PHOTOSENSITIZED DIMERIZATION OF PYRIMIDINES

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In a recent paper, (Greenstock et al., 1967) UV photodimerization of a large number of thymine, uracil and orotic acid derivatives in dilute aqueous solution has been shown to be the principal photochemical reaction. The dimer yield is strongly decreased by the presence of oxygen, or of paramagnetic ions (Beukers and Berends, 1961; Sztumpf-Kulikowska et al., 1967; Greenstock et al., 1967) implicating the involvement of the triplet state. Whether or not the singlet state (also quenched by oxygen) is involved in aqueous solution as it is thought to be in the frozen state by Eisinger and Shulman (1967) is still unresolved.

Sensitized dimerization by means of triplet ⟶ triplet energy transfer is a useful technique in this study (Charlier and Hélène, 1967; Elad et al., 1967; Lamola and Yamane, 1967; von Wilucki et al., 1967). In this paper, benzophenone ($Ø_2$C=0), acetophenone (ØAc) and acetone as water soluble sensitizers have been shown to produce progressively increasing sensitized yields (Øs) of dimers in uracil, thymine and orotic acid respectively. The dimer photoproducts separated chromatographically are identical with those formed in normal photolysis both in their mobilities in various solvents and in their relative proportions, unlike the case reported for coumarins (Morrison et al., 1966) in which a different dimer is formed from the triplet state sensitization by benzophenone as compared to that produced under normal photolysis reportedly from the singlet state.

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METHODS

Unbuffered samples in 2 mm or 1 cm quartz cells were degassed by bubbling with nitrogen and irradiated at wavelengths of 313 mμ or 334 mμ using the UV monochromator and irradiation techniques described previously (Johns and Rauth, 1965; Johns et al., 1964) together with a UV cutoff filter (Corning 7-59) to eliminate any direct photolysis of the pyrimidines.

^{14}C-cytosine, thymine, uracil and orotic acid were chromatographed by ascending thin layer chromatography (TLC) using Eastman cellulose plates in (I)n-butanol: H_2O(86:14,v/v), (II)n-butanol : acetic acid: H_2O(80:12:30,v/v/v), (III)iso-propanol: H_2O(3:1,v/v), (IV)n-propanol:H_2O(7:3,v/v) and (V)0.05M tartrate: iso-propanol:ethanol (3:4:3,v/v/v) and autoradiographed on Kodak No Screen X-ray film.

The sensitizers were purified by recrystallization and their molar extinction coefficients (ϵ) were carefully determined at the wavelengths of photolysis. Because the principal chromophore C=O undergoes an n, π^* transition - the absorption maximum of which is strongly blue shifted in polar solvents such as water (Lewis and Kasha, 1944) - the ϵ's were measured in dilute aqueous solutions at the concentrations used in the sensitized experiments (Table 2).

The efficiency of sensitized dimerization can be obtained from the parameter ϕ_s which is defined by:

$$\phi_s = \frac{\text{Number of molecules of acceptor dimerized}}{\text{Number of quanta absorbed by sensitizer}}$$

The number of acceptor molecules dimerized was determined chromatographically or from changes in absorbance and the number of quanta absorbed was obtained from the values of ϵ in Table 2.

RESULTS AND DISCUSSION

Reliable values of triplet state energies are difficult to find. Most determinations have been obtained from phosphorescent spectra recorded at 77°K in glasses; these values, shown in Table 1, will be used in the discussion, but it should be understood that they may not apply strictly to aqueous solutions at room temperature.

TABLE 1. Triplet Energies Determined from Phosphorescent Spectra at 77°K. E_T refers to the highest energy band maximum and E_{00} is the extrapolated highest energy transition between the 0th vibrational levels of the triplet and ground states.

Triplet Energies	Sensitizer			Acceptor			
	($\phi_2C{=}O$)	(ϕA_c)	Acetone	(C) Cytosine	(U) Uracil	(T) Thymine	(OA) Orotic Acid
$E_T(cm^{-1})$	24,000	26,000	-	25,000	24,400	23,200	-
$E_{00}(cm^{-1})$	24,800	26,500	28,200	27,900	27,500	26,300	21,300
Ref.	Kearns and Case, 1967		Borkman and Kearns, 1966	Lamola et al., 1967			Charlier and Hélène, 1967

For efficient triplet-triplet energy transfer to occur E_T-sensitizer must be greater than E_T-acceptor and the greater this energy gap, within limits, the more efficient is the transfer (Porter and Wilkinson, 1961). Consequently, for each sensitizer, the sensitized dimerization should increase in the order of C,U,T and OA. This is indeed the case experimentally (Table 2). Cytosine, however, shows no dimerization either by sensitization or by normal photolysis. For a given acceptor molecule, sensitization should also increase in going from $\phi_2C=0$ to ϕAc to Acetone according to the above theory. This is observed for uracil but for thymine and particularly for orotic acid, benzophenone is the most efficient sensitizer. Benzophenone however is unable to sensitize uracil appreciably as expected since its triplet energy lies below that of the uracil triplet.

TABLE 2. Quantum yields of Photosensitized Dimerization (ϕ_s) (x10^3) together with the molar extinction coefficients (ϵ) of the sensitizers at 313mμ and 334mμ.

			Acceptor (10^{-3}M)		
Sensitizer	$\epsilon_{313m\mu}$	$\epsilon_{334m\mu}$	U 313mμ	T 313mμ	OA 334mμ
Acetone 0.1M	.35	.02	4.1	4.2	35
ϕAc 10^{-3}M	105	27	.39	1.6	22
$\phi_2C=05x10^{-4}M$	380	195	.02	11	110

To show that the values in Table 2 were in fact due solely to sensitized dimer formation, the dimer yield was measured in the absence of sensitizer and was found to be 1% or less than that obtained with sensitizer under the same irradiation conditions (excluding the negligible benzophenone sensitization of uracil). The quantum yields for destruction of the sensitizers alone were measured and found to be less than 10^{-5}.

TLC was used throughout this work to separate ^{14}C dimer products (Table 3). This technique has the advantage of high resolution obtainable with short development times (3 hours). Of the five solvents used (III) and (IV) were able to resolve 4 uracil dimers ($\widehat{UU}_{1-4}$) and 3 thymine dimers ($\widehat{TT}_{1-3}$).

From Table 4, it can be seen that the same dimers are formed from normal photolysis as are produced by sensitization of the triplet state. Also, except for small fluctuations, the relative yields are the same. $\widehat{UU}_1$ and $\widehat{UU}_4$ are the principal dimer products in the two cases as is $\widehat{TT}_2$. It is interesting to note that the principal dimer in thymine ($\widehat{TT}_2$) is not the principal dimer formed in ice

TABLE 3. Rf Values of Dimers in Various Solvents Using Ascending TLC With Eastman Cellulose Plates.

Dimers	Solvents I	II	III	IV	V
$\widehat{UU}_1$	.04	.10	.07	.09	
$\widehat{UU}_2$			.11	.14	
$\widehat{UU}_3$			.14	.17	
$\widehat{UU}_4$	.15	.15	.20	.24	
Uracil	.35	.47	.56	.59	
$\widehat{TT}_1$	.10	.49	.40	.46	
$\widehat{TT}_2$	.18	.55	.49	.54	
$\widehat{TT}_3$	.27	.60	.56	.61	
Thymine	.46	.68	.66	.69	
$\widehat{OO}$		.03			.34
Orotic Acid		.16			.57

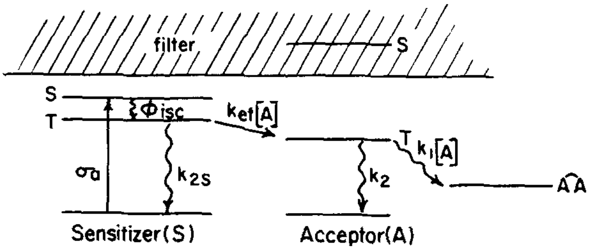
TABLE 4. A Comparison Of The Relative Sensitized Dimer Yields Measured As A % Of The Total ^{14}C Radioactivity In Each Dimer Spot, With Those Obtained From Photolysis Of Deaerated Aqueous Solutions At 265 mμ. The Dimers Formed In Frozen Solution Are Also Shown. There Is No Uracil Hydrate (U^*) Formed By Sensitization.

	Sensitizers			Photolysis of Deaerated	Photolysis of
Dimers	Acetone	Acetophenone	Benzophenone	Aqueous Solution	Frozen Solution
$\widehat{UU}_1$	4.6	6.6	.2	8.1	Secondary Dimer
$\widehat{UU}_2$	6.5	1.2	-	1.8	
$\widehat{UU}_3$	4.1	1.3	.5	3.6	
$\widehat{UU}_4$	6.8	3.1	.5	9.5	Principal Dimer
U^*		No hydrate formed		7.0	
$\widehat{TT}_1$	9.4	2.9	6.8	6.1	Principal Dimer
$\widehat{TT}_2$	21.0	6.5	17.0	11.8	Secondary Dimer
$\widehat{TT}_3$	7.0	2.9	4.5	10.0	

(Hariharan and Greenstock) suggesting that the ice dimer does not come principally from the triplet state but most likely from the singlet state as proposed by Eisinger and Shulman (1967). Further, no uracil hydrate has been detected in these experiments providing good evidence that the hydrate precursor is the excited singlet state of uracil and not the triplet, in agreement with the findings of Brown and Johns (1968).

In the case of orotic acid, only 1 dimer was isolated by TLC, so that the yield of dimer can be followed by measuring changes in absorbance. The orotic acid dimer yield was determined as a function of sensitizer and acceptor concentration to obtain the various kinetic parameters involved in the reaction.

Reaction Scheme



In this scheme, σ_a(cm^2 μmole^{-1}) is the absorption cross section for the sensitizer and K_{et}($M^{-1}sec^{-1}$)refers to the rate of triplet-triplet energy transfer. The rate of deexcitation of the sensitizer (acceptor) triplet state is K_{2s}(k_2) in sec^{-1}.

The cross section for acceptor triplet formation $\sigma_{TA} = \sigma_a \phi_{isc} \frac{K_{et}[A]}{(K_{et}[A]+K_{2s})}$

It follows that the initial rate of dimer formation is:

$$\left(\frac{d[\hat{AA}]}{dL}\right)_0 = \sigma_a \phi_{isc} [S] \frac{K_{et}[A]}{(K_{et}[A]+K_{2s})} \cdot \frac{K_1 [A]}{(K_1 [A]+K_2)}$$

$$\doteq \sigma_a \phi_{isc} [S]\left\{1+\left(\frac{K_{2s}}{K_{et}}+\frac{K_2}{K_1}\right)A^{-1}\right\}^{-1} \qquad (1)$$

The first graph shows that the initial dimer yield $\left(\frac{d[\hat{AA}]}{dL}\right)_0$ is directly proportional to [S] as predicted by equation (1) yielding $\phi_{isc} = 0.10$.

In the second graph, $\left(\frac{d[\hat{AA}]}{dL}\right)_0$ is plotted against $[A]^{-1}$. $\left(\frac{K_{2s}}{K_{et}}+\frac{K_2}{K_1}\right)$can be obtained from the slope of this line and $\sigma_a\phi_{isc}$ from the intercept to give

$(K_{2s}/K_{et} + K_2/K_1) = 10^{-4}M$; $\phi_{isc} \doteq \phi_s$ Dimer $= 0.12$

From direct photolysis of deaerated orotic acid at 280 mμ the ratio of the rates of spontaneous deexcitation and of dimer formation from the triplet state

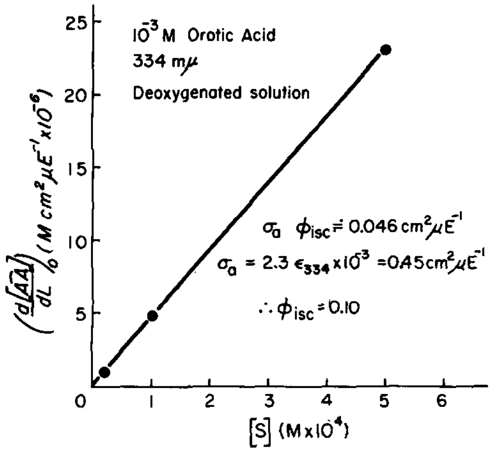


Fig. 1. Initial rate of orotic acid dimerization$(d[\widehat{AA}]/dL)_0$ as a function of benzophenone sensitizer concentration [S] following radiation at 334 mμ.

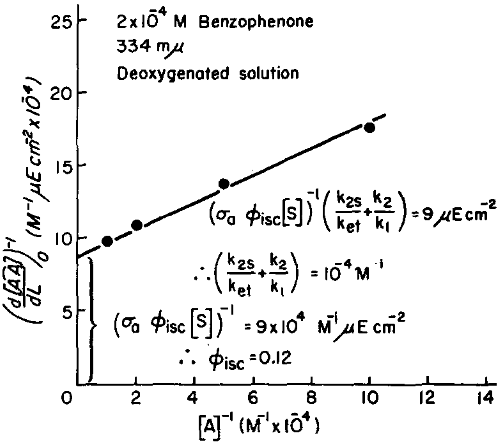


Fig. 2. A reciprocal plot of $(d[\widehat{AA}]/dL)_0^{-1}$ against $[A]^{-1}$ for orotic acid [A] using 2×10^{-4} M benzophenone sensitizer concentration, irradiating at 334 mμ.

is $K_2/K_1 = 0.9 \times 10^{-4}$M, and the quantum yield for intersystem crossing, a measure of the dimer yield $\phi_{dimer} = 0.06$.

Providing that the rate of energy transfer K_{et} is diffusion controlled, triple triplet energy transfer provides a convenient method of efficiently populating the acceptor triplet state, effectively increasing the dimer yield, by virtue of the efficient ϕ_{isc} of the sensitizer. In addition, there is no competing photoreversal of dimers at these long wavelengths, nor hydrate formation. The orotic acid dimer quantum yield is increased from 0.06 for photolysis at 280 mμ to 0.11 using benzophenone as sensitizer and a similar increase is evident for thymine.

The apparent ability of benzophenone to sensitize uracil to a small extent, even though $E_{T sens.} < E_{T acc.}$ may indicate the presence of energy transfer from vibrationally excited triplet states (hot bands) of the sensitizer. Acetone, acetophenone and benzophenone all produced 4 uracil dimers and 3 thymine dimers in similar proportions to those formed under normal photolysis providing strong support for the belief that the triplet state is their principal precursor in aqueous solution. The agreement obtained for k_2/k_1 in orotic acid, whether the triplet state is populated by intersystem crossing from the orotic acid singlet state or by energy transfer from the sensitizer triplet state, also supports this observation. The fact that the thymine ice dimer is not the principal dimer in aqueous solution suggests it comes from the singlet state.

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REFERENCES

Beukers, R. and Berends, W. Biochim. Biophys. Acta, 49, 181 (1961)
Brown, I.H. and Johns, H.E. Photochem. and Photobiol., In press.
Borkman, R.F. and Kearns, D.R. J. Chem. Phys. 44, 945 (1966).
Charlier, M. and Hélène, C. Photochem. and Photobiol. 6, 501 (1967).
Eisinger, J. and Shulman, R.G. Proc. Nat. Acad. Sci. (U.S.) 58, 895 (1967).
Elad, D., Krüger, C. and Schmidt, G.M.J. Photochem. and Photobiol. 6, 495 (1967).
Greenstock, C.L., Brown, I.H., Hunt, J.W. and Johns, H.E. Biochem. Biophys. Res. Comm. 27, 431 (1967).
Hariharan, P.V.P. and Greenstock, C.L. Unpublished results.
Johns, H.E., Pearson, M.L., LeBlanc, J.C. and Helleiner, C.W. J. Mol. Biol. 9,503 (1964)
Johns, H.E. and Rauth, A.M. Photochem. and Photobiol. 4, 673 (1965).
Kearns, D.R. and Case, W.A. J. Am. Chem. Soc. 88, 5087 (1966)
Lamola, A.A., Guèron, M., Yamane, T., Eisinger, J. and Shulman, R.G. J. Chem. Phys. 47, 2210 (1967).
Lewis, G.N. and Kasha, M. J. Am. Chem. Soc. 66, 2100 (1944).
Morrison, H., Curtis, M. and McDowell, T. J. Am. Chem. Soc. 88, 5415 (1966).
Porter, G. and Wilkinson, F. Proc. Roy. Soc. A264, 1, (1961).
Sztumpf-Kulikowska, E., Shugar, D. and Boag, J.W. Photochem. and Photobiol. 6, 41 (1967).
von Wilucki, I., Matthaus, M. and Krauch, C.M. Photochem. and Photobiol. 6, 497 (1967).