Tetrahedron Letters No.53, pp. 4683-4684, 1969. Pergamon Press. Printed in Great Britain

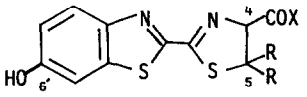
SYNTHESIS AND SPECTRAL PROPERTIES OF 2-(6'-HYDROXYBENZOTHIAZOL-2'-YL)-4-HYDROXYTHIAZOLE, A POSSIBLE EMITTING SPECIES IN THE FIREFLY BIOLUMINESCENCE

Nobutaka Suzuki, Masamitsu Sato, Kunisuke Nishikawa and Toshio Goto
Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan

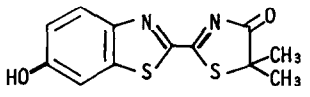
(Received in Japan 22nd September 1969; received in UK for publication 14th October 1969)

Firefly bioluminescence is produced by the enzymatic oxidation of firefly luciferyl adenylate (I) with molecular oxygen. Color of the normal firefly bioluminescence (in vitro at pH 7.6) is yellow-green (λ_{max} 565 nm with Photinus pyralis luciferase) (1), but under acidic conditions (pH 5.4) a red bioluminescence (λ_{max} 620 nm) is observed (2). Attempted isolation or identification of the reaction product(s), however, has been unsuccessful (3). McCapra et al. (4) reported that phenyl ester of 5,5-dimethylluciferin (II) in dimethyl sulfoxide (DMSO) with a base gave a red chemiluminescence (λ_{max} 605 nm) and that the emitter could be III, which was isolated from the reaction mixture. The same red chemiluminescence (λ_{max} 629 nm) was observed by the group of Seliger and White (5) with luciferyl adenylate (I), as well as 5,5-dimethyl-luciferyl adenylate (IV). They also made III by condensation of ethyl α-mercaptoisobutyrate with 2-cyano-6-hydroxybenzothiazole (V) and found that the fluorescence emission spectrum of the anion of III in DMSO is identical with the red chemiluminescence; thus confirming the emitter of the red color as the excited state of the monoanion of III.

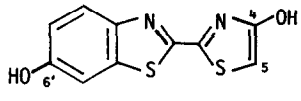
They further reported (6) that color of the chemiluminescence of luciferin derivatives is dependent to the base concentration; the phenyl ester of luciferin (VI), when treated with small amounts of potassium t-butoxide in DMSO, yields the red chemiluminescence and large amounts of base lead to a strong yellow-green emission (λ_{max} 555 nm, FWHM 2050 cm^{-1} ± 10%) (7). These two different emissions correspond closely to the two colors of bioluminescence, and they attributed the emitter of the yellow-green luminescence to the excited state of the dianion of VII. No



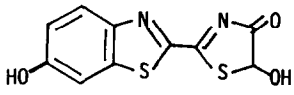
(I) R = H X = AMP
(II) R = CH_3 X = $-OC_6H_5$
(IV) R = CH_3 X = AMP
(VI) R = H X = $-OC_6H_5$



(III)



(VII)



(VIII)

report, however, has appeared in which isolation or synthesis of the proposed emitter (VII) is described. Derivatives of VII, such as 5,5-dimethyl (III), 5-methyl, 6'-O-methyl, and 6'-O-acetyl derivatives of VII (8), could be synthesized from the appropriate starting materials as described above without any special precautions (in ca 50% aq MeOH at pH ca 8 for a few hrs at room temp), but attempted condensation of ethyl thioglycolate and V under the similar condition

did not give the desired compound (VII), but a different compound (VIII) (9) containing one more oxygen atom in the molecule than the expected molecular formula. Physical data suggest that this compound has structure VIII. The desired compound (VII) (10) could be obtained in a good yield by extremely shortening the reaction time and lowering the reaction temperature (under ice-cooling for ca 2 min). The IR and NMR spectra of VII indicate the enol form, rather than the keto form.

Mixing of a DMSO solution of VII with a DMSO solution of potassium t-butoxide under vacuum ($1x10^{-5}$ mm Hg) gave a red solution, whose fluorescence spectrum showed its maximum at 557 ± 3 nm (FWHM 2200 cm^{-1} ± 10%), which is consistent with the maximum of the yellow-green chemiluminescence spectrum, confirming the emitter as the dianion of VII. When mixing is done in the presence of air, the resulting solution is yellow and shows a fluorescence maximum at 496 ± 3 nm. Fluorescence spectrum of VII in glycylglycine buffer (pH 7.6) has its maximum at 520 nm; addition of Luciola (Japanese firefly) luciferase (11), AMP, and/or Mg^{++}, in a preliminary experiment, however, did not change the spectrum. Further experiments are in progress.

REFERENCES AND FOOTNOTES

1. Different species of fireflies emit different colors of bioluminescence ranging from green to bright yellow (552 - 582 nm); H. H. Seliger and W. D. McElroy, Proc. Natl. Acad. Sci. U. S., 52, 75 (1964).
2. H. H. Seliger and W. D. McElroy, Arch. Biochem. Biophys., 88, 136 (1960)
3. H. H. Seliger and W. D. McElroy in F. H. Johnson and Y. Haneda Ed. "Bioluminescence in Progress", Princeton Univ. Press, Princeton, N. J. 1966.
4. F. McCapra, Y. C. Chang, V. P. Francois, Chem. Commun., 22 (1968).
5. T. A. Hopkins, H. H. Seliger, E. H. White and M. W. Cass, J. Amer. Chem. Soc., 89, 7148 (1967).
6. E. H. White, E. Rapoport, T. A. Hopkins and H. H. Seliger, J. Amer. Chem. Soc., 91, 2178 (1969).
7. FWHM = full band width between half-maximum intensity points of the spectrum.
8. 5-Methyl VII, m.p. 276-84° (dec.); 6'-O-methyl VII, m.p. 174-177°; 6'-O-acetyl VII, m.p. 195-198°; all compounds are in the enol form in DMSO. Satisfactory analyses were obtained for all compounds.
9. VIII: m.p. 192-3° (dec.); anal. found: C, 45.12; H, 2,31; N, 10.52 %; calcd. for $C_{10}H_6N_2O_3S_2$: C, 45.10; H, 2.27; N, 10.52 %; IR ν_{max} 1680 cm^{-1}; UV λ_{max} (MeOH) 388 nm (ε 6150), 305 (7125), 296 (8130), 286 (8130); (MeOH-KOH) 437 (8750), 322 (10000); (MeOH-HCl) 382 (9125), 305 (7125), 296 (8250), 282 (8630); fluorescence in DMSO containing t-BuOK: initially at 445 nm and finally at 495 nm.
10. VII: m.p. 169-171° (dec.); mass spec. m/e 250 (M^+); anal. found: C, 46.25, 46.52; H, 2.63, 2.63; N, 10.51, 10.46 %; calcd. for $C_{10}H_6N_2O_2S_2 \cdot 1/2H_2O$: C, 46.32; H, 2.72; N, 10.80; NMR (in DMSO-d_6, in ppm from int. TMS) 6.52 (1H, s), 7.05 (1H, q, J=2.6 and 8.8 Hz), 7.45 (1H, d, J=2.6 Hz), 7.90 (1H, d, J=8.8 Hz), 10.02 (1H, s), 10.95 (1H, s), the latter two signals disappear when D_2O is added; IR ν_{max} 1605, 1560 cm^{-1}; UV λ_{max} (MeOH) 371 nm (ε 18900); (MeOH-HCl) 371 (18000); (MeOH-KOH) 425 (18900); (pH 4) 372 (15500); (pH 7) 382 (11500); (pH 9) 420 (11700); (0.1N KOH) 427 (21300); (DMSO) 377 (20000); (DMSO-t-BuOK) 490 and 570 (10300 and 4800).
11. Y. Kishi, S. Matsuura, S. Inoue, O. Shimomura and T. Goto, Tetr. Lett. 2847 (1968).