Photosensitized Monomerization of 1,3-Dimethyluracil Photodimers

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The photomonomerization of the four 1,3-dimethyluracil (DMU) photodimers in the presence of a variety of photosensitizers is described. The reactions lead to the recovery of DMU in yields of up to 100%. The syn dimers are monomerized faster than the anti ones. An excited charge-transfer complex (exciplex) is proposed as the intermediate in these reactions. Monomerization of the photodimers could also be achieved with $AlCl_3$.

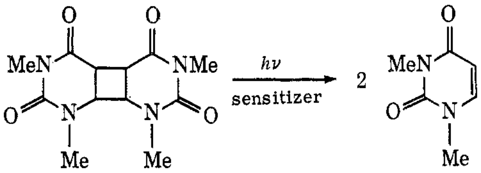
Irradiation of DNA or RNA with ultraviolet light has been reported to induce chemical and physical changes in these biopolymers.[2] These changes were found to be responsible for the damage caused to the living system, and the major known reaction was the formation of cyclobutane dimers between two adjacent pyrimidine moieties.[3] The lesion caused by ultraviolet light on the biological system is usually photoreactivable; that is, the effects caused by ultraviolet light are reversed in part by subsequent irradiation with light of wavelengths longer than 330 nm. For example, illumination of ultraviolet-inactivated transforming DNA in the presence of some enzyme extracts resulted in an increase in the transforming activity. Photoreactivating activity has been observed also in many living systems, such as bacteria, yeast, and fish.[4] It has been shown that during this photoreactivating process thymine dimers formed in irradiated DNA were cleaved to yield the thymine monomer moieties.[4]

The mechanism by which the enzymic photoreactivation process operates is still obscure.[5] Several attempts aiming at the clarification of this point have been made,[6-8] however, without any final conclusion. The aim of the present investigation is to study photochemical reactions which might be relevant to the photoreactivation process and shed light on its mechanism. We have chosen the DMU dimers and a variety of photosensitizers as suitable models for this study.[9] The availability of the four isomeric dimers enables also the study of the stereoselectivity of these photomonomerization reactions. The present publication includes details of some photosensitized monomerization reactions of DMU dimers, mainly with quinones, and a proposal for a mechanism for these reactions.

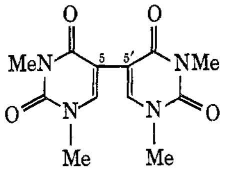
Results and Discussion

We have found that the four photodimers of DMU can be monomerized through irradiation of their solution with light of λ >290 nm in the presence of a photosensitizer. The reactions can be represented as described in Scheme I.

SCHEME I



The trans,syn (dimer B)[10] and the cis,syn (dimer D) dimers were monomerized faster than the trans,anti (dimer A) and cis,anti (dimer C) dimers with the same photosensitizer under similar reaction conditions. The quantum yields of the monomerization sensitized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) of the trans,syn and cis,syn dimers were 0.77 and 0.7, respectively, while those of the trans,anti and cis,anti dimers were 0.22 and 0.26, respectively. It has been observed, with sensitizers of the same series, that the higher the electron affinity (EA) of the sensitizer the higher the efficiency of the monomerization. Comparison of the photosensitized monomerization of the four DMU dimers with some benzoquinones in benzene, acetone, and acetonitrile indicated that the process is fastest in acetonitrile. Bis-5,5′-(1,3-dimethyl)uracil (I) was formed as a by-product in some of the reactions of the syn dimers, while none of this product could be observed in reactions of the anti dimers.[10] The reactions studied and the results obtained are summarized in Table I.



I

The progress of the reactions was followed through the increase in the absorption at the 265-nm region and by thin layer chromatography. Isolation of DMU and I, when formed, was achieved by column chromatography (silica gel). I was characterized by comparison with an authentic sample.[11] When chloranil was used as a photosensitizer, dihydrochloranil accompanied the formation of I (from the syn dimers), whereas none of the former could be detected in reactions where I was absent (with the anti dimers). Dihydrochloranil was isolated and characterized by comparison with an authentic sample. No reaction could be observed in the dark, even after heating of the reaction mixture to 50°; also light of λ >290 nm failed to monomerize the dimers in the absence of a photosensitizer. Mono-

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[1] In partial fulfillment of the requirements for a Ph.D. thesis submitted to the Feinberg Graduate School, 1971.

[2] For reviews see (a) K. C. Smith, *Radiat. Res. Suppl.*, **6**, 54 (1966); (b) R. B. Setlow, *Progr. Nucl. Acid Res. Mol. Biol.*, **8**, 257 (1968); (b) J. G. Burr, *Advan. Photochem.*, **6**, 193 (1968); (d) E. Fahr, *Angew. Chem., Int. Ed. Engl.*, **8**, 578 (1969).

[3] K. C. Smith and P. C. Hanawalt, "Molecular Photobiology," Academic Press, New York, N. Y., 1969, pp 62–68, and references cited therein.

[4] R. B. Setlow, *Progr. Nucl. Acid Res. Mol. Biol.*, **8**, 269 (1968).

[5] *Cf.* A. Muhammed, *J. Biol. Chem.*, **241**, 516 (1966).

[6] A. A. Lamola, *J. Amer. Chem. Soc.*, **88**, 813 (1966).

[7] A. Wacker, *et al.*, *Photochem. Photobiol.*, **3**, 369 (1964).

[8] C. Helene and M. Charlier, *Biochem. Biophys. Res. Commun.*, **43**, 252 (1971).

[9] Preliminary communication: I. Rosenthal and D. Elad, *ibid.*, **32**, 599 (1968).

[10] D. Elad, I. Rosenthal, and S. Sasson, *J. Chem. Soc. C*, 2053 (1971).

[11] H. Ishihara and S. Y. Wang, *Nature (London)*, **210**, 1222 (1966).

TABLE I

PHOTOSENSITIZED MONOMERIZATION OF THE FOUR 1,3-DIMETHYLURACIL PHOTODIMERS

Photosensitizer	$E_{1/2}^{\mathrm{red}}$,[a] eV	EA,[b] eV	E_T, kcal/mol	Solvent	Irradiation time, hr	Monomerization %[g] A	B	I	C	D	I
DDQ	−0.51	1.9	55.5[c]	Benzene	1[d]	0	60	0	34	50	0
				Acetone	1[d]	49	99	1	83	99	1
				Acetonitrile	1[d]	96	100	0	100	100	0
p-Chloranil	−0.01	1.37	57.2	Benzene	2[d]	0	26	29	0	22	10
				Acetone	2[d]	9	59	28	5	82	14
				Acetonitrile	2[d]	9	78	19	5	87	6
2,5-Dichloro-1,4-benzoquinone				Benzene	2[d]	0	3	31	0	1	0
				Acetone	2[d]	8	10	62	2	28	36
				Acetonitrile	2[d]	8	13	55	2	31	31
Tetracyanoethylene	−0.24	2.2		Acetonitrile	8[e]	44	59	0	59	76	0
o-Chloranil		1.5		Acetonitrile	8[e]	3	26	7	3	24	1
2,4,7-Trinitrofluorenone		1.1	64.4[c]	Acetonitrile	3[e]	5	41	27	2	64	11
				Acetone	18[d]	7	32	68	4	76	21
1,3,5-Trinitrobenzene		0.7		Acetonitrile	16[e]	25	13	9	1	14	6
				Acetone	18[d]	0	6	22	0	8	12
1,4-Benzoquinone	+0.51	0.7		Acetonitrile	8[e]	0	9	0	0.5	8	0
1,4-Naphthoquinone	+0.71	0.7	57	Acetonitrile	8[e]	10	35	40	1	31	3
2-Methyl-1,4-benzoquinone	+0.77	0.64		Acetonitrile	8[e]	0	12	8	0	12	4
				Acetone	18[d]	6	23	19	5	23	9
2-Bromo-1,4-naphthoquinone				Acetone	18[d]					32	33
2-Amino-1,4-naphthoquinone				Acetone	18[d]					4	0
9,10-Anthraquinone	+0.94	0.5	62	Acetonitrile	4[e]	3	58	23	3	56	11
				Acetone	18[d]	14	50	30	4	37	24
1,2,4-Tribromobenzene				Acetonitrile	22[e]	16	16	0	11	21	0
9-Fluorenone			53	Acetonitrile	16[e]	0	2	0	0	0.5	0
Hexachlorobenzene		0.5	70	Acetone	18[d]	3	20	0	0	18	0
Aluminum chloride				Acetonitrile	3[f]		60	0		34	0

[a] Half-wave reduction potential. See G. Briegleb, *Angew. Chem., Int. Ed. Engl.*, **3**, 617 (1964). [b] Electron affinity. [c] See Experimental Section. [d] Hanau Q81 high-pressure mercury vapor lamp (Pyrex filter). [e] Hanovia 200-W high-pressure mercury vapor lamp (Pyrex filter). [f] Hanovia 450-W high-pressure mercury vapor lamp (Pyrex filter). [g] A, trans,anti; B, trans,syn; C, cis,anti; D, cis,syn.

merization could be observed while leaving the reaction mixtures on the bench in the laboratory, due to the absorption of visible light by the appropriate sensitizer. It could also be achieved by exposure of powdered mixtures of the dimers with DDQ or *p*-chloranil to ultraviolet light or sunlight, resulting in good yields of DMU.

The reactions described operate through light absorption by the photosensitizer, as seen from the absorption spectra of the systems. The transfer of the excitation energy from the sensitizer to the dimer can be performed by several routes.

$$\mathrm{S} \xrightarrow{h\nu} \mathrm{S}^* \qquad \text{(a)}$$

$$\mathrm{S}^* + \mathrm{MM} \longrightarrow 2\mathrm{M}$$

$$\mathrm{S} + \mathrm{MM} \longrightarrow \{\mathrm{S\text{-}MM}\} \xrightarrow{h\nu} \{\mathrm{S\text{-}MM}\}^* \qquad \text{(b)}$$

$$\{\mathrm{S\text{-}MM}\}^* \longrightarrow 2\mathrm{M}$$

$$\mathrm{S} \xrightarrow{h\nu} \mathrm{S}^*$$

$$\mathrm{S}^* + \mathrm{MM} \longrightarrow \{\mathrm{S\text{-}MM}\}^* \qquad \text{(c)}$$

$$\{\mathrm{S\text{-}MM}\}^* \longrightarrow 2\mathrm{M}$$

S = photosensitizer; M = monomer; MM = dimer

The first route (a) involves an energy transfer process which may be of a singlet–singlet or a triplet–triplet nature. The singlet–singlet energy transfer process can be eliminated due to the absorption spectra of the dimers and the photosensitizers, the former absorbing at shorter wavelength than the latter.[12] The vertical triplet energy transfer process seems to be improbable, since sensitizers with high triplet energies, such as acetone, acetophenone, and benzene, did not lead to monomerization of any of the dimers. On the other hand, photosensitizers with relatively low triplet energies were effective in these reactions. The triplet energy of 5,6-dihydro-1,3-dimethyluracil has been used as the approximate triplet energies of the DMU dimers.[6,13] We derived the triplet energy of the former from its phosphorescence spectrum, and found it to be 67.5 kcal/mol. This number is higher by 10–12 kcal/mol than the triplet energies of the sensitizers (*p*-chloranil or DDQ) which were most efficient in the photomonomerization process. Therefore, we assume that a triplet–triplet energy transfer either vertical or nonvertical,[14] does not operate in these reactions, due to the large differences in the triplet energies of the reactants. The alternative mechanism (route b) involves the formation of a ground state complex between the quinone and the dimer. The ultraviolet, infrared, and nmr spectra of the reaction mixture do not indicate any formation of a ground state complex.[15] Similar results were obtained from an X-ray powder picture of the mixture of the quinone and the dimer. Further evidence against a ground state charge-transfer complex was derived from studies on the effect of temperature on the rate of the photomonomerization. We

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[12] A. A. Lamola in "Technique of Organic Chemistry," Vol. 14, P. A. Leermakers and A. Weissberger, Ed., Interscience, New York, N. Y., 1969, pp 37–42, and references cited therein.

[13] The direct determination of the triplet energies of the dimers involved difficulties, due to the instability of the dimers to light in their absorption region (see ref 10).

[14] N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, New York, N. Y., 1965, pp 182–183, and references cited therein.

[15] *Cf.* E. M. Kosower, *Progr. Org. Chem.*, **3**, 81 (1965).

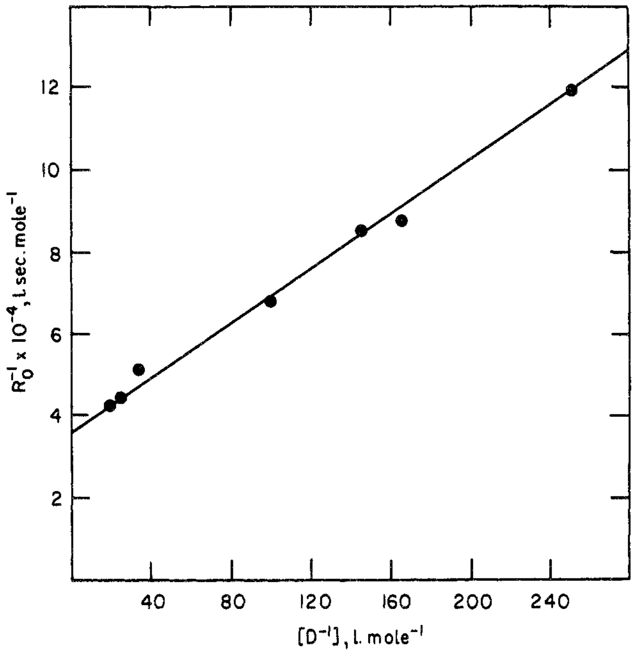

Figure 1.—Effect of initial concentration of dimer D on the rate of photomonomerization sensitized with DDQ.

have found that the latter increases with raise of temperature (temperature range 3–59°). This observation eliminates a mechanism involving a ground state charge-transfer complex, since the association constant of such a complex decreases with a rise in temperature.[16]

A plot of the reciprocal of the rate of photomonomerization (R_0, see Experimental Section) *vs.* the reciprocal of the concentration of the photodimer gave a straight line. An intercept at 3.57×10^4 and a slope of 3.37×10^2 were obtained with dimer D, while DDQ served as the photosensitizer (Figure 1). These results indicate that the photosensitized monomerization process involves an interaction of an excited species with a ground state molecule.[17] Since route a, which would involve such a process, is eliminated, it is most plausible that route c represents the mechanism of the monomerization, *i.e.*, that it proceeds through the formation of a complex between the excited photosensitizer and a ground state dimer molecule (exciplex).[18] Results obtained with the *p*-benzoquinones as sensitizers indicate that those sensitizers with higher electron affinities were more efficient in monomerizing the dimers (see Table I). Therefore, we assume that the process involves the transfer of an electron from the dimer to the excited quinones, so that the resultant complex is of a charge transfer nature. The enhancement in the monomerization with the increase in the polarity of the solvent presents additional evidence for such an intermediate, which is stabilized in polar solvents.[19,20] Further evidence for the trend of the DMU dimers to act as electron donors is given in our observation that aluminum chloride cleaves the DMU photodimers. We have found that the addition of aluminum chloride to a solution of dimer A or C resulted in the cleavage of the dimer into DMU. Monomerization of dimer B or D in the presence of aluminum chloride could also be achieved, however, only upon irradiation of the mixture. The addition of aluminum chloride to a solution of dimer B or D resulted in a downfield shift of the cyclobutane protons in the nmr spectrum, indicating the formation of a complex, which is concerned with the transfer of an electron from the dimer to aluminum chloride. Beresford, Lambert, and Ledwith[21] proposed a cation radical as an intermediate in the cleavage of *trans*-1,2-di(carbazol-9-yl)cyclobutane by tris(*p*-bromophenyl)amine cation or by cerium(IV). This intermediate results from the transfer of an electron from the cyclobutane compound to the amine cation or the cerium salt. A similar mechanism might fit very well for the reactions described.

The photosensitized monomerization reactions of the DMU dimers show some stereoselectivity,[22] as dimers B and D (syn type) are monomerized faster than dimers A and C (anti type). This results, most probably, from the steric factors involved in the complex formation between the dimer and the excited sensitizer. In the syn dimers the two carbonyl groups, which are near the cyclobutane ring, point to the same direction in space; this spatial arrangement might, perhaps, suit better for a sensitizer molecule to fit itself into a close contact with the dimer.[23] It is also noteworthy that *o*-chloranil, although possessing a higher electron affinity than *p*-chloranil, was less efficient than the latter in the photomonomerization process.

We feel that the present experiments shed some light on the possible mechanism of the photoreactivation process, in which a photosensitized monomerization of pyrimidine cyclobutane dimers occurs. It should be noted that various photosensitizers might cleave the dimers by different mechanisms, and that they do not necessarily follow the mechanism proposed by us for the photomonomerization with the *p*-benzoquinones. It appears that factors involving electron affinities and chemical structure play a role in determining the ability of a photosensitizer to cleave pyrimidine cyclobutane dimers; therefore, these should be evaluated in addition to triplet energy considerations.

Experimental Section

Kieselgel (0.05–0.20 mm, Merck) was used for chromatography. Petroleum ether refers to the fraction of bp 60–80°. Ascending thin layer chromatography was performed on Kieselgel G (Merck); a mixture of acetone–petroleum ether was used as eluent. Nmr spectra were determined with a Varian A-60 instrument as solutions in $CDCl_3$, unless otherwise stated.

Experiments in solution were carried out at room temperature in an immersion apparatus; Hanau Q81 or Hanovia 200-W and 450-W high-pressure mercury vapor lamps were used as the light source, and were cooled internally with running water. Pyrex filters were employed. Agitation was effected by bubbling oxygen-free nitrogen through the reaction mixtures as well as by magnetic stirring.

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[16] R. Foster, "Organic Charge-Transfer Complexes," Academic Press, New York, N. Y., 1969, p 189.

[17] O. L. Chapman and R. D. Lura, *J. Amer. Chem. Soc.*, **92**, 6352 (1970).

[18] *Cf.* S. L. Murov, R. S. Cole, and G. S. Hammond, *J. Amer. Chem. Soc.*, **90**, 2957 (1968).

[19] T. Foerster, *Angew. Chem., Int. Ed. Engl.*, **8**, 341 (1969), and references cited therein.

[20] Dr. A. A. Lamola informed us that he has reached a similar conclusion regarding the mechanism of anthraquinone-sensitized monomerization of thymine dimer. We are grateful to Dr. Lamola for disclosure of his results prior to publication.

[21] P. Beresford, M. C. Lambert, and A. Ledwith, *J. Chem. Soc. C*, 2508 (1970).

[22] *Cf.* E. Ben-Hur and I. Rosenthal, *Photochem. Photobiol.*, **11**, 163 (1970).

[23] *Cf.* N. C. Yang and W. Eisenhardt, *J. Amer. Chem. Soc.*, **93**, 1277 (1971), and ref 16, pp 195–201.

Photosensitized Monomerization of Dimer A with DDQ in Acetone.—A solution of dimer A (0.3 g, 1.07 mmol) and DDQ (0.3 g, 1.32 mmol) in acetone (150 ml) was irradiated (Hanau Q81) for 1 hr. The monomerization was followed by the increase in absorption at 265 nm. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (50 g). Acetone–petroleum ether (1:4) afforded 1,3-dimethyluracil (0.15 g) followed by the starting dimer (0.14 g, eluted with a 3:7 mixture).

Other reactions, described in Table I, were run under similar conditions in the appropriate solvent. I, when formed, was eluted after DMU with a 3:7 mixture of solvent, while dimer B, C, or D was eluted with 4:6, 1:1, or 3:7 mixtures, respectively.

Photosensitized Monomerization of Dimer A with DDQ on Silica Gel.—A solution of dimer A and DDQ (1:1 ratio) was spotted on a silica gel plate, dried, and irradiated (Mineralight lamp Model R-51) for 30 min. The product was eluted with a mixture of acetone–chloroform (2:1) and was found to be identical with DMU.

Similar results were obtained while employing dimer B, C, or D and DDQ or *p*-chloranil.

Photomonomerization of Dimer A with DDQ in the Solid State.—A powder mixture of dimer A (28 mg, 0.1 mmol) in DDQ (22.7 mg, 0.1 mmol) was irradiated (Westinghouse sun lamp, Pyrex filter, at a distance of 25 cm) for 90 hr. Work-up indicated 8% monomerization.

Similar experiments with dimer B, C, or D indicated 14, 9, and 22% monomerization, respectively. Irradiation under similar conditions in the absence of DDQ indicated 3% monomerization of dimer B or D and no monomerization of dimer A or C. Exposure of the mixture of dimer D and DDQ to sunlight afforded 55% monomerization after 4 hr. No monomerization was observed in the absence of DDQ.

Monomerization of Dimer A with Aluminum Chloride.—A solution of dimer A (28 mg, 0.1 mmol) and aluminum chloride (0.3 g, 2.26 mmol) in acetonitrile (10 ml) was left in the dark at room temperature. A maximum at 265 nm gradually appeared. Work-up after 22 hr indicated quantitative monomerization.

Similar results were obtained with dimer C.

Photomonomerization of Dimer B with Aluminum Chloride.—A solution of dimer B (28 mg, 0.1 mmol) and aluminum chloride (0.3 g, 2.26 mmol) in acetonitrile (10 ml) was irradiated externally (Hanovia 450-W lamp, Pyrex filter) under nitrogen for 3 hr. The solvent was removed under reduced pressure and the residue was treated with a saturated aqueous solution of sodium acetate and extracted with chloroform. Further work-up led to DMU (60% yield).

A similar experiment with dimer D led to 34% monomerization. No monomerization of either dimer B or D with $AlCl_3$ could be observed in the dark even after heating the mixture to 70° for 4 hr.

Quantum Yield Determination in Monomerization of Dimer A with DDQ.—A solution of dimer A (28 mg, 0.1 mmol) and DDQ (22.7 mg, 0.1 mmol) in acetonitrile (10 ml) was irradiated externally (Hanovia 200-W lamp, Pyrex filter). The intensity of the incident light was reduced by the use of a net (optical density of 0.7 was employed). The degree of monomerization was determined according to the increase in the 265-nm maximum. Actinometry measurements with ferrioxalate were taken before and after every run.[24] A quantum yield of 0.22 was observed for the monomerization of dimer A.

Similar determinations with dimers B, C, and D led to quantum yields of 0.77, 0.26, and 0.7, respectively.

Determination of the Triplet Energy of 1,3-Dimethyl-5,6-dihydrouracil.—The phosphorescence spectrum of the compound was determined on an Aminco-Bowman spectrophotofluorometer equipped with a Hg-Xe 200-W lamp and a photomultiplier (S-20, EMI 9558 QV). A solution of 1,3-dimethyl-5,6-dihydrouracil (2×10^{-2} *M*) in ethanol–methanol (1:4 mixture) was irradiated (Sovirel tube, 3 mm, λ 313 nm) at 77°K. The triplet energy was calculated according to the maximum emission at 430 nm (single maximum) and was found to be 67.5 kcal/mol.

Determination of the Triplet Energy of DDQ.—The phosphorescence spectrum of DDQ was determined on the Aminco-Bowman spectrofluorometer equipped with a photomultiplier (IP28). A solution of DDQ (10^{-3} *M*) in EPA (ethyl ether: isopentane:ethyl alcohol, 2:5:5) was irradiated (λ 295 nm) at 77°K. The triplet energy was calculated according to the maximum emission at 515 nm (single maximum) and was found to be 55.5 kcal/mol.

Determination of the Triplet Energy of 2,4,7-Trinitrofluorenone.—The determination was carried out as described above at 77°K using a 5×10^{-3} *M* solution in EPA (2:5:5) and λ 295 nm. The shortest wavelength maximum of emission was at 445 nm, corresponding to a triplet energy of 64.3 kcal/mol.

Dependence of the Rate of Monomerization on the Initial Concentration of the Dimer.—Solutions of dimer D (varying concentrations) and DDQ (22.7 mg, 0.1 mmol) in acetonitrile (10 ml) were irradiated externally (Hanovia 200-W lamp, Pyrex filter) while using a net of optical density of 0.7. The absorption at 265 nm was determined periodically. R_0, the reaction rates at $t = 0$, were obtained by plotting the amount of monomerization *vs.* time and extrapolation to $t = 0$. Experimental results are summarized in Table II.

TABLE II

PHOTOSENSITIZED MONOMERIZATION OF DIMER D WITH DDQ

Initial amount of dimer, mol $\times 10^{-5}$	R_0, mol l.$^{-1}$ sec^{-1} $\times 10^{-5}$
4	0.83
6	1.15
8	1.18
10	1.47
30	2.15
40	2.25
50	2.38

Effect of Temperature on the Rate of Photomonomerization of Dimer D with DDQ.—Solutions of the dimer (28 mg, 0.1 mmol) and DDQ (22.7 mg, 0.1 mmol) in acetonitrile (10 ml) were irradiated at different temperatures (3, 25, 36, and 59°). The amount of monomerization as well as R_0 were determined as described above. R_0 values for the various temperatures were plotted *vs.* $1/T$ (T = absolute temperature) to give a straight line (Figure 1).

Registry No.—Dimer A, 17237-77-3; dimer B, 17237-75-1; dimer C, 17237-76-2; dimer D, 17237-74-0; 1,3-dimethyl-5,6-dihydrouracil, 4874-13-9; DDQ, 84-58-2; 2,4,7-trinitrofluorenone, 129-79-3.

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[24] C. A. Parker, *Proc. Roy. Soc., Ser. A*, **220**, 104 (1953).