Tetrahedron Letters No.24, pp. 2847-2850, 1968. Pergamon Press. Printed in Great Britain.

LUCIFERIN AND LUCIOPTERIN ISOLATED FROM THE JAPANESE FIREFLY, LUCIOLA CRUCIATA

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(Received in Japan 21 February 1968; received in UK for publication 14 March 1968)

Firefly luciferin, a bioluminescent substrate, was first isolated from Photinus pyralis by Bitler and McElroy (1) and characterized as I by White et al. (2). Although the various species of American and Jamaican fireflies differ in the color of emitted light, ranging from green to yellow (λ_{max} 552-582 mμ), all the species examined (3), including Photinus, Photuris, Pyrophorus, Diphotus, and Lecontea, contain the same luciferin. This paper reports the isolation and characterization of luciferin and a fluorescent substance from the Japanese firefly, Luciola cruciata (Japanese name: genji-botaru), which emits light of maximum intensity at a shorter wave length (λ_{max} 544 mμ) than any of those mentioned above (4).

Acetone-dried and pulverized abdomens (233 g) of Luciola cruciata (12,000 individuals) were extracted with hot water. The aqueous extract was acidified with hydrochloric acid and extracted with ethyl acetate. The combined extracts were concentrated and the residue was chromatographed on cellulose powder using EtOAc-EtOH-H_2O (5:2:3) as the eluting solvent, resulting in a

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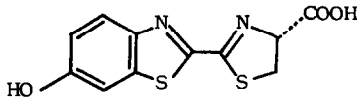
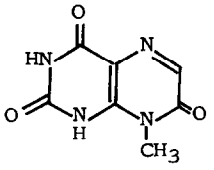
green-fluorescent fraction and a blue-fluorescent fraction. The former fraction was found to contain two fluorescent substances, made evident by paper chromatography; 1M NH_4OAc-95% EtOH (3:7) system: Rf 0.69 (major component, luciferin) and 0.41 (minor component), C_6H_6-MeOH-iso-AmOH-4% NH_4OH (35:35:17.5:12.5) system: Rf 0.23 (major) and 0.16 (minor). The mixture was further chromatographed on a DEAE-cellulose column, with gradient elution by aqueous sodium chloride, and the eluted major fluorescent fraction was acidified with hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract was concentrated and the residue was crystallized from methanol to give crystalline luciferin (5.5 mg), which was shown to be identical to the (synthetic) luciferin of other fireflies (2) by IR (ν^{KBr} 1702 cm^{-1})(5), UV, and mass spectral comparisons and ppc (6). By reaction with luciferase obtained from Luciola cruciata, no light and about a half of light yield of the luciferin obtained above were observed with synthetic L- and DL-luciferin, respectively, indicating that the luciferin obtained from Luciola has the D-configuration (formula I) identical with those from other fireflies.



(I)



(II)

The minor fluorescent component separated by the DEAE-cellulose chromatography shows a positive ninhydrin test and UV absorption characteristic of the 5-hydroxybenzothiazole moiety (λ_{max}^{MeOH} 322 mμ, $\lambda_{max}^{MeOH-NaOH}$ 378 mμ). It might be a precursor of luciferin or a product of the luminescence reaction.

The blue-fluorescent fraction from the first chromatography was adsorbed on

a Florisil column and eluted with water. Repetition of this chromatography (7) finally afforded fine white needles (salt of the component), which on treatment with dilute hydrochloric acid gave a strongly fluorescent compound, herein named luciopterin, as pale yellow micro-crystals, m.p. above 300°, $C_7H_6N_4O_3 \cdot 1/2H_2O$ (8), m/e 194 (mass spectr.); pKa' 3.69 and ca 12 in water (both acidic); $\lambda_{max}^{H_2O}$ (pH 1.5) 278 mμ (log ε 4.07), 330 (4.08), $\lambda_{max}^{H_2O}$ (pH 5.8) 253 (3.69), 288 (3.99), 350 (4.12), $\lambda_{max}^{H_2O}$ (pH 14) 261 (4.04), 365 (4.15); NMR (in D_2O, pH 6, ppm from int. DSS) 3.44 (3H, singlet), 7.55 (1H, singlet). These physical data suggest that luciopterin is one of the methyltrioxopteridines. It was identified by comparison with an authentic sample (9) as 8-methyl-1,2,3,4,7,8-hexahydro-2,4,7-trioxopteridine (II). Although this pteridine was synthesized in 1957, it has not been isolated from natural sources. Strehler (10) isolated from American fireflies a fluorescent substance, luciferesceine, but its properties differ from those of luciopterin, indicating non-identity between them.

The authors are grateful to professor Y. Hirata for his interest in this work. One of us (O.S.) wishes to thank the Japan Society for the Promotion of Science for support of this work.

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2. E. H. White, F. McCapra, G. F. Field and M. D. McElroy, J. Amer. Chem. Soc. 83, 2402 (1961); E. H. White, F. McCapra and G. F. Field, ibid. 85, 337 (1963).
3. H. H. Seliger and M. D. McElroy, Proc. Natl. Acad. Sci. U. S. 52, 75 (1964).
4. A part of this paper was reported at the Symposium on Bioluminescence in the Pacific Area of the 11th Pacific Science Congress, Tokyo, August, 1966.

5. The IR spectrum also shows a small peak at 1739 cm^{-1} (10-15% of intensity of the peak at 1702 cm^{-1}) attributable to DL-luciferin.
6. It is of interest to note that the same luciferin (1.5 mg and 0.5 mg, respectively) was also obtained from the thorax (850 g) and from the head (70 g). However, since the fireflies used were killed in dry ice-acetone mixture, whether or not the thorax and the head of live fireflies contained luciferin must await further investigation.
7. Non-fluorescent substance isolated from the eluate was identified as uric acid.
8. Satisfactory analysis was obtained.

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