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A STUDY OF PHOTOCHEMISTRY OF FLAVINS IN PYRIDINE AND WITH A DONOR*

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Abstract—Both anaerobic and aerobic photolysis of riboflavin in pyridine yielded several photoproducts, analogous to the photochemical reaction in aqueous solution. Lumiflavin was also photoreduced in pyridine (an electron donor) without decomposition of the isoalloxazine ring Differences in the reactivity of excited singlet and triplet states with respect to formation of photoproducts have been confirmed in pyridine. In the photoreduction of riboflavin by N,N′-dimethyl-N-benzylethylene diamine, an initial rate with a higher relative quantum yield than that of the reaction at a later stage was observed both in water and pyridine. Photolysis in D_2O with the donor showed no significant solvent isotope effect. These results strongly suggest that the photolysis of riboflavin in water does not involve the water molecule in the primary photochemical act. A detailed mechanism of flavin photoreaction in water to account for solvent oxygen incorporation into a photoproduct (benzaldehyde) from N,N′-dimethyl-N-benzylethylene diamine has been proposed without involving photochemical splitting of water based on our results and molecular orbital computations.

Preliminary results on the kinetics of free radical decay were obtained using an ESR spectrometer and the significance of the results are discussed.

INTRODUCTION

THE PHOTOCHEMISTRY of flavins has not been uniquely understood until recently, although photodecomposition of riboflavin has been well recognized. Earlier studies concerning primary photoprocesses of the photolysis of riboflavin were made by Nickerson's group[1–3] and others,[4,5] proposing that the anaerobic photobleaching (photoreduction) of riboflavin in water is due to splitting of the water molecule as an electron donor.

On the other hand, we proposed along with others that hydrogen for the photoreduction of the isoalloxazine ring is not from the splitting of water by an excited flavin but from the ribityl side chain (intramolecular photoreduction).[6–9]

Recently, Enns and Burgess[10] revived this controversial problem by supporting the Nickerson scheme that water was photochemically split by the excited riboflavin. While it was established in their work that oxygen in the photoproduct (benzaldehyde) from N,N′-dimethyl-N′-benzylethylene diamine originated from water, non-photochemical incorporation of oxygen due to a nucleophilic attack of water upon the electron-deficient primary photoproduct of the donor appears more likely.

With this point in mind, we have studied the photolysis of riboflavin and lumiflavin in pyridine in which the involvement of water is automatically excluded and the photoreaction

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pattern will be less complicated as far as the problem of the photo-splitting of water is concerned. It must be emphasized that care must be taken to select an organic solvent for the photolysis, as the solvent itself may complicate the photochemistry of flavins. In this connection, a few comments will be made on the recent work by KOZIOL[11] that the overall scheme of riboflavin photolysis in organic solvents is the same as in aqueous solution, lumichrome being the only major product.

MATERIALS AND METHODS

Materials

Riboflavin (RF, 6,7-dimethyl-9-(D-1'-ribityl)-isoalloxazine, hygroscopic), lumiflavin (LF, 6,7-dimethyl-9-(methyl)-isoalloxazine), formylmethylflavin (FMF, 6,7-dimethyl-9-(formyl methyl)- isoalloxazine), and lumichrome (LC, 6,7-dimethyl-alloxazine) were obtained as described elsewhere.[12,13]

Pyridine was "Baker-analytical" reagent grade and was further purified by refluxing over KOH pellets. Water was redistilled. (Deuterium oxide (99·85 mole%) was obtained from CalBiochem. Co.) N-benzyl-N,N'-dimethyl-ethylene diamine (NDB) was obtained from K & K Laboratories and was used without further purification. N,N'-dibenzyl-ethylenediamine (NDE) was obtained from Aldrich Co. Prepurified research grade nitrogen (99·998%) and oxygen (99·50%) were obtained from Matheson.

Methods

(a) *Kinetic measurement.* For aerobic photolysis, open colorimetric tubes or closed tubes (Pyrex) saturated with oxygen containing about 4 ml of RF solution were photolyzed at a distance of 1·2 cm from the light source. The visible light sources used in the photolysis were: (1) fluorescent illuminator, 15 W, Fisher Scientific Co., (2) photochemical grid lamp (PCQ-011 lamp coated with 400–500 nm phosphor, output at 405 and 436 nm, Ultraviolet Products, Inc.), and (3) photochemical grid lamp (PCQ-011 lamp coated with 366 nm phosphor, approximately 4·5 W, Ultraviolet Products, Inc.).

The rate of photolysis was followed spectrophotometrically by measuring the decrease in absorbance at 450 nm. Occasional shaking and aeration was required to ensure a complete aerobic condition of the solution in an open test tube. No significant difference in the rate of photolysis in open and closed (with oxygen) test tubes was noticed.

For anaerobic photolysis, about 4 ml of RF solution were placed in a test tube with a tight serum rubber stopper, and the solution was flushed with purified dry nitrogen through a hypodermic needle (12 cm) for about 20 min. The rate of photolysis was then followed as before. All the solutions were prepared in the dark or shielded from floor light with aluminium foil.

The pH of aqueous solutions was kept at 6·1 and pD of the D_2O solution was kept at 6·4 (measured on a Beckman pH meter by adding 0·4 to the pH meter reading[14]).

Most of the rate data were treated by plotting log $(I_o/I - 1)$ vs. time, according to the equation which was the integrated form of the usual photochemical rate law:

$$-\mathrm{d(RF)}/\mathrm{d}t = \phi \{I_{abs}[1 - \exp(-2{\cdot}3\,\epsilon\,(\mathrm{RF})\mathrm{l})]\} \quad ,$$

$$\log (I_o/I - 1) = \log (I_o/I - 1)_{t=0} - \phi\, I_{abs}\epsilon t$$

where log (I_o/I) is absorbance, I_{abs}, the actual intensity of light absorbed by a solution

(einsteins/sec), ϵ, the molar extinction coefficient of RF or LF in pyridine, l, the diameter of the tube, and ϕ, the quantum yield. Thus, the rate constant expressed in the first order unit (sec^{-1}) in this paper is equivalent to $\phi I_{abs}\,\epsilon$ (ϕ·einsteins sec^{-1} $mole^{-1}$ cm^{-1}), and the constant is then a measure of a relative quantum yield.

(b) *ESR measurements.* ESR spectra of the riboflavin ($3{\cdot}0 \times 10^{-4}$M) solution in pyridine in a nitrogen-flushed ESR cell after irradiating for 1–2 min were taken on a Varian ESR spectrometer. The same spectra were obtained with a light source (a Westinghouse mercury arc lamp (H38-4ab), 100 W) placed at about 40 cm from the ESR cavity slot and the light was collimated at the slot by means of a concave mirror. The relative rate of free radical formation and disappearance was followed by measuring the change in signal height as a function of time. The g-value of the spectrum was measured by the Dual Cavity method using peroxyl-amine disulfonate ion radical ($g=2{\cdot}0057$) as a reference.[15]

(c) *Thin-layer chromatography.* The photolyzed reaction mixture was concentrated by evaporation under mechanical vacuum in the dark. Two to three applications of the concentrate (approximately 1×10^{-3} M/l. of the original reaction mixture) on a thin-layer plate were then developed using either *n*-butanol : acetic acid : water (4 : 1 : 5, v/v) or *n*-amyl alcohol : acetic acid : water (3 : 1 : 3, v/v) for 3–4 hr. The spots were located under u.v. illumination using a mineralight (Model UVS-12, emission at 254 nm). LC has a distinctive blue fluorescence, while other flavins show yellow green fluorescence. FMF and LF which have been characterized previously by NMR and IR spectrometers can be identified by comparing with standard compounds.

(d) *Excitation and emission spectra.* "Apparent" singlet–singlet excitation and emission spectra of the reaction mixture and photoproducts from pyridine eluates of the TLC spots were taken on an Aminco–Bowman spectrophotofluormeter with a potted photomultiplier tube (1P21) and xenon arc lamp as a light source.

(e) *Molecular orbital computations.* The ω-SCF HMO was employed in computing electronic and reactivity indices from Fukui's frontier electron theory.[16] The computation on the IBM 7040 at the Texas Tech Computer Center was carried out using slightly modified programs originally written by Dr. C. E. Klopfenstein of the University of Oregon. The heteroatom parameters used were taken from Pullman & Pullman[17] and Streitwieser.[18]

RESULTS

Figures 1 and 2 show respectively the kinetics of anaerobic and aerobic photolysis of RF in pyridine, as well as of LF in chloroform. It appears that first order kinetics is satisfactory at constant intensity of irradiation, except for the rapid initial rate of anaerobic photolysis of RF in the absence of a quencher. However, it was also found from an initial-rate measurement that the rate up to 60 sec follows first order kinetics with a rate constant k_1. This rapid initial rate is absent in the presence of phenol or oxygen. Table 1 lists rate data estimated from Figs. 1 and 2.

In order to established whether or not water is the donor for the photoreduction of the flavin ring, the initial rate of anaerobic photolysis (linear portion of the rate curve) was followed as a function of varying concentration of water in pyridine solution. Results are shown in Fig. 3. It can be readily seen that the water molecule does not act as a hydrogen donor, since increase in the water concentration rather decreases the rate of photolysis.

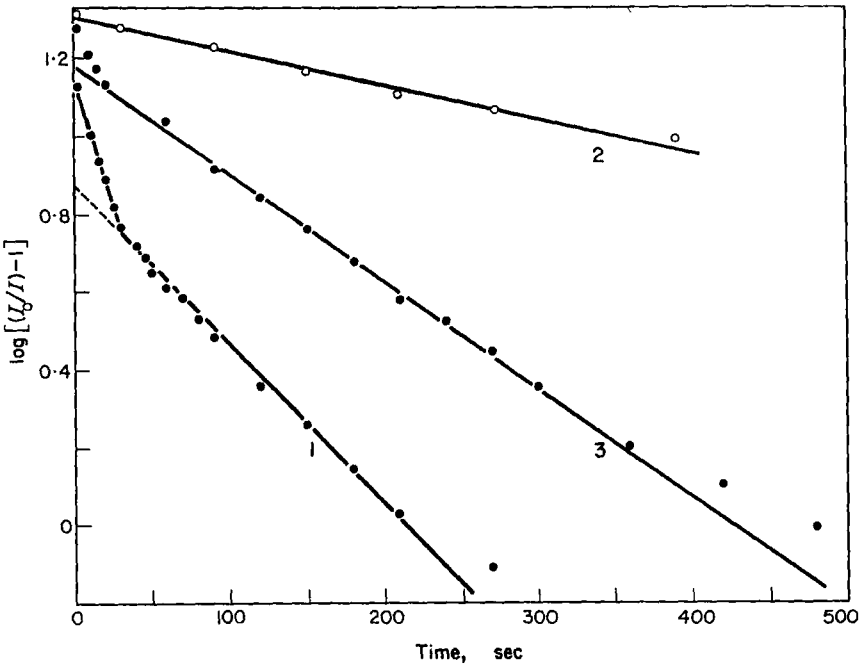


FIG. 1. Rate of anaerobic photolysis of (1) riboflavin (*ca.* 1·0 × 10^{-4}M), (2) lumiflavin (*ca.* 1·1 × 10^{-4}M), and (3) riboflavin (*ca.* 1·0 × 10^{-4}M) plus 0·012M phenol in pyridine. Photolysis was carried out with the fluorescence illuminator, and similar results were obtained with other light sources such as the photochemical grid lamp with 366 nm or 405/436 nm output.

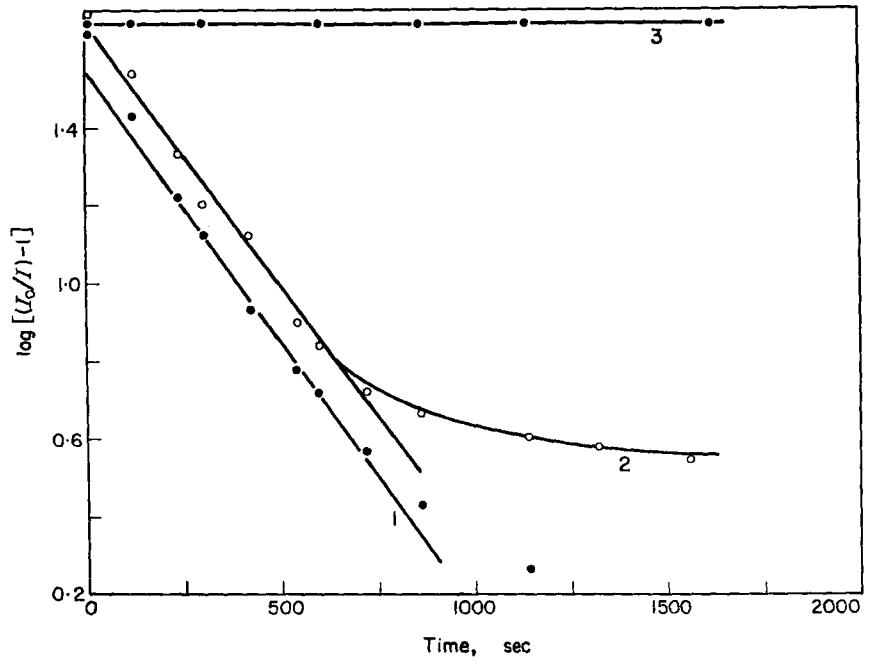


FIG. 2. Rate of aerobic photolysis of (1) riboflavin (*ca.* 1·5 × 10^{-4}M) in pyridine, (2) riboflavin (*ca.* 1·5 × 10^{-4}M) plus 0·0125 M phenol in pyridine, and (3) lumiflavin (*ca.* 1·0 × 10^{-4}M) in pyridine and/or in chloroform.

TABLE 1. RATE DATA FOR PHOTOLYSIS OF FLAVINS

Flavin	solvent	quencher	anaerobic k (sec^{-1})	anaerobic %photolysis* (time, sec)	anaerobic %color return†	aerobic k (sec^{-1})
RF	pyridine	—	$k_1=1{\cdot}28\times10^{-2}$ $k_2=4{\cdot}05\times10^{-3}$	83(325)	66	$1{\cdot}4\times10^{-3}$
RF	pyridine	phenol	$k_1=7{\cdot}0\times10^{-3}$ $k_2=2{\cdot}75\times10^{-4}$	54(270) 77(480)	28 26	$1{\cdot}32\times10^{-3}$
LF	pyridine	—	$9{\cdot}0\times10^{-4}$	26(680) 50(5500)	100 99	0·0
LF	chloroform	—	0·0	0	—	0·0

*Calculated from $(A_0-A_f)\ 100/A_0$, where A_0 and A_f are absorbance at time = 0 and at the end of photolysis, respectively.
†Calculated from $(A_a-A_f)\ 100/(A_0-A_f)$, where A_a is the absorbance after air oxidation.

Ratios of relative rate constants calculated from Fig. 3 are 1 (0% water) : 0·82 (3% water) : 0·63 (10% water) : 0·44 (100% water).

Figure 4 shows the rate of the photoreduction of RF in the presence of a donor (NDB or NDE). The general shape of the rate curve appears to be analogous to that of the photolysis in the absence of a donor. In other words, the sharp initial rate is common to the photolysis in the absence and presence of a donor. The values of k_1 are $3{\cdot}75\times10^{-2}$ sec^{-1} (NDB), $2{\cdot}63\times10^{-2}$ sec^{-1} (NDE), and $1{\cdot}5\times10^{-2}$ sec^{-1} (NDE plus phenol), respectively. The rate of the photoreduction of the flavin ring with NDB and NDE appears to be about the same. Figure 5 shows that the rate of the photoreduction of RF by NDB is slightly faster in D_2O ($k_1=3{\cdot}37\times10^{-2}$ sec^{-1}) than in H_2O ($2{\cdot}58\times10^{-2}$ sec^{-1}).

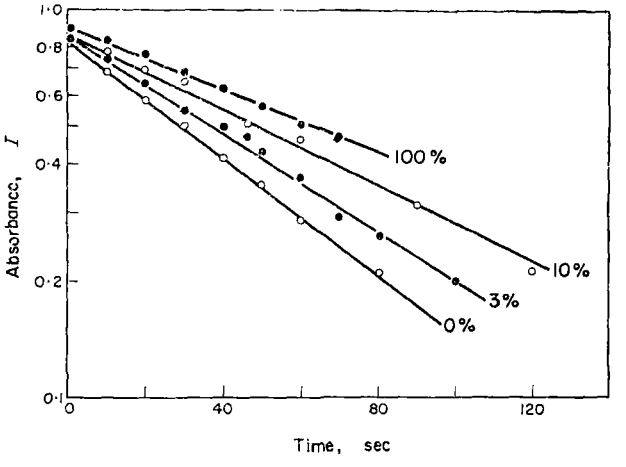


FIG. 3. Effects of water on the relative initial rate of photolysis of riboflavin (*ca.* $1{\cdot}5\times10^{-4}$M) in pyridine. Percentage of water in the pyridine solution is indicated for each rate curve. Photolysis was carried out with the fluorescence illuminator.

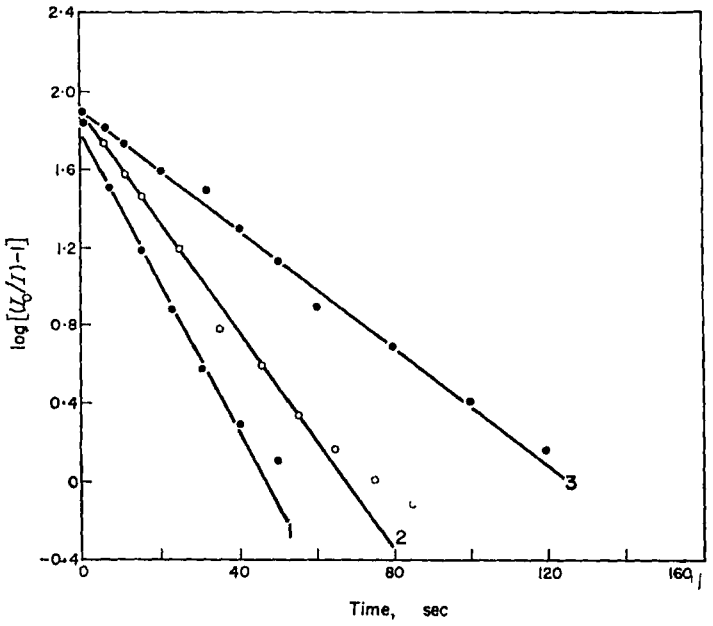


FIG. 4. Rate of anaerobic photolysis of riboflavin (*ca.* 1·5 × 10^{-4}M) in water (pH 6·1) in the presence of (1) *ca.* 0·03 M N,N'-dimethyl-N-benzyl-ethylenediamine (NDB), (2) *ca.* 0·03 M NDE plus 0·013 M phenol. Photolysis was carried out with the photochemical grid lamp with 405 and 436 nm output.

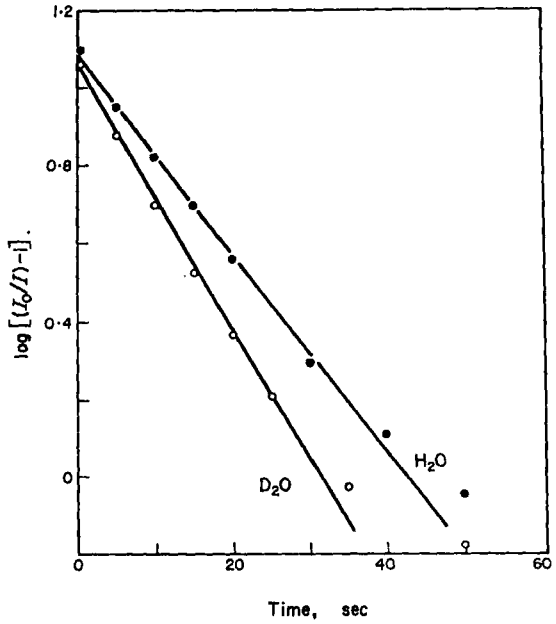


FIG. 5. Rate of the photoreduction of riboflavin (9·3 × 10^{-5}M) by N,N'-dimethyl-N-benzyl-ethylenediamine (3·5 × 10^{-2}M) in water (pH 6·1) and in deuterium oxide (pD 6·4). Photolysis was carried out using the photochemical grid lamp with 405/436 nm output.

Figure 6 shows ESR spectra of the semiquinone radical obtained from the irradiated solution of RF in pyridine and the g-value was found to be 2·00412. Included in the same figure is the decay curve of the free radical which is a flavin semiquinone with modified side chain similar to the one obtained from the photolysis of aqueous RF solution.[6,7] The radical concentration was seen to increase immediately after the irradiation, followed by a steady state concentration. The radical disappears quite slowly when the light is turned off. However, a non-photochemical steady state concentration (or an equilibrium concentration) of the radical remains even after 1 hr without irradiation. It seemed that the residual radical disappeared due to gradual air leakage into the ESR cell after a longer period of standing. The same was observed from the irradiated reaction mixture of RF and NDB.

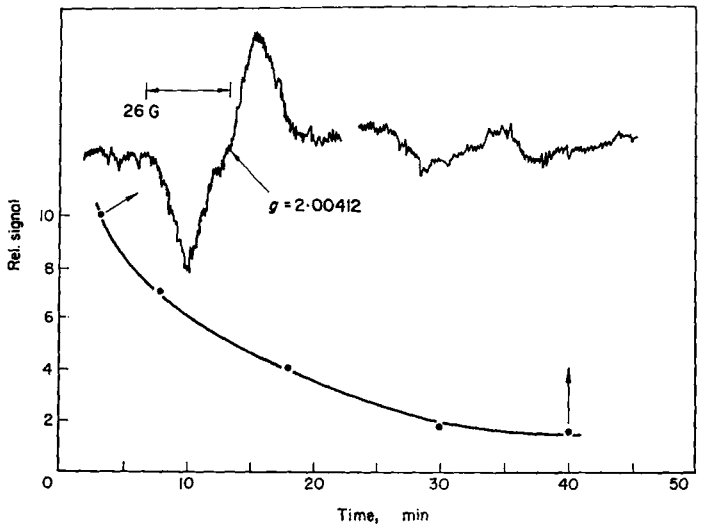


FIG. 6. Decay of flavin semiquinone radical after the irradiation of anaerobic riboflavin solution (*ca.* 3·0 × 10^{-4}M) in pyridine for 2 min. Spectra at 3 and 40 min after light was turned off are also shown. The modulation amplitude, signal level, and sweeptime in minutes were 100, 1000, and 5, respectively in the field of 3395 G.

Figure 7 shows thin-layer chromatograms of the photoproducts under different conditions of photolysis. It can be seen that FMF and another major product, Compound "A" [see ref. (7)] immediately above the RF spot, are either absent or present in trace amounts in the photoproducts obtained from aerobic and inhibited anaerobic photolysis of RF, judging from the intensity of fluorescence of the spots.

It is to be noted that LF which has slightly higher R_f value than that of compound "A" is not formed. In our previous radiochemical studies of photoproducts in aqueous solution, LF was anaerobically formed only at a high pH.[7] When the photolyzed solutions at various pH's prior to air oxidation were promptly neutralized, the formation of LF from alkaline hydrolysis of the photoproducts was prevented.[7,13]

Uncorrected singlet–singlet excitation (at shorter wavelength region) and emission (at longer wavelength region) spectra in pyridine of photoproducts isolated from the TLC in Fig. 7 were obtained. Emission peaks are at 511 nm (riboflavin), 507 nm (lumichrome

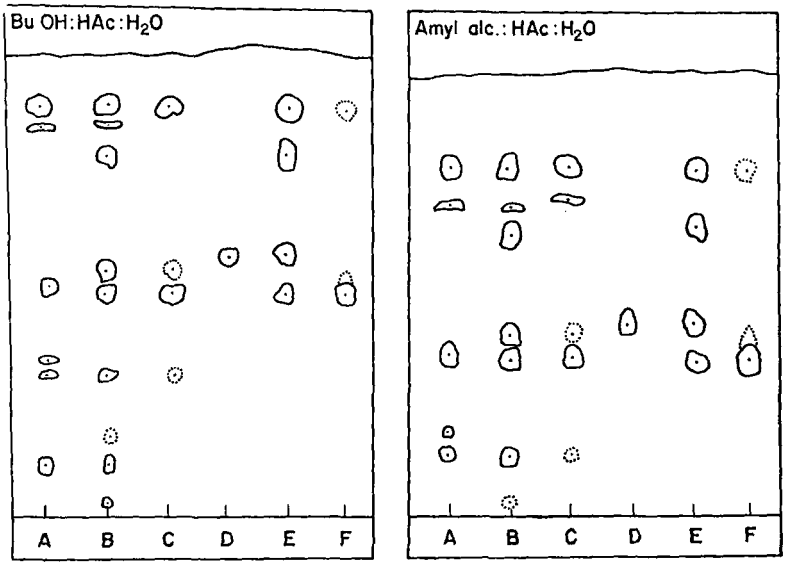


FIG. 7. Thin-layer chromatograms of fluorescent flavin photo-products from riboflavin and lumiflavin in pyridine. The developing solvents are indicated above the solvent front of the chromatograms.

(A) aerobic photolysis products of RF,
(B) anaerobic photolysis products of RF,
(C) anaerobic photolysis products of RF in the presence of 0·0125M phenol,
(D) anaerobic photolysis product (LF) from LF,
(E) mixture of authentic RF, LF, FMF, and LC in order of increasing mobility, and
(F) anaerobic photolysis products of RF in the presence of NDE or NDB (dark spots of NDE and NDB itself are not shown).

and formylmethylflavin), 510 nm (compound "A"), and 503 nm (lumiflavin), respectively. Excitation peaks are at 355/467 nm (riboflavin), 394 nm (lumichrome), 353/465 nm (formylmethylflavin), 352/468 nm (compound "A"), and 355/467 nm (lumiflavin), respectively. Six sharp peaks representing different vibrational transitions between 450 and 480 nm region can be resolved for riboflavin at 1 nm resolution. Each Spectrum was closely compared with that of the corresponding authentic compound (except "A").

Characteristic differences of each of the photoproducts can be used to distinguish photoproducts shown in Fig. 7, especially between compound "A" and LF. Finally, it is pointed out that anaerobic photolysis of RF in pyridine yields 8–9 products, depending upon separation and extent of photolysis. From Fig. 7, it can also be seen that the photoproduct from RF plus NBD is mostly reduced RF which upon air oxidation reoxidizes to give RF on the TLC. It is pointed out that the dependence of each of the photoproducts on oxygen which quenches the triplet state can be recognized from the TLC shown in Fig. 7.

DISCUSSION

The kinetic behavior of the photolysis of RF in pyridine appears closely similar to the photolysis in water, specifically with respect to the appearance of a faster initial rate followed by a slower rate curve and per cent color return.[6,7] This suggests that the nature of the photochemistry of RF involved is essentially unaffected by changing from water to pyridine

as a solvent. In an attempt to clarify the nature of the photoreaction kinetics in which two consecutive straight lines apparently appear in the plot of rate data (Fig. 1), the kinetics of the photolysis was also followed spectrophotofluorometrically. Three measurements gave a straight line when log (relative fluorescence) was plotted against time. In another attempt, TLC of the photoproducts after 90 sec and 28 min under anaerobic conditions gave no apparent qualitative differences in the number of photoproducts. A quantitative study of the photoproduct distribution as a function of irradiation time using RF-C^{14} is thus desirable.

Although it is still unclear why the two consecutive lines in Fig. 1 appear, two explanations are possible. First, the slower rate curve may be due to the absorbance at 450 nm contributed by a steady state concentration of intermediates such as semiquinone from RF, thus causing the rate curve to break as if the kinetics of the reaction is of consecutive first order. Such a semiquinone will have an excitation maximum much longer than 450 nm and an emission maximum at a longer wavelength than RF (520 nm), if it is fluorescent, and, spectrophotofluorometric measurements of rate should give no break, in agreement with the straight line obtained fluorometrically. However, the explanation is not as straightforward as it is suggested here, since the apparent rate constant measured is lower than k_1 from Fig. 1 (curve 1). Second, decrease in the quantum yield by products of the reaction may cause a gradual break in the rate curve.[9] In any case, the extent of the deviation from the initial straight line ($t = 0$) will also be apparent at the later stage of the reaction and is function of the concentration of RF, as can be seen in the rate expression (see Materials and Methods section) when I_{abs} cannot be approximated as constant.

In aerobic photolysis, the triplet state of RF is expected to be effectively quenched by the dissolved oxygen, LC being the major product under aerobic conditions. The mechanism for the formation of LC will be proposed later. It is interesting to note that the apparent rate constants of the aerobic photolysis and phenol-added photolysis of RF are about the same. Phenol was found to be an effective triplet quencher,[6,7] as shown in Table 1. Consequently, the same rate of photolysis is to be expected for the oxygenated aerobic and phenol-added aerobic photolysis. However, it was found, in Fig. 2, that the rate of phenol-added photolysis begins to level off due to appearance of a brown colored product, indicating an oxidation of phenol by an excited RF.

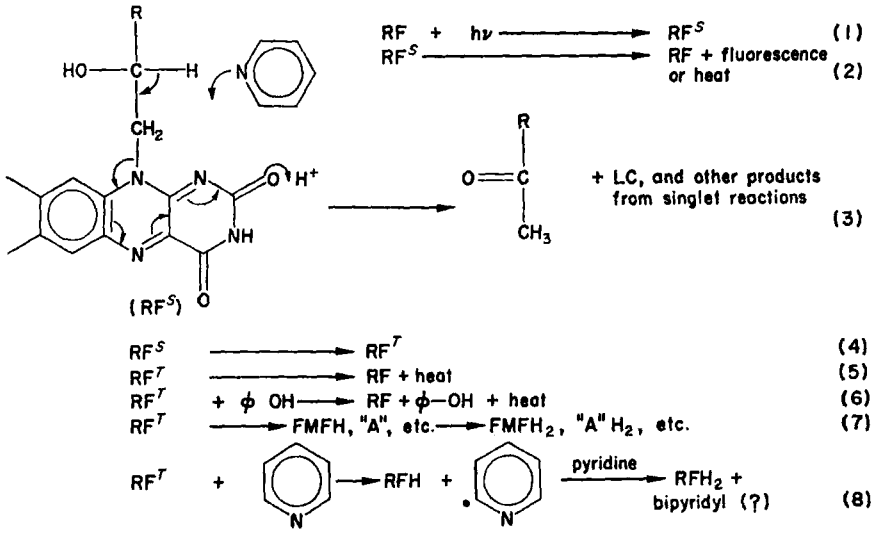
LF is also reduced in pyridine (Fig. 1), but not in water,[7,19] although the rate is much slower than the rate of photolysis of RF. It is, therefore, suggested that water cannot be photosplit by an excited flavin. It is obvious from Figs. 3 and 5 that water cannot serve as a donor, in contrast to the proposal made by some workers.[10] If the water molecule is involved in the primary photochemical act, an isotope effect in D_2O is to be expected, since the intermolecular hydrogen abstraction from NDB by the excited flavin is likely to be rate-determining. Figure 5 shows no apparent isotope effect in D_2O other than the difference in rate due to difference in pH and pD values.

In agreement with the reference,[10] RF is photoreduced by NDB, as can be judged from nearly 100 per cent recovery of the absorbance of the solution after the air-oxidation of the photobleached reaction mixture. TLC in Fig. 7 (F) also shows the preferred intermolecular photoreduction of the flavin. NDE is also found to be as good a donor as NDB.

It appears certain that the first product via the triplet RF is a semiquinone radical, as shown in Fig. 6. The slow decay of the radical may be due to disproportionation between semiquinones which are at low concentration. It has been proposed that such a radical arises by two-step one-electron transfer of the hydrogen (probably at C-2′) from the ribityl

side chain to the ring.[20,21] Recently, Terenin *et al.*[22] showed that the flash photolysis of RF and LF in the presence of diphenylamine yields initially the cation-radical of diphenylamine and the flavin semiquinone.

On the basis of the discussion above and the results described in some detail in conjunction with Fig. 7, a reasonable mechanism of the photolysis of RF in pyridine in the absence and presence of a donor can be proposed,



where S and T represent excited singlet and triplet states, respectively. The driving force for reaction (3) is the polarity of the excited singlet state. Reaction (3) also accounts for aerobic photolysis of RF in pyridine. Reactions (7, 8 and 11) are probably two-step one-electron processes. Reaction (8) can be disregarded, since pyridine is not as good a donor to the flavin ring (LF) as the side chain itself in RF (Table 1).

The site of the intramolecular hydrogen abstraction in reaction (7) can be suggested to be at nitrogen-1 of RF or its semiquinone from the molecular orbital computations shown in Fig. 8. Although nitrogen-10 is in a proper geometric position to abstract the 2'-hydrogen of the side chain in the molecular model, the reactivity of nitrogen-10 is considerably less than that of nitrogen-1.

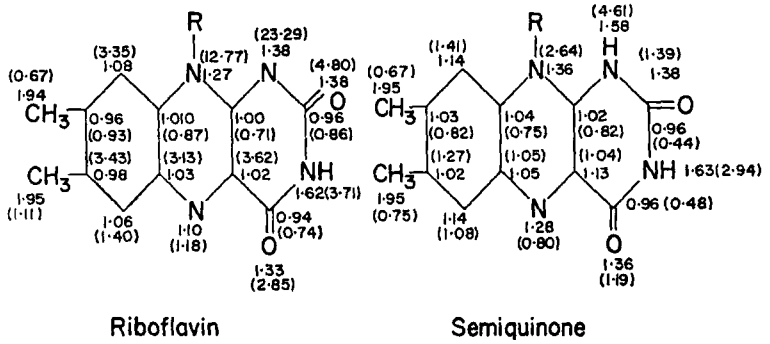


FIG. 8. Total π-electron densities and superdelocalizabilities (in parentheses) for a radical attack in riboflavin and its semiquinone. Nitrogen-1 has the highest superdelocalizability in both riboflavin and the semiquinone radical.

It is noteworthy that LF is anaerobically photo-reduced in pyridine, as shown in Table 1, possibly by α-hydrogen abstraction of pyridine. Complete recovery of LF is obtained after air oxidation of the photobleached LF solution. The mechanism of the photoreduction may be written as follows:

$$LF + h\nu \longrightarrow LF^s \tag{9}$$

$$LF^s \longrightarrow LF^t \tag{10}$$

$$LF^t + \text{pyridine(P)} \longrightarrow LFH + P\cdot \xrightarrow{P} LFH_2 + \text{bipyridyl (?)} \tag{11}$$

2,2'Bipyridyl is probably a likely product as the 2-carbon of pyridine shows the highest free valence (0·487) and superdelocalizability (0·841) for a radical attack among the three carbons. Triplet LF may act more likely as a free radical reagent. Ultraviolet irradiation of pyridine is known to yield 2,2'-bipyridyl.[23] Due to the low concentration of the product (theoretically less than 1×10^{-4} mole), proof of the presence of bipyridyl was not attempted.

It must be noted again that LF is not photoreduced in water,[19] and irradiation of LF in water does not produce the semiquinone radical.[6,7] Electrolytic or metallic reduction produces LF semiquinone,[24] however. The photoproduct distribution pattern between anaerobic photolysis in pyridine and water in Fig. 3 suggests a striking similarity, indicating essentially identical primary and secondary processes of the two systems. Recently, Koziol[11] concluded that LF produced in pyridine is an intermediate of the photolysis, LC being the major product from RF. However, separation on a paper chromatogram is not sensitive enough to distinguish LF from "A" which arises under incomplete aerobic conditions. For example, autoradiography of thin-layer chromatograms for aqueous photoproducts from RF-2-C^{14} gives at least ten clearly separated spots, whereas on paper only three or four products may be separated.[6] Furthermore, Figs. 2 and 3 indicate that LF cannot be an intermediate as it is not decomposed at all. LF can only be photoreduced anaerobically in pyridine (donor).

The implication of our studies is that the primary process of the photochemistry of riboflavin does not involve splitting of the water molecule. Results discussed thus far rule out photochemical splitting of the water molecule as a reducing agent. In brief, evidence from the present study against the Nickerson scheme may be summarized as follows:

(a) Primary and secondary photoprocesses of photolysis of RF in pyridine appear very similar to those in water.

(b) LF is photoreduced in pyridine, but not in water, and

(c) several flavins with a modified side chain are produced and are indicative of intramolecular reduction of the ring.

The result obtained by Enns and Burgess[10] that oxygen from water was incorporated into benzaldehyde as a photoproduct from N,N'-dimethyl-N'-benzylethylenediamine does not necessarily indicate primary photochemical splitting of water by excited RF. It is very likely that N,N'-dimethyl-N'-benzylethylenediamine does act as an electron donor to reduce RF, followed by nucleophilic attack of water upon the electron-deficient site of the oxidized intermediate of N,N'-dimethyl-N'-benzylethylenediamine.

Figure 9 shows that the electron transfer from NDB to the triplet RF originates probably from the nitrogen atom or methylene hydrogen, if the attack of the triplet RF is

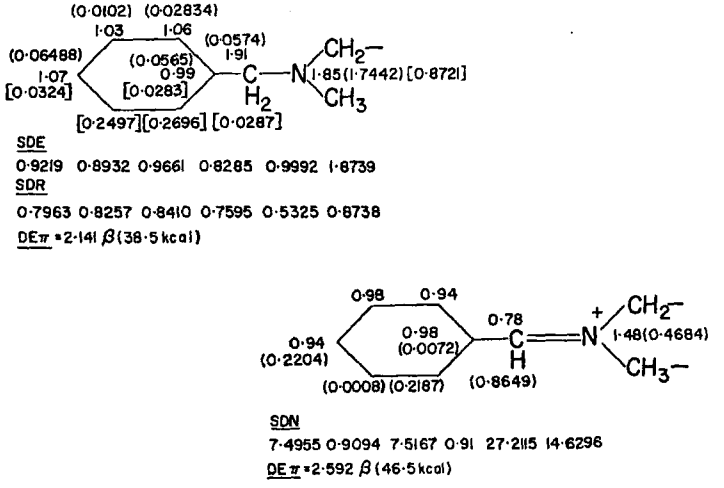
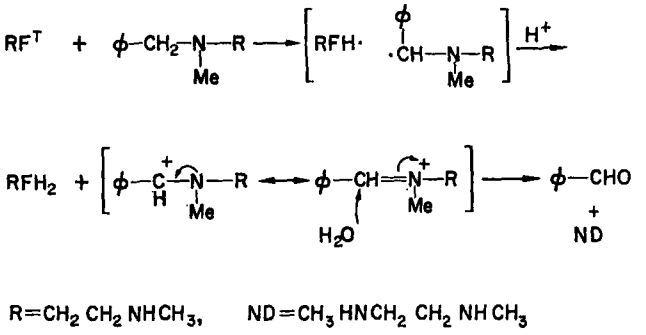


FIG. 9. Electronic properties of N,N′-dimethyl-N-benzyl-ethylene-diamine (NDB) and its oxidation product (NDB^+). For NDB: numbers at each atom are electron density; numbers in parentheses, frontier electron density; numbers in brackets, frontier radical density; SDE, superdelocalizability for electrophilic attack; SDR, superdelocalizability for radical attack; and $DE\pi$, delocalization energy.
For NDB^+: numbers in parentheses, frontier orbital density; and SDN, superdelocalizability for nucleophilic attack.

electrophilic in nature (i.e. one-step two-electron process). If the electron transfer occurs via a two-step one-electron process (i.e. the triplet RF as a biradical), the nitrogen atom with the highest SDR may preferentially lose electrons. The NDB cation with a considerable stability ($DE\pi = 46{\cdot}5$ kcal) can then be attacked at the carbon with the highest SDN by water to yield benzaldehyde. In the following mechanism (Fig. 10), the site of electron transfer is at the methylene hydrogen, but essentially the same mechanism can be written starting with the electron removal from the nitrogen atom in a two-step one-electron process. In the proposed mechanism, whether the electron transfer is a one-step or two-step process is not distinguished, as the overall stoichiometry would be the same. However, it is emphasized that the water molecule is not photo-split to reduce the flavin ring.



$R = CH_2\,CH_2\,NHCH_3$, $ND = CH_3\,HNCH_2\,CH_2\,NH\,CH_3$

FIG. 10. A possible mechanism of photooxidation of N,N′-dimethyl-N-benzyl-ethylene-diamine by riboflavin in water yielding benzaldehyde as a product.

The mechanism proposed then explains the results of Enns and Burgess[10] and the present work satisfactorily.

Finally, we wish to point out that pyridine may be an ideal model solvent for studying the photochemistry of flavins in water, since labile photoproducts such as FMF can be kept from hydrolysis for quantitative study of secondary processes of the photolysis. The aromatic solvent should also stabilize free radical intermediates arising from secondary processes.[25]

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