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FISH BIOLUMINESCENCE I. ISOLATION OF A LUMINESCENT SUBSTANCE FROM A MYCTOPHINA FISH, NEOSCOPELUS MICROCHIR, AND IDENTIFICATION OF IT AS OPLOPHORUS LUCIFERIN

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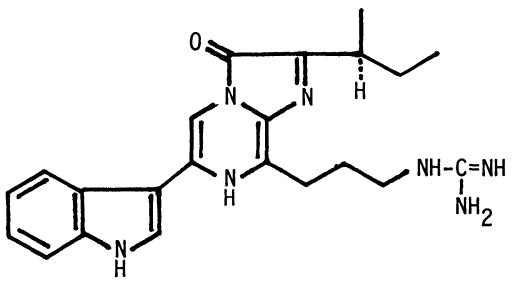
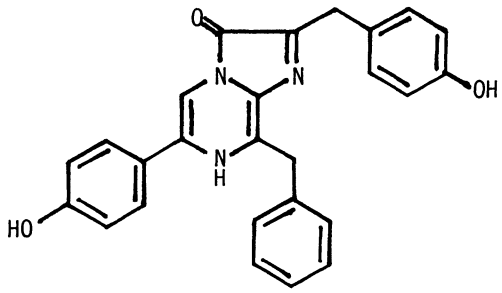
A luminescent substance was isolated from liver of N. microchir. It was proved to be identical with Oplophorus luciferin.

Bioluminescent fishes are divided into two groups:[1] one involves those luminescing by symbiosis of luminous bacteria, and the other by the luciferin-luciferase reaction with luminous substances. Johnson et al.[2] found that the bioluminescent fishes, Apogon ellioti and Parapriacanthus peryciformes store in their photophores Cypridina luciferin (I) which may be supplied from the crustacean Cypridina eaten as diet. Porichtys notatus (midshipman) also luminesces with the substance same as Cypridina luciferin (I).[3]

Most of the fishes belonged to Myctophina have photophores in their body. One of them, Diaphus elucens (Japanese name: suitō-hadaka) was recently reported by Tsuji and Haneda[4] to have luciferase which emits light with Cypridina luciferin (I), but luciferin itself has not been isolated. We wish to report here that one of the Myctophina fishes, Neoscopelus microchir Matsubara (Japanese name: sango-iwashi), contains in its liver not Cypridina luciferin (I), but a luminescent substance identical with Oplophorus luciferin (II).[5]



Cypridina luciferin (I)



Oplophorus luciferin (II)

Lyophylized livers (5.0 g) from 25 specimens of N. microchir were washed twice with oxygen-free dichloromethane and the residue was extracted thrice with oxygen-free methanol. The extracts were chromatographed on a Kiesel gel 60 column (1.5ϕ x 7 cm) using methanol-dichloro-

methane (1:7) as eluant. Yellow fractions were collected and evaporated to dryness to give a solid (4.8 mg), which was then purified by preparative TLC (Merck silica gel plate) using oxygen-free methanol-dichloromethane (1:10) as developing solvent. A yellow band at Rf 0.37 was eluted with methanol yielding a yellow substance (2.1 mg). This substance was proved to be identical with Oplophorus luciferin (II)[5] by the following evidence: (a) Rf values on the silica gel TLC using three different solvent systems [MeOH-CH_2Cl_2 (1:10) Rf 0.37, acetone-CH_2Cl_2 (1:10) Rf 0.09, and MeOH-benzene (1:5) Rf 0.29] were identical with synthetic II;[6] (b) mass spectrum gave an M^+ ion at m/e 423; (c) UV and NMR spectra were superimposable with those of II;[6] (d) the luminescence rate and spectrum (λ_{max} 455 nm) in the presence of the Oplophorus spinosus luciferase[5] were almost identical with those of II.

The luciferase, enzyme of luciferin-luciferase reaction, could not be extracted from N. microchir, but a closely related Myctophina fish, Diaphus elucens, gave the luciferase by extracting with phosphate buffer (pH 7.0). The latter fish, however, has almost no free II in its body (without photophores). These results indicate that Oplophorus luciferin (II) is possibly used for light emmission of these Myctophina fishes. It is not known whether the luminescent substance (II) comes from diet or is synthesized by the fishes.

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