Short Communications

SC 2249

On 1-methylthymine photoproduct*

Ultraviolet irradiation of a frozen solution of thymine produces thymine photoproduct[1]. When the solution is thawed the latter product reverts to thymine upon further ultraviolet irradiation[2]. The photoproduct has been shown to be a dimer with a cyclobutane ring formed by addition of 5,6 double bonds of the two thymines[3-6]. Uracil and N-substituted uracils and thymines behave similarly[1,5-10]. It is probable that formation of thymine photodimer is favored when thymine residues are close together in a suitable configuration. Thus the dinucleotide TpT readily forms the photoproduct in dilute solution[4,9-12]. The dimer is also formed, presumably mainly from adjacent thymines in the polynucleotide chain, upon irradiation of DNA in solution[10,13-16]. WANG proposes that microcrystalline aggregates of thymines form when thymine solutions are frozen and that in these crystals the thymines are favorably oriented for photodimerization. He was also able to prepare thymine dimers by irradiating thin films of thymine[5].

The photostationary state depends on wavelength. Formation of the dimer is favored by long wavelengths where only thymine absorbs; dissociation is favored at around 230 mμ where the absorption coefficients of the dimer are comparable to those of the monomer.

I wish to present evidence which supports the hypothesis that 2 thymine molecules held in a favorable configuration readily undergo the photochemical formation of the dimer. These fragmentary observations were made in the course of a study of the single crystal absorption spectra of purines and pyrimidines[17]. While measuring transmissions with light polarized perpendicular to the *b*-axis in the (102)-plane (the plane of the molecular layers[18]) of a 1-methylthymine single crystal, which was 0.1 μ thick, considerable bleaching took place. (The absorption coefficient for the first absorption band of the crystal perpendicular to the *b*-axis in (102) is about 10 times the absorption coefficient parallel to the *b*-axis in (102).) At the end of the experiment the birefringence of the crystal had fallen by about 30 % and its light transmission at 280 mμ had increased by 75 %.

An experiment to demonstrate that this bleaching was due to the formation of thymine photodimer was done as follows. A preparation of thin plates of 1-methylthymine was obtained by rapid evaporation of a hot solution spread out on the bottom of a Petri dish. The predominant form is (102), parallel to the molecular layers, and the thickness of the crystals ranged from 0.7–20 μ. The absorption maximum for the crystal is 273 mμ, the same as for solution. By irradiating the crystals mostly to the red of 270 mμ, a region where the dimer absorption coefficient is small relative to the monomer, a favorable photosteady state with major production of the photoproduct should be established. By employing a continuous source one is assured

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* Contribution No. 2621 from the Department of Chemistry of the California Institute of Technology.

of achieving appreciable photodamage in crystals the thicknesses of which vary from 1–20 μ. These conditions were approximately met by use of an Osram 500-W high-pressure xenon arc, for which the spectral intensity falls off markedly for wavelengths below 260 mμ. The crystals were irradiated at a solid angle of light gathered of 0.004 steradian (intensity about 10^{13} quanta·cm^{-2}·sec^{-1} in the interval $260\ \mathrm{m}\mu < \lambda < 300\ \mathrm{m}\mu$) for 16 h. The crystals were weighed, dissolved, and the ultraviolet absorption spectrum recorded. The solution was next irradiated for 15 min with a 4-W G.E. germicidal lamp at 5 cm. It is known that this irradiation will dissociate all of the photodimer in a dilute solution. The increase in the first absorption band was 78 %. This latter spectrum corresponded to the expected absorption of 1-methylthymine for the weighed sample of irradiated crystals. In view of all the work done previously[1–12], the above results show with high probability that about 44 % (78/178) of the 1-methylthymine was converted to 1-methylthymine photodimer by irradiation of the crystals and dissociated back to 1-methylthymine by irradiation of the solution. Crude calculations indicate that the crystals were not exposed to sufficient radiation to reach a photostationary state.

Polarized absorption measurements in (010) (the plane of the molecular layers) of a hydrogen-bonded complex of 1-methylthymine to 9-methyladenine[18,19] did not produce any detectable bleaching and presumably no 1-methylthymine photodimer was formed.

The 4 molecules per unit cell of the 1-methylthymine crystal structure are shown in Fig. 1. The 5,6 double bonds are indicated with double lines; the molecules in bold type (1 and 2) are in a plane 3.38 Å above the molecules in light type (3 and 4). The structure strongly suggests that Molecules 1 and 3 and Molecules 2 and 4 can form dimers upon ultraviolet irradiation. The center to center distance of the respective 5,6 double bonds is 3.7 Å. It can be seen that the dimers thus formed are a *d,l* pair and one expects a racemic mixture for the photoproduct. The dimer is

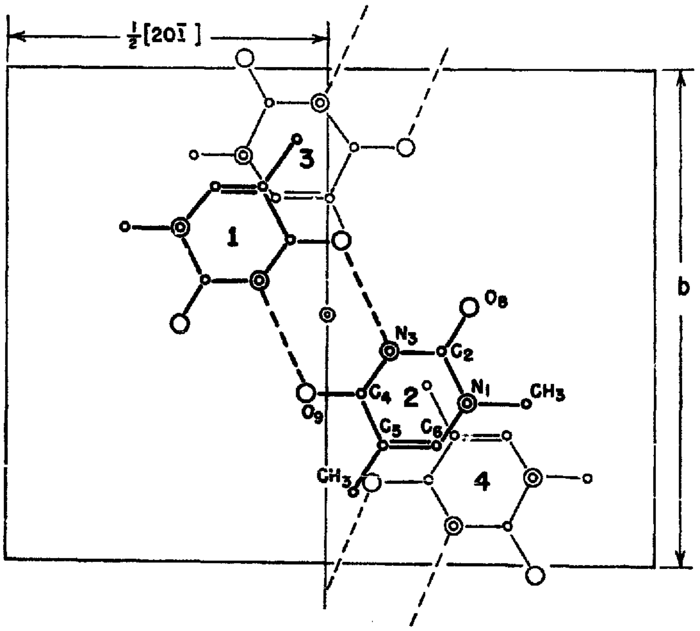

Fig. 1. Crystal structure of 1-methylthymine showing the 4 molecules per unit cell[12]. All molecules are coplanar to (102), the plane of the paper. Molecules in light type (3 and 4) are in a plane 3.38 Å below the molecules in bold type (1 and 2). The dimer from 1 and 3 is the mirror image of the dimer from 2 and 4.

a *trans*,5–5′,6–6′ type, which corresponds to Isomer II of the four possible isomers of thymine dimer reported by WULFF AND FRAENKEL[6].

Methylation of the N-3 positions of the photodimer obtained here would lead to an *N*,*N*′-dimethylthymine photoproduct which could then be compared to the two photoproducts isolated by WULFF AND FRAENKEL[6]. Such a comparison would help to identify some of the four possible isomers[6].

In the hydrogen-bonded complex of 1-methylthymine and 9-methyladenine, the thymine molecules are also stacked over one another but in this structure the 5,6 double bonds of adjacent 1-methylthymines are separated by 4.8 Å. Thus it was to be expected that no dimer would be formed by ultraviolet irradiation.

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Department of Chemistry, California Institute of Technology, ROBERT F. STEWART*
Pasadena, Calif. (U.S.A.)

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* Predoctoral Fellow of the U.S. Public Health Service. Present address: Department of Chemistry, University of Washington, Seattle, Wash. (U.S.A.).

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Equivalent pore radius in the frog gastric mucosa

The permeability of the frog gastric mucosa to water and potassium has been previously studied[1]. Two barriers, arranged in series, were proposed to explain the action of histamine. A first barrier, insensitive to histamine, was apparent in the water-diffusion experiments, and a second, affected by addition of histamine, in the water filtration and in the potassium diffusion experiments.

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