enhances the hydrogenation activity of the Pt/SiO_2 catalyst. At the conditions of the experiments, the activity of the Al_2O_3 or SiO_2 was small in comparison with that of any of the mixtures containing Pt/SiO_2 catalyst. Thus, at 153° and other conditions similar to those of Table I, the conversion of ethylene over 0.45 g. of Al_2O_3, the amount used in the $Pt/SiO_2 + Al_2O_3$ mixtures, was less than 0.10%. The conversion over SiO_2 was even lower. Therefore, the exceptionally high rate obtained on mixing Al_2O_3 with Pt/SiO_2 cannot be simply a matter of adding the separate catalytic contributions of the Pt/SiO_2 and the Al_2O_3. For, if we assume that the conversion over the mixture of Pt/SiO_2 and SiO_2 is due to the Pt/SiO_2 alone, the conversion over a mixture of Pt/SiO_2 and Al_2O_3 based on simple additivity considerations would only be 2.1 + 0.1 = 2.2%, which is many-fold lower than actually observed for the mixtures. The data clearly indicate that a cooperative effect of the Pt/SiO_2 and Al_2O_3 is involved.

Discussion

As indicated in the previous section, the results of the present study on ethylene hydrogenation over catalyst mixtures cannot be accounted for simply on the basis of additivity of separate catalytic contributions of the individual components of the mixtures. In the absence of a simpler explanation for the results, we conclude that the marked synergistic effect observed when Al_2O_3 is mixed with Pt/SiO_2 is due to a migration of reactive intermediates from one component of the mixture to the other. It appears reasonable that Pt centers activate hydrogen in some manner, and that the active hydrogen migrates to Al_2O_3 centers to react with chemisorbed ethylene. The fact that Al_2O_3 chemisorbs ethylene to a much greater degree than does SiO_2 then offers a reasonable explanation for the higher activity of the mixture of Pt/SiO_2 and Al_2O_3 when compared with the mixture of Pt/SiO_2 and SiO_2.

While the results of the present study suggest that migration of intermediates between different types of catalytic centers may be important, not much can be said about the detailed nature of these intermediates or the mechanism of migration. Experiments by Khoobiar[7] in this Laboratory have shown that WO_3, which is not reduced by hydrogen treatment at room temperature, can be readily reduced if a small amount of Pt/Al_2O_3 catalyst is mixed with it. The reduction of the WO_3 is accompanied by a color change from yellow to blue. It is known that hydrogen atoms will reduce WO_3,[8] and it has been suggested by Khoobiar that the role of the Pt/Al_2O_3 is to dissociate hydrogen molecules into atoms which can then migrate to WO_3. Applying this type of reasoning to the data obtained in the present study, one might speculate that Pt centers on the Pt/SiO_2 serve as a source of hydrogen atoms which migrate over the surface to centers on the Al_2O_3 to undergo further reaction. While this explanation appeals to us, there is some difficulty in reconciling the notion of migration of hydrogen atoms from Pt to Al_2O_3 centers with the work of Spenadel and Boudart,[9] who showed that the adsorption of hydrogen on Pt dispersed on Al_2O_3 corresponded to a 1:1 ratio of H to Pt atoms. If appreciable migration to Al_2O_3 centers had occurred, then one might have expected the ratio to be significantly higher than 1:1. There is also the question in the present experiments whether the extent of grain to grain contact is high enough for the rate of transport of active species by surface migration to be sufficient to account for the results. However, it is conceivable that a chain mechanism may be involved on the alumina. As an alternative to a mechanism involving surface migration of hydrogen atoms, there is the possibility that reactive intermediates may migrate through the gas phase, but more experimentation would be required to determine the nature of the intermediate species if this turned out to be the case.

(7) S. Khoobiar, unpublished data (1959).

(8) H. W. Melville and J. C. Robb, *Proc. Roy. Soc.* (London), **A196**, 445 (1949).

(9) L. Spenadel and M. Boudart, *J. Phys. Chem.*, **64**, 204 (1960).

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The Photochemistry of Riboflavin. I. The Hydrogen Transfer Process in the Anaerobic Photobleaching of Flavins[1]

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Several possible mechanisms for the anaerobic photobleaching of riboflavin have been eliminated on the basis of kinetic and polarographic studies. Simultaneous photolysis of solutions of riboflavin in deuterium oxide and water indicated no difference in the rate of photobleaching. Furthermore, polarographic studies of anaerobically photobleached solutions of riboflavin in the presence and absence of EDTA demonstrated that photoreduction does not occur in aqueous solutions in the absence of a hydrogen donor (such as EDTA) and that riboflavin is partially converted to lumichrome without the participation of oxygen. Rate studies on the anaerobic photolysis of the model flavins 9-(2′-hydroxyethyl)-isoalloxazine and 9-(2′-hydroxyethyl-2′,2′-d_2)-isoalloxazine showed a kinetic isotope effect of 2.5 for the ratio, k_H/k_D. This value agrees with that obtained for other photochemical hydrogen abstraction reactions. Spectrophotometric and polarographic data from the photolyzed solutions indicated that the products are alloxazine and an aldehyde, presumably acetaldehyde. A mechanism involving an intramolecular hydrogen transfer from the 2′-carbon to the 1-nitrogen is suggested as a pathway for the production of lumichrome and other photoproducts of the photobleaching of riboflavin.

Introduction

In recent years two irreconcilable mechanisms have been proposed to explain the anaerobic photobleaching of riboflavin (6,7-dimethyl-9-[D-1′-ribityl]-isoalloxazine) in aqueous solutions.[3,4] Strauss and Nickerson[3] have postulated that photoexcited riboflavin (Rb) can split water and abstract the equivalent of two hydrogen atoms to produce dihydroriboflavin (RbH_2) and hydrogen peroxide. This transfer, they suggest, is facilitated by the presence of an activator (M), such as

$$Rb + 2H_2O \longrightarrow Rb \cdot 2H_2O \xrightarrow{h\nu} RbH_2 + H_2O_2$$

$$RbH_2 + O_2 \longrightarrow Rb + 2 \cdot OH \longrightarrow H_2O_2$$

$$Rb + H_2O + M \longrightarrow Rb \cdot H_2O \cdot M \xrightarrow{h\nu} RbH_2 + MO$$

(1) Presented at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 8–13, 1963.

(2) Abstracted in part from the M.S. thesis of F. A. Raymond, Utah State University, 1963.

(3) G. Strauss and W. J. Nickerson, *J. Am. Chem. Soc.*, **83**, 3187 (1961).

(4) B. Holmström and G. Oster, *ibid.*, **83**, 1867 (1961).

methionine or EDTA, which reduces the energy required to break the O–H bond. Repeated cyclization of the photoreaction and oxidation reactions gave good recovery of riboflavin as measured spectrophotometrically. In the presence of an activator, complete recovery of riboflavin was obtained in the cyclization. No kinetic evidence has been presented to support this mechanism. Vernon[5] has obtained an apparent positive test for the presence of hydrogen peroxide in anaerobically photobleached solutions of riboflavin; however, there is some doubt as to the validity of the test.[5,10]

Holmström and Oster[4] have argued against the water splitting mechanism. They believe that the reaction is energetically unfavorable, and that the data can be explained in terms of a mechanism that involves the transfer of hydrogens from the ribityl side chain of riboflavin. The rate of photobleaching of the product, which was obtained from an oxidized photolyzed solution of riboflavin, was much faster than the original rate of photobleaching.[4] If the water-splitting mechanism were valid, the product should have been riboflavin, and the rate should have been the same as initially.

Karrer and Meerwein,[11] in one of the original mechanistic studies on the anaerobic photobleaching of riboflavin, postulated that a primary or secondary alcoholic group at the 2′-position of the side chain attached to the 9-nitrogen of the isoalloxazine nucleus was most favorable for photoreaction. This conclusion was based on the observation of the crude rates of photolysis for several substituted isoalloxazines. Halwer,[12] in a study of the effects of acids and buffers on the photobleaching reaction, reinvestigated the rates of photolysis for many of the same substituted isoalloxazines. Halwer's results confirmed those of Karrer and Meerwein[11] in neutral solution, but 9-(2′-hydroxy-2′-methylpropyl)-isoalloxazine (Id) was found to photolyze faster than 9-(2′-hydroxyethyl)-isoalloxazine (Ib) in acidic solutions. This was just the reverse of the situation in neutral solution where Ib photolyzed faster than any other isoalloxazine. Halwer concluded that an ionic mechanism involving the hydroxyl groups on the side chain was responsible for the photobleaching reaction. However, he overlooked the possibility of an acid-catalyzed rearrangement of the tertiary alcohol preceding the photoreaction, which would explain the strange behavior of Id.

A kinetic and polarographic study of the photoreaction of riboflavin and 9-(2′-hydroxyethyl)-isoalloxazine was undertaken with the intent of resolving some of the problems that have arisen in this field.

Experimental

Materials.—Riboflavin (6,7-dimethyl-9-[D-1′-ribityl]-isoalloxazine) from Nutritional Biochemicals Co. was tested for impurities by paper chromatography. When necessary, aqueous solutions of riboflavin were purified by column chromatography using cellulose packing and eluting with butanol–acetic acid–water mixtures.

Alloxazine was prepared by condensing alloxan with *o*-phenylenediamine using the method of Kuhling.[13] The crude product was recrystallized from alcohol.

9-(2′-Hydroxyethyl)-isoalloxazine (Ib) and 9-(2′-hydroxyethyl-2′,2′-d_2)-isoalloxazine (Ie) were synthesized by the method of Karrer, *et al.*[14] The 2-hydroxyethyl-2,2-d_2-amine was prepared by the reduction of glycine ethyl ester with lithium aluminum deuteride (Metal Hydrides Inc.) in ether.[15] The deuterated amine reacted with *o*-chloronitrobenzene in the presence of sodium acetate to produce N-(2′-hydroxyethyl-2′-2′-d_2)-*o*-nitroaniline which was isolated. The substituted *o*-nitroaniline was dissolved in absolute ethanol and was catalytically hydrogenated with platinum oxide. An aqueous solution of alloxan tetrahydrate was added to the reduction mixture and heated to boiling for a few minutes. Upon cooling, crystals of the isoalloxazine formed and the product was recrystallized from water. The ultraviolet spectra of Ib and Ie were identical with absorption maxima at 435, 350, 263, and 217 mμ. The presence and placement of the deuterium in Ie and its starting material, 2-hydroxyethyl-2,2-d_2-amine, was verified by infrared spectra. The former showed a new doublet at 2200 and 2100 cm.$^{-1}$, and the latter had a new peak at 2080 cm.$^{-1}$. These peaks agree with the theoretical shift predicted for C–D stretching vibrations. With the exclusion of these peaks for Ie, the infrared spectra of Ib and Ie were very similar to that of riboflavin (Ia).

Apparatus.—The photochemical reactor[16] consisted of a 800-C SAH Westinghouse mercury arc at the focal length of a double-convex quartz lens (diam. 2.5 in., f.l. 6 in.). A Beckman DU cell holder was located in the center of the collimated and filtered light beam. The light intensity incident on the two center compartments of the cell holder was uniform to within 3% as determined by thermopile measurements and simultaneous photolysis of identical solutions. Light intensities in the range of 10^{16} quanta-sec.$^{-1}$-cm.$^{-2}$ have been obtained with this system.

The photolysis cell used for kinetic studies consisted of a 10-mm. path length quartz spectrophotometer cell (Pyrocell Mfg. Co.) attached to a Pyrex degassing chamber by a Pyrex to quartz seal. The assembly could be connected to a vacuum system by a side arm that terminated in a ground glass joint.

Polarographic data were obtained with a Sargent Model XV recording polarograph, using an H-cell equipped with a calomel reference electrode. The precision of the half-wave potentials was found to be ±5 mv. Diffusion currents were measured at the top of the oscillations. Capillary characteristics, measured at open circuit, were: m = 2.32 mg./sec. t = 2.60 sec./drop for the waves in Fig. 3 and 6, and m = 1.39 mg./sec., t = 4.71 sec./drop for the waves in Fig. 4. The H-cell replaced the Beckman DU cell holder in the photochemical reactor for polarographic studies on the photoreactions.

Filter Systems.—The filter system used to transmit the mercury line at 366 mμ consisted of a saturated copper sulfate solution in a 2-cm. thick quartz cell and a Corning CS7-37 glass filter. An ultraviolet cut-off filter system which transmitted wave lengths longer than 400 mμ consisted of the copper sulfate filter and a Corning CS3-75 glass filter. A visible cut-off filter system which transmitted wave lengths less than 330 mμ consisted of a solution of nickel sulfate hexahydrate (690 g./l.) and cobalt sulfate heptahydrate (220 g./l.) in a 2-cm. thick quartz cell.

Procedure.—For the kinetic studies, the photolysis cells were filled with 3 ml. of solution and the air was removed by a minimum of four freeze–thaw cycles using Dry Ice–isopropyl alcohol mixtures under a reduced pressure of 10^{-3} torr. The cells were wrapped with opaque material during the degassing procedure to minimize photolysis. The cells were sealed with a torch, checked for leaks, and stored in the dark until photolyzed. The *in vacuo* solutions were irradiated for short intervals in the reactor and then were analyzed spectrophotometrically over the range 320 to 520 mμ with a Cary Model 15 spectrophotometer.

Solutions to be photolyzed in the polarographic H-cell were placed in the cell and helium (high purity nitrogen did not meet the necessary standards) was bubbled through for 30 min. to remove oxygen. A positive pressure of helium was maintained over the solution at all times. The presence of residual oxygen was checked by completely photoreducing an aqueous solution of riboflavin with EDTA and monitoring, polarographically, the amount of reduced riboflavin that was lost with time. Also the tightness of the gas system was easily checked by this procedure. Under the proper conditions, oxidation of the photol-

(5) L. P. Vernon, *Biochim. Biophys. Acta*, **36**, 177 (1959).

(6) The test depended upon the combination of two enzyme-catalyzed reactions. The final reaction involved the oxidation of DPNH by acetaldehyde, which was formed in the oxidation of ethyl alcohol by hydrogen peroxide. It has been shown that aldehydes are produced extraneously in the photobleaching reaction in every case that gave a positive test[7,8] and, furthermore, the enzyme (alcohol dehydrogenase) is a nonspecific catalyst for alcohols.[9]

(7) R. Brdička, *Chem. Listy*, **36**, 286, 299 (1942); *Collection Czechoslov. Chem. Commun.*, **14**, 130 (1949).

(8) W. R. Frisell, C. W. Chung, and C. G. Mackenzie, *J. Biol. Chem.*, **234**, 1297 (1959).

(9) S. P. Colwick and N. O. Kaplan, "Methods of Enzymology," Vol. I, Academic Press, New York, N. Y., 1955, p. 502.

(10) Strauss and Nickerson[3] claim to have evidence for the existence of hydrogen peroxide in anaerobically photolyzed solutions of riboflavin, but the results have not been presented.

(11) P. Karrer and H. F. Meerwein, *Helv. Chim. Acta*, **18**, 1126 (1935).

(12) M. Halwer, *J. Am. Chem. Soc.*, **73**, 4870 (1951).

(13) H. Kuhling, *Ber.*, **24**, 2362 (1891).

(14) P. Karrer, E. Schlittler, K. Pfaeller, and F. Benz, *Helv. Chim. Acta*, **17**, 1516 (1934).

(15) A. Weissbach and D. B. Sprinson, *J. Biol. Chem.*, **203**, 1031 (1953).

(16) W. M. Moore and M. Ketchum, *J. Am. Chem. Soc.*, **84**, 1368 (1962).

ysis products was found to be negligible during the time periods involved.

The reduction waves and half-wave reduction potentials for lumichrome, alloxazine, and acetaldehyde were obtained from authentic samples of these materials under anaerobic conditions. The pH of the solutions containing EDTA was adjusted with small amounts of concentrated NaOH to the desired value. All solutions used in polarography were 0.1 *M* in KCl.

Results and Discussion

Identical 10^{-4} *M* solutions of riboflavin in water and deuterium oxide were photolyzed *simultaneously*[16] *in vacuo* with the 400 mμ cut-off filter system. The solutions were removed from the reactor periodically and the absorption spectrum in the range 320 to 520 mμ was recorded (the absorption maximum at 445 mμ was used for analysis). There was no detectable difference in the rates of photobleaching for the two solutions. The kinetic data fit a pseudo-first-order relationship (except for a deviation in the first 5 min.) as shown in Fig. 1.

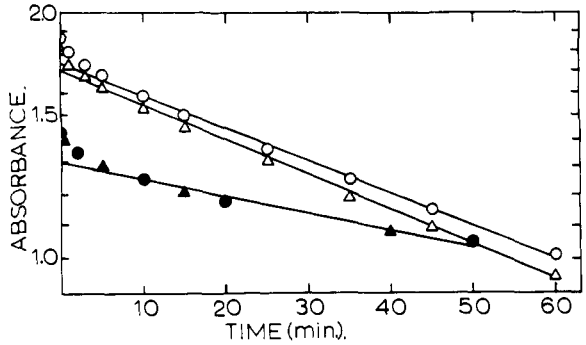


Fig. 1.—Anaerobic photobleaching of 10^{-4} *M* solutions of riboflavin in: O—O, D_2O with filter system > 400 mμ; Δ—Δ, H_2O with filter system > 400 mμ; ●—●, D_2O with filter system at 366 mμ; ▲—▲, H_2O with filter system at 366 mμ.

The data will also fit a relationship of the reciprocal of rate *vs.* absorbance as found by Holmström and Oster,[4] which indicates that the photoproducts quench the photoexcited riboflavin. The same result was obtained when the experiment was repeated with the 366 mμ filter system.

The consequences of the results prompted us to seek another photoreaction where the participation of solvent has also been postulated. Wang[17] has made a detailed study of the photoreaction of uridine to produce 6-hydroxyhydrouridine. On the basis of kinetic studies, he has proposed that water is involved in the rate-controlling process. We photolyzed 10^{-4} *M* solutions of uridine in water and deuterium oxide under the same conditions as employed for riboflavin, except that the 330 mμ cut-off filter system was used (uridine has an absorption maximum at 260 mμ). The kinetic data fit a zero-order rate law as shown in Fig. 2, and an average kinetic isotope effect of 2.1 for k_H/k_D was found. The results support the mechanism proposed by Wang[17] and demonstrate the sensitivity of the method for detecting isotope effects.

Aqueous solutions of riboflavin were photolyzed anaerobically in the polarographic H-cell placed in the photochemical reactor. Brdička[7] has reported half-wave potentials of: −0.47 v., −0.48 v., and −0.58 v. *vs.* s.c.e. for riboflavin, lumiflavin, and lumichrome, respectively, in neutral solution. We have confirmed these values.

We found that lumichrome is produced in the anaerobic photolysis of riboflavin (see Fig. 3.). Upon oxidation, the height of the lumichrome wave remained

(17) S. Y. Wang, *Photochem. Photobiol.*, **1**, 135 (1962).

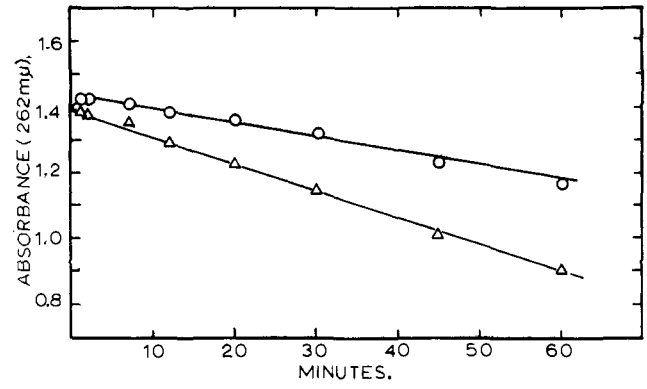


Fig. 2.—Photolysis of 10^{-4} *M* solutions of uridine with filter system < 310 mμ in: O—O, D_2O; Δ—Δ, H_2O.

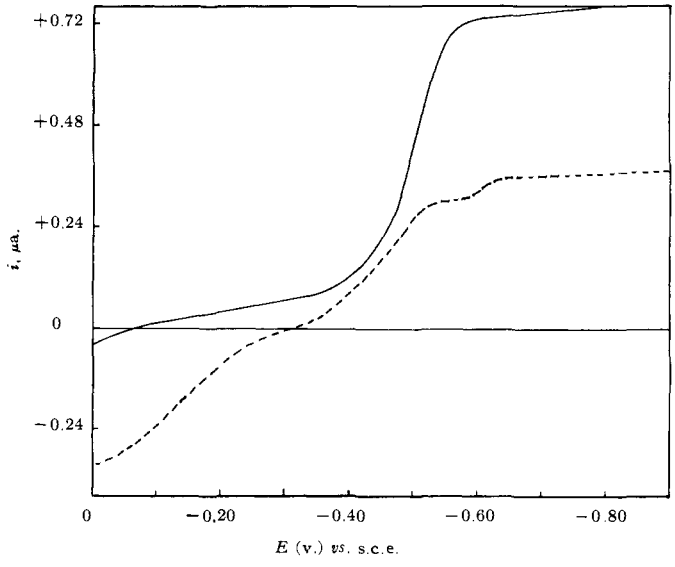


Fig. 3.—Polarographic waves for the anaerobic photobleaching of riboflavin; 1.00×10^{-4} *M* riboflavin, pH 6.85: ———, before photolysis; - - - -, after 30-min. photolysis.

constant, indicating that the formation of lumichrome from riboflavin in the photolysis does not involve oxygen, as previously suggested.[18] In addition to lumichrome, a reduced product is formed which gives an oxidation wave with half-wave potential of −0.20 v. *vs.* s.c.e. This has been suggested to be "leucodeuteroflavin."[7] It cannot be reduced riboflavin (RbH_2), since RbH_2 gives an oxidation wave with $E_{1/2}$ identical with $E_{1/2}$ for the reduction wave of Rb (the polarographic reduction of riboflavin is reversible). Upon oxidation with air, the oxidation wave for this product disappears. These results confirm the work of Brdička.[7]

In the presence of EDTA, Merkel and Nickerson[19] found that upon anaerobic photolysis riboflavin is reversibly reduced, giving RbH_2, and that upon oxidation with air riboflavin is reformed without loss. We have confirmed this work, as shown in the polarographic waves in Fig. 4, and have found the same result with methionine. It is obvious from Fig. 3 that the product formed in the anaerobic photobleaching of riboflavin in the absence of an "activator" is not dihydroriboflavin. This result contradicts the observation of Merkel and Nickerson.[19] The rates for the reactions differ greatly as a comparison of Fig. 3 and 4

(18) For a discussion of lumichrome formation, see G. Oster, J. S. Bellin, and B. Holmström, *Experientia*, **18**, 249 (1962).

(19) J. R. Merkel and W. J. Nickerson, *Biochim. Biophys. Acta*, **14**, 303 (1954).

will indicate. After 15 sec. the photoreduction of riboflavin is almost complete with only a slight change in the wave with extended irradiation. However, after 30 min., the aqueous solution of riboflavin is not completely photobleached. A rough comparison of the rates shows that the photoreduction proceeds 100 times faster than the photobleaching reaction with the same light intensity.

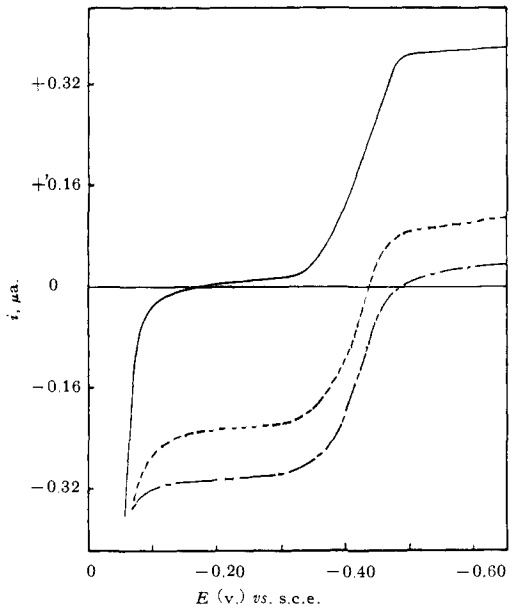


Fig. 4.—Polarographic waves for the anaerobic photobleaching of riboflavin in the presence of EDTA; 1.00×10^{-4} *M* riboflavin, 1.00×10^{-2} *M* EDTA, pH 6.85; ———, before photolysis; – – – –, after 15-sec. photolysis; — — —, after 30-min. photolysis.

Several conclusions can be drawn from these results. The hydrogen atoms on the hydroxyl groups of the ribityl side chain and on the 3-nitrogen of riboflavin are easily exchanged in deuterium oxide, as has been shown by infrared analysis.[20] Therefore, the absence of an observed kinetic isotope effect for the anaerobic photobleaching of riboflavin in deuterium oxide conclusively proves that *neither water nor the hydroxyl hydrogens of the ribityl side chain participate in the rate-controlling process.* Also, the hydrogen on the 3-nitrogen is not involved in the process. The photobleaching of riboflavin in neutral solutions gives at least two different products (lumichrome and "leucodeuteroflavin"), probably by different mechanisms, neither of which require oxygen. Furthermore, the anaerobic photobleaching of riboflavin in aqueous solution in the presence and absence of EDTA proceeds by two distinct mechanisms. Riboflavin is photoreduced in the presence of EDTA (*i.e.*, EDTA is a hydrogen donor for riboflavin), and that in the absence of such a donor, the anaerobic photobleaching process must involve only riboflavin.

The results for the aqueous anaerobic photobleaching of riboflavin gives information about what mechanisms are invalid, but it does not give much evidence as to a plausible mechanism. A detailed study of a simpler isoalloxazine as suggested by the work of Karrer and Meerwein[11] was undertaken as the best approach to this problem. Accordingly, we have re-examined the anaerobic aqueous photobleaching of 9-(2′-hydroxyethyl)-isoalloxazine (Ib) on the supposition that the nature of the 2′-position on the side chain is vital to the photoreaction.

(20) T. Kanzawa and T. Masuda, *Pharm. Bull.* (Tokyo), **4**, 316 (1956).

Identical 10^{-4} *M* solutions of Ib and 9-(2′-hydroxyethyl-2′-2′-d^2)-isoalloxazine (Ie) were irradiated *simultaneously in vacuo* and the rate of isoalloxazine disappearance was followed spectrophotometrically as described previously. The data fit a pseudo-first-order rate law as shown in Fig. 5, and an average kinetic isotope effect of 2.5 was obtained for the slope (H)/slope (D). The magnitude of the isotope effect agrees well with the values of 2.7 and 2.5 found for the photoreduction of benzophenone with benzhydrol-2-*d*[21] and 2-propanol-2-*d*,[22] respectively. Suelter and Metzler[23] found an isotope effect of 3.16 for the dark reaction of riboflavin with 1-propyl-1,4-dihydronicotinamide-4,4-d_2. The photoreduction of benzophenone is known to proceed *via* a free-radical mechanism,[21] but the latter has been postulated to involve a hydride ion transfer.[23]

During the irradiation of Ib and Ie, isosbestic points at 390 and 373 mμ were observed, and toward the end of the photolysis, a new peak in the vicinity of 380 mμ formed. In contrast, during the photobleaching of riboflavin, no isosbestic points were observed, but the formation of absorbing products was evident (see ref. 4 for spectra). Paper chromatographic analysis of the oxidized solution showed one new bluish white fluorescent spot indicative of an alloxazine.

Polarographic analysis of the anaerobic photobleaching of Ib by the method previously described gave the data shown in Fig. 6. The photoreaction is fairly

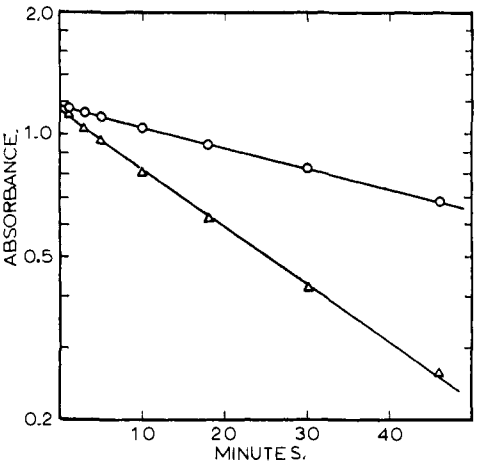


Fig. 5.—The anaerobic photobleaching in aqueous solutions of: O—O, 9-(2′-hydroxyethyl-2′,2′-d_2)-isoalloxazine; Δ--Δ, 9-(2′-hydroxyethyl)-isoalloxazine.

simple as indicated by the spectral data and the polarogram. The 9-(2′-hydroxyethyl)-isoalloxazine reduction wave disappeared as another reduction wave characteristic of alloxazine (V) (−0.56 v.) appeared. In addition, another more negative reduction wave formed with half-wave potential (−1.38 v.) which corresponds to the half-wave potential of acetaldehyde.[24] Of particular importance, no "leucodeuteroflavin" oxidation wave appeared, as with riboflavin. Alloxazine and the anaerobic photoproduct from the photobleaching of Ib gave the same ultraviolet absorption maxima at 378, 343, and 257 mμ in neutral solution, and their polarograms corresponded. McDonald and Metzler[25] have

(21) W. M. Moore, G. S. Hammond, and R. P. Foss, *J. Am. Chem. Soc.*, **83**, 2789 (1961).

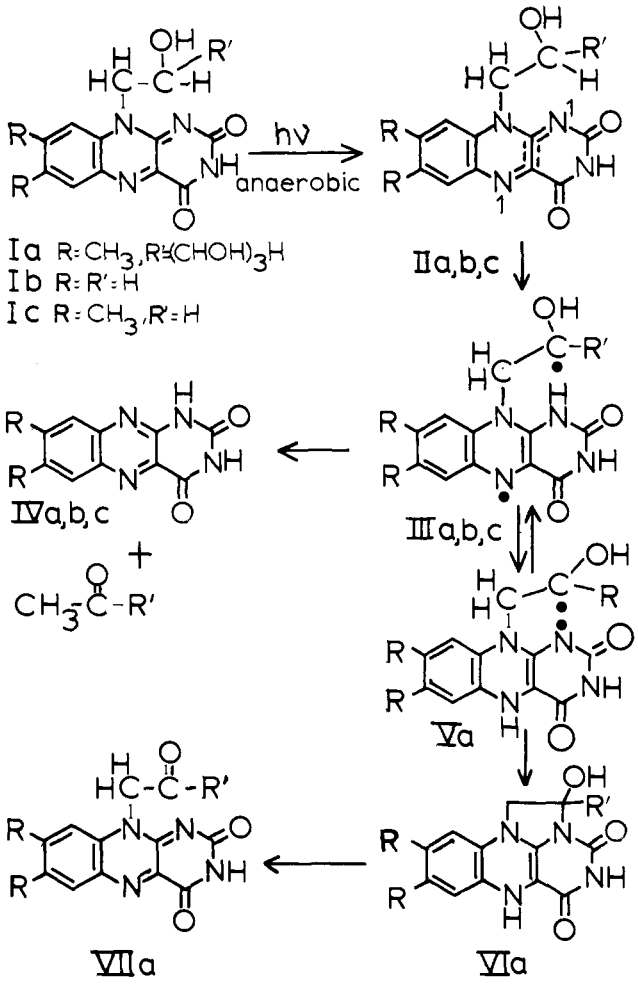
(22) W. M. Moore and M. D. Ketchum, unpublished results.

(23) C. H. Suelter and D. E. Metzler, *Biochim. Biophys. Acta*, **44**, 23 (1960).

(24) The small height of the acetaldehyde wave is due to its removal by helium which was bubbled through the solution continuously during photolysis.

(25) M. McDonald and D. E. Metzler, private communication.

found a similar cleavage with 6,7-dimethyl-9-(2′-hydroxyethyl)-isoalaloxazine (Ic).



From these results, the beginning of a rational mechanism can be formulated for the photoreactions of flavins. In the case of Ib a simple intramolecular rearrangement involving the aliphatic hydrogen atoms on the 2′-carbon explains the kinetic isotope effect and the products. The pathway Ib–IIb–IIIb–IVb can be visualized as a possibility for a free-radical mechanism,[26] or the products might result from a one-step process not involving IIIb. The process I to II is most likely the result of a singlet to singlet excitation followed by conversion to the triplet.[28] The hydrogen abstraction process involving a triplet state is well known and the kinetic isotope effect substantiates that the step II to III is rate controlling. Intermediate III, if it exists, is a diradical that can undergo several reactions. From the products observed for Ib, it is evident that the electrons rearrange in IIIb causing cleavage of the 9 N–1′-C bond and forming alloxazine (IVb) and acetaldehyde. The formation of lumichrome (IVa) in the anaerobic photobleaching of riboflavin can also be explained by this mechanism.

The photoproduct that Terao[31] has obtained from the anaerobic irradiation of riboflavin, which is tentatively the 2′-carbonyl derivative of riboflavin (VIIa), can be explained by the pathway Ia–IIa–IIIa–Va–VIa–VIIa, which is a modification of the mechanism first proposed by Brdička.[7] Intermediate IIIa, the diradical formed by the hydrogen abstraction of photoexcited riboflavin can, in addition to cleavage, undergo a proton exchange to give Va, a diradical that readily couples to yield VIa. Leucodeuteroflavin would most appropriately fit the properties of VIa. Upon air oxidation VIa would form VIIa. If VIIa is the elusive "deuteroflavin,"[32] the outlined mechanism then accounts for most of the photoproducts of riboflavin photobleaching except lumiflavin. Lumiflavin may result from the same type of process operating on the 3′-carbon position of the ribityl side chain.

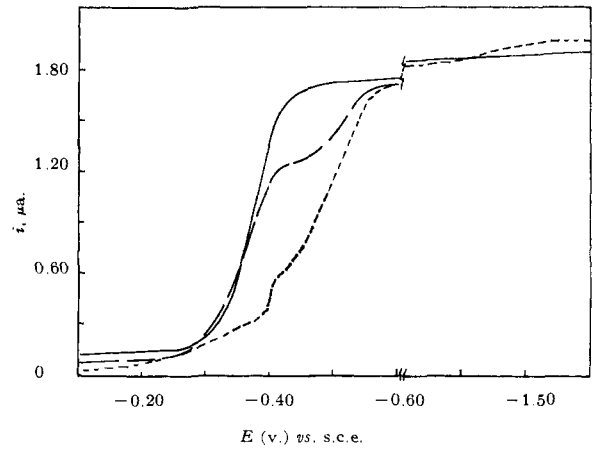


Fig. 6.—Polarographic waves for the anaerobic photobleaching of 2.5×10^{-4} *M* solutions of 9-(2′-hydroxyethyl)-isoalloxazine, at pH 6.50: ———, before photolysis; — — — —, after 20-min. photolysis; - - - -, after 90-min. photolysis.

The complexity of the photoreactions involving riboflavin alone may result from the various choices for hydrogen abstraction by the isoalloxazine nucleus. A model of riboflavin shows that all the aliphatic hydrogens of the ribityl side chain can be placed in proximity of the 1-nitrogen, while only the aliphatic hydrogens at the 3′-, 4′-, and 5′- positions can be placed in proximity to the 2-carbonyl group. The difference in the photoreactions of riboflavin in acidic and basic solutions may be due to a change in the reactive site on the isoalloxazine nucleus. Further work is in progress to resolve the nature of the competing reactions.

The photoreduction of riboflavin in the presence of methionine, EDTA, or other amines must be an entirely different reaction from the photoreactions of riboflavin alone.[33] There is still the question as to whether the primary process for photoreduction of riboflavin involves a one- or two-electron transfer. The excellent work of Frisell, Chung, and Mackenzie[8] has shown that the over-all process must be equivalent to an ionic reaction, but much more mechanistic work is required.

(26) This mechanism will not give the observed experimental rate law, and some other factors must be known. The light intensity absorbed (I_A) will follow the relationship, $I_0(1 - 10^{-A})$; where I_0 is the incident light intensity and A is the absorbance of the solution. This expression cannot be simplified in the absorbance range covered by our kinetic experiments and more rate studies are needed to test the proposed mechanism. Crude rate data from polarographic studies[27] where light absorption was almost complete gave a pseudo-zero-order rate law.

(27) Steven D. Colson, Senior Thesis, Utah State University, 1963.

(28) Several workers have found effects which suggest that the photochemically reactive state is a triplet.[29,30]

(29) H. Theorell, *Biochem. Z.*, **279**, 186 (1935).

(30) J. Posthuma and W. Berends, *Biochim. Biophys. Acta*, **51**, 392 (1961).

(31) M. Terao, *Tohoku Med. J.*, **59**, 441 (1959).

(32) Deuteroflavin, the name given by the early workers for the unknown oxidation product of riboflavin photobleaching, should not be confused with the deuterated flavin used in this study.

(33) It is regrettable that this interesting photoreaction of riboflavin was introduced into the controversy concerning the participation of water in the photobleaching of riboflavin.

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The Condensation–Polymerization of Pentamethyldisilanyl Cyanide[1] and Related Compounds[2]

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Pentamethyldisilanyl cyanide and heptamethyltrisilanyl cyanide have been found to undergo a condensation–polymerization reaction on heating to give trimethylsilyl cyanide and higher methylcyanopolysilanes. Pentamethyldisilanyl chloride and a mixture of methylchlorodisilanes were also found to undergo analogous reactions when heated with silver cyanide.

In a previous paper[4] it was shown that pentamethyldisilanyl cyanide, $(CH_3)_3SiSi(CH_3)_2CN$, decomposed upon heating to give trimethylsilyl cyanide and less volatile products. The present investigation was carried out in order to study this reaction in greater detail.

Experimental

Materials.—Pentamethyldisilanyl chloride and cyanide were prepared as previously described[4] and were of a similar state of purity. The cyanide was obtained in improved yields (77%) and it was found to melt sharply at 25.0–25.5°. The mixture of methylchlorodisilanes[5] employed boiled at 153–156° and consisted mainly of $Cl_2CH_3SiSiCH_3Cl_2$ and $Cl_2CH_3SiSi(CH_3)_2Cl$.[6,7] Unless otherwise stated all reactions were carried out in an atmosphere of dry nitrogen.

Condensation–Polymerization of Pentamethyldisilanyl Cyanide. I.—A Nester gold-plated monel metal semimicro spinning band distillation column (23 theoretical plates) was employed. When $(CH_3)_3SiSi(CH_3)_2CN$ (12.2 g.) was refluxed (oil-bath temperature 175°) in a 25-ml. flask attached to the column it began to turn dark brown. After heating at this temperature for 7 hr. all volatile material was removed by distillation. The maximum oil-bath temperature employed was 230°.

The most volatile fraction consisted of $(CH_3)_3SiCN$ (5.7 g., b.p. 116–117.5°, $n^{26}D$ 1.3899, d^{30}_4 0.7830). The infrared spectrum was essentially identical with that reported in the literature.[8] The reported values are: b.p. 117.8°,[8] $n^{25}D$ 1.3910,[9] $n^{26}D$ 1.3883,[9] and d^{30}_4 0.7834.[8] The next most volatile fraction was unreacted $(CH_3)_3SiSi(CH_3)_2CN$, (0.8 g., b.p. 85° at 34 mm., reported[4] 87° at 34 mm.; confirmed by infrared spectrum[4]).

Redistillation of a 4.0-ml. fraction of an orange colored oil (b.p. 87–95° at 34–0.6 mm.) produced: (a) $(CH_3)_3Si[Si(CH_3)_2]_4CN$, (1.5 ml., b.p. 67.5–71.0° at 0.2 mm. *Anal.*[10] Calcd. for $C_{12}H_{33}Si_5N$: C, 43.43; H, 10.02; Si, 42.32; N, 4.22; mol. wt., 331.8. Found: C, 43.37; H, 10.83; Si, 42.09; N, 4.27; mol. wt.,[10] 330). (b) $(CH_3)_3Si[Si(CH_3)_2]_{4.5}CN$ (2.0 ml., b.p. 92.5–100.0° at 0.25 mm. *Anal.* Calcd. for $C_{13}H_{36}Si_{5.5}N$: C, 43.26; H, 10.05; Si, 42.80; N, 3.88; mol. wt., 360.9. Found: C, 43.21; H, 11.11; Si, 42.01; N, 4.11; mol. wt., 360).

The brown tarry residue which remained was dissolved in diethyl ether and the solution was passed through an alumina chromatography column. After removing the ether, the oil which remained was molecularly distilled in a micro sublimator. This produced $(CH_3)_3Si[Si(CH_3)_2]_7CN$ (0.5 ml. *Anal.* Calcd. for $C_{18}H_{51}Si_8N$: C, 42.70; H, 10.15; Si, 44.38; N, 2.77; mol. wt., 506.3. Found: C, 41.35; H, 10.01; Si, 44.38; N, 2.72; mol. wt., 494).

II.—$(CH_3)_3SiSi(CH_3)_2CN$ (12.79 g.) was heated in the apparatus described above at oil-bath temperatures from 100 to 160° but no major reaction occurred until the temperature was maintained at 175–180°. The material was heated at this temperature until distillation produced 3.94 g. of $(CH_3)_3SiCN$, b.p. 116–118°, $n^{25}D$ 1.3915. Fractionation of the dark colored less volatile material produced: (a) Impure $(CH_3)_3SiSi(CH_3)_2CN$, 2.11 g., b.p. 71–73° at 21 mm., $n^{30}D$ 1.4301, m.p. 17–20°; pure $(CH_3)_3SiSi(CH_3)_2CN$, b.p. 73.1° at 18 mm., $n^{30}D$ 1.4373,[4] m.p. 25.0–25.5°). (b) $(CH_3)_3Si[Si(CH_3)_2]_2CN$, 2.52 g., b.p. 36–40° at 0.4 mm., mol. wt. found 223, mol. wt. calcd. 215.4. A liquid of similar b.p. (0.55 g.) which was partly hydrolyzed during preparation for analysis was also obtained. *Anal.* Calcd. for $C_8H_{21}Si_3N$: C, 44.60; H, 9.78; Si, 39.12; N, 6.50. Found: C, 44.58; H, 9.78; Si, 39.17; N, 6.34. It is believed that in this compound the cyanide group is attached to a terminal silicon atom since the proton magnetic resonance spectrum of this material gave three peaks in the ratio 2:2:3. The chemical shifts with respect to $CHCl_3$ (upfield) were 7.16, 7.30, 7.34 p.p.m.[11] By analogy, it is assumed that the cyanide group is present on a terminal silicon atom in other members of the series $(CH_3)_3Si[Si(CH_3)_2]_xCN$. (c) $(CH_3)_3Si[Si(CH_3)_2]_3CN$, 1.16 g., b.p. 65–75° at 0.4 mm. *Anal.* Calcd. for $C_{10}H_{27}Si_4N$: C, 43.88; H, 9.95; Si, 41.05; N, 5.12; mol. wt., 273.7. Found: C, 44.14; H, 10.00; Si, 41.20; N, 4.97; mol. wt., 286. (d) $(CH_3)_3Si[Si(CH_3)_2]_4CN$, 0.91 g., b.p. 81–85° at 0.4 mm. *Anal.* Calcd. for $C_{12}H_{33}Si_5N$: C, 43.43; H, 10.02; Si, 42.32; N, 4.22; mol. wt., 331.8. Found: C, 43.40; H, 9.82; Si, 42.48; N, 4.30; mol. wt., 333. (e) A fraction, 0.10 g., b.p. 27–35° at 0.4 mm., which was not analyzed but was believed to be a mixture of methylcyanopolysilanes. (f) A dark brown tarry residue (0.86 g.) which upon molecular distillation produced: (1) A viscous yellow oil which had the composition $NC(CH_3)_2Si[Si(CH_3)_2]_7CN$, 0.24 g. *Anal.* Calcd. for $C_{18}H_{48}Si_8N_2$: C, 41.79; H, 9.35; Si, 43.44; N, 5.42; mol. wt., 517.3. Found: C, 40.84; H, 10.15; Si, 43.73; N, 5.26; mol. wt., 507. This substance, which represented 2.1% by weight of the volatile material produced in the reaction, could have been formed from a small amount of $NC(CH_3)_2SiSi(CH_3)_2CN$ (approximately 0.6%) impurity present in the starting material. This could have resulted from the presence of a very small amount of $Cl(CH_3)_2SiSi(CH_3)_2Cl$ impurity in the $(CH_3)_3SiSi(CH_3)_2Cl$ used to prepare $(CH_3)_3SiSi(CH_3)_2CN$. (2) A dark gummy nondistillable residue (0.62 g.) which represented 4.86% of the weight of the starting material. It had the appearance and consistency of plycine wax. It was soluble in ether and when warmed it melted to a gummy resin.

The total weight of material recovered from the reaction was 94.2% of the weight of the $(CH_3)_3SiSi(CH_3)_2CN$ employed.

Condensation–Polymerization of Heptamethyltrisilanyl Cyanide.—$(CH_3)_3Si[Si(CH_3)_2]_2CN$ (0.9850 g.) was sealed under vacuum in a magnetic break-seal tube and was heated in an oil bath at 175–195° for approximately 60 hr., by which time it had turned dark brown. On opening the tube on a high vacuum

(1) No assumption is made as to whether this compound or any other organosilicon cyanide described in this communication has the normal cyanide or the isocyanide structure. For convenience they are all written as normal cyanides.

(2) This report is based on portions of a thesis to be submitted by Joseph V. Urenovitch to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The work is, in part, a contribution from the Laboratory for Research on the Structure of Matter, University of Pennsylvania, supported by the Advanced Research Projects Agency, Office of the Secretary of Defense.

(3) Alfred P. Sloan Research Fellow.

(4) A. D. Craig, J. V. Urenovitch, and A. G. MacDiarmid, *J. Chem. Soc.*, 548 (1962).

(5) The sample of mixed methylchlorodisilanes was kindly presented by the General Electric Co., Waterford, N. Y.

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(10) All analyses and molecular weight measurements were performed by Galbraith Laboratories, Knoxville, Tenn. Molecular weights were determined by vapor pressure osmometry in benzene solution. The precision of the measurements was approximately 3% in the molecular weight range investigated.

(11) The proton magnetic resonance spectra of a number of pentamethyldisilanyl compounds will be reported in greater detail elsewhere.