# PHOTOREVERSIBLE PHOTOPRODUCT OF THYMINE[1]

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A deamination product of cytosine-thymine adduct ($P_2B$ or PO-T) from acid hydrolysates of DNA irradiated with ultraviolet light has recently been identified (Wang and Varghese, 1968; Varghese and Wang, 1967). More recently, thymine-thymine adduct has been characterized as a photoproduct of thymine (Varghese and Wang, 1968). Together, these findings have brought recognition of a new type of photoreaction common to some pyrimidine bases. This new reaction may be of more biological importance than cyclobutyl dimerization (Wang, 1960, 1961, 1964; Beukers and Berends, 1960, 1961) and hydration (Sinsheimer and Hastings, 1949; Moore and Thomson, 1955; Wang, 1956) in the understanding of the photochemistry of nucleic acids (reviewed: Gunther and Prusoff, 1967a; Wang, 1965; Grossman _et al_., 1965; McLaren and Shugar, 1964; Smith, 1964; Wacker, 1963). The purpose of this communication is to describe the isolation and properties of a photoproduct of thymine which has the characteristics of the adducts, the photoreversibility of the cyclobutyl dimers and the instability of the hydration products.

Ultraviolet irradiation of 2-$C^{14}$ or 5-methyl-$H^3$-thymine in frozen aqueous solution produces two detectable photoproducts (Smith, 1963; Wang _et al_., 1967; Gunther and Prusoff,

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1967b). The photoproduct with $R_f$ = 0.15 in solvent A (n-butanol-acetic acid-water: 80/12/30, v/v/v) is designated $PT_1$ and the other, with $R_f$ = 0.29, as $PT_2$ (Wang et al., 1967). Contrary to the general belief that $PT_2$ is cis-syn thymine dimer (T=T), it has recently been shown to be a mixture of cis-syn T=T and T-T adduct (Varghese and Wang, 1968).

Aliquots of 10 ml of 3 mM solution of twice recrystallized thymine were placed in petri dishes (diam 9 cm), frozen over dry ice and irradiated for one hour with a germicidal lamp at a distance of 40 cm. The irradiated solution was thawed, lyophilized, redissolved in water, applied on Whatman No. 3 papers and developed in solvent A. From the chromatograms, strips between $R_f$ 0.1 - 0.2 were cut out and extracted with water; the combined extracts were lyophilized. The material was then rechromatographed, the desired product extracted as before and crystallized from water. In radioactive labeled study, 0.1 ml aliquots of the T-2-$C^{14}$ solution (10 μc/μl; 0.3 mg/ml) were frozen and irradiated. The irradiated solution was thawed, applied on Whatman No. 3 paper strips and developed with the same solvent system. The dried chromatograms were analyzed for distribution of radioactivity with a Vanguard Autoscanner 880. The peak areas of radioactivity were cut out and eluted with water for quantitation, (Wang et al., 1967).

Unlike T=T, $PT_1$ is not stable in acid or alkaline solutions as shown by the gradual changes in the UV absorption spectra, depending on the pH of the solution.

An aqueous solution of $PT_1$ shows two UV absorption maxima after UV-irradiation (Fig. 1). Equimolar proportions of T and T-T adduct, not T=T, have been isolated from the irradiated solution. These products have been characterized by UV, IR, NMR spectra and chromatographic mobilities in several solvent systems. T-T adduct has been further characterized by isolating its dehydration product (Varghese and Wang, 1968).

On boiling, an aqueous solution of $PT_1$ becomes pink in color. The resulting solution has two absorbancy maxima (265 mμ

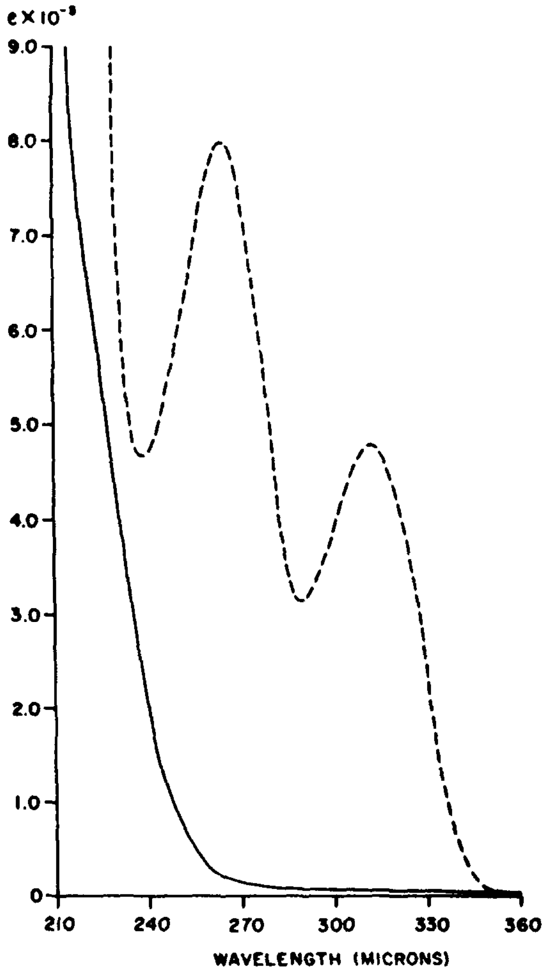

FIG. 1

UV spectra of PT$_1$ in water (———) and after irradiation (----)

and 316 mμ), characteristic of T and T-T adduct, respectively.

In 2 N HCl or trifluoroacetic acid at 25°, a product having $\lambda_{max}$ at 285 mμ (Fig. 2) is formed from PT$_1$. This product behaves in the same manner as PT$_1$ on re-irradiation with 2537 A light, forming T and T-T adduct but not T=T (Fig. 2). On refluxing the acid solution for 15 minutes, a major compound having $\lambda_{max}$ at 268 mμ is obtained among other products. The characteristics of this compound, together with the IR (Fig. 3), mass and NMR spectra of PT$_1$, have been used for the structural elucidation of $PT_1$, apparently a trimer of thymine (in preparation).

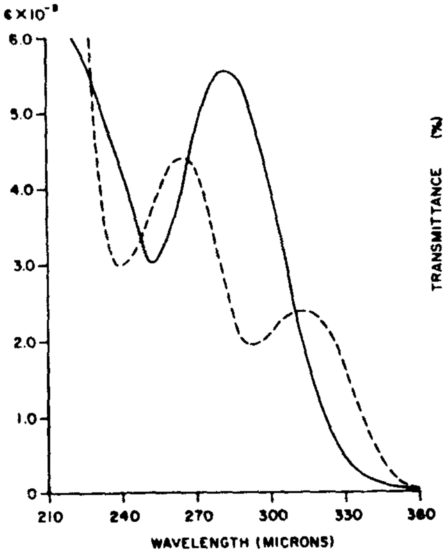

Fig. 2

Ultraviolet spectra of $PT_1$ in acid (———) and after irradiation (----)

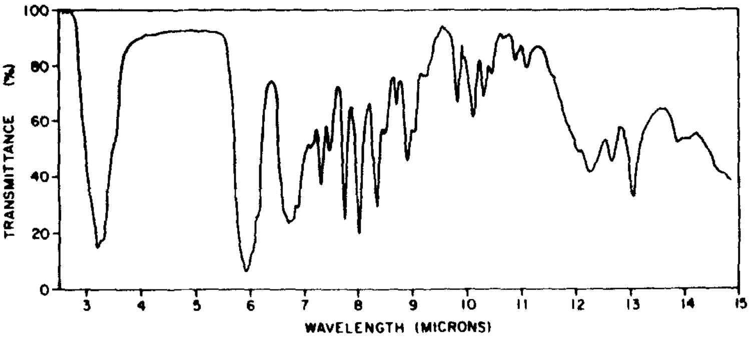

Fig. 3

Infrared spectrum of $PT_1$ in potassium bromide pellet

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In 1 N $NH_4OH$, the UV absorption of $PT_1$ changes gradually to one with $\lambda_{max}$ at 244 mμ. This reaction yields three detectable products, with $R_f$ values of 0.15 (main product), 0.29, and 0.60 (least product), in solvent A. The second product not only has the same $R_f$ value (0.29) but also reverses to thymine with 2537 A light in aqueous solution as *cis-syn* T=T.

Thus, $PT_1$ is an interesting and intriguing intermediate, which can be converted under different conditions to T, T-T adduct and possibly T=T. These reactions may be summarized as follows:

$$PT_1 \xrightarrow[H_2O]{h\nu} \text{T-T adduct + T}$$

$$PT_1 \xrightarrow[\text{room temp.}]{\text{2 N HCl or TFA}} \lambda_{max}\ 285\ \text{m}\mu \xrightarrow[HOH]{h\nu} \text{T-T adduct + T}$$

$$PT_1 \xrightarrow{1\text{ N }NH_4OH} \text{T=T} + 2\text{ other products}$$

$$PT_1 \xrightarrow[\text{refluxing}]{2\text{ N HCl}} \lambda_{max}\ 267\text{ m}\mu\text{ product} + \text{T} + \text{T-T adduct}$$

The characterization of T-T adduct (Varghese and Wang, 1968) is necessary for the understanding of $PT_1$, but it also points out the importance of photoproducts other than cyclobutyl dimers in UV irradiated DNA. Since these products are all interrelated, the following table lists some of their properties for comparison:

TABLE 1. SOME PROPERTIES OF THYMINE PHOTOPRODUCTS

| | T=T | T-T Adduct | $PT_1$ |
|---|---|---|---|
| $R_f$ values: | | | |
| A | 0.29 | 0.29 | 0.15 |
| B* | 0.15 | 0.13 | 0.05 |
| C* | 0.37 | 0.40 | 0.28 |
| UV spectrum | end absorption | $\lambda_{max}$ 316 mμ | end absorption |
| Irradiation (254 mμ) | T | no change | T-T adduct, T |
| Acid (TFA, 2 N HCl) | stable | dehydration | T, others |
| Base (1 N $NH_4OH$) | stable | stable | T=T, others |

*B: n-butanol-water (86/14); C: t-butanol-methyl ethyl ketone-water-formic acid (40/30/15/15).

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Whether the formation of a photoproduct such as $PT_1$ is a common photochemical reaction of certain pyrimidines is currently under study. However, the isolation and characterization of $PT_1$ cautions us not to draw conclusions based solely on kinetics or mechanistics or chromatographic mobilities of labelled compounds of pyrimidine bases.

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