 mainly from the analysis of the spectroscopic data. For the further studies on the jellyfish bioluminescence, the structure confirmation of AF-350 is necessary. In this communication we would like to report a synthesis of the 2-aminopyrazine (5) and the identification of the synthesized material with AF-350.

p-Methoxyphenylglyoxal aldoxime (1)[2] was heated with α-amino-hydrocinnamonitrile (2)[3] in pyridine in the presence of titanium tetrachloride[4] at 80° for 2.5 hr, to yield 2-amino-3-



(3)



(4) R = CH_3

(5) R = H

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benzyl-5-(4'-methoxyphenyl)pyrazine 1-oxide (3)[5] in 72 % yield [yellow plates with mp 181-3°; ms 307, 291 and 276; λ_{max} in MeOH or MeOH-NaOH 253nm (log ε 4.18), 285 (4.42) and 361 (3.86) λ_{max} in MeOH-HCl 288 (4.43) and 364 (3.96); δ_{ppm} in $CDCl_3$ 3.89 (3H, s), 4.27 (2H, s), 5.4 (2H, broad), 6.99 (2H, AB, J=9), 7.31 (5H, s), 7.84 (2H, AB, J=9) and 8.37 (1H, s)]. The 2-aminopyrazine 1-oxide (3) was reduced to the corresponding 2-aminopyrazine (4) by a treatment with Raney nickel in ethanol under a hydrogen-atmosphere[6]. The 2-aminopyrazine (4)[5] was isolated as yellow needles with mp 153-4° in 92 % yield [ms 291, 276 and 249; λ_{max} in MeOH or MeOH-NaOH 280nm (log ε 4.36) and 348 (4.01) λ_{max} in MeOH-HCl 284 (4.40) and 374 (3.86); δ_{ppm} in $CDCl_3$ 3.84 (3H, s), 4.15 (2H, s), 4.4 (2H, broad), 6.94 (2H, AB, J=9), 7.24 (5H, s), 7.84 (2H, AB, J=9) and 8.29 (1H, s)]. Hydrolysis of the methoxy group in (4) was performed by a treatment with pyridine hydrochloride at 200° for 15 minutes. The product (5)[5] was isolated as yellow needles with mp 217-9° in 87 % yield (corrected yield: 93 %) [ms 277, 261 and 249; λ_{max} in 50% aq. EtOH 278nm (log ε 4.31) and 350 (3.96) λ_{max} in 50% aq. EtOH-HCl 283 (4.36) and 367 (3.82) λ_{max} in 50% aq. EtOH-NaOH 298 (4.34) and 373 (3.93); δ_{ppm} in DMSO-d_6 4.12 (2H, s), 6.23 (2H, s), 6.84 (2H, AB, J=9), 7.2 - 7.6 (5H), 7.81 (2H, AB, J=9), 8.38 (1H, s) and 9.56 (1H, s)].

2-Amino-3-benzyl-5-(4'-hydroxyphenyl)pyrazine (5) thus synthesized was identified with AF-350 obtained from the photoprotein, aequorin, by a comparison of spectroscopic data (ir in KBr disc, uv, nmr[7], ms, fluorescence[8] and pKa) and of tlc behavior.

We are indebted to Drs. Shimomura and Johnson, Princeton University, for their sending us the pre-print of the paper appeared in Biochemistry and the copies of spectroscopic data of AF-350 and also for their carrying out the identification of the synthesized material with AF-350 (tlc and fluorescence spectrum).

REFERENCES AND FOOTNOTES

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4. T. P. Karpetsky and E. H. White, J. Am. Chem. Soc., 93, 2333 (1971)
5. Satisfactory analytical and spectroscopic data were obtained on this compound.
6. E. Ochiai, "Aromatic Amine Oxide", Elsevier, New York, N. Y., page 184
7. The spectrum in $CDCl_3$ obtained by time-averaging of 64 scans [δ_{ppm} in $CDCl_3$ 4.16 (2H, s), 4.47 (2H, broad), 6.91 (2H, AB, J=9), 7.27 (5H, s), 7.83 (2H, AB, J=9) and 8.30 (1H, s)] was compared with the spectrum[1] of AF-350.
8. Fluorescence spectrum (λ_{max} in EtOH 434 ± 1nm, excited at 350nm) was recorded on an Aminco-Bowman Spectrophotofluorometer by Dr. Shimomura, Princeton University.