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THE LIGHT-EMITTER IN BIOLUMINESCENCE OF THE SEA CACTUS CAVERNULARIA OBESA

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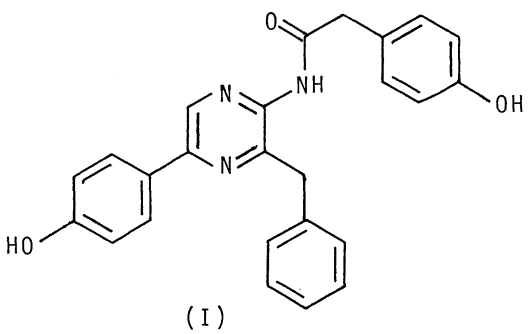
The structure of the light-emitter extracted from the sea cactus Cavernularia obesa was determined as 2-(p-hydroxyphenylacetamido)-3-benzyl-5-(p-hydroxyphenyl)pyrazine. This result strongly suggests the structure of coelenterate luciferyl sulfate.

It was reported that the components required for luminescence in a number of bioluminescent coelenterates such as Aequorea, Renilla, Cavernularia, Ptilosarcus, Stylatula, Acanthoptilum, Parazoanthus and Mnemiopsis are similar.[1] Most of them have luciferin-luciferase system —— luciferyl sulfate, luciferase, and luciferin sulfokinase —— as well as photoprotein system, except Aequorea which contains only photoprotein, aequorin.[2,3] It has been suggested, but not proved, that light-emitter in both systems may be identical.[1] The light-emitter of Aequorea photoprotein, aequorin, was elucidated as I.[2] The luciferin-luciferase system has been extensively studied in the case of Renilla, but structure of the luciferyl sulfate has been elucidated only partially as II (R = unknown; possibly a similar group to benzyl).[4] We found that the bioluminescence of the sea cactus, Cavernularia obesa, also involved both compounds, luciferyl sulfate and photoprotein, and the both systems afforded the same light-emitter I. This result strongly indicates that Cavernularia luciferyl sulfate, and possibly other coelenterate luciferyl sulfate, have the structure II in which R is p-hydroxybenzyl group.

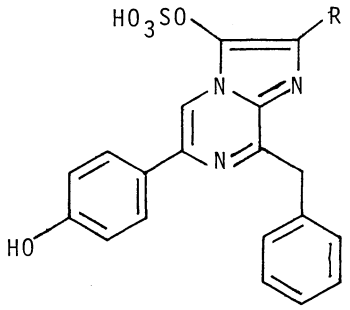
Three specimens of Cavernularia in a contracted state were cut in slices 3 mm thick, and stimulated to a bright luminescence by putting into 100 ml of 0.8M KCl containing 0.01M $CaCl_2$. The slices in this solution were repeatedly pressed and squeezed until the luminescence finally died away, and the whole preparation was mixed with 500 ml of methanol and then filtered. The filtrate was evaporated to 100 ml of aqueous solution, extracted with ether, and the ether layer was re-extracted with 0.025N NaOH. The alkaline aqueous layer, after washing 4 times with ether, was neutralized with CO_2 and extracted with ether. The ether extract contained a blue fluorescent compound which was further purified by two successive tlc (silica gel, water-saturated ether). The purified compound was indistinguishable from synthetic I[2] as evidenced by the behavior in tlc, absorption spectra (λ_{max} in methanol 277, 292 and 333 nm; in water 272 and 330 nm; in 0.01N NaOH 302 and 364 nm) and mass spectrum

(m/e 411, 304 and 277), thus leading to the conclusion that this fluorescent compound has the structure of I. The yield was roughly 5 μg per large specimen of Cavernularia based on the absorbance of synthetic I.

When slices of specimen were extracted with methanol omitting pretreatment with KCl solution to elicite luminescence, this methanol extract was capable of chemiluminescence[5] in dimethylsulfoxide or in dimethylformamide after acid-treatment of the sample, indicating that this extract contained a luciferyl sulfate.[1] Although the methanol extract under these conditions did not afford a detectable amount of I after purification by the same method as described above, the spent solution of chemiluminescence, carried out in dimethylformamide, afforded compound I (ca. 1 μg per large specimen), which is obviously the product of chemiluminescence.[6] In despite of the presence of two types of bioluminescent systems in Cavernularia —— photoprotein and luciferin-luciferase —— no other comopund similar to I was found in any of the extracts mentioned above.



(I)



(II)

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REFERENCES

1) M. J. Cormier, K. Hori, Y. D. Karkhanis, J. M. Anderson, J. E. Wampler, J. G. Morin, and J. W. Hastings, J. Cell. Physiol., 81, 291 (1973).
2) O. Shimomura and F. H. Johnson, Tetrahedron Letters, 2963 (1973).
3) O. Shimomura and F. H. Johnson, Biochemistry, 13, 3278 (1974).
4) K. Hori, J. E. Wampler, J. C. Matthews, and M. J. Cormier, Biochemistry, 12, 4463 (1973).
5) K. Hori, J. E. Wampler, and M. J. Cormier, Chem. Commun., 492 (1973).
6) As expected from the mechanism,[3] a trace of 2-amino-3-benzyl-5-(p-hydroxylphenyl)pyrazine was also isolated from the medium.

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