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RESEARCH ARTICLES

Effect of Certain Additives on the Photochemistry of Riboflavin

CHUNG TECK SHIN*, B. J. SCIARRONE, and C. A. DISCHER

Abstract □ The quantum efficiency of riboflavin under aerobic conditions was determined by using a microirradiation method. It was found that the initial quantum efficiency was constant and independent of intensity of light, wavelength of light, and concentrations employed. The quantum efficiency of riboflavin in the presence of phenols and other compounds was also determined. Only in the presence of phenols was the quantum efficiency decreased yielding a linear relationship between the Hammett's sigma values and the rate of photodecomposition. Benzyl alcohol and benzoic acid were found to be relatively ineffective as photochemical stabilizers compared to phenols. Cinnamyl alcohol, as an electron donor, enhanced the photodecomposition of riboflavin. It appears, from the compounds tested, that the hydroxyl group should be either attached to the benzene ring or be in conjugation with the benzene ring in order to be an effective photochemical stabilizer. The effects of temperature and pH on the system were also determined. Kinetic studies were made to elucidate the reaction mechanism.

Keyphrases □ Riboflavin, photochemistry—additives, temperature, pH effects □ Microirradiation—riboflavin, quantum efficiency □ Kinetic studies—riboflavin degradation □ Quantum riboflavin, efficiency—equations derived □ Spectrophotometry—analysis

The photosensitivity of riboflavin was first observed in 1932 by Warburg and Christian (1). Since then its photochemistry has been the subject of extensive investigation, and can be followed by recent reviews (2, 3). Although the fact that riboflavin will undergo photoreduction in the absence of an electron donor has been generally accepted, there have been conflicting views concerning the actual mechanism of the reaction. However, the presently accepted theory for the photodecomposition of riboflavin proposes that the reaction proceeds from the lowest triplet state of the flavin and involves intramolecular hydrogen-transfer from the ribityl sidechain with the subsequent formation of lumichrome and/or lumiflavin depending on basicity of the solution (4–6). The photolysis of riboflavin and several other flavins in acid or neutral solution is subject to general acid and base catalysis (6).

The formation of molecular complexes between riboflavin and various compounds has also been observed (7–11). Particularly, studies of the charge-transfer complexes between riboflavin and phenol derivatives have received considerable attention, because interactions of donor-acceptor type may be quite common in biological systems (12). Thus, an understanding of the correlation of these properties with the photochemical behavior of riboflavin might provide an insight into some of the energy transfer and storage mechanisms of living organisms.

Stabilization of riboflavin to light in the presence of additives is pharmaceutically important, since certain additives were observed to have considerable influence on the light stability of the system (13–22). Despite this importance, very few photochemical kinetic studies have been published for riboflavin in the presence of complexing agents. Therefore, the present study was undertaken

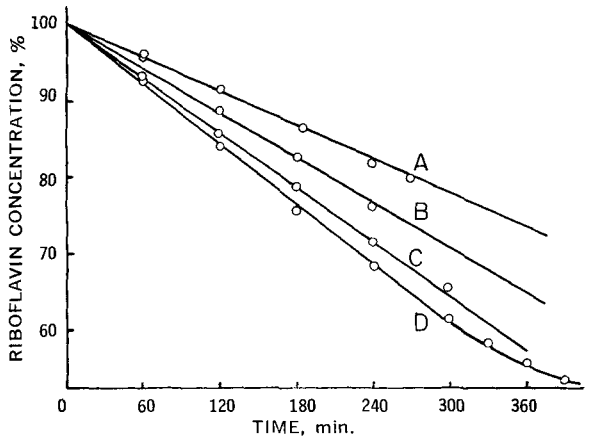

Figure 1—*The effect of light intensity (wavelength = 363 mμ) on the photodecomposition of riboflavin at 30°. Riboflavin concentration = 5.0 × 10⁻⁵ M. Key: Light intensity (photons/sec. × 10⁻¹⁴) A = 7.63; B = 10.46; C = 12.62; D = 13.66.*

to investigate the effect of certain additives, particularly, phenol derivatives and other compounds on the quantum efficiency of riboflavin. The phenol derivatives employed were phenol, *p*-chlorophenol, *p*-methoxyphenol, resorcinol, and hydroquinone. Benzyl alcohol, cinnamyl alcohol, and benzoic acid were also included to note the effect of the hydroxyl group conjugated with the benzene ring. Kinetic studies were also made to elucidate the mechanism of photodecomposition of riboflavin in the presence of additives. Temperature, pH, wavelength, and concentration were varied during these studies.

There is considerable information on the photodecomposition products and other compounds related to riboflavin using high intensity light sources. Under such conditions the photodecomposed products of riboflavin complicate the kinetic pattern and the calculation of quantum efficiency. Thus a microirradiation method developed by Dischер *et al.* (23–25) was chosen for this study.

EXPERIMENTAL

Instrumentation and Equipment—The irradiation instrumentation employed was basically that developed by Dischер *et al.* (23–25). Since the photodecomposition study was carried out at an elevated temperature, slight modifications were necessary on the reaction chamber; it was insulated with urethan foam and covered with aluminum foil to better maintain temperature at 30 ± 0.1°. Irradiation studies were performed only after 1-hr. temperature equilibration.

All solutions were analyzed in the visible region using a Beckman DU spectrophotometer, and the absorption spectra of the solutions were obtained with the Beckman DK-2 recording spectrometer.

Materials—Riboflavin[1] was used without further purification. Other materials employed were phenol, *p*-chlorophenol, *p*-methoxyphenol, resorcinol, hydroquinone, benzyl alcohol, cinnamyl alcohol, and benzoic acid. All phenol derivatives and aromatic alcohols were purified by recrystallization or distillation.

Photodecomposition Studies—These were conducted under aerobic conditions since most pharmaceutical products including riboflavin are stored in this manner. Few photochemical kinetic studies have been reported for these conditions.

Fresh solutions of riboflavin with and without additives were prepared in 0.05 *M* phosphate buffer, pH = 6.8. A 3-ml. sample of solution was pipeted into a Teflon-stoppered 1-cm. cell. The irradiation sample was analyzed by measuring its absorbance at 445 mμ on the Beckman DU spectrometer immediately before and after irradiation.

The mercury lamp employed showed two intense and sharp emission spectral lines at 363 and 433 mμ. Since these lines are close to the maximum absorbance of riboflavin, photodecomposition studies were undertaken at these wavelengths. The intensity of the light falling on the reaction cell was determined by measuring the galvanometer response as described by Dischер *et al.* (23–25). Since the intensity of the mercury lamp changed over a period of about 5 hr. by only 1.4% of the initial intensity, it was considered essentially constant for the period of irradiation. The quantum efficiency of riboflavin was calculated as described also by Dischер *et al.* (23–25). The initial rate of photodecomposition of riboflavin was determined from the linear portion of a zero-order plot of concentration *versus* time. All irradiation measurements were made two or three times.

Table I—The Effect of Intensity of Light on the Quantum Efficiency of Riboflavin at 30°. Riboflavin Concentration = 5.0 × 10⁻⁵ *M* (9.0345 × 10¹⁴ molecules/3 ml.)

Wavelength, mμ	Intensity of Light Photons/sec. ×10⁻¹⁴	Photons Absorbed/sec. ×10⁻¹⁴	Molecules Decomposed/sec. ×10⁻¹²	Average Quantum Efficiency ×10³
363	13.66	9.49	1.98	2.08
	12.62	8.67	1.78	2.05
	10.46	6.83	1.39	2.04
	7.63	5.32	1.09	2.05
			Average =	2.06
433	10.09	7.62	1.54	2.02
	8.72	6.46	1.30	2.02
	7.49	5.59	1.11	1.99
	5.34	4.03	0.79	1.95
			Average =	2.00

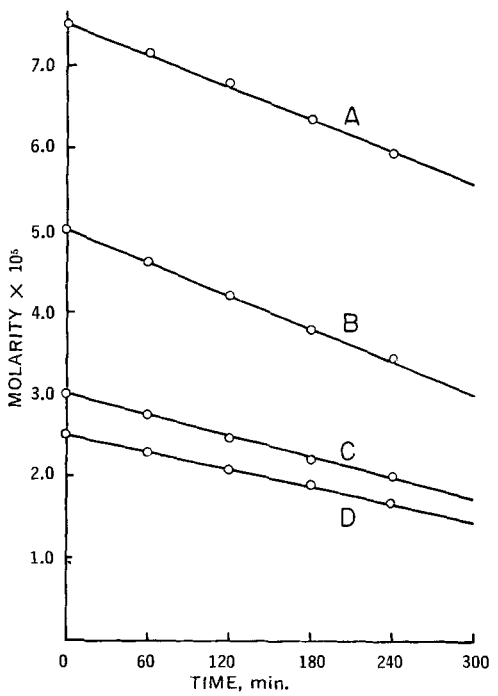

Figure 2—*Kinetic studies for the photodecomposition of riboflavin at various concentrations at 363 mμ. Key: Concentration (molarity × 10⁵) A = 7.5; B = 5.0; C = 3.0; D = 2.5.*

[1] The authors wish to thank Hoffmann-La Roche, Inc., Nutley, N. J., for supplying the necessary riboflavin.

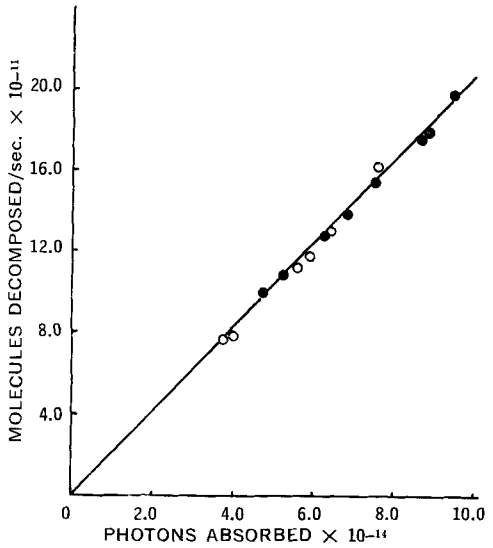

Figure 3—*Evaluation of quantum efficiency from a plot of molecules decomposed/sec. as a function of photons absorbed/sec. Key:* O, *light of wavelength 433 mμ; and* ●, *light of wavelength 363 mμ.*

RESULTS AND DISCUSSION

Kinetic Studies—The results of the kinetic studies at 363 mμ in the presence and absence of additives all fit the zero-order kinetic pattern rather than a pseudo first-order process. This is illustrated in Figs. 1 and 2. However, a linear zero-order relationship deviated after 5 hr. of irradiation, *i.e.*, when riboflavin was decomposed by more than 35% of initial concentration. This is illustrated in Fig. 1. Therefore, the length of exposure time was limited to 5 hr. so that the rate of decomposition of riboflavin was calculated from only the linear portion of the zero-order plot. When the 433 mμ wavelength of irradiating light was used a similar zero-order kinetic pattern was obtained.

Basic Quantum Efficiency Studies—The effect of the light intensity on the quantum efficiency of riboflavin was studied. The results of these studies are summarized in Table I (see Fig. 1). According to these data the quantum efficiency of riboflavin is independent of intensity of light. The number of molecules decomposed per unit time is directly proportional to the number of photons absorbed per unit time at each wavelength.

The effect of concentration of riboflavin on its quantum efficiency was also investigated. The data are summarized in Table II (see Fig. 2). These data indicate that quantum efficiency of riboflavin is constant within the concentration range tested. As shown in Table II, the higher the concentration of riboflavin solution the more photons were absorbed and subsequently more riboflavin decomposed. According to the above two series of studies the quantum efficiency of riboflavin was found to be constant at each wavelength employed.

It was deemed important to note the influence of irradiating time on the quantum efficiency of riboflavin. It was found that

Table II—The Effect of Concentration of Riboflavin on the Quantum Efficiency of Riboflavin at 30°

Wavelength, mμ	Concentration, Molarity ×10⁵	Molecules Decomposed/sec. ×10⁻¹²	Photons Absorbed/sec. ×10⁻¹⁴	Average Quantum Efficiency ×10³
363	7.5	1.80	8.88	2.03
	5.0	1.56	7.58	2.05
	3.0	1.28	6.29	2.03
	2.5	1.02	4.74	2.15
			Average =	2.07
433	7.5	1.64	7.63	2.15
	5.0	1.18	5.93	1.99
	3.0	0.77	3.75	2.04
			Average =	2.06

Table III—The Quantum Efficiency of Riboflavin in the Presence of Various Additives at 30° under Aerobic Conditions. Riboflavin Concentration = 2.5×10^{-6} *M*

Additives	Concentration of Additives, Molarity 2.5×10^{-5}	1.0×10^{-4}	5.0×10^{-4}
	Quantum Efficiency ×10³		
Phenol	1.59	1.28	0.74
p-Chlorophenol	1.46	1.13	0.70
p-Methoxyphenol	1.65	1.39	0.70
Resorcinol	1.34	0.35	—
Hydroquinone	2.94	2.75	1.56
Cinnamyl alcohol	1.86	2.03	3.22
Benzyl alcohol	1.98	1.98	1.94
Benzoic acid	1.82	1.77	1.73

quantum efficiency of riboflavin initially varied slightly in each study, but decreased after 5 hr. of irradiation. Such a result might be expected if a photodecomposition product or an intermediate quenches excited molecules or if a product of the reaction or an intermediate absorbs incident light.

The quantum efficiency of riboflavin can be evaluated from the slope of the plot of the molecules decomposed per unit time as a function of photons absorbed per unit time. All data in Tables I and II are plotted in Fig. 3. A linear relationship was observed between molecules decomposed and photons absorbed. This indicates that the riboflavin photodecomposition, under conditions used in this experiment, obeys the first law of photochemistry. The slope of the straight line was found to be 2.04×10^{-3}. Standard deviation for the measurement of quantum efficiency was 0.04×10^{-3} in this study. Theoretical and experimental rates of decomposition were compared as functions of the number of photons absorbed resulting in a superimposition of the straight line plots (not shown). Slopes of both lines were 6.76×10^{-23}.

Effect of Additives on the Quantum Efficiency—The quantum efficiency of riboflavin in the presence of various additives at

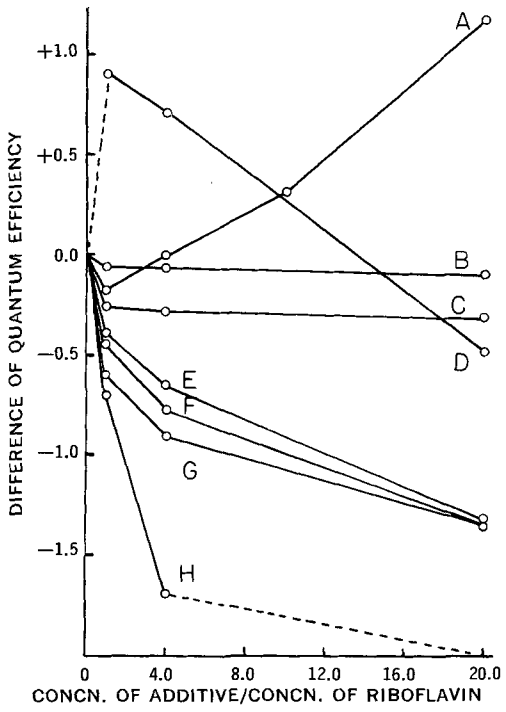

Figure 4—*Plot of difference in quantum efficiency of riboflavin in the presence and absence of additives as a function of the ratio of additive concentration to riboflavin concentration at 30°. Riboflavin concentration = 2.5 × 10⁻⁵ M. Key:* A = *cinnamyl alcohol;* B = *benzyl alcohol;* C = *benzoic acid;* D = *hydroquinone;* E = p-*methoxyphenol;* F = *phenol;* G = p-*chlorophenol;* H = *resorcinol.*

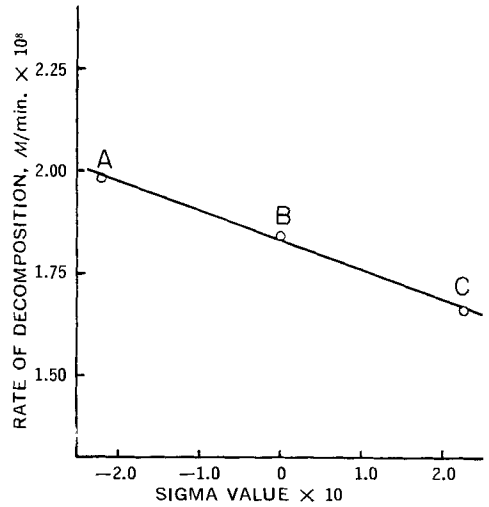

Figure 5—*A plot of rate of photodecomposition of riboflavin in the presence of phenols as a function of Hammett's sigma value for each phenol. Concentration of riboflavin = 2.5 × 10⁻⁵ M; concentration of phenols = 1.0 × 10⁻⁴ M. Key:* A = p-*methoxyphenol;* B = *phenol;* C = p-*chlorophenol.*

different concentrations are summarized in Table III. As shown in this table the quantum efficiency of riboflavin was either decreased or increased or remained constant depending on the physical and/or chemical properties of the additives. Any decrease in quantum efficiency in the presence of additives would be indicative of a photochemical stabilization process. A plot of the difference in quantum efficiencies of riboflavin in the presence and absence of additives as a function of the ratio of additive concentration to riboflavin concentration is shown in Fig. 4. The quantum efficiency of riboflavin in the presence of each phenol decreased gradually in the same fashion as the concentration of the given phenol was increased. This is probably due to the formation of the same type of complex between riboflavin and the different phenols. Therefore, the mechanism involved in the photodecomposition of riboflavin in the presence of monophenols may be considered to be similar.

The *p*-chlorophenol, which has an electron attracting chlorine atom (Hammett's sigma value = +0.227), lowered the quantum efficiency more than *p*-methoxyphenol, which has an electron donating methoxy group (Hammett's sigma value = −0.268). This indicates that the inductive and conjugative effects of substituents may influence both the stability of the complexes and the rate of photodecomposition of riboflavin. The rate of decomposition of riboflavin in the presence of phenols are plotted in Fig. 5 as a function of their respective sigma values. A linear relationship is observed in this plot and can be expressed as:

$$K_1 = 7.11 \times 10^{-9}\,\sigma + 1.83 \times 10^{-8} \qquad \text{(Eq. 1)}$$

where K_1 = the rate of decomposition of riboflavin in the presence of 1.0×10^{-4} *M* phenol (per min.) and σ = Hammett's substituent constant for phenols.

A linear relationship was observed between the pK values of phenols in the ground state and the quantum efficiency of riboflavin in the presence of the phenol. However, this linear relationship was not observed when pK* for the excited phenols was similarly plotted. In both the ground and excited states phenols which have lower pK and pK* values were found to be more effective in lowering the quantum efficiency of riboflavin. This appears to be due to the electron attracting chlorine atom in the *p*-chlorophenol which causes the molecule to be a better proton donor and subsequently giving it a greater tendency to form a stable hydrogen bond-type complex than *p*-methoxyphenol. It has been known that the excited molecules in many cases have a greater tendency to form hydrogen bonds and that a new equilibrium is reached during the life time of the excited states (26). However, none of the phenols showed absorbance at the wavelength of irradiating light used.

The quantum efficiency of riboflavin in the presence of resorcinol was significantly decreased as the concentration of resorcinol was increased. A possible explanation for this observation would be that if the excited molecules have a greater tendency to form a hydrogen bond-type complex (26) resorcinol would have a better chance to form complexes than a monohydroxyphenol since it has two hydroxyl groups. Resorcinol would then be much more effective in lowering the quantum efficiency of riboflavin. However, in the case of hydroquinone the quantum efficiency of riboflavin first increased and then decreased gradually at higher concentrations. These phenomena indicate that two different reaction mechanisms are involved depending upon the concentration of hydroquinone—*viz*, redox-reaction as well as complexation. In the presence of lower concentration of hydroquinone the redox-reaction mechanism is favored, *i.e.*, hydroquinone reduced the riboflavin, which was more easily decomposed through a triplet state. Therefore, the quantum efficiency of riboflavin was increased in the presence of lower concentration of hydroquinone. However, as the concentration of hydroquinone was increased, more of the complex(es) form(s), thereby stabilizing riboflavin.

It is interesting to note that when a riboflavin (2.5×10^{-5} *M*) solution was irradiated in the presence of a higher concentration (5.0×10^{-4} *M*) of resorcinol a red-brown colored solution resulted. The irradiation of a resorcinol solution (5.0×10^{-4} *M*) alone did not produce the colored product under the same conditions. Furthermore, in the ground state the colored products did not appear. Thus it is considered that the reaction involves interaction of riboflavin with resorcinol under irradiation. The colored solution had a maximum absorbance at 477 mμ. It was found that the photodecomposition product of resorcinol (1.0×10^{-3} *M*) formed after 78 hr. of irradiation also had this absorbance maximum. It is concluded, therefore, that the photooxidation of resorcinol was sensitized tremendously by the presence of riboflavin.

On the basis of these experiments it is clear that the photodynamic behavior of riboflavin in the presence of phenols is complicated and several other explanations are possible for the reduction of the quantum efficiency of riboflavin in addition to the complexation mechanism. It may be speculated that there might exist a direct transfer of the exicitation energy from riboflavin to phenols, probably a triplet-triplet transfer may be an acceptable explanation of the phenomena observed.

The quantum efficiency of riboflavin in the presence of 2.5×10^{-5} *M* concentration of cinnamyl alcohol was found to decrease but it then increased as the concentration of cinnamyl alcohol was increased. In the case of benzyl alcohol the quantum efficiency of riboflavin was very slightly decreased initially and remained essentially constant as the concentration of benzyl alcohol was increased. When in the presence of benzoic acid the quantum efficiency of

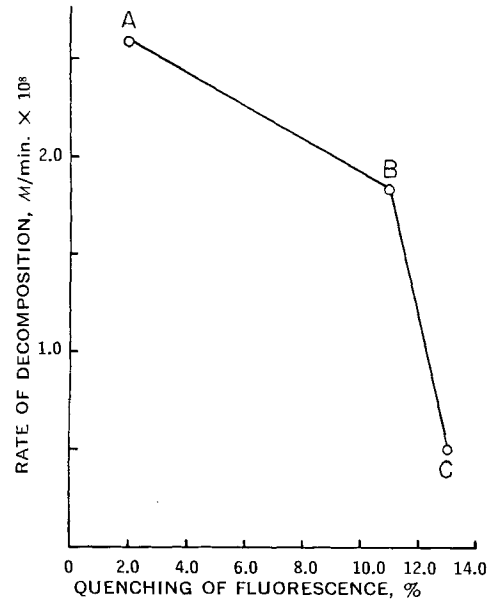

Figure 6—*A plot of rate of decomposition of riboflavin in the presence of benzoic acid, phenol, and resorcinol as a function of quenching efficiency (from* Reference 2*) of fluorescence of riboflavin. Key:* A = *benzoic acid;* B = *phenol;* C = *resorcinol.*

riboflavin was found to be sightly lower than that of riboflavin in the presence of benzyl alcohol and also remained essentially constant as the concentration of benzoic acid was increased. All three compounds were found to be less effective than the phenols in lowering the quantum efficiency. Therefore, it is considered that the hydroxyl group attached to the benzene ring must be conjugated with the benzene ring in order to be an effective stabilizer for the photodecomposition of riboflavin.

The cinnamyl alcohol, which has a conjugated double bond with the benzene ring, behaves entirely differently from benzyl alcohol and phenol in these irradiation studies. It is considered that cinnamyl alcohol is photochemically interacting with riboflavin as an electron donor. It is well known that alcohols are good electron donors and are photooxidized in the presence of riboflavin (21). However, in the case of benzyl alcohol there was no photochemical interaction between riboflavin and benzyl alcohol except possibly hydrogen bonding or a solvent effect. It has been reported that benzyl alcohol does not form complexes and does not quench the fluorescence of riboflavin (2, 9). However, benzoic acid does quench the fluorescence of riboflavin slightly (2). Therefore, it is obvious that the addition of benzoic acid can affect the excited state of riboflavin and lower its quantum efficiency more effectively than benzyl alcohol.

The rate of photodecomposition of riboflavin in the presence of resorcinol, phenol, and benzoic acid are plotted in Fig. 6 as a function of their quenching efficiency (2) of the fluorescence of riboflavin. There appears to be a direct correlation between the rate of decomposition and quenching efficiency, *i.e.*, the rate of decomposition of the riboflavin is decreased in the presence of a substance which has a higher quenching efficiency. Mataga (26) and El-Bayoumi (27) ascribed this quenching effect to the formation of the hydrogen bond type of complex.

The Effect of Temperature—The quantum efficiency of riboflavin in the absence and presence of phenol as a function of temperature is shown in Fig. 7. Separately an Arrhenius plot was constructed over the temperature range of 20 to 30°, which showed that the reaction had a greater temperature dependence in the absence of phenol. The activation energies, calculated from the slope of the Arrhenius plots, were 8.94 kcal. in the absence of phenol and 6.25 kcal. in the presence of phenol (1.0×10^{-4} *M*).

The Effect of pH—Since phosphate buffer also affects the rate of decomposition of riboflavin all samples were prepared in water by adjusting pH with hydrochloric acid and sodium hydroxide. Figure 8 presents the pH effect on the quantum efficiency of riboflavin first in the absence and then the presence of phenol as an additive. When phenol was added to a riboflavin solution at constant pH it was found that the quantum efficiency decreased. These phenomena indicate that the reduction of the quantum efficiency is not merely due to a pH effect but that the phenol itself is hindering the excitation process of riboflavin and thereby stabilizes it. Since the protonated riboflavin is essentially stable to light and does not fluoresce, the riboflavin becomes quite stable toward photodecomposition below pH 3.

Kinetic Analysis—Oster *et al.* (18) proposed a kinetic scheme for the photoreduction of riboflavin illustrating the role of the triplet state in the reaction. Such a scheme can be applied to the present study with certain modifications. On the basis of the present investigation the following reaction mechanism is proposed.

Riboflavin absorbs light to give the first electronically excited species, Rf*,

$$\mathrm{Rf} \xrightarrow[k_1]{} \mathrm{Rf}^* \qquad \text{(Eq. 2)}$$

This excited Rf* may fall to the ground state with the emission of fluorescence and the production of heat,

$$\mathrm{Rf}^* \xrightarrow[k_{2a}]{} \mathrm{Rf} + h\nu \qquad \text{(Eq. 3}a\text{)}$$

$$\mathrm{Rf}^* \xrightarrow[k_{2b}]{} \mathrm{Rf} + \text{heat} \qquad \text{(Eq. 3}b\text{)}$$

Equations 3*a* and 3*b* indicate deactivation of the first excited singlet state by fluorescence (k_{2a}) and a radiationless process (k_{2b}), respectively. For the purpose of simplification of the kinetic mechanism, these two processes can be expressed as follows:

$$\mathrm{Rf}^* \xrightarrow[k_2]{} \mathrm{Rf} + h\nu/\text{heat} \qquad \text{(Eq. 3}c\text{)}$$

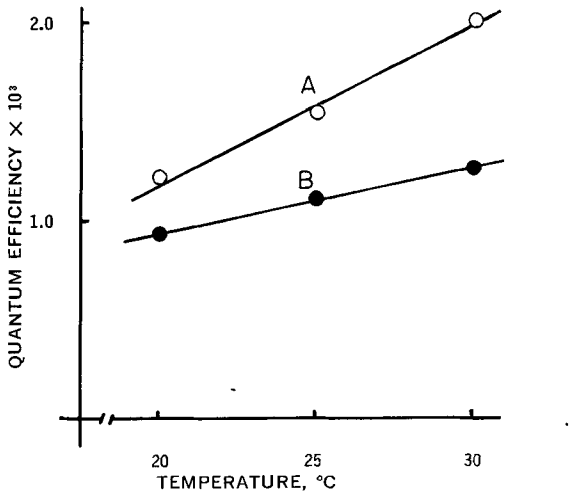

Figure 7—*Quantum efficiency as a function of temperature. Key:* A = *quantum efficiency of riboflavin in the absence of phenol;* B = *quantum efficiency in the presence of phenol* (1.0×10^{-4} M).

where $k_2 = k_{2a} + k_{2b}$

Alternatively the singlet excited species, Rf*, undergoes transition to a long-lived (triplet) state, Rf′, through electronic vibration.

$$\mathrm{Rf}^* \xrightarrow[k_3]{} \mathrm{Rf}' \qquad \text{(Eq. 4)}$$

This long-lived species may fall to the ground state, with the evolution of heat, and possibly phosphoresce,

$$\mathrm{Rf}' \xrightarrow[k_{4a}]{} \mathrm{Rf} + h\nu \qquad \text{(Eq. 5}a\text{)}$$

$$\mathrm{Rf}' \xrightarrow[k_{4b}]{} \mathrm{Rf} + \text{heat} \qquad \text{(Eq. 5}b\text{)}$$

Equation 5*b* indicates deactivation of the triplet state by a radiationless transition (k_{4b}). Again in order to simplify the kinetic mechanism the two above processes can be expressed as follows:

$$\mathrm{Rf}' \xrightarrow[k_4]{} \mathrm{Rf} + h\nu/\text{heat} \qquad \text{(Eq. 5}c\text{)}$$

where $k_4 = k_{4a} + k_{4b}$

The long-lived species may undergo transition to a more reactive species or may react directly to form various photodecomposed products. The kinetic mechanisms are simplified at this point to include all possible reactions with the triplet state in one rate constant, k_5,

$$\mathrm{Rf}' \xrightarrow[k_5]{} \text{products} \qquad \text{(Eq. 6)}$$

The addition of quencher, *Q*, reduces the rate of decomposition of riboflavin by affecting the singlet and triplet excited states. It

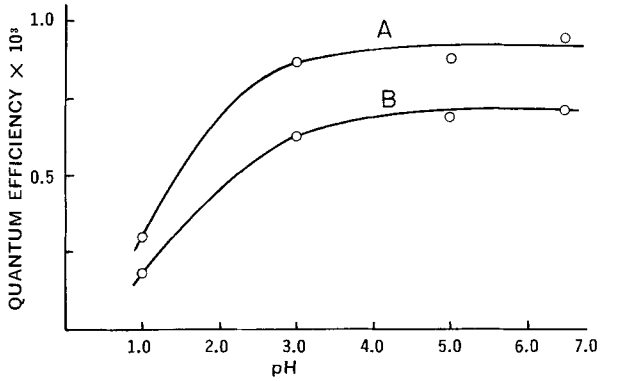

Figure 8—*The effect of pH on the quantum efficiency of riboflavin in the presence and absence of phenol. Key:* A = *riboflavin only* (2.5×10^{-5} M); B = *riboflavin with phenol* (5.0×10^{-4} M).

will probably quench the fluorescence and phosphorescence of the riboflavin and the excited species may fall to the ground state. During these processes there might exist a direct transfer of the excitation energy from the riboflavin to the quencher with the resulting formation of an excited complex. These new species may then dissociate to riboflavin and the excited quencher. The conjugative and inductive effects of substituents of a quencher (phenol) might show an influence on the stability of the excited complex. The excited quencher may finally be decomposed. The photosensitization of resorcinol in the presence of riboflavin can be explained by this mechanism.

$$Rf^* + Q \rightleftharpoons (Rf\text{-}Q)^* \rightarrow Rf + Q^* \qquad \text{(Eq. 6}a\text{)}$$

$$Rf' + Q \rightleftharpoons (Rf\text{-}Q)' \rightarrow Rf + Q' \qquad \text{(Eq. 7}a\text{)}$$

If the excited complex is assumed to be short-lived the overall reactions can be simplified as follows:

$$Rf^* + Q \xrightarrow[k_6]{} Rf + Q^* \qquad \text{(Eq. 6}b\text{)}$$

$$Rf' + Q \xrightarrow[k_7]{} Rf + Q' \qquad \text{(Eq. 7}b\text{)}$$

Using steady state assumptions regarding the transient species Rf* and Rf', the overall rate of photodecomposition of riboflavin can be expressed as follows:

$$\frac{d(Rf)}{dt} = \frac{-k_1k_3k_5(I)}{[k_2 + k_3 + k_6(Q)][k_4 + k_5 + k_7(Q)]} \qquad \text{(Eq. 8)}$$

where I = intensity of irradiating light.

The quantum efficiency of the reaction, ϕ, is equal to the overall rate divided by the initial rate. Therefore, the quantum efficiency can be expressed in terms of the rate constants:

$$\phi = \frac{k_3\,k_5}{[k_2 + k_3 + k_6(Q)][k_4 + k_5 + k_7(Q)]} \qquad \text{(Eq. 9)}$$

This equation indicates that the quantum efficiency of riboflavin is proportional to k_3 and k_5, *i.e.*, the mechanism of the photodecomposition of riboflavin involves mainly the long-lived (triplet) state. This equation also expresses the quantum efficiency of riboflavin to be inversely proportional to the concentration of quencher. This relationship is well demonstrated experimentally in Fig. 4.

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