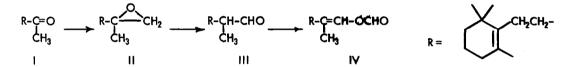
SYNTHESIS OF LATIA LUCIFERIN

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The novel enol formate formula IV has been proposed recently¹ as the constitution of <u>Latia</u> luciferin, the specific substrate in the bioluminescence enzyme (luciferase) system of the fresh water limpet <u>Latia neritoides</u>. By procedures summarized below, this structure has now been confirmed by synthesis, constituting the third example of the synthesis of a luciferin extracted from a luminescent organism, the other two being those of the firefly² and the ostracod crustacean Cypridina³. A possible mechanism for the Latia system has been suggested ⁴.



Dihydro- β -ionone (1)⁵ was added dropwise to dimethylaxosulfonium methylide⁶, both in dimethylsulfaxide, stirring 18 hr at 25°, then heating 1 hr at 50°. Chromatography of the product on silicic acid yielded epoxide II (67%) as a viscous oil; ir (film): 3025, 1260, 900, and 796 cm⁻¹; nmr (CDCl₃): τ 7.44 (d, J=5 Hz, 1 H), 7.26 (d, J=5 Hz, 1 H), 8.21 (s, 3 H), 8.65 (s, 3 H), and 9.0 (s, 6 H); calc. for C₁₄H₂₄O: C, 80.71; H, 11.61; found: C, 80.52; H, 11.52. Treatment of II in ether with BF₃-etherate at 0° and chromatography of the oily product on silicic acid with elution by hexane gave luciferin aldehyde III in 25% yield; ir (neat): 2700, 1725, 1475, 1450, 1378, 1373, and 1358 cm⁻¹; nmr (CDCl₃): τ 0.15 (d, J=2 Hz, 1 H), 8.39 (s, 3 H), 9.00 (s, 6 H), and 8.84(d, J=7 Hz, 3 H). The ir and mass spectra of III were identical with those of luciferin aldehyde obtained by hydrolysis of natural luciferin, confirming the structure assigned by Shimomura and Johnson ¹.

Aldehyde III was treated with an excess of freshly prepared formic acetic anhydride⁷, added stepwise at 8-htt-internala-fet-c-period-of-32-htt. The mixture-west-powed-enterise-and-guistly-autrasted-with-pentane. The extracts were drived over sodium sulfate, concentrated, and chromatographéd on silicic acid. Elution by hexane

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containing 25% benzene gave an oily material which included about 3% of active luciferin IV as judged by luminescence assay with luciferase. Thin layer chromatography with hexane containing 20% 1,2-dichlorohexane revealed only two substances in the oily material; one with $R_f = 0.48$, identical with that of aldehyde III, and the other with $R_f = 0.51$, identical with that of luciferin IV. The two were satisfactorily separated in this manner on a thicker layer of silicic acid using the same solvent pair.

The mass spectrum (70 eV, indirect sample introduction, M^+ : m/e 236) of the synthetic enol formate IV was identical with that of natural luciferin; both spectra show minor peaks characteristic of aldehyde III (M^+ : m/e 208), which, as noted earlier¹, results from the breakdown of the unstable luciferin both during and prior to mass spectrographic analysis. Synthetic IV was active in light emission with Latia luciferase.

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