Triplet state of orotic acid and orotic acid methyl ester in solution[1, 2]

R. W. Yip and W. D. Riddell[3]
Division of Chemistry, National Research Council of Canada, Ottawa, Canada

AND

A. G. Szabo
Division of Biology, National Research Council of Canada, Ottawa, Canada

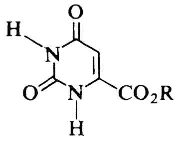
Received October 14, 1969

Flash photolysis of orotic acid and orotic acid methyl ester in aqueous solution was studied. The transients were identified as triplet excited states on the basis of their lifetimes, sensitization, quenching, and self-quenching experiments. The rate constants for self-quenching and for decay of the triplet were measured and discussed in terms of dimerization and competing unimolecular and pseudo-unimolecular processes. A triplet energy of 60 kcal mole^{-1} was deduced for both orotic acid and orotic acid methyl ester from triplet energy transfer measurements.

Canadian Journal of Chemistry, **48**, 987 (1970)

The photochemistry of pyrimidines (1) is interesting both biologically and chemically. In the first instance ultraviolet (u.v.) irradiation of microorganisms (2) causes mutagenic and lethal effects and is a powerful probe to investigate repair mechanisms operating in the cell (3). Photodimerization of the pyrimidines is one of the reactions which contributes to these effects. Chemically, the mechanisms of photodimerization of pyrimidines bear directly on the general and important problem of the nature of the photochemically induced cycloaddition process of unsaturated carbonyl compounds. Specific questions yet to be answered concern the particular spin state in a given solvent and state of aggregation (4), the extent of self-quenching caused by dimer formation, and the rate constant for reaction by triplet excited states. On a slightly more subtle level, questions regarding the intervention of complex formation preceding dimer formation have lately been raised (5, 6).

Sztumpf and Shugar (7) have shown that orotic acid (**1***a*) and orotic acid methyl ester (**1***b*) (methyl orotate) dimerize in aqueous solution, and that the rate of dimerization is reduced by oxygen, confirming the earlier observations of Beukers and Berends (8) on the oxygen effect. From a quantitative analysis of the oxygen quenching (assuming a quenching rate constant, k_q, of oxygen as 10^{10} M^{-1} s^{-1}) Sztumpf-Kulikowska *et al.* (9) inferred a lifetime of 2 μs for the reactive state, almost certainly the triplet. We therefore felt that there was a good probability of directly observing the triplet state of orotic acid and of methyl orotate. Subsequently, we have learned that Boag and Godfrey (10) had observed a short-lived transient prior to our investigation. In this paper we have identified the triplet state of orotic acid in aqueous solution by sensitization, quenching, and self-quenching experiments. We have measured the rate constants for dimerization and for decay of the triplet (in the absence of dimerization) and for triplet energy transfer in aqueous solution. The experimental rate constants for energy transfer are important in themselves in connection with the theory of triplet energy transfer and especially if reasonable values of triplet lifetimes are to be obtained by Stern–Volmer type quenching experiments. Most experiments were carried out with the methyl ester to avoid the complication of dissociation of the acid to its anion since we have no information on the p*K* of the triplet excited state. During the course of our investigation, Herbert *et al.* (11) reported the observation of the triplet state of orotic acid in water at p*H* 5 by flash photolysis and measured the rate constants for reactions under this condition.



1

a R = H
b R = CH_3

---

[1] Issued as NRCC No. 11124.

[2] Presented in part at the Northeast Photobiology Group, Cornell University, Ithaca, New York, on May 10, 1969.

[3] NRCC Postdoctoral Fellow, 1968–1969.

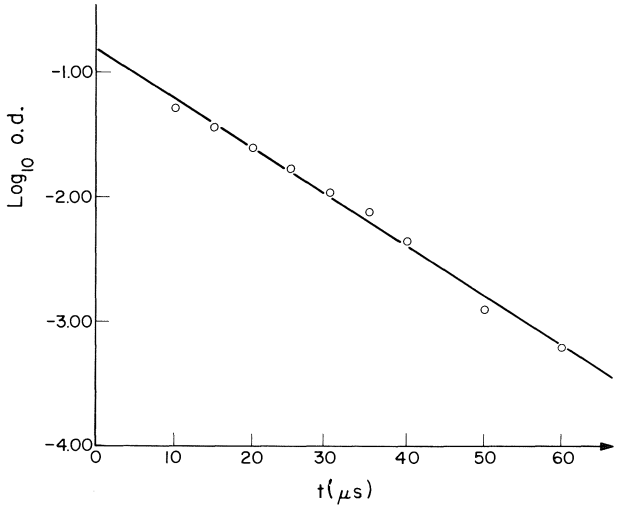


FIG 1. Plot of $\log_{10}$ o.d. vs. time (μs); [methyl orotate] = 4×10^{-5} *M*; monitoring wavelength, 340 mμ; data obtained from Fig. 2.

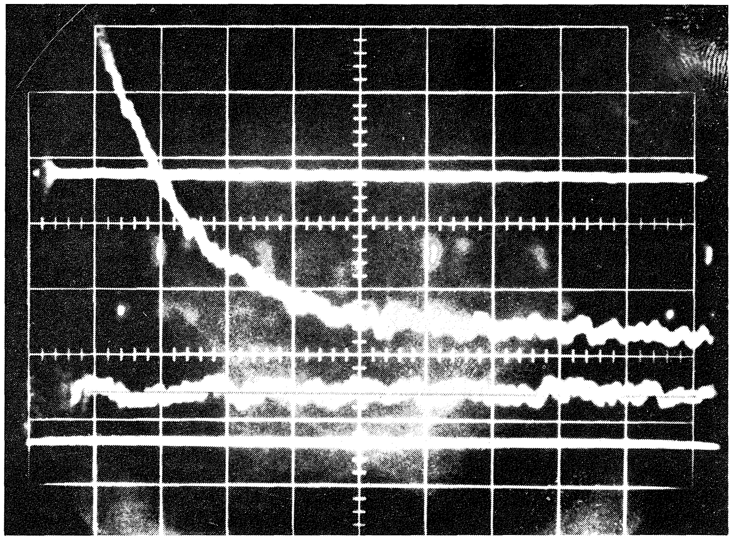


FIG. 2. Typical oscilloscope trace of transient observed on flashing aqueous solutions of methyl orotate or orotic acid; [methyl orotate] = 4×10^{-5} *M*; monitoring wavelength, 340 mμ; timebase, 10 μs/cm; transient voltage, 0.05 V/cm; photoflash energy, 67 J.

Results

Flash excitation of either orotic acid or its methyl ester in aqueous solution at p*H* 5 produced a transient which decayed by first order kinetics (eq. [1]) (Fig. 1). A typical trace is shown

$$[1] \quad 2.303 \log(\text{o.d.}) = k_{\text{obs}}t + \text{constant}$$

in Fig. 2. Flash excitation of aqueous solutions of orotic acid and HCl (p*H* 0.6) (or to a lesser extent with H_2SO_4, p*H* 0.7), produced a long-lived transient in addition to the principal first order transient. The long-lived transient could not be detected when the photoflash was filtered with 1 cm of neat acetic acid. Flash excitation of an HCl (p*H* 0.6) solution alone without filter gave a strong long-lived transient. Therefore the long-lived transient observed with orotic acid at p*H* 0.6 most probably originates from photolysis of the HCl. The transient observed on photolysis of either orotic acid or its methyl ester was assigned as the triplet state of these pyrimidines on

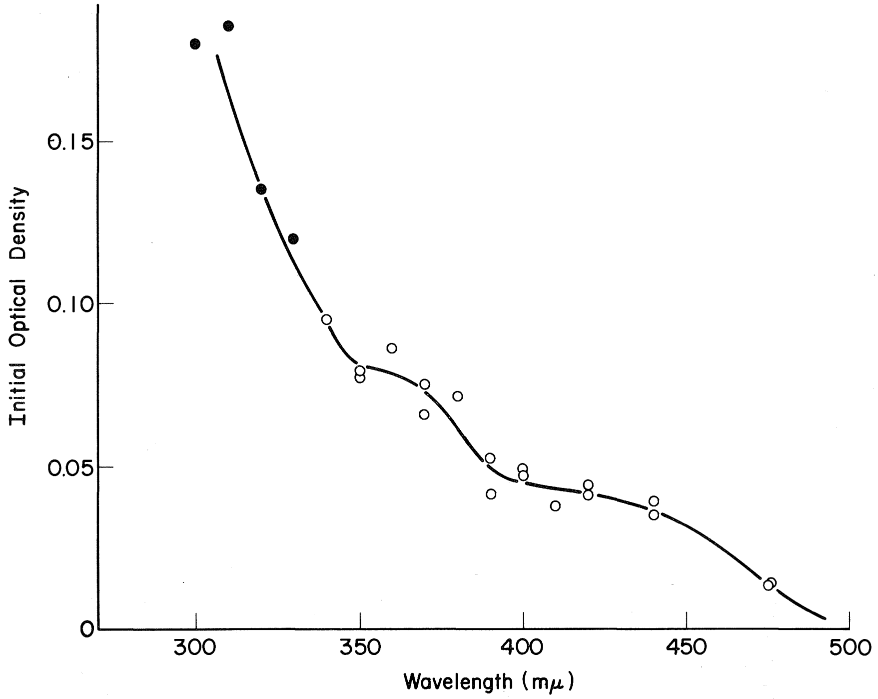


FIG. 3. Absorption spectrum of triplet orotic acid in aqueous solution, p*H* 5; ○ [orotic acid] = 2×10^{-5} *M* (half bandwidth, 11 mμ); ● [orotic acid] = 0.5×10^{-5} *M* (half bandwidth, 20 mμ); effective pathlength 16 cm; photoflash filtered through 2 mm Vycor glass; photoflash energy, 108 J.

the basis of the following experiments and observations.

Transient Absorption Spectra

The absorption spectra of the transients from flash excitation of orotic acid and methyl orotate in water at p*H* 5 are shown in Figs. 3 and 4. Both are very broad, extending from about 475 mμ, no clear maxima being evident in the region scanned. To obtain reliable data from wavelengths below 320 mμ, it was necessary to work with very dilute solutions. Because of ground state absorption, it was not possible to scan much below 300 mμ. Our results on the spectrum of the transient from orotic acid are not in agreement with a maximum at 320 mμ as reported by Herbert *et al.* (11).

Acetone Sensitization

Acetone was chosen as a sensitizer on the basis of its triplet energy (12), solubility, and ground state absorption. In addition, there is little overlap of the absorption spectrum of triplet acetone (13) with that of the acceptor transient. Moreover the lifetime of triplet acetone in aqueous solution, 18 μs, (13) is sufficiently long to permit efficient transfer to methyl orotate at low ester concentrations. At orotate concentrations of greater than 1–2 × 10^{-4} *M*, the transient lifetime lies beyond the time resolution of the instrument because of self-quenching.

Flash excitation, through a 2 mm Pyrex filter, of an aqueous solution of 0.05 *M* acetone and 2×10^{-5} *M* methyl orotate gave a strong signal at 340 mμ (Fig. 5*a*) which decayed by first order kinetics with a lifetime of 19 μs. Note the slow rise time (compared with the direct photolysis shown in Fig. 2) of the sensitized transient which is expected for an acceptor triplet (14). While the greater part of the transient absorption was due to sensitization, a small part of the absorption was the result of direct excitation of the methyl ester, as was revealed in a separate control experiment. In this experiment, 2×10^{-5} *M* methyl orotate alone was flashed under identical conditions (Fig. 5*b*). Finally, to demonstrate that the acetone triplet absorption does not interfere, a solution containing 0.05 *M* acetone alone was flashed under the same conditions and as can be seen in Fig. 5*c* gave no measurable transient at 340 mμ.

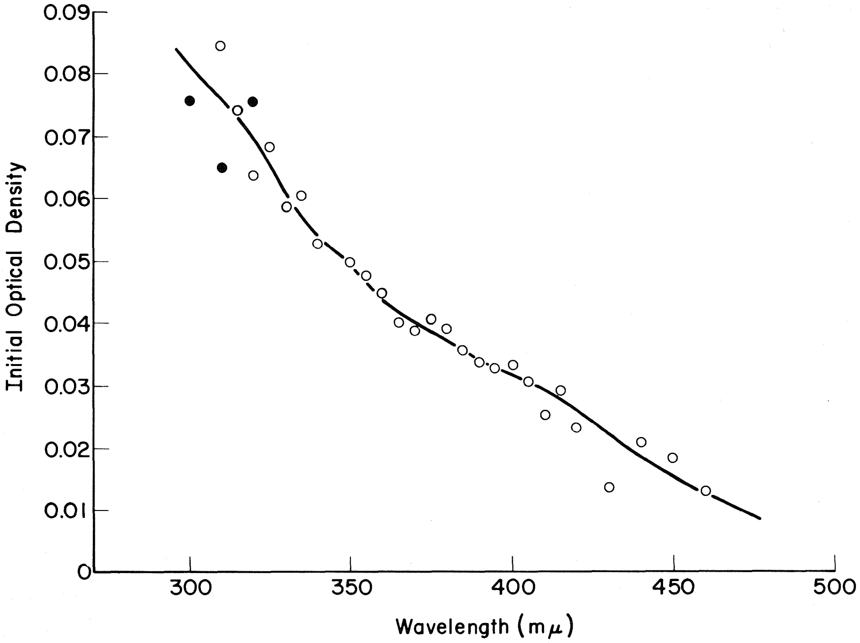


FIG. 4. Absorption spectrum of triplet methyl orotate in aqueous solution, pH 5; ○ [methyl orotate = 2×10^{-5} M (half bandwidth, 11 mμ); ● [methyl orotate] = 0.25×10^{-5} M (half bandwidth, 20 mμ); effective pathlength, 20 cm; photoflash energy, 92 J.

Quenching of Sensitizer Triplets by Methyl Orotate in Aqueous Solution

The rates of decay of sensitizer triplets (T) under certain conditions can be represented by eq. [2],

$$\frac{\mathrm{d}[\mathrm{T}]}{\mathrm{d}t} = k_o + k_q [\mathrm{Q}], \tag{2}$$

where [Q] is the concentration of added quencher and k_0 is a composite pseudo first order rate constant. In the present work, low sensitizer concentrations ($1 \times 10^{-5} - 3.7 \times 10^{-5}$ M) and low flash energies (~ 100 J) were used to reduce the likelihood of complex formation and self-quenching by the sensitizer, and triplet–triplet annihilation which would introduce a term which is second order in triplet-concentration. In all samples except one, excellent first order rate plots were obtained. The exception was the case of naphthalene with no added methyl orotate. Here, long triplet lifetime and lack of competitive quenching from the presence of added quencher resulted in considerable deviation from first order decay due to triplet–triplet annihilation. However, first order kinetics was easily restored in this case by adding a Corex filter to reduce the flash intensity and hence the triplet concentration.

The results of these quenching experiments, recorded in Table 1, show a clear correlation between k_q and the triplet energy of the sensitizer.

Quenching of Methyl Orotate Transient in Aqueous Solution

The lifetime of the transient observed on flash excitation of methyl orotate in water was reduced by the addition of 2,4-hexadien-1-ol and dimethyl fumarate (E_T 59.5 and ~ 60 kcal mole^{-1} respectively (15, 16)). By varying the concentration of the quenchers, rate constants of $2.88 \pm 0.15 \times 10^9$, and 2.3×10^9 M^{-1} s^{-1} were obtained for quenching by hexadienol and by fumarate respectively. The extent of consumption of ground state methyl orotate in the presence and absence of hexadienol is shown in Fig. 6. Clearly, the rate of decomposition of methyl orotate is markedly reduced in the presence of hexadienol. Hexadienol quenching of the transient from orotic acid in water was also carried out and a k_q of $2.93 \pm 0.12 \times 10^9$ M^{-1} s^{-1} was obtained (Fig. 7). It was not possible to obtain a rate constant for hexadienol quenching of the transient from orotic acid in water at pH 0.6 because

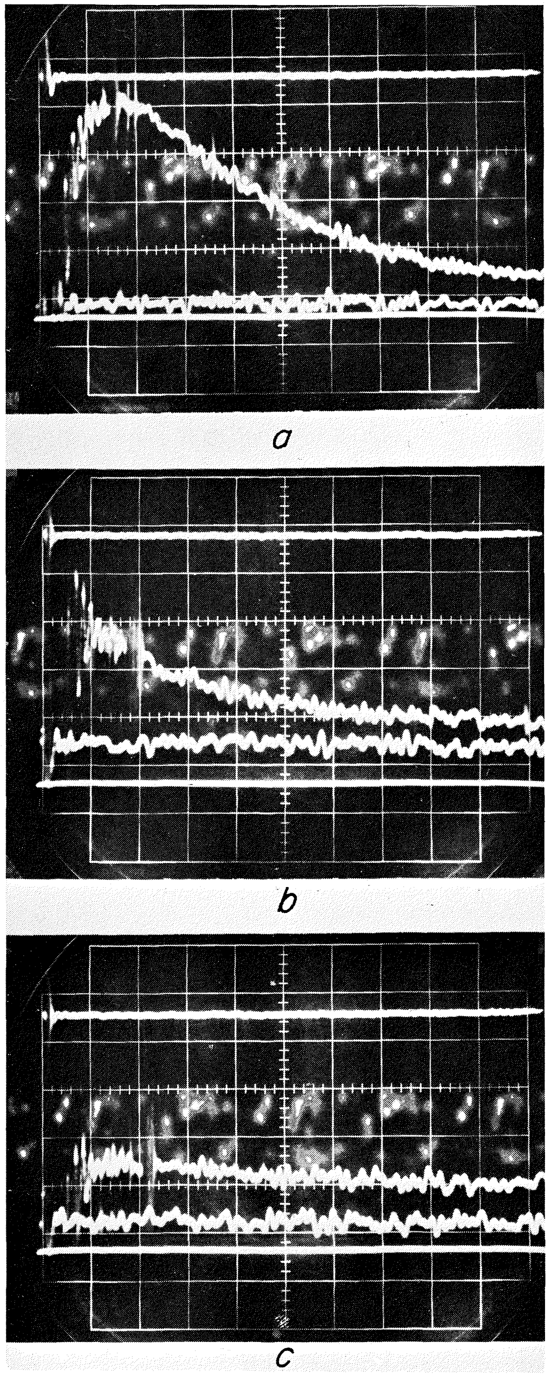


FIG. 5. Oscilloscope traces showing acetone sensitized formation of triplet methyl orotate in aqueous solution. Conditions for all 3 traces are identical. Monitoring wavelength, 340 mμ, optical half bandwidth, 45 mμ; photoflash filtered through 2 mm Pyrex glass; timebase, 10 μs/cm; in each trace the lowest line is the baseline; uppermost line is the initial transmission, 0.1 V/cm; transient voltage, 0.01 V/cm. *a*, (upper trace) [acetone] = 0.05 *M*, [methyl orotate] = 2 × 10^{-5} *M*; *b*, (middle trace) [methyl orotate] = 2 × 10^{-5} *M* (acetone absent); *c*, (lower trace) [acetone] = 0.05 *M* (methyl orotate absent).

of the rapid dehydration of hexadienol to hexatriene under these conditions.

For comparison of the above-mentioned quenching rate constants with those of triplet energy transfer in aqueous solution, the rate constants for quenching of triplet excited 2-acetonaphthone (E_T 59.3 kcal mole^{-1} (17, 18)) and 1-acetonaphthone (E_T 58 kcal mole^{-1}, (19)) by hexadienol in aqueous solution were also measured. The hexadienol quenching results are summarized in Table 2.

Rate Constants for Photodimerization of Orotic Acid and Methyl Orotate in Aqueous Solutions

The observed pseudo first order rate constant k_{obs} for decay of the transient in the presence of orotic acid (OA) (or its methyl ester) can be written as eq. [3], where k_0 is the rate constant at

$$[3] \qquad k_{obs} = k_0 + k_{dim}[OA]$$

infinite dilution of orotic acid. Values of k_{dim} were obtained from the gradients of plots of k_{obs} against [OA] (Figs. 8, 9) and are recorded together with values of k_0 in Table 3. The results show that these values of k_{dim} for orotic acid at p*H* 0.6 and methyl orotate at p*H* 5 (both neutral species in the ground state), are very similar (1.91 × 10^9 and 2.20 × 10^9 M^{-1} s^{-1}, respectively) and are significantly higher than that for orotic acid at p*H* 5 (1.13 × 10^9 M^{-1} s^{-1}) where the acid is fully dissociated in the ground state. In absolute terms, these rate constants are exceedingly large and are almost diffusion-controlled.

Discussion

Identification of the Transient

A transient lifetime of 78 μs observed for flash excitation of orotic acid at p*H* 5 is considerably longer than, but of the correct order of magnitude expected for, the lifetime of the triplet inferred on the basis of the oxygen quenching measurements of Sztumpf-Kulikowska *et al.* (9) and of the experimentally measured k_q for oxygen quenching of the transient (11). First order decay kinetics are interpretable in terms of a triplet excited state rather than a radical intermediate. Quenching by

TABLE 1

Rate constants for quenching of sensitizer triplets by methyl orotate in aqueous solution*

Sensitizer (concentration M)	E_T (kcal mole^{-1})	Analysis wavelength (mμ)	τ_0† (μs)	$k_q \times 10^{-9}$ (M^{-1} s^{-1})
Xanthen-9-one§ (1.14×10^{-5})	74	600	37.7	2.78 ± 0.24
Naphthalene¶ (1.72×10^{-5})	60.9	415	246	2.35 ± 0.16 (2.22 ± 0.07)‡,¶
2-Acetonaphthone‖ (1×10^{-5})	59.3	430	244	0.71 ± 0.09
1-Acetonaphthone‖ (1×10^{-5})	56.4	500	277	0.82 ± 0.04
9-Fluorenone (3.7×10^{-5})	53.3	430	110	0.026 ± 0.01

*pH 5.
†Lifetime of unquenched sensitizer.
‡Rate constant for quenching of naphthalene triplet by orotic acid in aqueous solution at pH 5. Naphthalene concentration 2.7×10^{-5} M.
§Soft glass as filter.
‖Pyrex as filter.
¶Corex as filter.

ground state orotic acid (or methyl orotate) referred to here as self-quenching implicates the transient in the photodimerization process and the exceedingly high self-quenching rate constants of 10^9 M^{-1} s^{-1} (see Table 3) demonstrate that the transient is an extremely reactive species as might be expected of an excited state molecule.

The assignment of the triplet state to the observed transient is further supported by the hexadienol and dimethyl fumarate quenching results. The quenching rate constants, which appear to represent a limiting value for triplet energy transfer characteristic of the solvent, are essentially identical with those obtained for the quenching of triplet 1-acetonaphthone and 2-acetonaphthone by hexadienol and for the quenching of triplet xanthenone by methyl orotate. As well, substantial quenching by a

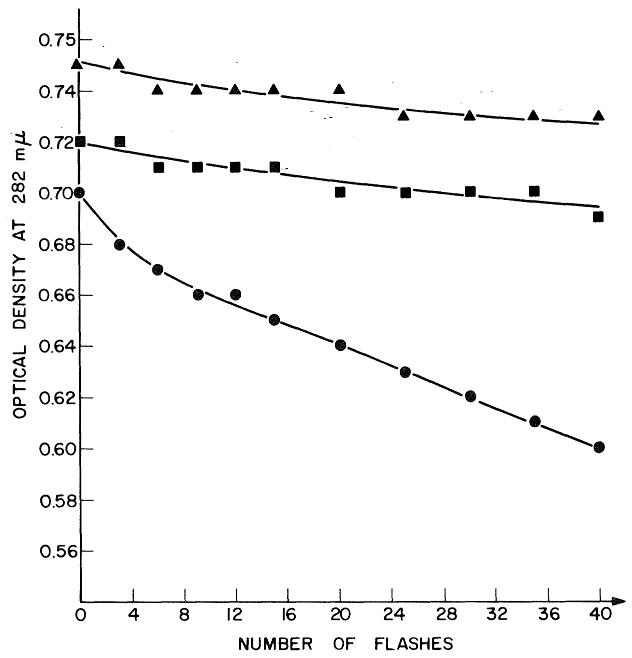


FIG. 6. Plot of decrease in optical density at 282 mμ with successive flashes of aqueous solutions of [methyl orotate] = 1×10^{-5} M and varying [hexadienol]; ● [hexadienol] = 0; ■ [hexadienol] = 3×10^{-5} M; ▲ [hexadienol] = 1×10^{-4} M. (The variation in optical density at 0 flash results from minor variations in [methyl orotate] and minor absorption from the added hexadienol.)

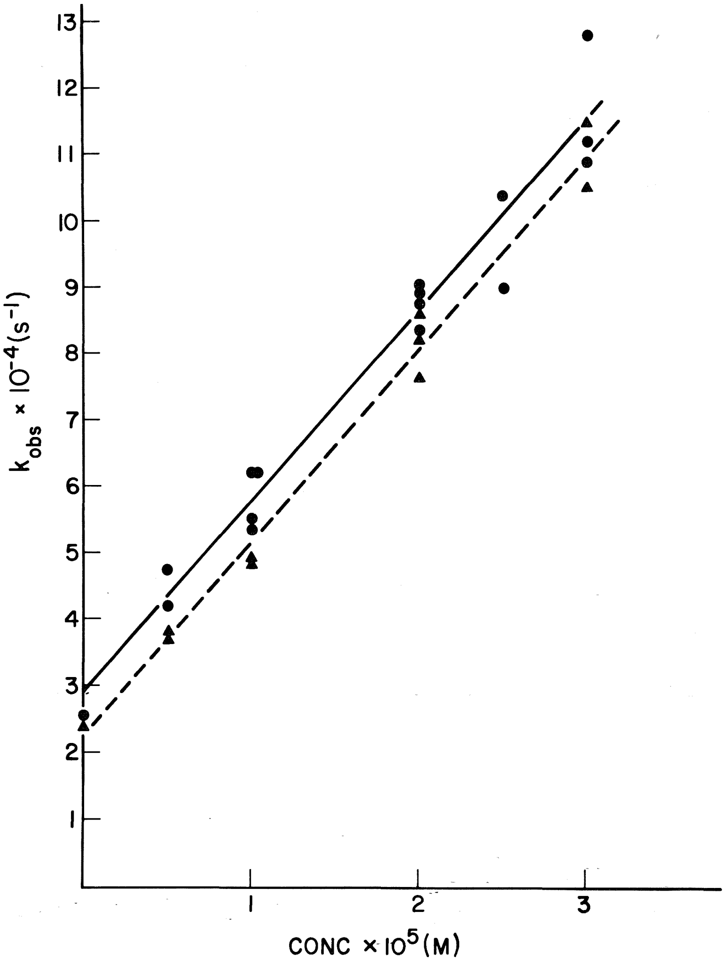


FIG. 7. Plot of k_{obs} vs. [hexadienol] for quenching studies of methyl orotate and orotic acid, pH 5, triplets; ● [methyl orotate] = 1 × 10^{-5} M; ▲ [orotic acid] = 1 × 10^{-5} M.

chemical reaction between triplet methyl orotate and hexadienol is eliminated by the negligible rate of decrease of ground state methyl orotate in the presence of hexadienol (see Fig. 6).

TABLE 2
Quenching of pyrimidine transients and sensitizer triplets by 2,4-hexadien-1-ol*

Sensitizer†	E_T (kcal $mole^{-1}$)	$k_q \times 10^{-9}$ (M^{-1} s^{-1})
Orotic acid	—	2.93 ± 0.12
Methyl orotate	—	2.88 ± 0.15 (2.3 ± 0.2)‡
2-Acetonaphthone	59.3	2.69 ± 0.08
1-Acetonaphthone	56.4	2.41 ± 0.08

*In aqueous solution at pH 5.
†Concentration of sensitizer 1 × 10^{-5} M.
‡Rate constant for quenching of methyl orotate transient by dimethyl fumarate in aqueous solution at pH 5.

The results of the acetone sensitization confirm the triplet assignment. The control experiments show that the transient observed on sensitization arises mainly from excitation of acetone but is not triplet acetone. The lifetime of the sensitized species is, within experimental error, identical with the lifetime of the transient observed from directly excited methyl orotate. Since singlet energy transfer is unlikely due to the low concentration of methyl orotate used (2 × 10^{-5} M), the sensitized species is triplet methyl orotate formed by triplet energy transfer. The slow risetime of the sensitized species is consistent with this view.

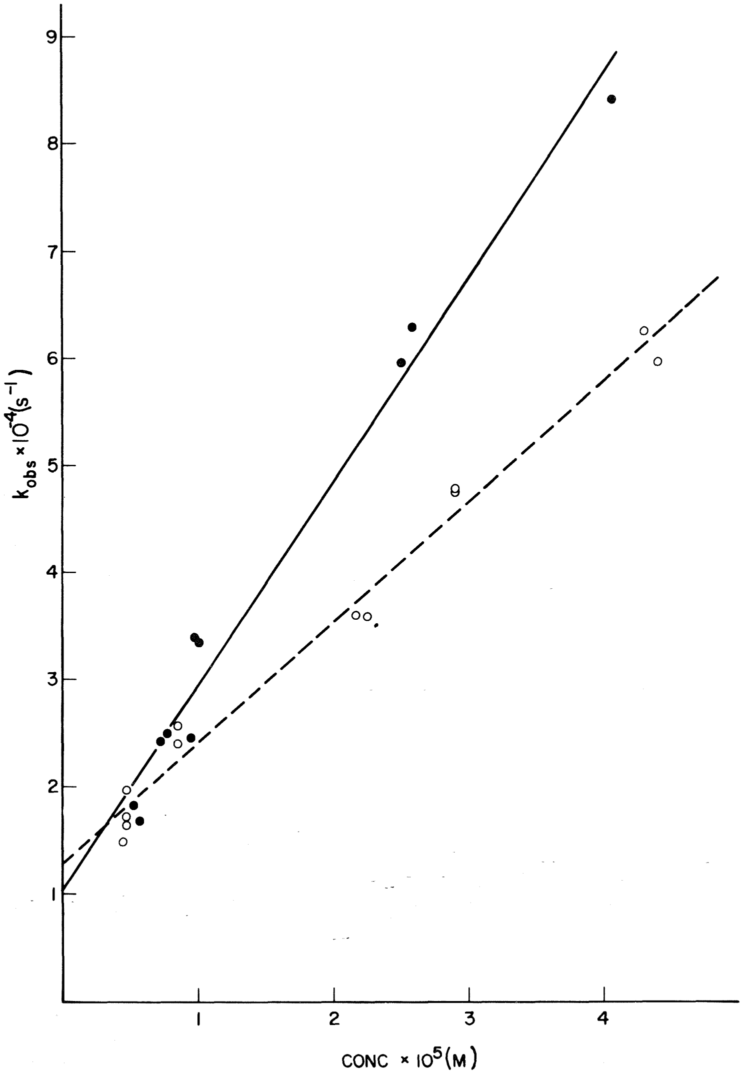


FIG. 8. Plot of k_{obs} vs. [orotic acid]; ● pH 0.6; ○ pH 5.

Triplet Energy Transfer in Solution and Triplet Energy of Methyl Orotate and Orotic Acid

The measured rate constants for methyl orotate quenching of triplet xanthenone (E_T 74 kcal mole^{-1}) and naphthalene (E_T 61 kcal mole^{-1}) shown in Table 1 are $\sim 2.5 \times 10^9$ M^{-1} s^{-1} and this suggests that in both cases, triplet energy transfer is exothermic. It appears to be the general case, at least for non-viscous solvents, that the experimental value of k_q is lower than the value calculated by the Debye equation (7×10^9 M^{-1} s^{-1} for water at 23 °C) by a factor of at least 3 (20). With sensitizers having triplet energies of 59 kcal mole^{-1} or less, quenching by methyl orotate becomes substantially less efficient. On the basis of these quenching results, we infer that the energy of the lowest triplet state of methyl orotate is close to 60 kcal mole^{-1}. Since orotic acid at pH 5 (which exists in the anion form in the ground state) quenches naphthalene triplet with a rate constant of 2.22×10^9 M^{-1} s^{-1}, almost identical with that of the ester, therefore the triplet energy of orotic acid is most probably the same as the ester. The triplet energies thus deduced for the neutral species and for the anion, are in

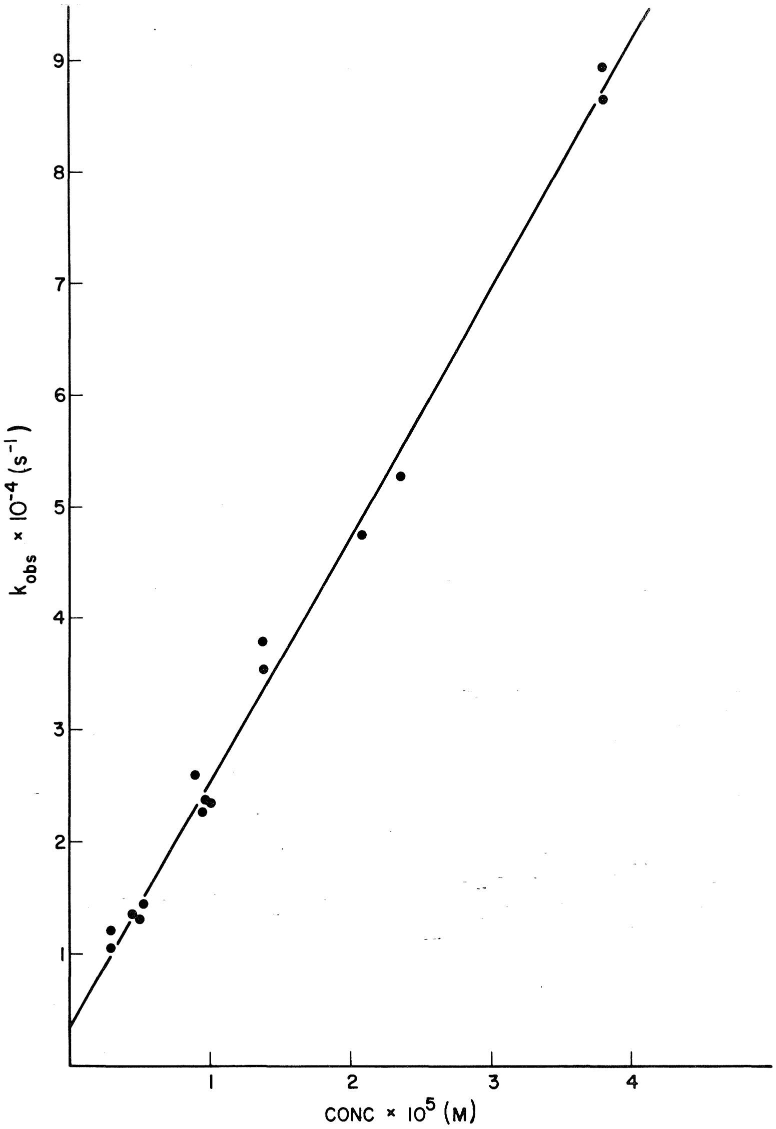


FIG. 9. Plot of k_{obs} vs. [methyl orotate].

agreement with the value obtained by Haug and Douzou (21) for orotic acid from phosphorescence measurements (see Table 4).

Hexadienol quenching of triplet methyl orotate and orotic acid at p*H* 5 is surprisingly efficient ($k_q \sim 3 \times 10^9$ M^{-1} s^{-1}). This value taken together with the quenching rates of triplet 2-acetonaphthone (E_T 59.3 kcal $mole^{-1}$) and 1-acetonaphthone (E_T 56.4 kcal $mole^{-1}$) by hexadienol indicates that the reported E_T of 2,4-hexadienol of 59.6 kcal $mole^{-1}$ (15) should be revised to a lower value. It should be kept in mind however, that the optical triplet energies of dienes are known with less certainty than are those of aromatic molecules. Chemical quenching is unlikely to account for the large k_q because of the effectiveness with which hexadienol suppresses the consumption of methyl orotate (Fig. 6). This implies that if products are formed between methyl orotate and hexadienol, the quantum yield of formation is low. The rate constants for quenching of triplet orotic acid at p*H* 1.1 and 5

TABLE 3

Rate constants for photodimerization* of orotic acid and methyl orotate in aqueous solution

Compound	pH	% Dissociated in the ground state	$k_0 \times 10^{-4}$ (s^{-1})	τ_0 (μs)	$k_{dim} \times 10^{-9}$ (M^{-1} s^{-1})
Orotic acid	5	100	1.27 ± 0.11	78 ± 6	1.13 ± 0.05
Orotic acid	0.6	6	1.03 ± 0.19	97 ± 18	1.91 ± 0.1
Methyl orotate	5	—	0.35 ± 0.09	287 ± 72	2.20 ± 0.05

*Vycor as filter.

TABLE 4

Triplet energy of orotic acid and methyl orotate

Compound	Solvent	E_T (kcal mole^{-1})	Method	Reference
Orotic acid	Propylene glycol/H_2O 1:1 (v/v)	69.8	Phosphorescence*	23
Orotic acid	Propylene glycol/H_2O 1:1 (v/v) (in HCl)	60.9	Phosphorescence*	23
Orotic acid	Ethanol	60.9	Phosphorescence*	22
Orotic acid	DMF/EtOH/ether‡ 1:1:1.7 (v/v)	60.9	Phosphorescence*	21
Orotic acid	H_2O§	~ 60	Energy transfer†	This work
Methyl orotate	H_2O§	~ 60	Energy transfer†	This work

*77 °K.
†300 °K.
‡pH 12 ref. (24).
§pH 5.

by oxygen reported by Whillans and Johns (25) are considerably lower than our values for hexadienol. These workers also found a different k_q for the neutral form as compared with the anion. In the case of oxygen quenching of triplet fluorescein in aqueous solution, Kasche and Lindqvist (26) obtained a k_q of 1.2×10^9 M^{-1} s^{-1} for both the monoanion and the neutral molecule. It is interesting that the quenching rate of 1-acetonaphthone triplet by methyl orotate is slightly greater than that of 2-acetonaphthone (0.82×10^9 cf. 0.71×10^9 M^{-1} s^{-1}). In view of the inefficiency of endothermic energy transfer in this case other processes may compete more effectively, such as chemical reaction.

Lifetime and Reactivity of the Triplet State

The lifetime of triplet orotic acid determined in this work by flash photolysis (97 μs at pH 0.6, Table 3) is considerably longer than the value obtained by Sztumpf–Kulikowska *et al.* (9) from their oxygen quenching study of the rates of disappearance of orotic acid in aqueous solution. If we recalculate their results using the k_q determined by Whillans and Johns (25) instead of 10^{10} M^{-1} s^{-1} used by Sztumpf–Kulikowska and co-workers (9), a lifetime of 5 μs can be inferred from the oxygen quenching studies. The discrepancy between the recalculated lifetime and the directly measured lifetime would seem large but is perhaps not unexpected. All triplet lifetimes indirectly inferred from triplet quenching studies depend on a reaction of the triplet excited state and it therefore requires a relatively long extrapolation to obtain the lifetime in the absence of reaction. On the other hand, lifetimes obtained by flash photolysis are more accurate in the absence of reaction when the lifetime is longest and the extrapolation to infinite dilution least.

The rate constant k_0 for decay of triplet orotic acid or methyl orotate in the absence of self-quenching does not reflect any impurity quenching. We can represent the composite rate constant k_0 by eq. [4], where the term k_q(Q) represents impurity quenching of the triplet, k_{chem} represents a unimolecular reaction of the triplet, and k_i, the intrinsic rate of decay of the triplet.

$$[4] \qquad k_0 = k_q(\mathrm{Q}) + k_{chem} + k_i$$

If k_q(Q) were to dominate, we would expect identical decay times for triplet orotic acid and methyl orotate in water, contrary to the results shown in Table 3. In addition, if k_i were independent of phase, the phosphorescence results of Haug and Douzou (21) would suggest that k_i

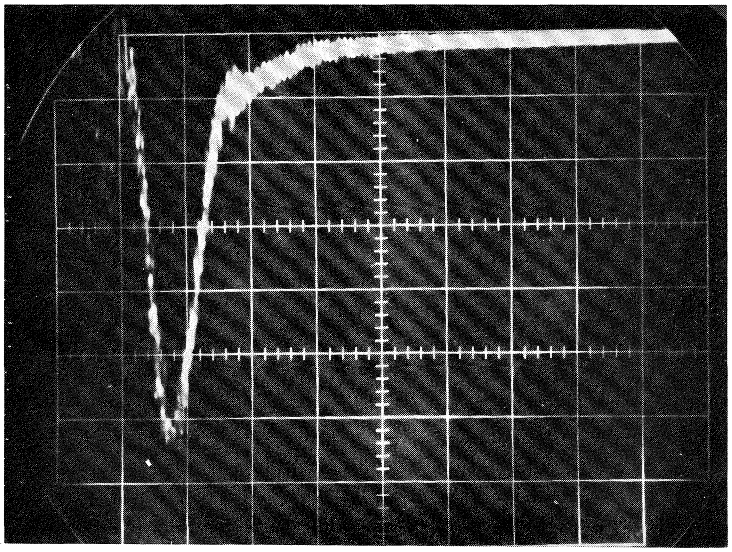


FIG. 10. Oscilloscope trace of photoflash pulse; monitoring wavelength, 350 mμ; timebase, 1 μs/cm; voltage, 0.5 V/cm.

might be small compared to k_0. We might therefore speculate that k_0 is in fact a reflection of unimolecular reaction(s) of the triplet state, for instance, decarboxylation or ring cleavage.

At present we have no evidence to suggest that the self-quenching rate constant k_{dim} does not represent the rate constant for dimerization. The value of k_{dim} for triplet orotic acid at p*H* 5 is approximately a factor of 2 smaller than either the values for orotic acid at p*H* 0.6, or methyl orotate at p*H* 5. The smaller value would seem reasonable if triplet orotic acid exists in the anion form at p*H* 5. Since ground state orotic acid is fully dissociated at p*H* 5, the rate constant for reaction between two like charged species would be expected to be slower than for reaction between neutral species.[4] Wagner has reported rate constants for dimerization of uracil and thymine obtained from quenching studies and found a smaller value for thymine as compared to uracil. He attributed the differences to steric effects. Comparison of his values with ours shown in Table 3 would suggest that such steric effects are not important and that the electronic distribution of the molecule is the more important factor controlling reactivity (6).

Experimental

Apparatus

The flash apparatus was identical with the 5 μs dual beam flash photoelectric apparatus already described (27), except for the discharge circuit and lamps. Instead of series connections of the lamps, each of the 2 lamps was operated from 2 separate capacitor-spark gap circuits which are triggered synchronously. Each circuit used a 0.3 μF 25 kV capacitor. The lamps had a cross-sectional area of 3.74 mm^2 and an arc length of 100 mm and were filled with 4 cm argon and 1 cm hydrogen. A light pulse width of 0.9, 1.1, and 2.3 μs at 1/2, 1/3, and 1/10 of its peak value respectively was obtained (Fig. 10). The absorption cells were all quartz in construction, 22 mm o.d. and 100 mm path length. For degassing they were connected to a Pyrex vessel by means of a graded seal.

Instruments

Gas–liquid chromatography (g.l.c.) was carried out using a Hewlett–Packard 5754 gas chromatograph with a flame ionization detector. Infrared (i.r.) spectra were determined on a Perkin–Elmer 257 i.r. spectrophotometer. Ultraviolet (u.v.) spectra were measured on a Cary 15, dual beam u.v. spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were recorded on a Varian A-100 spectrometer, using tetramethylsilane as an internal standard. Melting points are uncorrected and were measured on a Fisher–Johns melting point apparatus. Low p*H* values were determined spectrophotometrically using *p*-nitroaniline according to the method outlined by Albert and Serjeant (28), otherwise p*H* was measured using a Radiometer p*H* meter.

Procedure

Prior to being filled with the sample solution the photolysis cell and degassing chamber were pumped continuously under high vacuum. During the pumping,

---

[4] The Debye treatment for diffusion rates predicts a factor of 0.45 or 0.60 for spheres of 5 or 7.5 Å, respectively, of similar unit charge as compared with 2 uncharged species (20*b*).

the apparatus was briefly flamed. Solutions were degassed under vacuum through several freeze–pump–thaw–shake cycles and the cell sealed off with a flame. No noticeable difference in lifetime was found using an oil diffusion pump as compared with a mercury diffusion pump.

The concentration of orotic acid or methyl orotate was monitored before and between flashes by u.v. absorption measurement of the 10 cm path photolysis cell using a Bausch and Lomb single beam double grating spectrophotometer.

Hexadienol solutions were degassed immediately upon preparation to prevent oxidation to the aldehyde. Neat hexadienol keeps well but very dilute solutions were easily oxidized. Dilute aqueous solutions of hexadienol dehydrate rapidly to the triene at p*H* 0.6.

The transient trace was shifted from the baseline to facilitate measurement. Sufficiently long sweep times were used to allow unambiguous definition of the baseline from the transient trace alone. Any variation in the shape of the baseline was used to correct the transient trace. In general, data were taken out to at least 2 half-lives of the transient. For each sample, at least 5 traces were taken and the results averaged. Data taken from the Polaroid photographs, or from their enlargements, were processed using an IBM 360/50 computer from which best value rate constants were obtained. The decay kinetics were plotted on an X–Y recorder linked to the output of the computer.

Materials

Orotic acid was obtained from Sigma Chemical Co. and was recrystallized from water and dried in a desiccator prior to use. Its pK_a was determined spectrophotometrically to be 1.7 (29).

Methyl orotate was prepared according to the method of Fox *et al.* (30) and was recrystallized from water and dried prior to use, m.p. 248–249° (lit. (30) m.p. 243–245°, (31) m.p. 248–250°); ν_{max} ($CHCl_3$), 3380, 1730, 1705, 1600 cm^{-1}; λ_{max} (H_2O) (log ε), 284 (3.89) mμ; δ (DMSO-d_6) 3.86 (3H, s), 6.04 (1H, d, $J = 2$ c.p.s.), 11.09 (1H, br), 11.36 (1H, br) p.p.m.

The sensitizers xanthen-9-one, 2-acetonaphthone, 1-acetonaphthone, and fluoren-9-one were obtained from J. T. Baker Chemical Co., and were used without further purification. Naphthalene was zone refined by Koch Light Laboratories. The 2,4-hexadienol was obtained from Aldrich Chemical Co., and distilled prior to use, b.p. (22 mm) 83–84°. The g.l.c. on C-20M, 6 ft, 100°, indicated it was largely a single isomer (90%). Dimethyl fumarate was prepared according to the method of Fischer Spiers (32) and purified by distillation, m.p. 102°. The g.l.c. on C-20M, 6 ft, 80°, indicated it to be free of dimethyl maleate and no impurity with concentration greater than 0.5% could be detected.

NOTE ADDED IN PROOF: We have identified and measured the kinetics of the triplet state of thymine in acetonitrile solutions and found that k_{dim} was $5.3 \times 10^8\ M^{-1}\ s^{-1}$ and the lifetime was 14 μs (Can. J. Chem. In press).

One of us (W.D.R.) wishes to thank the National Research Council of Canada for the award of a postdoctoral fellowship (1968–1969). We are grateful to Mr. Peter Tolg for his excellent technical assistance, and to Mr. R. Lauzon for assistance in the data processing. We are indebted to Mr. P. Clay for the development of the computer program.

1. (*a*) J. G. BURR. *In* Advances in photochemistry. Vol. 6. *Edited by* W. A. Noyes, Jr., G. S. Hammond, and J. N. Pitts. Interscience Publishers, Inc., New York. 1968. p. 193. (*b*) A. D. MCLAREN and D. SHUGAR. Photochemistry of proteins and nucleic acids. Pergamon Press, Inc., New York. 1964.
2. J. K. SETLOW. *In* Comprehensive biochemistry. Vol. 27; Photobiology, ionizing radiations. *Edited by* M. Florkin and E. H. Stotz. Elsevier Publishing Co., Amsterdam. 1967. p. 157.
3. B. S. STRAUSS. *In* Current topics in microbiology and immunology. Vol. 44. Springer-Verlag, Berlin. 1968. p. 1.
4. R. LISEWSKI and K. L. WIERZCHOWSKI. Chem. Commun. 348 (1969).
5. R. O. LOUTFY, P. DE MAYO, and M. F. TCHIR. J. Amer. Chem. Soc. **91**, 3986 (1969).
6. P. J. WAGNER and D. J. BUCHECK. J. Amer. Chem. Soc. **90**, 6530 (1968).
7. E. SZTUMPF and D. SHUGAR. Photochem. Photobiol. **4**, 719 (1965).
8. (*a*) R. BEUKERS and W. BERENDS. Biochim. Biophys. Acta, **38**, 573 (1960). (*b*) B. BEUKERS, J. IJLSTRA, and W. BERENDS. Rec. Trav. Chim. **78**, 879 (1959).
9. E. SZTUMPF–KULIKOWSKA, D. SHUGAR, and J. W. BOAG. Photochem. Photobiol. **6**, 41 (1967).
10. J. W. BOAG and T. GODFREY. Private communication, August, 1968.
11. M. A. HERBERT, J. W. HUNT, and H. E. JOHNS. Biochem. Biophys. Res. Commun. **33**, 643 (1968).
12. R. F. BORKMAN and D. R. KEARNS. J. Chem. Phys. **44**, 945 (1966).
13. G. PORTER and R. W. YIP. Unpublished observations.
14. S. ARAI and L. M. DORFMAN. J. Phys. Chem. **69**, 2239 (1965).
15. R. E. KELLOGG and W. T. SIMPSON. J. Amer. Chem. Soc. **87**, 4230 (1965).
16. G. S. HAMMOND, J. SALTIEL, A. A. LAMOLA, N. J. TURRO, J. S. BRADSHAW, D. O. COWAN, R. C. COUNSELL, V. VOGT, and C. DALTON. J. Amer. Chem. Soc. **86**, 3197 (1964).
17. J. G. CALVERT and J. N. PITTS, JR. Photochemistry. John Wiley and Sons, Inc., New York. 1967. p. 298.
18. W. G. HERKSTROETER, A. A. LAMOLA, and G. S. HAMMOND. J. Amer. Chem. Soc. **86**, 4537 (1964).
19. G. N. LEWIS and M. KASHA. J. Amer. Chem. Soc. **66**, 2100 (1944).
20. (*a*) P. J. DEBYE. Trans. Electrochem. Soc. **82**, 265 (1942). (*b*) E. F. CALDIN. Fast reactions in solution. Blackwell Scientific Publications, Oxford. 1964. p. 12.
21. A. HAUG and P. DOUZOU. Z. Naturforsch. **20*b***, 509 (1965).
22. M. CHARLIER and C. HÉLÈNE. Photochem. Photobiol. **6**, 501 (1967).
23. M. CHARLIER, C. HÉLÈNE, and M. DOURLANT. J. Chim. Phys. **66**, 700 (1969).
24. R. G. SHULMAN and R. O. RAHN. J. Chem. Phys. **45**, 2940 (1966).
25. D. W. WHILLANS, and H. E. JOHNS. Photochem. Photobiol. **9**, 323 (1969).

26. V. Kasche and L. Lindqvist. J. Phys. Chem. **68**, 817 (1964).
27. R. W. Yip. Rev. Sci. Instr. **40**, 1035 (1969).
28. A. Albert and E. P. Serjeant. Ionization constants of acids and bases. Methuen and Co. Ltd., London. 1962. p. 78.
29. D. Shugar and J. J. Fox. Biochim. Biophys. Acta, **9**, 199 (1952).
30. J. J. Fox, N. Yung, and I. Wempen. Biochim. Biophys. Acta, **23**, 295 (1957).
31. G. Biscaro and E. Belloni. Ann. Soc. Chim. Milano, **18** II, 71 (1905).
32. A. I. Vogel. Elementary practical organic chemistry. Longmans, Green and Co., Ltd. London. 1958. p. 310.