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Dinoflagellate Bioluminescence: Chemical Behavior of the Chromophore towards Oxidants

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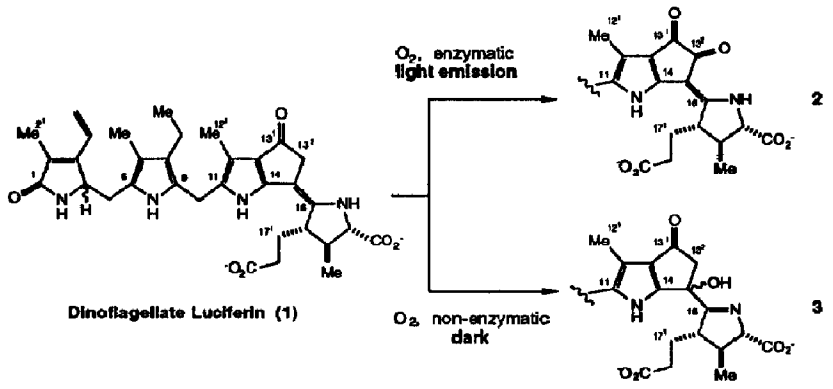
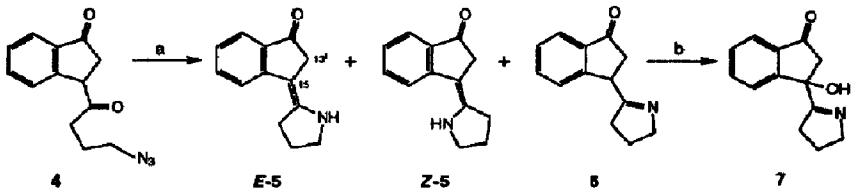
Abstract: The chromophore model **5** of dinoflagellate luciferin was synthesized, and its behavior towards oxidants was studied. Molecular oxygen at high substrate concentrations, superoxide anion, and Fenton reagent effected oxidation of **5** at the C.13[2] position. On the basis of these results, a possible mechanism for these oxidations and for bioluminescent air-oxidation of dinoflagellate luciferin, is suggested.

The structure of dinoflagellate luciferin has recently been elucidated as **1**.[1] In the presence of dinoflagellate luciferase, **1** is air-oxidized to **2** with light emission, whereas in the absence of enzyme, **1** is air-oxidized to **3** without light emission. In the preceding paper,[2] we outlined a synthesis of the chromophore portion of dinoflagellate luciferin and its behavior towards molecular oxygen in the absence of the luciferase. In this paper, we report oxidation of the chromophore model **5**, yielding products corresponding to **2**. Based on this observation, we propose a sequence of chemical steps which might be involved in the dinoflagellate bioluminescence.



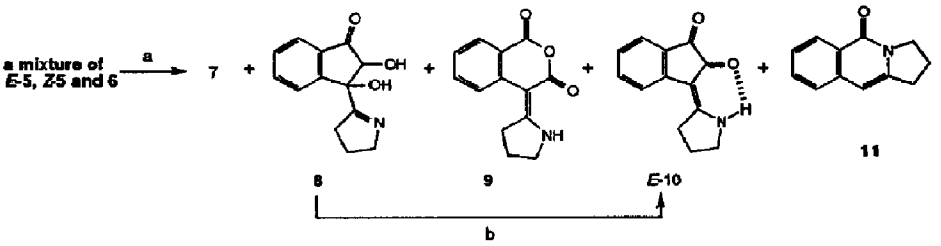
A noticeable difference between the light emitting and dark processes is the site of air-oxidation: dinoflagellate luciferin **1** is oxidized at C.13[2] in the former process, but at C.15 in the latter.[3] The chromophore model reported in the preceding paper appeared suitable for investigating the difference in enzymatic and non-enzymatic air-oxidative pathways. However, because of the technical difficulties encountered,[4] we opted to use the enamine **5** as the model compound for this study. Thus, the azido ketone **4** was synthesized in four steps from 1-indanone.[5] Reductive cyclization of **4** by triphenylphosphine,[6] with rigorous exclusion of air, generated cleanly a mixture of ***E*-5**, ***Z*-5**, and **6** in the ratio of 5:1:2 in C_6D_6.[7] Exposure of this mixture to air

produced hydroxy imine **7** quantitatively, demonstrating that this model adequately mimics the non-enzymatic air-oxidation of dinoflagellate luciferin.[2] It is worth noting that the C.13[2] protons are acidic; these protons rapidly exchanged with deuterium in CD_3OD (^{1}H-NMR). The rate of H-D exchange was sharply concentration-dependent, which may suggest that **5** itself acts as a catalyst.



Scheme 1. Reagents and Reaction Conditions: a. PPh_3, PhH or H_2 (1 atm), Pd (5%) on C, EtOH (degassed). b. O_2.

Encouraged by these observations, we then studied oxidation of the model chromophore **5** with superoxide anion and Fenton reagent ($K_3Fe(CN)_6$, H_2O_2).[8] The mixture of ***E*-5**, ***Z*-5** and **6** generated by triphenylphosphine reduction was contaminated with the reducing agent and because of its extreme air-sensitivity, the pure product was difficult to isolate. Therefore, we looked for alternative means of generating the enamine **5** without contaminants which could interfere with our oxidation reactions. Catalytic hydrogenation of **4** with rigorous exclusion of atmospheric oxygen was satisfactory. The ^{1}H-NMR (C_6D_6) spectrum indicated that the purity of this mixture was slightly less than that of the mixture obtained by triphenylphosphine reduction.[9] However, upon exposure to molecular oxygen in ethanol, this mixture yielded only the hydroxy imine **7**.[10]

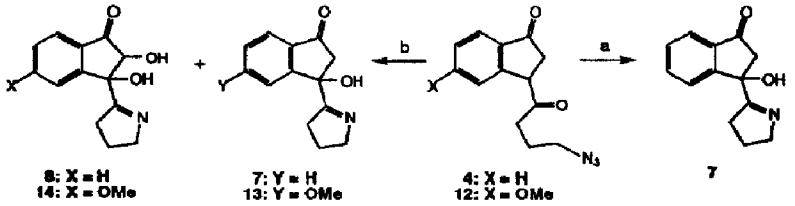


Scheme 2. Reagents and Reaction Conditions: a. 1. $K_3Fe(CN)_6$, H_2O_2, NaOH, EtOH or KO_2, 18-C-6, DME. 2. $NaHSO_3$. b. evaporation of toluene solution.

The crude product obtained from catalytic hydrogenation was treated with Fenton reagent or superoxide anion to give a complex mixture. The major product was the hydroxy imine **7** (ca. 20-30% overall yield from **4**). Importantly, the products, which were oxidized at C.13[2], were also identified. Dihydroxy imine **8**[11] and anhydride **9** were isolated by silica gel chromatography in low yields. In addition, on comparison of ^{1}H-NMR and TLC with independently synthesized samples,[12] α-diketone **10**[11,13] and lactam **11**[14] were definitively detected in the crude mixture of products.

Catalytic hydrogenation of **4** in non-degassed ethanol at low concentrations (2 mg/mL), followed by slow exposure to air, yielded cleanly hydroxy imine **7**. Interestingly, at higher concentrations (> 20 mg/mL), it

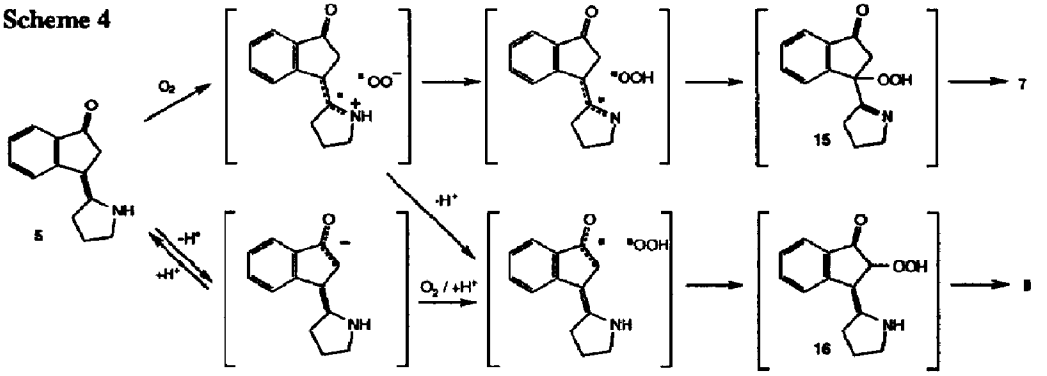
consistently gave a mixture of **7** and dihydroxy imine **8** (10-30% yield). In order to exclude the possibility that **8** was formed via **7**, a cross reaction was carried out; when reduction of **4** was done in the presence of **13**, **8** was detected as the only dihydroxy imine formed. Furthermore, reduction of azido ketone **12**[15] in the presence of **7** yielded **14** as the only dihydroxy imine, eliminating the possibility that an electronic effect might have controlled the cross reaction.



Scheme 3. Reagents and Reaction Conditions: a. H_2 (1 atm), Pd (5%) on C, EtOH (non-degassed, 2 mg/mL), then O_2. b. H_2 (1 atm), Pd (5%) on C, EtOH (non-degassed, > 20 mg/mL), then O_2.

We suggest a possible mechanism to explain these results (Scheme 4). The enamine **5** might react with molecular oxygen, yielding the radical cation and superoxide radical anion.[16] The latter can deprotonate the radical cation either at the nitrogen or at C.13[2]. Deprotonation at the nitrogen would yield the hydroperoxide imine **15**, eventually giving hydroxy imine **7** (the upper half of Scheme 4). On the other hand, deprotonation at C.13[2], and collapse of the resultant radical pair, would yield the peroxide **16**, leading to dihydroxy imine **8** (the lower half of Scheme 4). The dihydroxy imine **8** could also be formed via air-oxidation of the enolate formed from **5** (or the corresponding enol). The concentration-dependent H-D exchange observed for the C.13[2] protons (*vide ante*) may provide an explanation for partition of the air-oxidation to either **7** or **8**.

Scheme 4



It is tempting to suggest the same mechanism for the enzymatic, bioluminescent air-oxidation of **1**. This suggestion is appealing because the bioluminescence spectrum is very close to the fluorescence spectrum of dinoflagellate luciferin **1**.[1] A radical recombination during the enzymatic air-oxidation of luciferin might yield an excited state intermediate, cf. **16** in the model system, that could be either the emitter itself or could transfer energy to an unreacted dinoflagellate luciferin **1**. An important role of the enzyme would be to facilitate deprotonation of the C.13[2] proton(s).

Acknowledgements: Financial assistance from the National Science Foundation (CHE-9408247) is gratefully acknowledged.

References and Footnotes

1. For the background of dinoflagellate bioluminescence, see references 1 and 2 in the preceding paper.

2. Stojanovic, M. N.; Kishi, Y. the preceding paper.

3. The numbering system of dinoflagellate luciferin is adopted for all compounds in this paper.

4. These included poor solubility and high sensitivity towards molecular oxygen. Retrospectively, we realized that **5** might have been a better model compound for another reason as well. The acidity of the C.13[2] protons in the pyrrole model compound was found to be lower than those in **5** (H-D exchange experiments by NMR); and the C.13[2] position in the pyrrole model compound might not be as sensitive towards oxidation as that observed for **5**.

5. 1. 1-indanone, LiHMDS, TESCl, THF. 2. BuLi, THF, then RCHO. 3. HCl, THF, H_2O. 4. PDC, CH_2Cl_2.

6. (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.

7. The stereochemistry assignment for ***E*-5** and ***Z*-5** was based on nOe experiments.

8. Goto, T. In *The Role of Oxygen in Chemistry and Biochemistry*, Ando, W.; Moro-oka, Y., Eds.; Elsevier: Amsterdam, 1988; p. 367.

9. The ^{1}H-NMR spectrum of **5** in C_6D_6 was sharply dependent on the presence and concentration of aromatic compounds such as PPh_3 and PPh_3O.

10. Exposure to air in benzene and ethyl acetate gave a 1:1 mixture of **7** and the corresponding C.15 hydroperoxide **15**. All attempts to isolate this intermediate resulted in isolation of **7** as the sole product. Thus, **15** was identified based on (1) the ^{1}H-NMR spectrum of the crude product and (2) its instantaneous conversion to **7** upon treatment with PPh_3.

11. The mass spectrum of **8** gave a peak corresponding to m/e=M^+-18. This inspired us to pyrolize **8** by evaporating toluene in an open flask almost to dryness, to yield cleanly **10**. Furthermore, the fragmentation pattern in the mass spectra of **8** and **10** were almost identical.

12. The authentic sample of **10** was synthesized from 1,2-indanedione in three steps: 1. $CuBr_2$, $CHCl_3$, Δ. 2. thiopyrrolidinone, CH_2Cl_2. 3. toluene, Δ. The authentic samples of **9** and **11** were obtained by treatment of **10** with $ZnCl_2$ in aged dioxane, and with K_2CO_3 in MeOH, respectively.

13. Structurally, α-diketone **10** corresponds to oxyluciferin **2**. Based on a nOe experiment, the *E*-stereochemistry was assigned to **10**---note that this *E*- and *Z*-notation does not follow the Cahn-Ingold-Prelog sequence rule but is used in consistence with the configuration of dinoflagellate luciferin. All attempts to obtain the corresponding *Z*-isomer, by chemical and photochemical isomerization of **10** or by structural modification such as introduction of substituents on the D ring, failed. These observations may suggest the same stereochemistry to dinoflagellate oxyluciferin **2**. Apparently, the intramolecular hydrogen bond stabilizes the *E*-isomer, cf. **10**.

14. Haimova, M. A.; Ognyanov, V. I.; Mollov, N. M. *Synthesis* **1980**, *10*, 845.

15. Synthesized from 5-methoxy-1-indanone in the same way as **4**.[5]

16. Malhotra, S. K.; Hostynck, J. J.; Lundin, A.F. *J. Am. Chem. Soc.* **1968**, *90*, 6565.

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