DETECTION OF THE TRIPLET STATE IN OROTIC ACID BY FLASH PHOTOLYSIS

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A good deal of indirect evidence has recently been obtained suggesting that when orotic acid (and other pyrimidines) are irradiated in solution with ultraviolet (UV) light dimerization occurs via the interaction of a molecule in a triplet state with a ground state molecule. We have now detected this triplet state by flash photolysis. The triplet state has peak absorption at 320 nm and decays in dilute solutions in the absence of oxygen with a mean life[+] of 79 μsec.

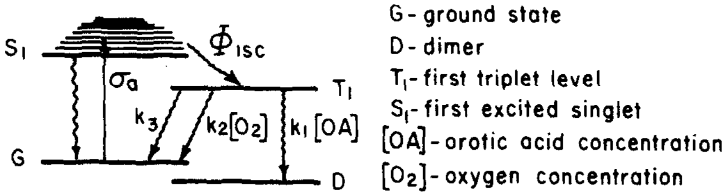

Fig. 1. Schematic diagram for the reaction of the triplet state in orotic acid.

Sztumpf-Kulikowska, Shugar and Boag (1967) suggested a model for pyrimidine dimerization as illustrated in Fig. 1. In this figure, k_1 is the rate constant in liters moles^{-1} sec^{-1} for the reaction of a triplet state molecule with a ground state one to form a dimer, k_2 is the rate constant in the same units for deexcitation by oxygen and k_3 is the rate constant in sec^{-1} for radiationless relaxation to the ground state. Recent work in our laboratory, Greenstock, Brown, Hunt, Johns (1967); Greenstock and Johns (1968); Brown and Johns (1968); Whillans and Johns (1968); has

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[+] Mean life = 1.44 times the half life.

amplified this scheme and extended it to uracil. According to this scheme, a fraction f of the triplet states leads to dimers, where f is given by:

$$f = \frac{k_1 [OA]}{k_1 [OA] + k_2 [O_2] + k_3} \qquad (1)$$

By studying the variation of f, both as a function of oxygen concentration, and as a function of orotic acid concentration, Whillans and Johns (1968) were able to determine the ratio of the rate constants and the results are given in Table I. They were, however, unable to determine the absolute values for k_1, k_2, and k_3.

Table I. Ratio of rate constants as determined by Whillans and Johns (1968) at pH5.

k_3/k_1	2.0×10^{-5}
k_2/k_1	1.2

The mean life time τ of the triplet state is:

$$\tau = \frac{1}{k_1 [OA] + k_2 [O_2] + k_3} \qquad (2)$$

At low enough concentrations of orotic acid and O_2, no quenching takes place and the maximum lifetime τ_o is obtained. This is given by

$$\tau_o = \frac{1}{k_3} \qquad (3)$$

Combining equations (2) and (3) we obtain

$$\tau = \left\{ \frac{1}{\frac{k_1}{k_3} [OA] + \frac{k_2}{k_3} [O_2] + 1} \right\} \tau_o = R \, \tau_o \qquad (4)$$

where R is the factor by which the triplet state lifetime is shortened by quenching by orotic acid and by oxygen.

By flash photolysis a number of transient species in orotic acid have been observed and one of these has a lifetime which depends upon $[OA]$ and $[O_2]$ in the manner predicted by equation (4) using the values for k_1/k_3 and k_2/k_3 given in Table I. This transient species has a maximum lifetime τ_o = 79 μsec in H_2O at pH5 and is the precursor state giving rise to the dimer. From the properties of this state, as discussed by Whillans and Johns (1968), we conclude that it is the triplet state.

METHODS

Orotic acid was irradiated in a cylindrical jacketed cell, 3 cm in diameter, and

15 cm long. The flash was produced by the discharge of a 0.75 μF condenser charged to 20 kv. The transient species was detected by passing light from an Osram XBO-150 w/l Xenon lamp lengthwise through the cell into a Spex monochromator. The radiation was detected by a Hamamatsu HTV-R136 photomultiplier. The transient was observed by photographing the trace on a Hewlett-Packard 180 A oscilloscope. The solutions of orotic acid(Cyclo Chemical Grade I) were freshly mixed using triply distilled H_2O for each experiment and were degassed by bubbling with extra high purity N_2 for 40 min. The flash had a half width of 3 μsec at an energy of 150 J and more than 90% of the light was emitted before 20 μsec had elapsed. Typical traces are shown in Fig. 2.

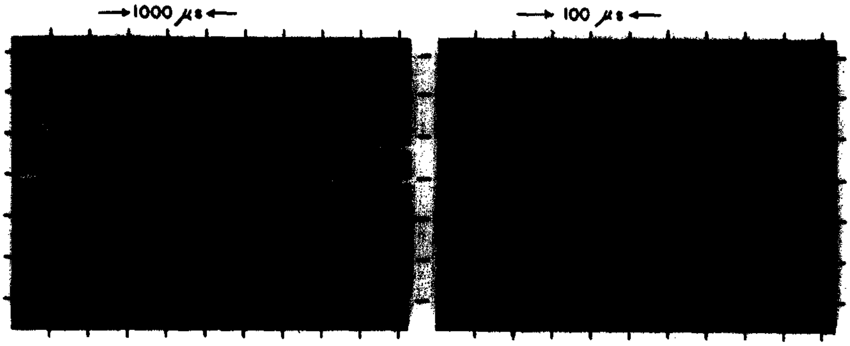

Fig. 2. Typical flash photolysis traces for a transient observed at 320 nm. Trace (a) shows the transient over a long time interval; Trace (b) over a short interval. Orotic acid at 4.8×10^{-6} Molar in water, pH5. (AC gain = 0.5 v/cm, DC gain = 0.05v/cm).

RESULTS

From Fig. 2 it is clear that one is observing a short lived transient superimposed on a long lived one. Trace 2(b) is shown schematically in Fig.3(a). In this set of traces, S is the scattered light from the flash observed with the analysing light off, B is the base line, B' is the base line after subtracting the long-lived transient, T_o is the steady light through the cell before the flash at a sensitivity 0.1 that of the signal traces, and X is the observed transient. The absorbance A is given by the equation shown in Fig. 3(a). In this expression S is negative so (B-S) is positive. The absorbance is plotted on a log scale versus time in Fig. 3(b), and it decreases exponentially with time.

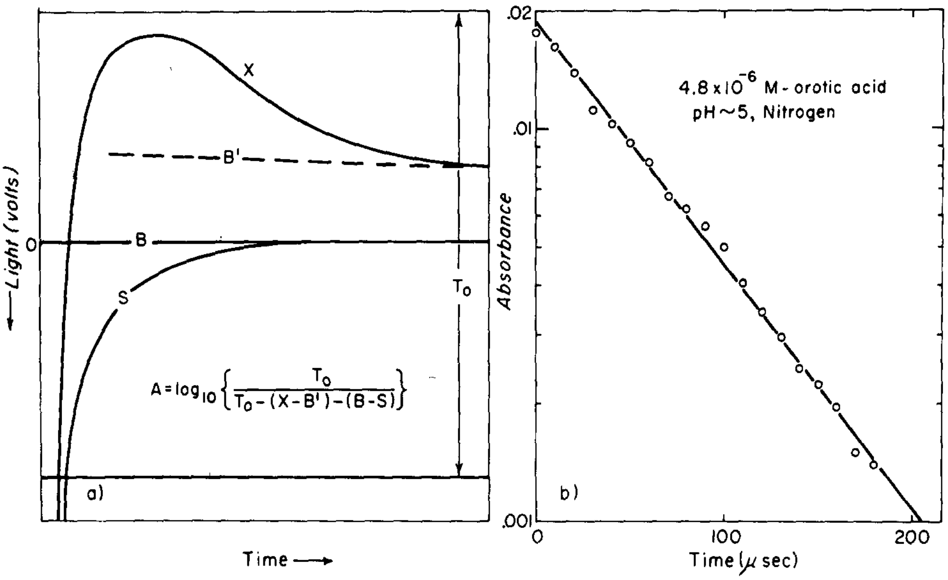

Fig. 3. (a) Schematic diagram taken from an actual trace such as shown in Fig. 2(b). (b) Plot of the absorbance of the short lived species as a function of time.

The transient has its peak absorption at 320 nm and extends from about 290 to 400 nm. It is broad with a half width of about 50 nm. The transient is quenched by oxygen, but not by N_2O or acidic pH's and its lifetime decreases as the orotic acid concentration increases. At very low concentrations of orotic acid ($\sim 10^{-6}$ M) its lifetime approaches a constant value of about 70 μsec. Since N_2O or acid does not quench it, this transient is not the one formed by e_{aq}^- attack which is expected to absorb at this wavelength(Theard et al 1968).The reaction of this transient species both with oxygen and with orotic acid strongly suggests that it is the triplet state which is responsible for the formation of dimers.

Table 2. Calculation of τ_0 for different concentrations of orotic acid and oxygen

Orotic Acid Concentration	Fraction R	Measured τ (μs)	Calculated τ_0 (μs)
5.3×10^{-5} M	.28	21	77
2.1×10^{-5} M	.49	37	76
1.1×10^{-5} M	.64	52	80
1.1×10^{-5} M	.64	54	83
4.8×10^{-6} M	.81	65	80
4.8×10^{-6} M	.81	61	75
2.5×10^{-6} M	.89	67	78

Average $\tau_0 = 79 \pm 4$ μs

Orotic Acid Concentration = 1.1×10^{-5} M

Oxygen Concentration	Fraction R	Measured τ (μs)	Calculated τ_0 (μs)
1.3×10^{-3} M	0.14	Too short to measure	
1.3×10^{-5} M	0.45	34	76
6.5×10^{-6} M	0.54	39	72
1.3×10^{-6} M	0.65	50	75
N_2	0.66	54	81

A critical test of this conclusion is shown in Table 2. The lifetime of the transients was measured for different concentrations of orotic acid under $[O_2] = 0$ and τ_0 was calculated using equation (4), and the values for the rate constants given in Table I. The fact that τ_0 calculated in this way is constant shows that the lifetime obeys equation (4) and that the observed species is the one responsible for dimerization.

In a similar way, experiments were carried out by varying the O_2 concentration. The observed and calculated lifetimes are given at the bottom of Table 2, and are in good agreement.

Using this value of $\tau_0 = \frac{1}{k_3}$ it is now possible to use the rate constant ratios in Table I to obtain absolute values for the reaction of the orotic acid triplet state with orotic acid and with oxygen. These for pH 5 are as follows:

Table 3. Rate Constants for Reactions of Orotic Acid Triplet State at pH 5

k_1 M^{-1} sec^{-1}	6.5×10^8
k_2 M^{-1} sec^{-1}	7.8×10^8
k_3 sec^{-1}	1.3×10^4

The rate constant, k_2, for the quenching of the orotic acid triplet by oxygen is 7.8×10^8 at pH 5 which is in good agreement with recent values in other systems (Porter, 1954). The extinction coefficient of the triplet absorption, the effects of pH and the properties of the other transients are now being investigated.

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