Tetrahedron Letters No. 22, pp. 2021-2024, 1971. Pergamon Press. Printed in Great Britain.

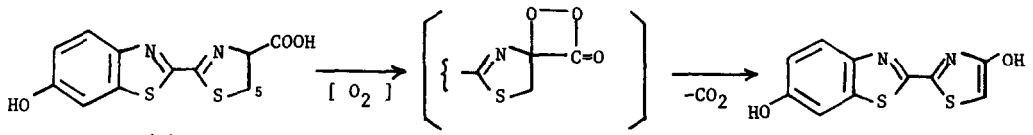
FIREFLY BIOLUMINESCENCE II. IDENTIFICATION OF 2-(6'-HYDROXYBENZOTHIAZOL-2'-YL)-4-HYDROXYTHIAZOLE AS A PRODUCT IN THE BIOLUMINESCENCE OF FIREFLY LANTERNS AND AS A PRODUCT IN THE CHEMILUMINESCENCE OF FIREFLY LUCIFERIN IN DMSO

Nobutaka Suzuki and Toshio Goto

Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan

(Received Japan 2 April 1971; received in UK for publication 30 April 1971)

2-(6'-Hydroxybenzothiazol-2'-yl)-4-hydroxythiazole (firefly oxyluciferin) (III) has been suggested as the emitter in the firefly bioluminescence and also in the chemiluminescence of firefly luciferin (I) in dimethyl sulfoxide (DMSO) from the consideration of the proposed reaction mechanism, which involves dioxetane intermediate II, and from an analogy with the product of chemiluminescence reaction of 5,5-dimethylluciferin derivatives of I (1-4). However, attempted isolation of the expected product (III) in the chemiluminescence reaction (1,3,4) and in the in-vitro bioluminescence of firefly luciferin (5) by White et al. has been unsuccessful; they have recognized, instead, on the chromatograms of Sephadex LH 20 or silica gel three distinct compounds designated as A, B, and C, but none of them corresponded to III.



(I) (II) (III)

They assumed that oxyluciferin (III) was first formed as a direct product of the bioluminescence reaction but it was too unstable to be isolated and was rapidly converted into the three compounds. Recently, DeLuca and Dempsey (6) reported that analysis of ^{18}O content in CO_2 formed during the in-vitro bioluminescence of I conducted in $^{18}O_2$ and $H_2{}^{18}O$ gave results which contradict the mechanism involving dioxetane II. To clarify the reaction mechanism it is highly desirable to isolate and identify the product other than CO_2.

In the previous paper (7) we reported a successful synthesis of a compound having the structure corresponding to III and coincidence of its fluorescence spectrum with the chemiluminescence spectrum of luciferin. We now wish to report that III is indeed produced during the in-vivo bioluminescence of firefly and also during the chemiluminescence reaction of firefly luciferin (I) in DMSO containing t-butoxide.

Isolation of oxyluciferin (III) from a spent solution of chemiluminescence of luciferin (I).

Pottasium t-butoxide (BuOH soln.) was added to a solution of L-luciferin (optical antipode of natural luciferin) in DMSO (light produced) and the mixture was evaporated in vacuo. Tlc (Avicel; MeOH-H_2O 1:1) of the residue gave a blue fluorescent spot (Rf 0.10) identical in Rf and color of fluorescence with those of synthetic III. After elution with methanol, the spot showed uv spectrum identical with those of III (Fig. 1). Incidentally, without addition of t-butoxide light was not produced and III was not detected. Further characterization was carried out as follows: the reaction mixture of the above chemiluminescence was treated with pyridine and acetic anhydride and the solution evaporated in vacuo. Ether extracts of the residue were subjected to tlc separations on silica gel (Et_2O-CH_2Cl_2 10:1) and then four times on Avicel (MeOH-H_2O 4:5). The product thus obtained showed uv (Fig. 2) and mass spectra identical with those of oxyluciferin diacetate (IV) (8).

The above product : m/e 334 (15%) (M^+), 292 (47), 250 (100), 177 (62)

Oxyluciferin diacetate (IV): m/e 334 (14%) (M^+), 292 (50), 250 (100), 177 (55)

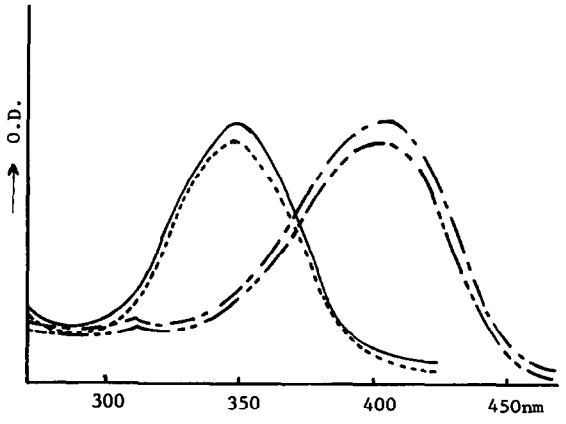


Fig. 1. Uv spectra of oxyluciferin (III)

From chemilumi.: ——— (MeOH); — - — (MeOH-KOH)

Synthetic: ----- (MeOH); — -- — (MeOH-KOH)

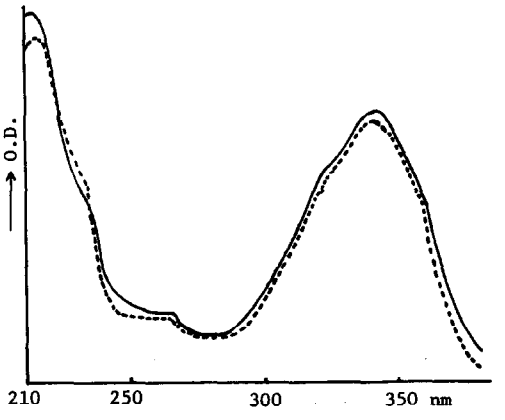


Fig. 2. Uv spectra of oxyluciferin diacetate

From chemilumi.: ——— (MeOH)

Synthetic: ----- (MeOH)

Evidence for the formation of oxyluciferin (III) during in-vivo bioluminescence of firefly.

Live fireflies (Luciola cruciata) (9) were killed by dipping them in powdered dry ice and stored at -20°. Seven individuals of the firefly were allowed to stand at room temp. to produce in-vivo bioluminescence. After the light emission ceased (ca 15 min.) the lanterns were cut off and extracted with methanol containing dry ice. Tlc of the methanol extracts on Avicel gave the spots shown in Table 1 (after bioluminescence). On the other hand, the same experiment was done without allowing bioluminescence (before bioluminescence) for comparison. Identification was made by using the following three solvent mixtures as the developing solvents, and by comparisons in Rf and color of fluorescence with the authentic specimens.

(a) MeOH-H_2O (1:1) (b) n-BuOH-HOAc-H_2O (4:1:4) (c) 95% EtOH-1N NH_4OAc (7:3)

Table 1

		Luciferin (I)	Oxyluciferin (III)	Dehydroluciferin	Luciopterin(9)
Rf and color of	a	0.80 Y	0.10 B	0.57 Y	0.78 V
fluorescence*	b	0.68 Y	0.60 B	0.45 Y	0.09 V
(365 nm lamp)	c	0.55 YG	0.50 YG	0.23 YG	0.29 V
before biolumines.		++	-	±	++
after biolumines.**		++	+	±	++

Y: yellow YG: yellowish green B: blue V: violet

* I, III, and dehydroluciferin have similar fluorescence intensity on the tlc.

** One or two unidentified fluorescent spots, though being very weak (±), were also detected.

Table 1 indicates the formation of oxyluciferin (III) during the in-vivo bioluminescence.

Attempted isolation of III directly from firefly lanterns was unsuccessful since repeated tlc of the oxyluciferin fractions led to the decomposition of III. Hence, the isolation was done after treatment with pyridine and acetic anhydride. Thus, after allowing to stand at room temp. for 15 min. the firefly lanterns (100 indiv.) were ground in a mortor with pyridine and acetic anhydride under cooling with dry ice. The resulted slurry was allowed to stand at room temp. for 1.5 hr and then evaporated in vacuo. The residue was extracted with ether and the extracts were subjected to tlc separations on Avicel (MeOH-H_2O 1:1; Rf 0.70) and then on silica gel (Et_2O-CH_2Cl_2 10:1; Rf 0.84; repeated three times). The oxyluciferin fractions showed uv spectrum identical with that of authentic oxyluciferin (III) (Fig. 3).

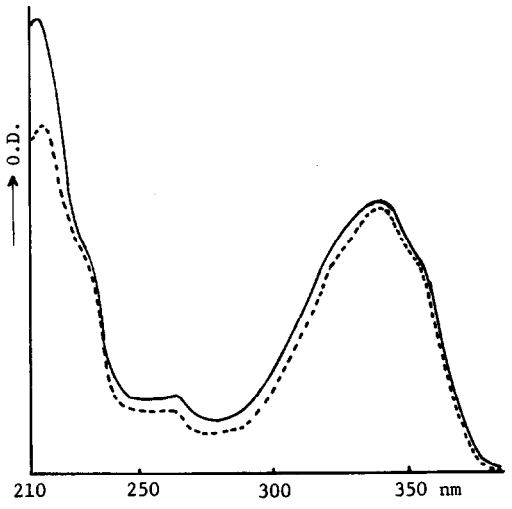


Fig. 3.

Uv spectra of oxyluciferin diacetate (IV)

From biolumi.: ——— (MeOH)

Synthetic: ----- (MeOH)

REFERENCES AND FOOTNOTE

1) T. A. Hopkins, H. H. Seliger, E. H. White and M. W. Cass, J. Amer. Chem. Soc., 89, 7148 (1967).

2) F. McCapra, Y. C. Chang and V. P. Francois, Chem. Commun., 22 (1968).

3) E. H. White, E. Rapoport, T. A. Hopkins and H. H. Seliger, J. Amer. Chem. Soc., 91, 2178 (1969).

4) W. D. McElroy, H. H. Seliger and E. H. White, Photochem. Photobiol., 10, 153 (1969).

5) P. J. Plant, E. H. White and W. D. McElroy, Biochem. Biophyl. Res. Commun., 31, 98 (1968).

6) M. DeLuca and M. E. Dempsey, Biochem. Biophys. Res. Commun., 40, 117 (1970).

7) Firefly bioluminescence I. N. Suzuki, M. Sato, K. Nishikawa and T. Goto, Tetr. Lett., 4683 (1969).

8) Oxyluciferin diacetate (IV) : oxyluciferin (III) (7) was acetylated with acetic anhydride and pyridine under nitrogen atm. to give yellow leaflets, mp 178-181° (from methanol). Found: C, 50.30; H, 2.78; N, 8.10. $C_{14}H_{10}N_2O_4S_2$ requires: C, 50.29; H, 3.01; N, 8.38%. Ir: ν(KBr) 1760 cm^{-1}; uv: λ(MeOH) 341 nm (ε 19600); nmr:δ in ppm ($CDCl_3$) 2.36 (3H, s), 2.39 (3H, s), 7.27 (1H, dd; J=2.5 and 9.0 Hz), 7.35 (1H, s), 7.73 (1H, d; J=2.5 Hz), 8.08 (1H, d; J=9.0 Hz); ms: m/e 334 (M^+).

9) Y. Kishi, S. Matsuura, S. Inoue, O. Shimomura and T. Goto, Tetr. Lett., 2847 (1968).