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An Isomer of the Cyclobutane-Type Thymine Dimer Produced in the Presence of Adenine.*

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There are four possible structural isomers of the cyclobutane-type thymine dimer originally described by Beukers and Berends (1961). Two of these forms are optically active thus yielding a total of six forms of the dimer (Figure 1). According to the Watson and Crick structure for DNA the Type I dimer would be expected to arise from the photo-dimerization of adjacent thymine rings in one strand of deoxyribonucleic acid (Wulff and Fraenkel, 1961). Other isomers of the dimer might be formed in intermolecular and intramolecular interstrand crosslinks (Wulff and Fraenkel, 1961).

Wulff and Fraenkel (1961) provided evidence for the existance of isomeric forms of dimers when they isolated, by chromatography, two different dimethylthymine dimers from irradiated 1,3-dimethylthymine. Two different dimers of thymine have been produced in irradiated thymidylyl-thymidine (TpT) (Johns et al., 1964). The irradiation of crystals of 1-methylthymine leads to the formation of a dimer believed to be Type II in Figure 1 (Stewart, 1963). After complete methylation (Stewart, 1964), this dimer behaves differently from either of the

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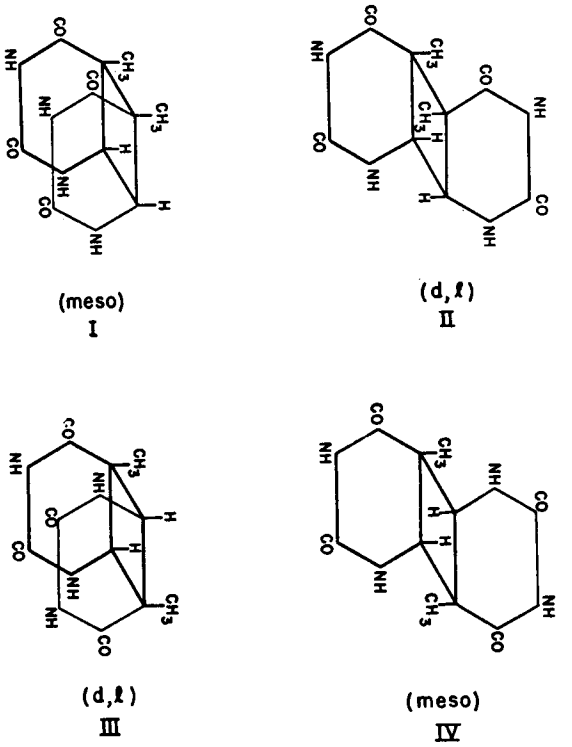

Figure 1. Isomeric Forms of the Cyclobutane-Type Thymine Dimers. (Modified from Wulff and Fraenkel, 1961).

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dimers of dimethylthymine produced by Wulff and Fraenkel (1961). More recently Weinblum and Johns (1966) have isolated five of the six possible dimers of thymine by irradiation of TpT, frozen thymidine, DNA and frozen thymine.

Some years ago, we reported that a new photoproduct of thymine was produced when thymine was irradiated in frozen solution in the presence of adenine (Smith, 1963). A new photoproduct of thymine has also been observed in irradiated bacterial spores (Smith and Yoshikawa, 1966; Donnellan and Setlow, 1965). These two photoproducts of thymine have very similar Rf values in several solvents and at first the possibility was considered that they might be identical (Smith and Yoshikawa, 1966). That they are <u>not</u> identical, however, is shown by

the fact that the spore photoproduct is not converted back to thymine by reirradiation in solution (Smith and Yoshikawa, 1966), whereas the in vitro product is converted back to thymine as is the original thymine dimer (Beukers and Berends, 1961). The photoproduct of thymine formed in irradiated spores remains to be identified but the photoproduct of thymine produced in vitro in the presence of adenine appears to be the Type III (Figure 1) isomer of the thymine dimer. This paper describes some of the unique properties of this isomer and some of the conditions favoring its photochemical formation in frozen solution.

Methods

The general techniques for the U.V. irradiation of frozen samples and their subsequent chromatography and analysis are those previously described (Smith, 1963).

Results

Formation of the Thymine Dimers: When thymine-2-C-14 (0.25 mg/ml) was irradiated in frozen solution about 76% of the thymine was converted to the Type I cyclobutane-type thymine dimer (Rf 0.24) and about 9% to an unknown photoproduct at Rf 0.13 (in n-butanol/acetic acid/water; 80/12/30) (Smith, 1963). When thymine was irradiated frozen in the presence of an equal weight of adenine very little of the Type I thymine dimer was formed but a new photoproduct appeared at Rf 0.33 (Smith, 1963). By increasing the molar concentration of adenine (A) relative to that of thymine (T) the production of the Type I thymine dimer decreased rapidly from 75% (T/A = 1/0) to a plateau of about 5% (T/A = 1/2) (Table 1). Conversely the production of the Rf 0.33 material went from 0.00% (T/A = 1/0) to a plateau of about 6% (T/A = 1/1). Increasing the ratio T/A to 1/8 did not significantly alter the yield (Table 1). This new photoproduct does not contain adenine since reciprocal experiments using adenine-8-C-14 plus non-labeled thymine showed the formation of

Molar Ratio	Product Yield	
T/A	iso $\overset{\frown}{TT}$	$\overset{\frown}{TT}$
1/0	0%	75%
1/.5	4.5%	26%
1/1	6.1%	13%
1/2	6.5%	5%
1/4	6.5%	5%
1/8	6.5%	5%

Table 1. The Effect of the Molar Ratio of Thymine to Adenine on the Yield of Thymine Dimers. Different amounts of Thymine-2-C^{14} (0.5 mg/ml and 2.5 μc/ml) were mixed with H_2O and/or adenine (1.2 mg/ml) to a total volume of 200 μl to give the listed molar ratios. The solutions stood for 30 min at room temperature before freezing, irradiating and chromatography as previously described (Smith, 1963). The results are the average of two experiments.

no labeled material with an Rf of 0.33. The same yield of Rf 0.33 material was obtained whether thymine-2-C-14 or thymine-methyl-H-3 was used.

Acid Stability and Photochemical Reversibility of the Dimers: The Type I thymine dimer (produced in frozen solution) is stable to hydrolysis (60 min at 155°C) in trifluoroacetic acid (conditions used to isolate the Type I dimer from irradiated DNA and bacteria; Smith, 1964), but the Rf 0.33 material is 50% converted back to thymine by this treatment. Hydrolysis for 150 min quantitatively converted this photoproduct back to thymine. The identity of the thymine was established by co-chromatography with non-labeled thymine and the resultant coincidence of radioactivity (from photoproduct conversion) and U.V. absorbing material (marker thymine) on the chromatograms.

The absorption spectrum of the Rf 0.33 material was identical with that of Type I dimer isolated from the same chromatograms (when normalized to equal amounts of radioactivity) and both were similar to the published spectra for the Type I dimer (Setlow, 1961; Johns, Rapaport and Delbruck, 1962). The characteristic feature of the spectra of these dimers is the

absence of a U.V. absorbing peak in the region of 260 mμ indicating that no double bonds remain in the pyrimidine rings.

When samples of the Type I dimer and the Rf 0.33 material were re-irradiated in solution the production of thymine (as judged by the re-appearance of U.V. absorption at 260 mμ and confirmed by paper chromatography with unlabeled thymine) proceeded at essentially the same rate for the two photoproducts.

Separation of Monomer and Dimer on Sephadex G-10: Thymine (molecular weight: 126) and the Type I thymine dimer (molecular weight : 252) can be separated from each other using short columns of the molecular sieve

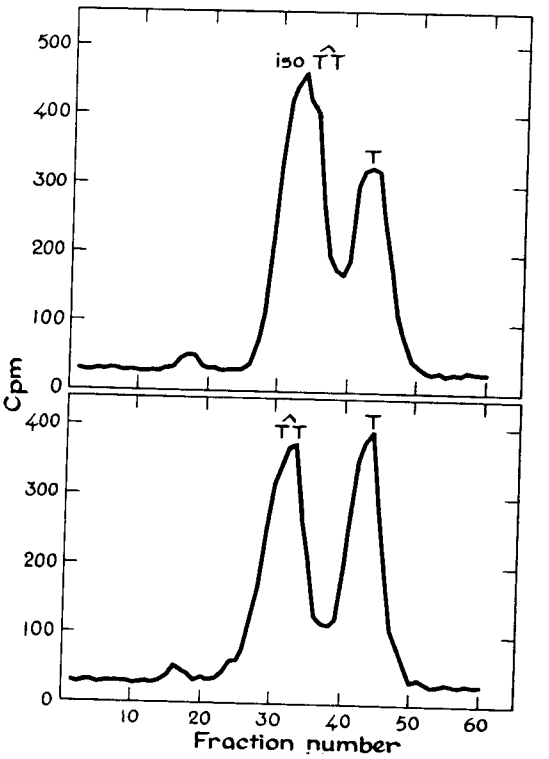

Figure 2. Separation of Thymine and Thymine Dimer on Sephadex G-10. A column (0.8 x 35.5 cm) of Sephadex-G10 (40-120μ) was used. The samples were dissolved in, and the column was equilibrated with water. 0.5 ml samples were collected every 2 min. T stands for thymine; $\hat{TT}$ for the cyclobutane-type thymine dimer produced when T is U.V. irradiated in frozen solution; and iso $\hat{TT}$ for the new photoproduct of thymine produced when thymine is U.V. irradiated in frozen solution in the presence of adenine.

material Sephadex G-10. The Type I dimer elutes at tube #30 while thymine comes off at tube #40. The Rf 0.33 material also comes off at tube #30 (Figure 2). The dimeric nature of the Rf 0.33 material is therefore indicated.

Discussion

The Rf 0.33 material is similar to the Type I, cyclobutane-type, thymine dimer (Rf 0.24 material) in U.V. absorption properties, in the conversion to thymine by re-irradiation in solution and in behavior on Sephadex G-10. It differs from the Type I dimer, however, both in Rf on paper chromatograms (Table 2) and in its sensitivity to acid. The Rf 0.33 material would appear to be an isomeric form of the cyclobutane-type thymine dimer.

Weinblum and Johns (1966) have assigned their isomeric dimers to the structures shown in Figure 1 on the basis of chromatographic properties, acid stability, optical rotation and infrared absorption spectra. Our photoproduct has chromatographic (Table 2) and acid stability properties

Compound	Chromatographic Solvent				
	A	B	C	D	E
Thymine	.60	.55	.78	.72	.59
Uracil Dimer	.12	.03	--	--	--
Thymine - Uracil Dimer	.19	--	--	--	--
Thymine Dimer - (Type I)(2)	.24	.14	--	--	.49
		.12(1)			.49(1)
Thymine Dimer (Type III)(2)	.33	.17	.62	.40	.56
(R_f 0.33 Material)		.17(1)	--	--	.56(1)

Table 2. R_f Values. Chromatographic Solvents: A. n-Butanol/Acetic Acid/Water (80/12/30); B. n-Butanol/Water (86/14); C. Isopropanol/HCl/Water (68/15.5/16.5); D. Sec. Butanol saturated with water; E. Isopropanol/NH_3/Water (7/1/2).
(1) Data of Weinblum and Johns (1966).
(2) Structure given in Figure 1.

similar to the isomer that these authors have assigned to be the Type III thymine dimer. We therefore conclude that the Rf 0.33 material is in fact an isomeric form of the cyclobutane-type thymine dimer whose structure is depicted in Figure 1 as the Type III dimer.

The role of adenine in causing the formation of the Type III thymine dimer and inhibiting the formation of the Type I thymine dimer can only be surmised. The base pairing interaction of adenine and thymine may so direct the stacking of the bases during the freezing step as to lead to the observed results. It is therefore of interest that the major effect of the presence of adenine was reached when the adenine and thymine were present in essentially equimolar amounts (Table 1). Furthermore, no Type III thymine dimer was formed in the presence of an equal molar amount of hypoxanthine and the yield of the Type I dimer was only depressed by 20% as compared to 83% for adenine (Table 1).

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References

Beukers, R. and Berends, W., (1961). Biochim Biophys. Acta 49, 181.

Donnellan, J.E., Jr. and Setlow, R.B., (1965). Science 149, 308.

Johns, H.E., Pearson, M.L., LeBlanc, J.C., and Helleiner, C.W., (1964). J. Mol Biol. 9, 503.

Johns, H.E., Rapaport, S.A., and Delbruck, M., (1962). J. Mol. Biol. 4, 104.

Setlow, R.B., (1961). Biochim. Biophys. Acta. 49, 237.

Smith, K.C. (1963). Photochem. Photobiol. 2, 503.

Smith, K.C., (1964). Photochem. Photobiol. 3, 1.

Smith, K.C., and Yoshikawa, H., (1966). Photochem. Photobiol. 5,

Stewart, R.F., (1963). Biochim. Biophys. Acta 75, 129.

Stewart, R.F., (1964). Personal communication cited in Johns et al., 1964.

Weinblum, D., and Johns, H.E., (1966). Biochim. Biophys. Acta 114, 450.

Wulff, D.L., and Fraenkel, G., (1961). Biochim. Biophys. Acta 51, 332.