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FISH BIOLUMINESCENCE II.[1] TRACE CHARACTERIZATION OF THE LUMINESCENCE SYSTEM OF A MYCTOPHINA FISH, DIAPHUS ELUCENS

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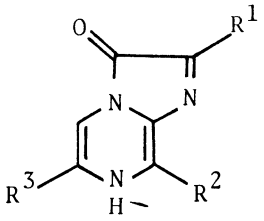
Oplophorus luciferin (Ia) was characterized in a pair of nasal photophores of a myctophina fish, Diaphus elucens. Liver of the fish contains luciferin in a bound form, possibly as its enol ether. A luciferase active toward O. luciferin was extracted from flesh of the fish. From these results it is concluded that the luminescence system of D. elucens is similar to that of Oplophorus and not to that of Cypridina.

Most myctophina fishes have photophores in their body. Among them Diaphus elucens (Japanese name: suitō-hadaka) has a pair of exceptionally large photophores (ca 10 x 3 mm) on both sides of the snout.[2] Tsuji and Haneda[2] reported that extracts of the photophores showed cross luciferin-luciferase (L-L) reaction[3] with Cypridina system and suggested that Diaphus elucens has a luminescence system similar to, if not identical, that of Cypridina. We have recently, however, isolated[1] a luminescent substance from liver of a myctophina fish, Neoscopelus microchir (Japanese name: sango-iwashi) and identified it as Oplophorus luciferin (Ia), although no luciferase could be extracted from the fish. This apparent contradiction between the myctophina fishes has stimulated us to reinvestigate the Diaphus luminescence system.

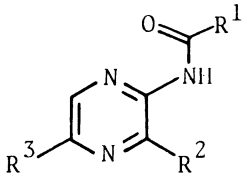
D. elucens is rarely found in nets for collecting a large amount of decapod shrimp, Sergia lucens, at Suruga Bay. We have extracted from a pair of the nasal photophores a luminescent substance and identified it as Oplophorus luciferin (Ia).[4] We have already obtained[1] from flesh of D. elucens a luciferase that is active toward the luciferin (Ia). Thus, it is established that D. elucens has a luciferin-luciferase system[3] similar to Oplophorus and not to Cypridina.

A pair of nasal photophores of D. elucens was lyophilized (dry weight 6.1 mg) and extracted with ether. The extract gave a blue fluorescent spot on thin layer chromatography (tlc) [Rf values: MeOH-CH_2Cl_2 (1:10) 0.52; MeOH-benzene (1:5) 0.34].[12]

The Rf values were identical with those of synthetic Oplophorus oxyluciferin[4] (=coelenteramide[5]) (IIa). The residue was further extracted with methanol. The extract clearly showed positive L-L reaction with Oplophorus luciferase, but negative with Cypridina luciferase. The methanol extract was concentrated in vacuo and developed on a tlc plate giving several fluorescent spots; one of which having yellow fluorescence gave the completely identical Rf values with that of Oplophorus luciferin (Ia) [MeOH-CH_2Cl_2 (1:10) 0.37; acetone-CH_2Cl_2 (1:1) 0.09; MeOH-benzene (1:5) 0.29].[12]



(I)



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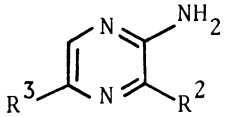
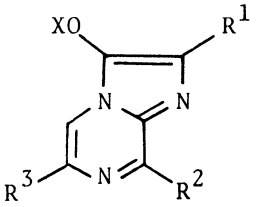


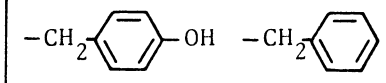
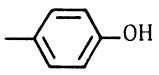
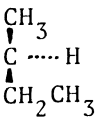
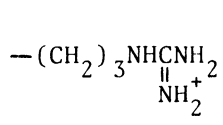
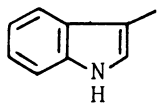
(III)



(IVa) X=SO_3H

(Va) X=unknown

side chains

	R^1	R^2	R^3
a			
b			

The spectrum (λ_{max} 455 nm) and the rate of bioluminescence of this yellow-fluorescent substance were same as those of O. luciferin (Ia) and the product of chemiluminescence in dimethyl sulfoxide was identical with O. oxyluciferin (IIa) [mass spec. m/e 411 (M^+)]. Two of other fluorescent spots were identified as O. oxyluciferin (IIa) and O. etioluciferin[4] (=coelenteramine[6]) (IIIa) [Rf values: MeOH-CH_2Cl_2 (1:10) (IIa) 0.52, (IIIa) 0.78; MeOH-benzene (1:5) (IIa) 0.34, (IIIa) 0.52; ether (IIa) 0.30, (IIIa) 0.55].[12]

No fluorescent spot corresponding to Cypridina luciferin (Ib),[7] C. oxyluciferin (IIb)[8] or C. etioluciferin (IIIb)[7] was detected. Total amounts of O. luciferin (Ia) in the pair of nasal photophores were determined to be ca 5 μg by measurement of total light yield in the L-L reaction with O. luciferase.[9] Standard light yield was obtained using synthetic O. luciferin.

Contrary to the case of N. microchir, liver of D. elucens contained no free O. luciferin (Ia), but strong luminescence was observed with O. luciferase on the extract of the liver with 70% methanol, when heated in 2% methanolic HCl at 80°C for 2 min and neutralized with sodium bicarbonate; indicating the presence of luciferin in a bound form (Va) similar to the luciferyl sulfate (IVa).[10] It could not be, however, hydrolyzed with methanolic 0.1M HCl at room temperature for 2 min nor with 1M NaOH, while luciferyl sulfate[10] and acetate[11] were easily hydrolyzed in the above conditions, respectively; Rf values of the bound luciferin on tlc were much smaller than that of the sulfate [Rf values: EtOH-AcOEt (1:8) (IVa) 0.40, (Va) ∿0.00; MeOH-CH_2Cl_2 (1:5) (IVa) 0.20, (Va) ∿0.00].[12] These results suggest that the bound luciferin (Va) is not the luciferyl sulfate, but may have an enol ether linkage to an unknown molecule with high polarity.

It is concluded that bioluminescence system of Diaphus elucens as well as Neoscopelus microchir is similar to that of Oplophorus and not to that of Cypridina.

It is, however, not known whether the luciferin comes from diet or is synthesized by the fish.

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12 For tlc analysis throughout this work Merck TLC aluminum sheet (silica gel 60 F_{254} pre-coated, layer thickness 0.2 mm, 5 x 20 cm) were used.

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