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Dinoflagellate Bioluminescence: The Chromophore of Dinoflagellate Luciferin

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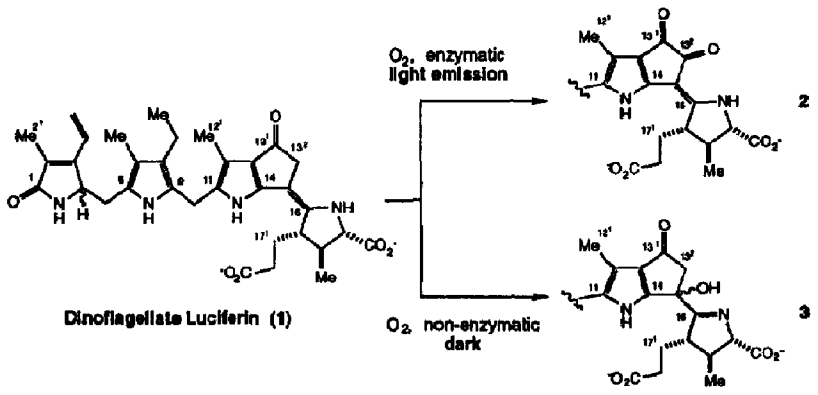
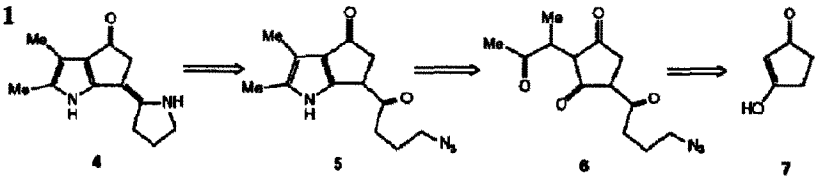
Abstract: A concise synthesis of the chromophore proposed for dinoflagellate luciferin was accomplished. The chemical behavior towards molecular oxygen and spectroscopic characteristics of the synthetic chromophore were found to match well with those observed for dinoflagellate luciferin. Comparison of the ^{13}C chemical shifts observed for the *E*- and *Z*-isomers of the synthetic model with those observed for dinoflagellate luciferin suggested the natural luciferin to have an *E*-configuration about its C.15-C.16 olefinic bond.

Many species of dinoflagellates are bioluminescent; they are ubiquitous in the oceans of the world and responsible for much of the sparkling luminescence elicited by disturbing surface waters, which is recognized as the "phosphorescence" of the sea. The chemical process of light emission involves air-oxidation of dinoflagellate luciferin catalyzed by dinoflagellate luciferase.[1] Based on spectroscopic and degradation studies, the structure of dinoflagellate luciferin was recently elucidated as **1**.[2] It has been shown that the chromophore of **1** plays a central role in its air-oxidation. In the presence of dinoflagellate luciferase, **1** is air-oxidized to yield **2** accompanied with light emission. Air-oxidation of **1** in the absence of dinoflagellate luciferase yields **3** without light emisssion. The proposed structure of the chromophore of **1** is unprecedented. In this letter, we report a synthesis of the chromophore model **4** and its spectroscopic and chemical properties.

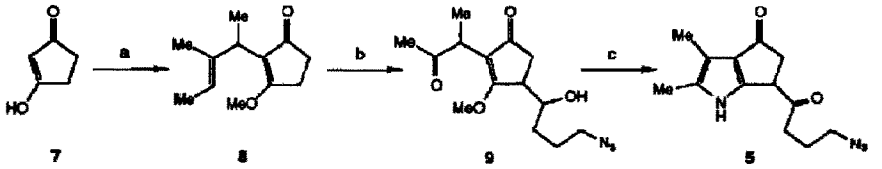


In the analysis of possible synthetic routes to the chromophore model **4** (Scheme 1), we were most concerned with the extraordinary instability observed for the chromophore of dinoflagellate luciferin towards molecular oxygen. In this respect, we viewed reductive cyclization of the azido ketone **5** to be an attractive choice. Furthermore, we envisioned that the pyrrole ring of **5** should be available via Paal-Knorr pyrrole synthesis, cf. **6**→**5**. The requisite starting material should be obtainable from 1,3-cyclopentadione (**7**).

Scheme 1

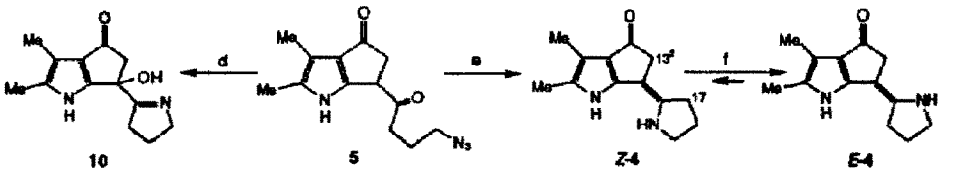


Scheme 2 summarizes the synthesis of the azido ketone **5** from 1,3-cyclopentadione (**7**). Noteworthy is the Claisen rearrangement used to avoid the difficulties encountered in direct *C*-alkylation of **7** with 3-bromo-2-butanone, cf. steps a.1 and a.2. The vinylogous ester **8** was deprotonated at the γ-position using LiHMDS[3] and condensed with 4-azidobutanal.[4] Ozonolysis of the products,[5] followed by cyclization with ammonia and oxidation, produced the azido ketone **5**. The regiochemistry of **5** was unambiguously established by chemical correlation.[6]



Scheme 2. Reagents and Reaction Conditions: a. 1. RX, DBU, THF. 2. Δ, xylenes (80% yield over 2 steps). 3. CH_2N_2, Et_2O (95% yield). b. 1. LiHMDS, THF, -78 °C, then RCHO (80% yield). 2. O_3, CH_2Cl_2, MeOH, -78 °C, then Me_2S (80% yield). c. 1. NH_3-MeOH (1:3), sealed tube, 60 °C, 48 hours (55% yield). 2. DMSO, DCC, py, TFA (60% yield).

Upon hydrogenation (H_2, Pd (5%) on C, RT, 2 h) in non-degassed ethanol, the azido ketone **5** was cleanly converted into a single product, with spectroscopic data (^{1}H- and ^{13}C-NMR, HR-MS, UV, and IR) fully consistent with the hydroxy imine **10** (Scheme 3). Presumably, **10** was formed through reductive cyclization of **5**, followed by tautomerization to the chromophore model **4**, and then O_2-oxidation. Hydrogenation under the rigorous exclusion of atmospheric oxygen did not give encouraging results.[7]



Scheme 3. Reagents and Reaction Conditions: d. H_2 (1 atm), Pd (5%) on C, EtOH (non-degassed), RT. e. PPh_3, MeCN-C_6H_6 (10:3, degassed), RT. f. CD_3OD (degassed), -20 °C, 2-3 days.

The azido ketone was reduced by triphenylphosphine[8] in a rigorously degassed 10:3 mixture of acetonitrile and benzene. The desired product **4** precipitated out as a fine yellow powder virtually free of any side-products, except for triphenylphosphine and triphenylphosphine oxide. The UV ($\lambda_{max\ (MeOH)}$ = 389 nm) and fluorescence ($\lambda_{max\ (MeOH)}$ = 466 nm) spectra agreed well with those observed for dinoflagellate luciferin (UV: 388nm; fluorescence spectrum: 474 nm). The ^{1}H NMR spectrum (CD_3OD) revealed that the product was a 3:1 mixture of *Z*- and *E*-isomers immediately after sample preparation.[9] A 2D NOESY experiment on this mixture established the major component to be ***Z*-4** and the minor to be ***E*-4**; definitive cross-peaks were detected between the H-13^2 (δ 3.15 ppm, s) and H-17 (δ 2.53 ppm, t) resonances for the major isomer, whereas no cross-peaks were observed between the H-13^2 (δ 3.08 ppm, s) and H-17 (δ 2.68 ppm, t) signals for the minor isomer.[10] After one day at room temperature in CD_3OD, the ratio of ***Z*-4** and ***E*-4** changed from 3:1 to 1:3, favoring the *E*-isomer, and appeared to represent the thermodynamic ratio for these isomers.[11]

Table 1. ^{13}C-NMR (125 MHz, CD_3OD) Data of 1, *E*-4, *Z*-4, 3, and 10

	C.10	C.11	C.12	C.12'	C.13	C.13^1	C.13^2	C.14	C.15	C.16	C.17	C.18	C.19
1	23	138	124	9	109	194	45	150	88	161	50	42	70
***E*-4**	nd[a]	nd	nd	nd	nd	194[b]	45[c]	nd	88[b]	160[b]	30[f]	nd	48[g]
***Z*-4**	nd	nd	nd	nd	nd	195[d]	47[e]	nd	87[d]	158[d]	31[h]	nd	49[i]
3	23	139	128	10	109	198	57	156	74	181	58	44	84
10	11	135	128	9	111	196	57	158	74	182	35	24	61

[a]not determined. [b]established through HMBC from H-13^2 (δ 3.08, s). [c]established through HMQC from H-13^2 (δ 3.08). [d]established through HMBC from H-13^2 (δ 3.15, s). [e]established through HMQC from H-13^2 (δ 3.15). [f]established through HMQC from H-17 (δ 2.68, t). [g]established through HMQC from H-19 (δ 3.32, t). [h]established through HMQC from H-17 (δ 2.53, t). [i]established through HMQC from H-19 (δ 3.42, t).

The C.15-C.16 olefinic stereochemistry of dinoflagellate luciferin had not yet been determined. We hoped that comparison of the spectroscopic characteristics, especially ^{13}C-NMR, of ***Z*-4** and ***E*-4** with those of the natural product might provide some evidence to define its stereochemistry. Although sufficient quantities of ***Z*-4** and ***E*-4** were available via the current synthetic route, its poor solubility (ca. 0.5 mg in 1 ml of CD_3OD) presented a technical problem for directly recording the ^{13}C-NMR spectrum. However, we could observe both direct (HMQC[12]) and long range (HMBC[13]) ^{1}H-^{13}C correlations. By using these techniques, we were able to determine the chemical shifts for the relevant carbons for each of the two isomers (Table 1). As seen from Table 1, the ^{13}C chemical shifts obtained for ***E*-4** were consistently closer to those for the corresponding carbons of dinoflagellate luciferin. This fact strongly suggests the configuration of the C.15-C.16 olefinic bond in dinoflagellate luciferin to be *E*. This assignment is also consistent with the observation that dinoflagellate luciferin did not exhibit any tendency for isomerization of this olefinic bond.[2a]

Under the same air-oxidation conditions that gave **3** from dinoflagellate luciferin **1**, a mixture of ***E*-4** and ***Z*-4** was cleanly converted into **10**. The ^{13}C chemical shifts observed for the relevant carbons in **10** were very close to those for the corresponding carbons in **3** (Table 1). Finally, it is worth noting that, although very faint, light emission was detected upon air-oxidation of a mixture of ***E*-4** and ***Z*-4** in the presence of dinoflagellate luciferase.[14]

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References and Footnotes

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2. (a) For the gross structure of dinoflagellate luciferin, see: Nakamura, H.; Kishi, Y.; Shimomura, O.; Morse, D.; Hastings, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 7607. (b) For the absolute stereochemistry of the D-ring moiety, see: Nakamura, H.; Oba, Y.; Murai, A. *Tetrahedron Lett.* **1993**, *34*, 2779.

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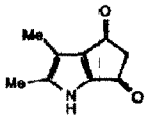
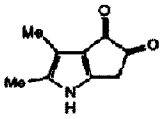
5. Syn and anti diastereomers were easily separated by silica gel chromatography and were carried on separately. They showed no significant difference in reactivity.

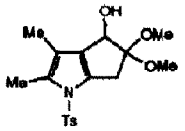
6. PDC oxidation for the step c.2 in Scheme 2 gave the dione A. The isomeric dione B was independently synthesized. Its regiochemistry and consequently that of A, was established from the ^{1}H-NMR spectrum of C, a derivative of B---note that C contained isolated CH_2 and CH spin systems, whereas the corresponding substance derived from A should have a CH_2-CH spin system.



A



B



C

7. Apparently, overreduction was a problem; mass spectral analysis of the crude mixture showed a molecular ion corresponding to m/e=(molecular ion of **4**)+2.

8. (a) Lambert, P. H.; Vaultier, M.; Carrie, R. J. *J. Chem. Soc., Chem. Commun.* **1982**, 1224. (b) Mitchell, D.; Liebeskind, L. S. *J. Am. Chem. Soc.* **1990**, *112*, 291.

9. The NMR sample was prepared in a glove box (the oxygen level was below 2 ppm), and the NMR tube containing the sample was sealed with a flame under vacuum. The first data collection was done approximately 0.5 hours after having dissolved the sample in CD_3OD. Thus, we could not exclude the possibility that the yellow precipitate obtained was pure *Z*-isomer.

10. The numbering system adopted for dinoflagellate luciferin is used.

11. The 1:3 ratio did not change over one week. Because of their high sensitivity towards molecular oxygen, we were unable to separate ***E*-4** from ***Z*-4**. Thus, the experiment using pure or predominantly ***E*-4** was not done.

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14. We thank Dr. Osamu Shimomura for this experiment.

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