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COELENTRATE BIOLUMINESCENCE III.[1] TRACE CHARACTERIZATION OF LUMINESCENCE SUBSTANCES OF CAVERNULARIA OBESA AND PTILOSARCUS GUERNEYI

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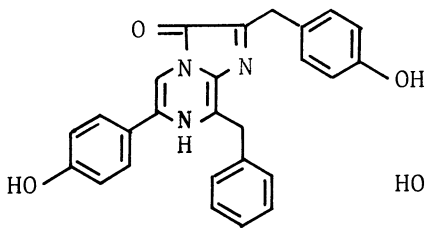
Trace amount of luminescent substances of Cavernularia obesa and Ptilosarcus guerneyi were characterized to be Renilla luciferin and luciferyl sulfate using 100 g of C. obesa in quiescent state and 200 g of P. guerneyi in frozen state.

It has been suggested that the anthozoan coelenterates such as Renilla, Cavernularia etc., have similar type of luminescent systems.[2] In the previous paper,[1] we reported isolation of Renilla luciferin and luciferyl sulfate from frozen specimens of Renilla mülleri and determined their structure to be I and II, respectively. In the cace of Cavernularia obesa we could not, however, obtain its luciferin from frozen specimens. When we put the live specimens into 0.8M KCl solution containing 0.01M $CaCl_2$, bright luminescence was observed. After the luminescence ceased, we could isolate from the solution coelenteramide (III), the possible light emitter (ca 5 μg per a large specimen),[3] suggesting that C. obesa may contain a luminescence system similar to Renilla. The probable reason of difficulty in obtaining the luciferin from the frozen specimens is that it may be largely consumed during storage in dry ice and/or extraction with methanol.

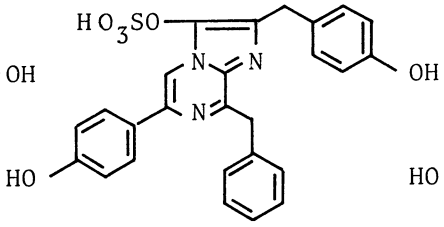
Indeed, during quick freezing in powdered dry ice the live animals gave strong luminescence. We found, however, that the live animals could be kept in quiescent state by putting them in Tris buffer containing $MgCl_2$, Using such quiescent specimens we have succeeded in isolating the luciferin (I) and luciferyl sulfate (II) by extraction with methanol.

Live specimens of sea cactus, C. obesa (200-600 g per specimen), were put on filter-paper undisturbed when they shrunk with discharge of sea water to 20-40 g body weight (contents of sea water are variable according to individuals and biorhythm). The shrunk specimens were soaked undisturbed for about 20 min in chilled Tris buffer (pH 7.2 at ca 10°C) containing 0.3M $MgCl_2$, 0.02M EDTA, and 0.04M NaCl.

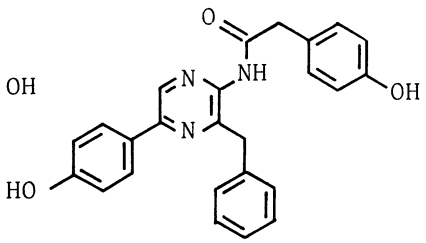
Three specimens (ca 100g) of live animals in quiescent state thus obtained were sliced (5-10 mm) and put into chilled methanol (-20°C), and the mixture was homogenized immediately (15,000 rpm, 3 min) and filtered. After repeating of this extraction, the combined extracts (ca 200 ml) were evaporated in vacuo below 20°C to dryness. The residue was extracted with methanol-CH_2Cl_2 (1:7) and the extract chromatographed on a silica gel column using the same solvent system into three fractions, A, B, and C [fraction C was obtained by elution with MeOH-CH_2Cl_2 (1:3)].



(I)



(II)



(III)

Fraction A contained a trace of oxyluciferin (III) as reported previously.[3] Fraction B gave a yellow fluorescent substance (ca 5 μg) by preparative tlc first using MeOH-CH_2Cl_2 (1:10) and then MeOH-benzene (1:7) as solvent.[6] The substance showed positive cross luciferin-luciferase (L-L) reaction with Renilla luciferase and Oplophorus luciferase,[4] and identical Rf values with synthetic Renilla luciferin (I)[1,5] on tlc[6] using the following solvent systems: MeOH-CH_2Cl_2 (1:10) Rf 0.37; MeOH-benzene (1:5) Rf 0.29; and acetone-CH_2Cl_2 (1:1) Rf 0.09; as well as identical UV spectra with that of I. Further evidence was obtained by adding it in dimethyl sulfoxide, when chemiluminescence was observed[5] and from the spent solution was isolated coelenteramide (III) [m/e 411 (M^+), UV λ_{max}^{MeOH} 277, 292, and 333 nm].[3] Fraction C gave a blue fluorescent substance by preparative tlc separations using

MeOH-CH_2Cl_2 (1:5) and ethanol-ethyl acetate (1:8) as solvent.[6] The fluorescent substance showed UV absorption at 271 nm (in water at pH 7.5) and Rf values on tlc using several solvent systems coincided with synthetic Renilla luciferyl sulfate.[1] When treated with 1% HCl-MeOH at room temp. for 2 min and purified by the similar procedure used for fraction B, the fluorescent substance afforded Renilla luciferin (I) [m/e 423 (M^+); UV spectrum was identical with that of synthetic I].

The above experiments established that the luminescent system in C. obesa involves Renilla luciferin (I) and luciferyl sulfate (II) same as in Renilla.

Sea pen, Ptilosarcus guerneyi, showed positive cross L-L reaction with Renilla and with Cavernularia, suggesting that the luminescent system of P. guerneyi is similar to that of Renilla and Cavernularia. Extraction of frozen P. guerneyi (200 g) by the same procedure used for extraction of Renilla[1] gave Renilla luciferin (I) and luciferyl sulfate (II), which were identified similarly as described above.

Quantities of luciferin (I) and luciferyl sulfate (II) in the three coelenterate, Renilla mülleri, Cavernularia obesa and Ptilosarcus guerneyi, were determined as shown in Table 1 by the L-L reaction with Oplophorus luciferase.[4]

Table 1. Contents of Renilla luciferin and luciferyl sulfate in anthozoa

Animal	Luciferin (I)[a] (in 100 g of animal)	Luciferyl sulfate (II)[b]
Renilla mülleri (frozen)	5 μg	14 μg
Cavernularia obesa (live)	15 μg	12 μg
Ptilosarcus guerneyi (frozen)	9 μg	6 μg

(a) The specimens were extracted with methanol (20 times by v/w) below 0°C and the extract concentrated in vacuo below 20°C to one third volume. The concentrate (10 μl) was transfered into 3 ml of solution of Oplophorus luciferase dissolved in 10 mM Tris-HCl buffer (pH 8.3) containing 50 mM NaCl. The concentration of the luciferase was so adjusted that the luminescence reaction finished within 1 min. Synthetic Renilla luciferin (I) (=Watasenia preluciferin)[5] was used as standard for light yield.

(b) To 10 μl of the concentrate described above was added 3 drops of 1% HCl and the mixture was heated in a boiling water-bath for 1 min under argon atmosphere

to hydrolyze the luciferyl sulfate. The solution was cooled immediately, neutralized with sodium bicarbonate solution and determined its luminescence activity as described in (a). Contents of luciferyl sulfate (II) was calculated by subtracting the luciferin contents in (a) from the value obtained after acid hydrolysis.

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REFERENCES AND FOOTNOTE

1. Preceding paper; S. Inoue, H. Kakoi, M. Murata, T. Goto and O. Shimomura, Tetrahedron Lett., 2685 (1977).
2. 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).
3. O. Shimomura, S. Inoue and T. Goto, Chem. Lett., 247 (1975).
4. O. Shimomura, T. Masugi, F. H. Johnson and Y. Haneda, Biochem., 17, 994 (1978).
5. S. Inoue, S. Sugiura, H. Kakoi, K. Hashizume, T. Goto and H. Iio, Chem. Lett., 141 (1975).
6. For tlc analysis throughout this work,Merck TLC aluminum sheets (silica gel 60 F_{254} pre-coated, layer thickness 0.2 mm, 5 x 20 cm) were used. For preparative tlc,glass plates (20 x 20 cm) coated with silica gel (Merck 60 F_{254}, layer thickness 0.25, 0.5, or 2mm) were used.

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