

the products obtained from the *cis* isomer we suggest that fission of the carbon-carbon bond of the heterocyclic ring is occurring first. Measurements of the bond distances and bond angles for ethylene oxide can probably be used without introducing any important error in this qualitative discussion of the ethylenimine ring. Cunningham has pointed out that the carbon-oxygen distance is near normal but the carbon-carbon distance is considerably shortened, and consequently most of the ring strain must be accounted for in the carbon-carbon bond.¹⁴ It is suggested that the crowded *cis*-ethylenimine relieves its strain in the excited state by the initial cleavage of the carbon-carbon bond.¹⁵

Experiments designed to provide further information on the identity of the excited states and other transients responsible for these reactions are in progress.

Acknowledgment. We gratefully acknowledge support of this work by the National Science Foundation (Grant GP-3972).

(14) G. L. Cunningham, A. W. Boyd, and W. D. Gwinn, *J. Chem. Phys.*, **17**, 211 (1949).

(15) A steric interaction between two groups attached to the carbon atoms of a number of related ethylenimine ketones has been suggested by Cromwell to account for the lower polarity and less single bond character of the carbonyl group for the *cis* isomer in contrast with the *trans* isomer (N. H. Cromwell, R. E. Bambury, and J. L. Adelfang, *J. Am. Chem. Soc.*, **82**, 4241 (1960), for leading references).

(16) National Institute of Health Predoctoral Fellow; 1964-present.

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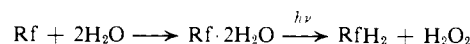
The Photochemical Splitting of Water by Riboflavin

Sir:

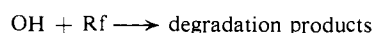
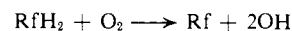
When air-free aqueous solutions of riboflavin are exposed to visible light, the yellow color slowly fades. Subsequent admission of air partly restores the yellow color and the solution is found to contain a complex mixture including riboflavin, lumichrome, lumiflavin, and aldehydes.¹⁻³ If, however, certain tertiary amines or sulfur compounds (*e.g.*, EDTA or methionine) are present in solution, fading is rapid and it is possible by admitting air to restore completely the yellow color and recover all of the riboflavin.^{4,5} To account for

(1) R. Brdicka, *Collection Czech. Chem. Commun.*, **14**, 130 (1949).
 (2) G. Oster, J. S. Bellin, and B. Holmstrom, *Experientia*, **18**, 249 (1962).
 (3) E. C. Smith and D. E. Metzler, *J. Am. Chem. Soc.*, **85**, 3285 (1963).
 (4) J. R. Merkel and W. J. Nickerson, *Biochim. Biophys. Acta*, **14**, 303 (1954).

these and other observations, Nickerson, *et al.*^{5,6} have proposed a reaction scheme that postulates the splitting of water.



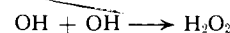
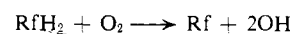
On addition of air



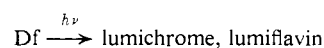
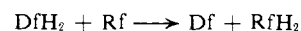
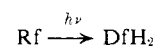
In the presence of a suitable nitrogen- or sulfur-containing compound (M)



On addition of air



On the other hand, Oster, *et al.*,^{2,7} have suggested a reaction scheme in which water plays no direct role but which, in the absence of M, postulates the existence of a light-sensitive intermediate deuterioflavin (Df).



In the presence of M



In this investigation air-free solutions of riboflavin were irradiated in a Pyrex polarographic cell with a General Electric H100 SP38-4 mercury arc lamp. With this arrangement it is possible to carry out polarographic analysis at any time during or after irradiation without exposing the solution to air. Polarographic analysis is especially suited for studying the photochemistry of riboflavin because it is capable of detecting and determining quantitatively such pertinent compounds as riboflavin, reduced riboflavin, lumichrome, aldehydes, oxygen, and hydrogen peroxide.¹ The solutions were 0.2 mM in riboflavin, 0.1 M in KCl and in phosphate buffer, and at pH 7.8. The values of the absorption maxima and $E_{1\%}^{1\text{cm}}$ of the riboflavin, Eastman White Label grade, agreed with those reported in the literature. Therefore the riboflavin was used without further purification. The solutions were deaerated by flushing with nitrogen which had been passed through a train consisting of three flasks containing acidified chromous chloride and amalgamated zinc and two flasks containing distilled water.

Polarograms of riboflavin solutions, which had been irradiated in the absence of nitrogen- or sulfur-containing compounds and to which no air had been admitted, showed the presence of riboflavin, reduced riboflavin, lumichrome, an aldehyde, and possibly other products. There was no evidence of hydrogen peroxide. These results agree with those of Brdicka¹ and Smith, *et al.*,³ and tend to support Oster's reaction scheme. The Nickerson scheme requires the formation of hydrogen

(5) W. J. Nickerson and G. Strauss, *J. Am. Chem. Soc.*, **82**, 5007 (1960).

(6) G. Strauss and W. J. Nickerson, *ibid.*, **83**, 3187 (1961).

(7) B. Holmstrom and G. Oster, *ibid.*, **83**, 1867 (1961).

peroxide and does not account for the presence of riboflavin degradation products before exposure to air. However, it should be pointed out that the Nickerson scheme was proposed for solutions 0.025 mM in riboflavin and 0.05 M in acetate at pH 4.8.

Figure 1 shows three polarograms of a riboflavin solution which was 6 mM in N,N'-dimethyl-N'-benzylethylenediamine (NBD). Polarogram 1 was obtained before irradiation and polarogram 2a after a 75-sec. exposure to the mercury arc lamp. The two polarograms show that in the presence of NBD riboflavin is reduced without photolysis and as a consequence a new wave with an $E_{1/2}$ of -1.30 v. is formed. Polarogram 2b was obtained after adding benzaldehyde to the irradiated solution. It is seen that the height of the new wave was increased without change in the $E_{1/2}$. This suggests the new wave is due to benzaldehyde. The almond odor of the solution also suggested benzaldehyde.

To verify the formation of benzaldehyde in the photo-reaction, a relatively large amount of the photooxidation product of NBD was prepared. A solution, 0.3 mM in riboflavin and 0.05 M in NBD and buffered with phosphate at pH 7.8, was irradiated in the presence of air for 2 hr. After irradiation the solution, which had a strong almond odor, gave a polarogram similar to 2b except that the riboflavin wave was entirely above the zero current line and the new product wave was about ten times higher. Nitrogen was passed through the irradiated solution and expurgated photooxidation product was absorbed in a 0.1 M phosphate buffer. The ultraviolet spectrum of the phosphate buffer proved to be identical with that of a phosphate buffer to which pure benzaldehyde had been added.

Since the photooxidation of NBD by riboflavin yields benzaldehyde in the absence of air, photofission of water must occur to supply oxygen. It might be argued that oxygen is supplied by the sugar side chain of riboflavin, or through an air leak, or from the phosphate buffer. However, polarographic analysis showed no evidence of riboflavin photolysis or of an air leak during or after irradiation. To make sure that the phosphate buffer played no direct role irradiations were carried out without buffer. The results were the same as in the presence of buffer.

Measurement of the wave heights in the polarograms of riboflavin-NBD solutions showed that 1 mole of benzaldehyde is formed per mole of riboflavin reduced. It therefore appears that in the absence of air the overall reaction between riboflavin and NBD is

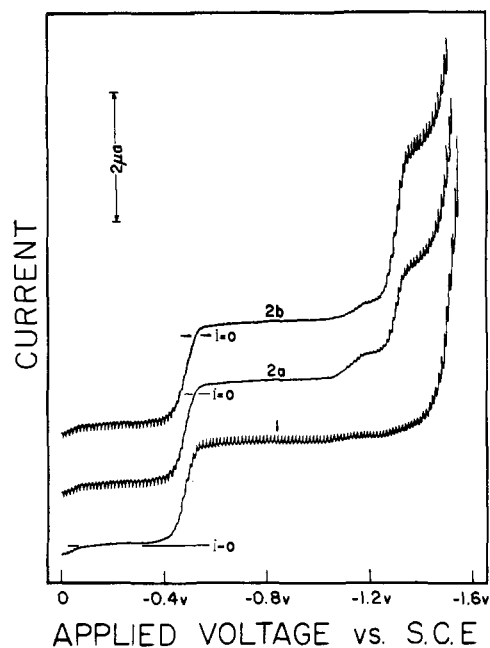
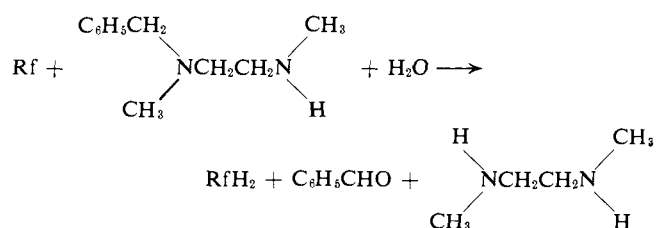


Figure 1. Polarograms of solutions 0.3 mM in riboflavin, 6.0 mM in N,N'-dimethyl-N'-benzylethylenediamine, 0.1 M in KCl and phosphate buffer, pH 7.8. (1) Before irradiation, (2a) after irradiation, (2b) after irradiation and addition of benzaldehyde.



This reaction is consistent with the Nickerson scheme for the irradiation of riboflavin solutions in the presence of M.

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Book Reviews

Essays in Coordination Chemistry. Edited by W. SCHNEIDER, G. ANDEREGG, and R. GUT. Birkhauser Verlag, Basel, Switzerland. 1964. 305 pp. 17 × 25 cm. 48.--s Fr.

It is particularly fitting that a group of his students and associates should have chosen to honor Professor Gerold Schwarzenbach on his sixtieth birthday through the compilation and publication of

this series of papers in the area to which he has contributed so significantly. It is perhaps too soon to assess completely the importance of Professor Schwarzenbach's work to all areas of chemistry, both practical and theoretical, but all persons will agree that his work has influenced substantially the pattern of current research in coordination chemistry and related topics and has led to developments