A PROPOSED SYMMETRY FORBIDDEN OXIDATION MECHANISM FOR THE BACTERIAL LUCIFERASE CATALYZED REACTION

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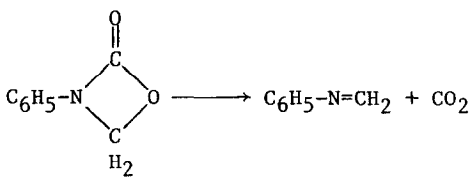
SUMMARY

2-Oxo-3-phenyl-1,3-oxazetidine was found to thermally undergo a 2,2-cycloreversion reaction with an enthalpy of activation of 30.8 Kcal. This suggests that an oxazetidine fused to a flavin could be the labile light producing intermediate in the bacterial luciferase reaction since the reaction would be favored by ring fusion and the lower excited state of flavin.

INTRODUCTION

Several mechanisms have been proposed for the bacterial luciferase reaction (1,2,3), $FMNH_2 + RCHO + O_2 \xrightarrow{\text{bacterial luciferase}} FMN + RCOOH + H_2O + h\nu$. A mechanism is proposed here which not only includes all experimental facts concerning the reaction but would be expected to produce light. The mechanism involves the cleavage of a 1,3-oxazetidine which were thought to be relatively stable. However, we have found that 2-oxo-3-phenyl-1,3-oxazetidine thermally undergoes a 2,2-cycloreversion reaction with an activation enthalpy of only 30 Kcal.



(I) (II)

TABLE I

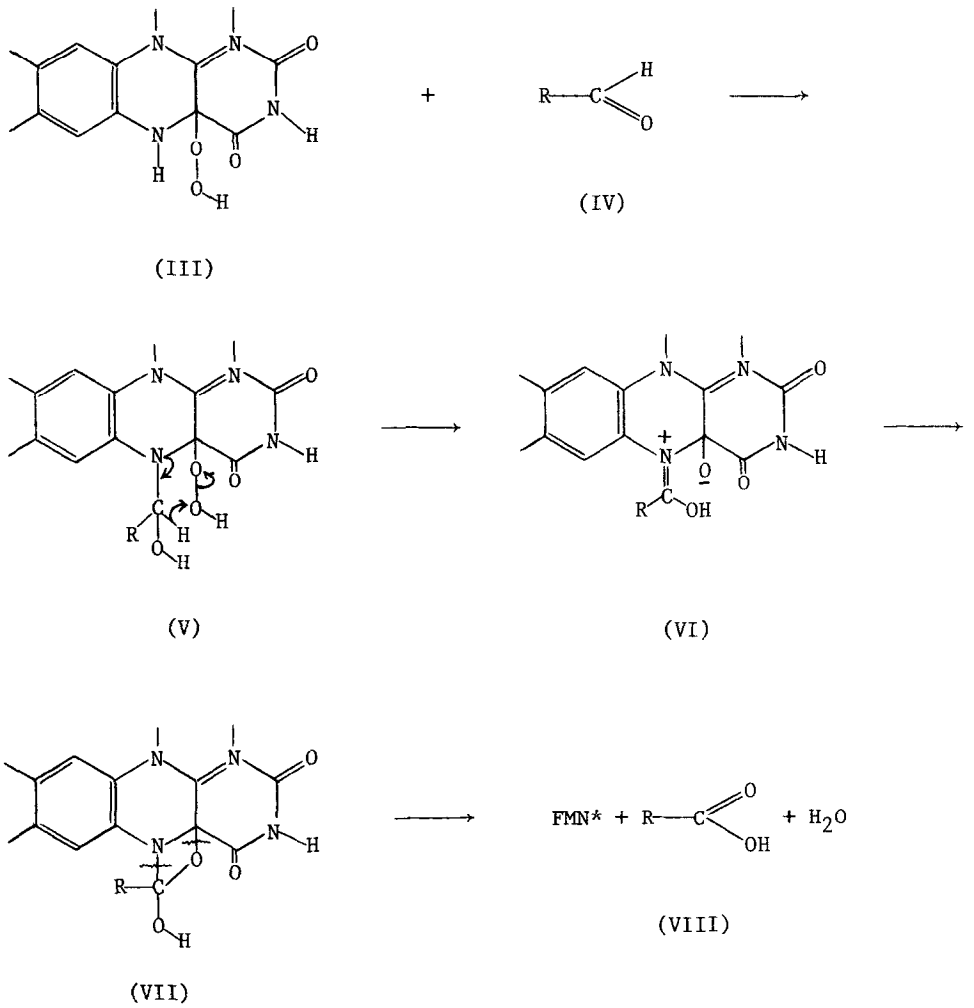
Temperature	k (min^{-1})
114.0	0.00156
118.5	0.00197
123.5	0.00349
128.5	0.00525
135.5	0.0118
138.5	0.0161
144.5	0.0284

EXPERIMENTAL RESULTS

2-Oxo-3-phenyl-1,3-oxazetidine (I) was prepared by the method of Ozaki (4). The mass spectra obtained were identical in position to that reported by Ozaki but the relative abundance varied considerably with the heat used to introduce the sample into the spectrometer. The peak at m/e = 149 corresponding to the cation of I decreased and the peaks at m/e = 105 and m/e = 44 corresponding to the cations of II and of carbon dioxide increased at higher temperatures. Heating I neat at the melting point (127°) under 20 mm N_2 (to prevent sublimation) produced bubbles in the melt. The gas above the melt gave major mass peaks at m/e = 44 (CO_2^+) and m/e = 28 (N_2^+). Solutions of I (15% weight) in dimethyl formamide in a JEOL-JNM-MH-100 spectrophotometer with a JES-VT-3 controller gave a sharp methylene singlet which was slowly displaced at 114° or above by a new sharp singlet 0.52 ppm upfield. The residue after evaporation of dimethyl formamide was very low in oxygen; C, 77.76%; H, 6.54%; N, 12.94%. Calc. for II (or polymer); C, 79.96%; H, 6.71%; N, 13.32%. Rates of thermal cleavage were measured by comparing the two singlet methylene peaks. The rates given in Table I give an activation enthalpy of 30.8 ± 1.2 Kcal.

DISCUSSION

These results allow a mechanism for the bacterial luciferase reaction via a 1,3-oxazetidine. The formation of the 1,3-oxazetidine and its 2+2 cycloreversion would proceed as shown in Scheme I.



Scheme I

Compound III has been identified as the first step in the enzyme catalyzed reaction by Hastings et al. (5) and in a non-enzymatic model reaction by Kemal and Bruice (6). The reaction of the basic nitrogen of (III) with aldehyde (IV) to give the carbinolamine (V) has many organic analogues and is implicated (7) in another flavin reaction. The conversion V → VI is analogous to the peracid

oxidation (8) of ketals to carboxylic acids. The hydride shift V → VI is favored by a good electron donating substituent, N, and by a cyclic six-membered transition state. In VI, the relative positions of the nucleophilic oxygen and the electrophilic carbon and also a hydrophobic enzyme center would favor rapid closure to the strained 1,3-oxazetidine (VIII). The conversion of V → VII could be concerted or free radical.

The reaction VII → VIII would be driven by angle strain and an increase in resonance energy. This reaction is a 2,2-cycloreversion reaction thermally forbidden to produce ground state products in a four center transition state by the Woodward-Hoffman rules (9) but would produce a product in an excited state providing the free energy is favorable and the activation energy is sufficiently low to proceed. This is an extension of the chemiluminescent theory of McCapra (10,11,12,13) for 1,2-dioxetane 2,2-cycloreversions.

Four centered transition states are favored by equal electronegativities of the reaction products (14). Any imbalance in VII could be corrected by protonation of VII. The emitted light appears to be the fluorescence of the protonated flavin (15). Light was not observed nor would be expected to be observed in I → II because II would lose energy rapidly by internal conversion. A rigid product like a flavin is needed for fluorescence.

The activation enthalpy would be much lower than the value of 30.8 Kcal both because of the strain in fusing the oxazetidine to the flavin ring and also because of the lower excited state of flavin compared to II. A molecular orbital calculation (for methodology c.f. reference 16) showed that the LEMO[1] of flavin is at -0.734 ev whereas II lies at 0.909 ev. The corresponding HOMO[2] are at -7.90 ev and -8.52 ev. Thus the two LEMO differ by approximately 38 Kcal strongly favoring[3] the formation of excited flavin over excited II. Consequently VII may kinetically be a quite labile intermediate.

[1]LEMO = lowest empty molecular orbital.

[2]HOMO = highest occupied molecular orbital.

[3]This is only a rough estimate. No correction can be made for the relative levels of sigma electrons in II and VII and not all of this energy difference could occur at the transition states.

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