CYTOSINE-THYMINE ADDITION PRODUCT FROM DNA IRRADIATED WITH ULTRAVIOLET LIGHT[1]

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A thymine derived product, P_2B, observed in the acid hydrolysates of DNA irradiated with ultraviolet light has been reported earlier (Varghese and Wang, 1967a). Its absorbancy (O.D.) maxima[3] are at 316 (E=5,380), 316 (E=5,380), and 304 (E=7,000) mμ in neutral, pH 2 and pH 11 aqueous solutions, respectively. The UV spectrum of P_2B converts to one similar to that of thymine when P_2B is irradiated with 360 or 313 mμ light. The quantity of P_2B in acid hydrolysates of UV irradiated DNA was reduced if the irradiated DNA was photoreactivated with the yeast enzyme prior to hydrolysis (Herriott and Wang, 1967). P_2B has been isolated from DNA irradiated with doses of UV light (ca. 5×10^4 ergs/mm^2) comparable to that employed in biological studies. The IR, UV, NMR and mass spectra suggest that 6-4'-[pyrimidin-2'-one]-thymine (PO-T) is the probable structure of P_2B. The pyrimidone (PO) moiety could be derived either from the demethylation of thymine or deamination of cytosine. Our evidence indicates that PO-T is formed from an intermediate, consisting of cytosine and thymine, whose formation involves a new type of photoreaction.

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[3]The absorbancy maximum for P_2B was reported as 312 mμ and should be corrected.

Isolation

The conditions of irradiation and hydrolysis were the same as those used for the isolation of cis-syn thymine homodimer (T=T) from UV irradiated DNA (Varghese and Wang, 1967b). From the dried chromatograms developed with n-butanol-acetic acid-water (80/12/30), strips (R_f 0.24-0.36) were cut out and were extracted thoroughly with water. The combined extract was concentrated, applied on a 5 x 45-cm column of Dowex 50W-X12 (H^+, 100-200 mesh), and was eluted with water. Fractions of 50 ml each were collected and the fractions (55-65) having O.D. maximum at 316 mμ were combined and evaporated to dryness. The residue was dissolved in water and its volume was reduced to 3 ml using a stream of nitrogen. After refrigeration overnight, needlelike crystals were formed. They were collected by suction filtration and dried in vacuo over P_2O_5. From 5 g of calf thymus DNA, 4.5 mg of the P_2B (m.p. > 300°) was obtained.

P_2B isolated in similar yields from perchloric acid hydrolysates is identical to that from trifluoroacetic acid hydrolysates as shown by the IR spectra (Fig. 1). Formic acid hydrolysis, however, gave unsatisfactory results because P_2B underwent further degradation.

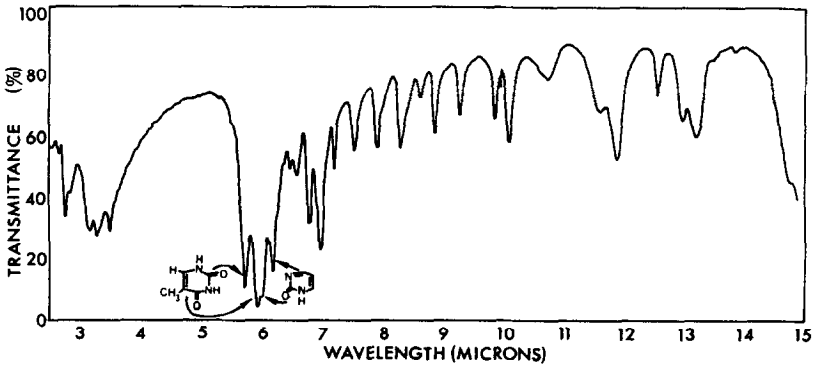

Fig. 1 Infrared spectrum of P_2B in potassium bromide pellet

Structure

P_2B possesses the characteristic absorption in the ultraviolet (Fig. 2) for the chromophore of PO and its derivatives

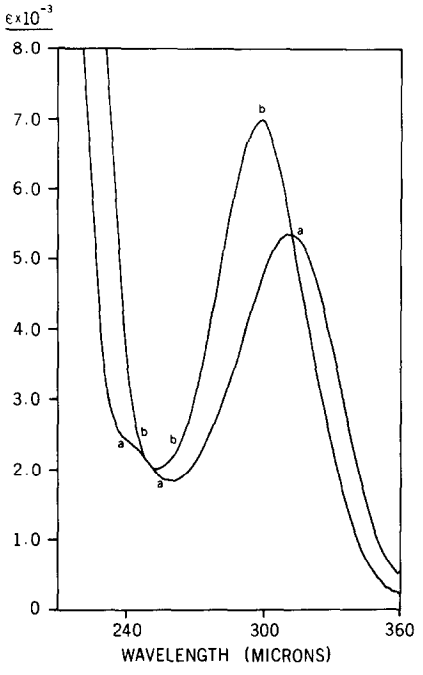

Fig. 2 Ultraviolet spectra of P_2B (a) in water and in N/10 HCl and (b) in N/10 NaOH

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which have O.D. maxima above 300 mμ and little absorption between 230-300 mμ (Brown *et al.*, 1955; Laland and Hanssen, 1964). Based on this, P_2B may have a PO moiety in which C_4 is linked to a C or H atom.

Nuclear magnetic resonance spectrum of P_2B in $(CD_3)_2SO$ at 100 Mc/sec (Fig. 3) shows the following signals: a strong singlet at δ 1.68 (3H) indicates the presence of a vinyl-CH_3 with no proton on the β-carbon atom. Two other protons at δ 6.46 (1H, doublet) and δ 8.06 (1H, doublet) with J=3 cps are due to the group -CH=CH- as observed in uracil and cytosine. A broad peak at δ 11.2 (ca 2H, singlet) is from the protons of NH groups. Thus, the data agree with the structure I for P_2B.

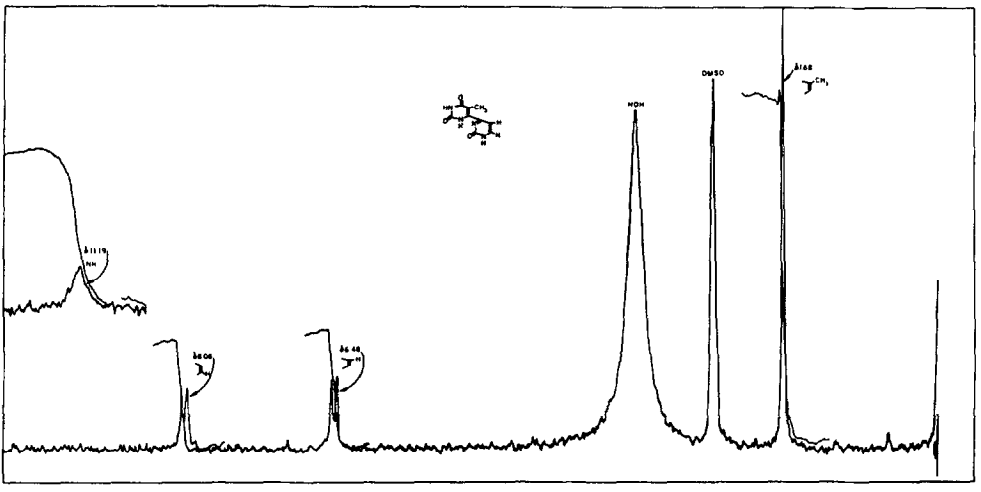


Fig. 3 NMR spectrum of P_2B in $(CD_3)_2SO$ at 100 Mc

The mass spectrum (Fig. 4) further supports the proposed structure. The strong molecular ion peak at 220 corresponds to the expected molecular weight of $C_9H_8N_4O_3$. Fragmentation pattern is perfectly analogous to that known for 5,6-disubstituted uracils (Rice *et al.*, 1965). In addition, the fragment ion of M/e 95 confirms the presence of PO nucleus from which the thymine moiety is cleaved.

The absorption bands at 3587 cm^{-1} and 1208 cm^{-1} in the IR spectrum indicates the presence of an aromatic OH group. Also, the peak at m/e 203 (M-17) and the metastable ion at m/e 187.3 in the mass spectrum supports the loss of an OH group from an aromatic nucleus. Furthermore, the NMR spectrum accounts for only two NH groups. Therefore, it is probable that the lactam group of PO exists as a lactim (Ia).

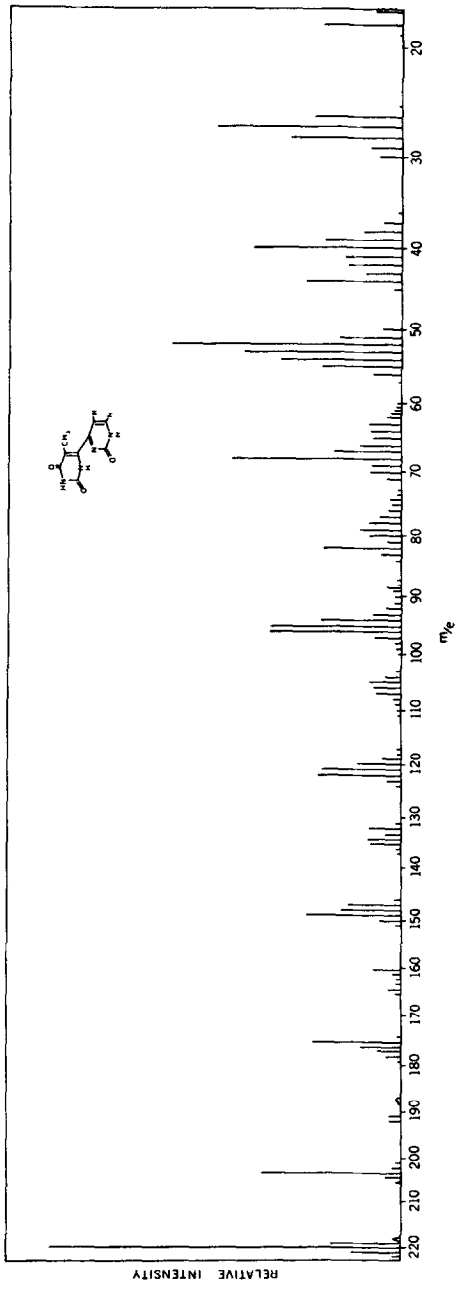
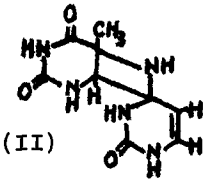


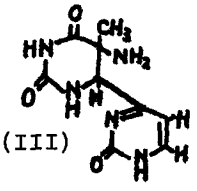
Fig. 4 Mass spectrum of P_2B at 70 e.v.*

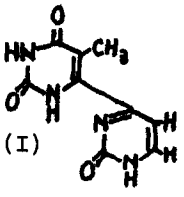
*It was determined by Morgan Schaffer Corp., Quebec, Canada.

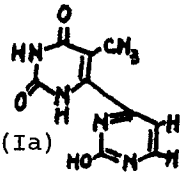
Mechanism

While the above evidence suggests that P_2B probably is 6-4'-[pyrimidin-2'-one]-thymine, the mechanism for its formation is not immediately obvious. However, our studies of the photochemistry of thymine (Varghese and Wang, 1967c) suggest the following reaction sequence of its formation. Ultraviolet irra-


(II)


(III)


(I)


(Ia)

diation of DNA brings about the formation of the azetidine derivative (II) from thymine and cytosine. Considering the Watson-Crick structure of DNA, this reaction is stereochemically more favored than T=T formation (Varghese and Wang, 1967c). Presumably, II is unstable and rearranges to a thymine adduct (III). Upon acid hydrolysis, PO-T (I) is formed by the elimination of NH_3. Although a report of the photochemical formation of azetidine has not been made previously, the formation of the analogous oxetane is known (Rabinovich and Schmidt, 1967). Furthermore, we have isolated the photoproducts analogous to III and PO-T from a similar study with thymine (Varghese and Wang, 1967c).

Importance in Photobiology

At present, there is no definite proof to show that T=T is formed directly in UV irradiated DNA. On the other hand, an increase in O.D. at 316 mμ has been observed when DNA is irradiated with UV light (Wang, 1962; Setlow, 1963; Varghese and Wang, 1967). With the knowledge of the structures and hence molar extinction coefficients of PO-T and analogous compounds, we realize that the increase in O.D. at 316 mμ upon irradiation of DNA with UV can account for all the "biological UV damage" as estimated by various laboratories (Wacker, 1963; Smith, 1964; Setlow, 1966). Thus, it can be said that the increase in O.D. at 316 mμ represents one if not the only major photochemical change in DNA as a result of UV irradiation. It may be noted that the formation of an

intermediate such as the azetidine derivative requires the photo-tautomerization of cytosine moiety which is largely inhibited at low pH (Wang, 1959) and explains the observation that the photo-reactivable UV lesions are not detected when DNA is irradiated at pH 3 (Rupert, 1964). Also, the reaction mechanism discussed above can explain some of the biochemical and chemical effects of UV irradiated cytidylic acids (Ono et al., 1965; Johns et al., 1965).

Acknowledgments

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