Crystal and Molecular Structure of Photodimer A of 1,3-Dimethylthymine (the Isomer in Irradiated Deoxyribonucleic Acid)

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Abstract: The elucidation of the crystal structure of photodimer A of 1,3-dimethylthymine confirms that this is the *cis*-5,5:6,6 stereoisomer. This is the same isomer as that obtained from ultraviolet-irradiated DNA; hence the structure of the thymine dimer in irradiated DNA is proved. The cyclobutane ring is puckered, each atom lying 0.5 Å out of the plane of the other three, and the thymine nuclei are rotated by 26° away from a position of direct overlap of the nuclei atoms. The thymine residues are planar if C(6) of one ring and C(5) of the other are omitted from the planes; the angle between these planes is 33.5°. The crystals are monoclinic, space group $P2_1/c$, with cell dimensions $a = 8.41$, $b = 14.01$, $c = 14.55$ Å, $\beta = 117°15'$; $Z = 4$ dimers per cell. The dimers are split into monomers under exposure to X-rays; thus the molecular dimensions obtained include some contribution from monomers in the crystals. A stereochemical explanation is proposed for the relative stabilities of the thymine photodimers. The structure was solved by the symbolic addition procedure.

There has been much interest in pyrimidine photodimers since the isolation and identification of dimers of thymine as photoproducts of ultraviolet irradiation of aqueous solutions of DNA and of frozen aqueous solutions of thymine.[1] The photodimerization of thymine in DNA is of considerable biological importance: evidence has been obtained for the production of dimerized thymine in irradiated bacteria *in vivo*,[2] and it has been shown that this is the major factor in the inactivation of microorganisms by ultraviolet irradiation.[3]

There are four stereoisomeric dimers possible by *cis* linkage of two thymines to form a cyclobutane ring across their 5,6 double bonds: *cis-syn*, *cis-anti*, and *trans-syn*,*trans-anti*, where *cis* and *trans* refer to the geometrical arrangement of the thymines with respect to the cyclobutane ring, and *syn* and *anti* to 5,5:6,6 and 5,6:5,6 (or head-to-head and head-to-tail) linkages, respectively. Only one isomer is obtained from the ultraviolet irradiation of DNA, and chemical and spectroscopic evidence[4] has shown that one of the two dimers obtained from the uv irradiation of 1,3-dimethylthymine, dimer A, is the same isomer as the photodimer from DNA. Thus the determination of the structure of this photodimer of dimethylthymine would give unequivocal proof of the conformational structure of the thymine dimer formed in DNA; accordingly, we have undertaken a single-crystal X-ray diffraction analysis of photodimer A of dimethylthymine.[5]

Experimental Section

Photodimer A of 1,3-dimethylthymine was prepared by uv irradiation of a frozen aqueous solution of dimethylthymine and colorless platy crystals with well-developed $\{20\bar{4}\}$ forms were obtained from water. The crystals are monoclinic and systematic absences indicated space group $P2_1/c$. The unit cell dimensions, from least-squares refinement of diffractometer measurements (Cu Kα radiation, $\lambda = 1.5418$ Å) are $a = 8.41 \pm 0.02$, $b = 14.01 \pm 0.01$, $c = 14.55 \pm 0.01$ Å, $\beta = 117°15' \pm 10'$; there are 4 dimers per cell.

Intensity data were collected on an automated Picker four-circle diffractometer with Ni-filtered Cu radiation. The moving-crystal–moving-counter technique was employed with stationary counts for background on each side of the reflection. During data collection it was observed that the intensities of the six standard reflections were varying with time, generally decreasing but not all at the same rate. After collection of all independent hkl and $h0l$ reflections in the range $0 < 2\theta \leq 133°$ the intensities of the standards had fallen by as much as 33%; accordingly, a second crystal was used to collect all the $h\bar{k}l$ reflections in this range. Nine standard reflections were frequently remeasured during this part of the data collection and upon termination of collection their intensities had fallen by as much as 50% (though one of them had actually increased in intensity by 75%). It was also noted that during data collection the length of the a axis varied somewhat with time. These phenomena were interpreted to mean that the dimer was being monomerized by the X-radiation, and the crystals were, with time, being converted to a mixture of dimers and monomers. This postulation was proved correct when uv spectra of irradiated crystals showed the presence of monomer peaks. Though the rates of change of intensity of the individual standard reflections varied considerably, plots of *average* rates of decrease with time of the standards were fairly linear; as a first approximation linear scale factors were derived for and applied to each set of data to put both sets on a "time-zero" scale. The two data sets were then scaled together by comparing 16 $h0\bar{l}$ and 72 $0kl$ medium-strong reflections which had been measured with both crystals. Lorentz and polarization corrections were applied to the data, and structure amplitudes $|F|$ and normalized structure amplitudes $|E|$ were derived. A total of 1650 reflections were in the range recorded, 1258 of these having intensities above background.

Structure Determination. The symbolic addition procedure[6] was used to determine the phases. Application of the Σ_2 formula

$$\mathrm{s}E_h \sim \mathrm{s}\sum_k E_k E_{h-k} \qquad (1)$$

(where s means sign of) to three origin specifying planes, introduc-

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(1) R. Beukers, J. IJlstra, and W. Berends, *Rec. Trav. Chim. Pays-Bas* **79**, 101 (1960).

(2) A. Wacker, H. Dellweg, and D. Weinblum, *Naturwissenschaften*, **20**, 477 (1960).

(3) For a review, see J. K. Setlow in "Current Topics in Radiation Research," Vol. II, M. Ebert and A. Howard, Ed., North Holland Publishing Co., Amsterdam, 1966, p 196.

(4) D. L. Wulff and G. Fraenkel, *Biochim. Biophys. Acta*, **51**, 332 (1961); D. Weinblum, *Biochem. Biophys. Res. Commun.*, **27**, 384 (1967); A. J. Varghese and S. Y. Wang, *Nature*, **213**, 909 (1967).

(5) Preliminary results of this investigation were given in N. Camerman and A. Camerman, *Science*, **160**, 1451 (1968).

(6) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

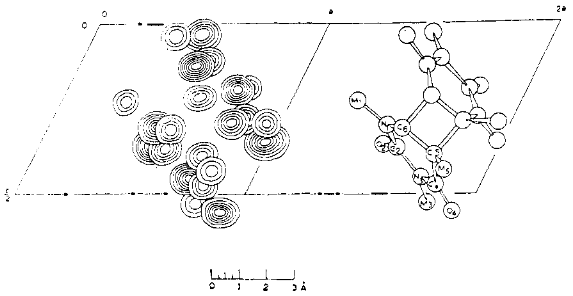

Figure 1. Superimposed sections of the electron density distribution calculated at the atomic centers, parallel to (010): C, carbon; N, nitrogen; O, oxygen; M, methyl-group carbon.

tion of one symbol (Table I), and iterative recycling led to signs (+, −, or $\pm a$) for 73 planes with $|E| > 2$, all with probability at least 0.97. These reflections were then used in the Σ_2 formula to generate signs for planes with $2 > |E| \geq 1.5$; after a number of cycles a total of 205 signs were determined for planes with $|E| \geq 1.5$. A very strong indication that the symbol *a* was obtained from the $\Sigma_{1'}$ test

$$\mathrm{s}E_{2h,0,2l} \sim \mathrm{s}\sum_{k} (-1)^{h+k}(E^2{}_{hkl} - 1) \qquad (2)$$

A three-dimensional *E* map was computed from the 205 data and could be interpreted to give a plausible structure for the dimer in which 17 of the 22 (nonhydrogen) atoms were visible. However, when a three-dimensional Fourier synthesis was calculated, with phases based on the partial structure, no additional atoms appeared on the map. Thus we concluded that the *E* map was incorrect.

Table I. Phases and Symbols Assigned to Implement Eq 1

	$\vec{h}$			$E(\vec{h})$	$\phi(\vec{h})$
	2	1	$\bar{4}$	3.91	0
	1	2	10	3.05	0
	3	3	7	2.84	0
First attempt	2	0	$\bar{4}$	3.81	*a*
Second attempt	3	10	3	3.19	*a*
	3	5	8	2.82	*b*
	4	8	1	3.15	*c*

A new attempt at phase determination was initiated by choosing a different basis set (Table I) consisting of the same three origin-specifying planes but three new symbol-assigned planes. Several cycles of the Σ_2 procedure led to signs and symbols for most of the same 205 reflections, and gave a very strong indication that the symbol *b* was negative, and also a fair number of indications that *c* was negative and *a* positive. A three-dimensional *E* map computed with these assignments revealed the entire molecule clearly.

With the knowledge of the correct signs, an analysis of the first try at phase determination was made to discover where it had failed. It was found that fairly early in the sign determination procedure the Σ_2 formula had indicated the sign of the 2 1 3 reflection ($|E| = 2.47$) to be negative, with a probability of 0.977. This sign was incorrect (though the signs of the two planes which had contributed to its determination *via* the Σ_2 formula were correct); its adoption led to incorrect signs for 75 reflections in the ensuing phase determination. This experience emphasizes the need for extreme care in the early stages of phase determination by the symbolic addition method.

Refinement of the Structure. The atomic coordinates and thermal parameters were refined by full-matrix least squares using a modified version of the ORFLS program.[7] The function minimized was $\Sigma(F_o - F_c)^2$ and the atomic scattering factors were taken from the International Tables for X-Ray Crystallography.[8] Several cycles of refinement with isotropic and then anisotropic temperature factors resulted in the discrepancy factor, $R = 15.6\%$, for the observed reflections. Bond lengths were calculated at this stage, and all were near normal values except the interpyrimidine cyclobutane lengths, which were 1.72 and 1.78 Å, giving further evidence of the splitting of these bonds during irradiation of the crystals, and remonomerization of the dimers.

Because of the effects of X-rays on these crystals, we felt that from the nature of the data collection processes, photographic methods would be preferable to diffractometer methods for recording the diffraction intensities. The intensities are collected in sections through the reciprocal lattice photographically, and on any one section the effects of the X-rays can be taken as uniform, to a first approximation, and the various layers can then be interrelated by a least-squares scaling procedure; the sequential method of data collection with a diffractometer on the other hand, has a larger error inherent in correlating the data under conditions where the X-radiation affects the molecular structure. Accordingly, further refinement of the photodimer was carried out using intensity data which had been collected photographically.[9] These data were obtained from Weissenberg photographs of the levels *h*0*l* through *h*9*l* and an 0*kl* precession photograph; the intensities were measured with a densitometer and consisted of 864 independent reflections of which 623 were above background levels. The Weissenberg films, integrated in one direction, were collected using Cu Kα radiation and the levels scaled to each other by exposure times, which ranged from 70 to 100 hr. Scales were refined in the least-squares procedure. The crystals used for the photographs were checked after 6 months and were found to have deteriorated completely with no intensities evident other than powder rings.

Several cycles of least-squares refinement of the atomic and thermal parameters, and subsequent difference Fourier maps, allowed the hydrogen positions to be determined, and dropped *R* to 8.8% (hydrogen parameters included). The final parameters are given in Table II, in which the β_{ij} are coefficients in the expression

$$\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{23}kl + 2\beta_{13}hl + 2\beta_{12}hk)]$$

The refined hydrogen isotropic thermal parameters (in the usual form, B) are given for the sake of completeness, but no significance should be attached to them. The table of final observed and calculated structure factors has been deposited as Document No. NAPS-00886 with ASIS National Auxiliary Publication Service.[10]

The ΔF maps, from which the hydrogen positions were located, were examined for evidence of minor sites for the positions of C(5) and C(6) in the monomer. No such evidence could be found. Maximum unexplained background in the final difference map was less than ±1 electron/Å^3.

Discussion

Figure 1 shows superimposed sections of the electron density distribution taken through the atomic centers, parallel to (010), along with a perspective drawing of the molecule. The electron-density distribution confirms that photodimer A has a 5,5:6,6 thymine-thymine cyclobutane linkage with the thymines *cis* to each other (*i.e*, it is a *cis-syn* dimer), as would be expected from the dimerization of adjacent thymines in DNA.

The cyclobutane ring is puckered, with dihedral angles of 152 and 153° (defined as the angle between planes having the transannular distances in common), similar to the values of 153 and 154° in the *cis-anti* photodimer of dimethylthymine,[11] and 155° in the *cis-syn* dimer of uracil;[12] each atom of the cyclobutane ring lies 0.5 Å out of the plane defined by the other three

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(7) W. R. Busing, K. O. Martin, and H. A. Levy, ORFLS, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962.

(8) "International Tables for X-Ray Crystallography," Vol. III, The Kynoch Press, Birmingham, England, 1962.

(9) R. F. Stewart and L. H. Jensen, unpublished data.

(10) A copy may be secured by citing the document number and by remitting $2.00 for microfiche or $5.00 for photocopies to the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.

(11) N. Camerman, D. Weinblum, and S. C. Nyburg, *J. Amer. Chem. Soc.*, **91**, 982 (1969).

(12) E. Adman, M. P. Gordon, and L. H. Jensen, *Chem. Commun.*, 1019 (1968).

Table II. Fractional Coordinates,[a] Anisotropic (for Nonhydrogen Atoms) Thermal Parameters ($\times 10^5$), and Isotropic Thermal Parameters (Hydrogen Atoms)

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	0.5069 (13)	0.2536 (12)	0.1235 (8)	2621	492	514	390	109	10
M(1)	0.3436 (26)	0.3001 (24)	0.0386 (13)	3406	1227	662	1094	114	−22
C(2)	0.5484 (18)	0.1669 (15)	0.1124 (11)	2403	480	686	208	19	−148
O(2)	0.4694 (14)	0.1171 (10)	0.0350 (8)	3812	1037	930	185	−230	−274
N(3)	0.7058 (13)	0.1254 (12)	0.1953 (7)	2144	819	573	7	175	31
M(3)	0.7577 (38)	0.0258 (19)	0.1852 (17)	5498	412	1251	544	936	−117
C(4)	0.8228 (18)	0.1779 (16)	0.2832 (10)	1823	674	684	−112	506	34
O(4)	0.9594 (12)	0.1390 (9)	0.3462 (7)	2330	894	867	367	58	92
C(5)	0.7816 (18)	0.2789 (14)	0.2930 (9)	2843	493	600	−480	370	156
M(5)	0.9319 (29)	0.3398 (18)	0.2935 (15)	3203	609	917	−184	889	277
C(6)	0.5869 (17)	0.3074 (13)	0.2160 (10)	2514	757	628	492	63	−48
N(1,2)	0.4546 (15)	0.1875 (13)	0.3003 (8)	2566	484	643	141	117	−7
M(1,2)	0.2652 (17)	0.1662 (14)	0.2237 (11)	1372	1187	800	−7	−13	−262
C(2,2)	0.5404 (21)	0.1187 (19)	0.3668 (13)	2713	504	913	−464	553	−44
O(2,2)	0.4831 (15)	0.0365 (12)	0.3584 (7)	3159	696	692	−13	402	57
N(3,2)	0.7089 (14)	0.1352 (13)	0.4536 (7)	1634	1040	528	−353	138	−197
M(3,2)	0.7952 (25)	0.0500 (21)	0.5221 (15)	2937	1256	726	−283	181	459
C(4,2)	0.7894 (22)	0.2216 (17)	0.4710 (11)	2607	938	675	−711	313	−161
O(4,2)	0.9280 (12)	0.2357 (10)	0.5510 (6)	2747	1482	626	−1061	99	61
C(5,2)	0.7268 (17)	0.2940 (13)	0.3838 (9)	3036	778	632	−194	294	178
M(5,2)	0.7677 (36)	0.3943 (18)	0.4311 (18)	5078	581	1310	−969	1393	−361
C(6,2)	0.5222 (16)	0.2834 (12)	0.3057 (9)	1757	548	866	213	194	−145
H(6)	0.528 (11)	0.387 (9)	0.229 (7)	5.8					
H(M1)	0.210 (36)	0.285 (24)	0.032 (19)	20.8					
H(M1)	0.359 (7)	0.273 (6)	−0.013 (4)	0.7					
H(M1)	0.325 (12)	0.344 (9)	0.032 (7)	5.4					
H(M3)	0.670 (21)	−0.022 (14)	0.110 (12)	14.7					
H(M3)	0.928 (27)	−0.041 (18)	0.211 (15)	15.6					
H(M3)	0.726 (15)	0.003 (10)	0.228 (8)	8.0					
H(M5)	1.050 (8)	0.307 (6)	9.343 (5)	1.5					
H(M5)	0.962 (14)	0.315 (10)	0.228 (8)	8.1					
H(M5)	0.896 (9)	0.391 (7)	0.308 (5)	2.9					
H(6,2)	0.391 (8)	0.330 (6)	0.307 (5)	2.0					
H(M1,2)	0.205 (17)	0.159 (12)	0.275 (10)	10.5					
H(M1,2)	0.257 (12)	0.137 (9)	0.158 (7)	6.6					
H(M1,2)	0.195 (19)	0.261 (14)	0.207 (11)	12.5					
H(M3,2)	0.725 (10)	0.028 (7)	0.567 (5)	2.6					
H(M3,2)	0.897 (27)	0.048 (20)	0.509 (18)	18.9					
H(M3,2)	0.867 (15)	0.085 (10)	0.570 (8)	7.7					
H(M5,2)	0.793 (13)	0.473 (9)	0.387 (7)	6.0					
H(M5,2)	0.697 (11)	0.375 (9)	0.472 (6)	5.2					
H(M5,2)	0.937 (19)	0.405 (13)	0.470 (10)	12.0					

[a] Standard deviations in parentheses.

Table III. Least-Squares Planes[a] and Deviations

Plane 1		Plane 2	
Atom	Deviation, Å	Atom	Deviation, Å
N(1)	−0.02	N(1,2)	−0.01
C(2)	0.03	C(2,2)	0.01
N(3)	−0.01	N(3,2)	0.00
C(4)	−0.01	C(4,2)	0.00
C(5)	0.01	C(6,2)	0.01
M(1)	0.03	M(1,2)	−0.25
O(2)	0.18	O(2,2)	0.04
M(3)	0.05	M(3,2)	0.06
O(4)	0.04	O(4,2)	−0.12
M(5)	1.31	M(5,2)	−0.32
C(6)	−0.32	C(5,2)	0.37

[a] Plane 1: $0.8272x + 0.2983y - 0.4762z = 3.1694$; plane 2: $0.7949x - 0.2730y - 0.5419z = 1.3619$, where x, y, z are coordinates in ångströms referred to orthogonal axes a^*, b, and c.

atoms. The thymine residues are not planar, but least-squares planes calculated through all combinations of five atoms taken at a time showed that one of the rings is approximately planar if C(6) is omitted from the calculations of its plane (maximum deviation from the plane = 0.03 Å, and deviation of C(6) = − 0.32 Å), and the other thymine nucleus is planar if C(5) is omitted (maximum deviation = 0.01 Å, C(5) deviation = 0.37 Å). This stereochemical feature is similar to that in the *cis-anti* dimer of dimethylthymine,[11] where C(6) of each ring (corresponding *in position* to C(6) of one ring and C(5) of the other in this isomer as the linkage is 5,6:5,6 in that molecule *vs.* 5,5:6,6 here) are out of the planes of the other atoms; the angle between five-membered planes in that molecule is 33°, similar to the value here of 33.5°. Table III shows the deviations from the least-squares planes.

The two thymine residues are rotated with respect to each other (away from a position of direct overlap of corresponding atoms in each ring) by about 26° (calculated by averaging the angles between normals to planes through C(6), C(6,2), N(1,2), and C(6), C(6,2), N(1); C(5), C(5,2), C(4,2), and C(5), C(5,2), C(4); and other equivalent sets); this is close to the value of 24° in the same dimer of uracil,[12] and 29° in the *cis-anti* dimer of dimethylthymine.[11] Since this angular displacement is thought to be 36° for two adjacent thymines in the Watson–Crick model of DNA, only a small angular twist (a maximum of 5° for each thymine) probably accompanies photodimerization in DNA.

Table IV. Comparison of Some Bond Lengths in Pyrimidine Dimers

	N(1)–C(2)	C(2)–N(3)	N(3)–C(4)	C(4)–C(5)	C(5)–C(6)	C(6)–N(1)	C(5)–C(5,2)	C(6)–C(6,2)
Dimethylthymine dimer A (*cis-syn*)	1.305	1.43	1.38	1.50	1.565	1.435	1.60	1.66
Uracil dimer A[a] (*cis-syn*)	1.333	1.394	1.362	1.498	1.537	1.438	1.572	1.563
Dimethylthymine dimer C[b] (*cis-anti*)	1.343	1.416	1.378	1.506	1.531	1.440		
Thymine dimer D[c] (*trans-anti*)	1.334	1.390	1.357	1.508	1.547	1.440		

[a] Reference 12. [b] Reference 11. [c] N. Camerman and S. C. Nyburg, *Acta Crystallogr.*, *B*, **25**, 388 (1969).

The bond distances and angles are shown in Figures 2 and 3. Because of the impossibility of obtaining high-accuracy intensity data due to the monomerization of the dimers by X-rays, the standard deviations of the bond lengths and angles are rather high: 0.025–0.035 Å and 1–2°. Table IV lists a comparison between corresponding pyrimidine ring bonds in the various photodimers thus far structurally elucidated (values averaged over the corresponding bonds in the two halves of each dimer) and indicates that these standard deviations are, in most cases, probably overestimated.

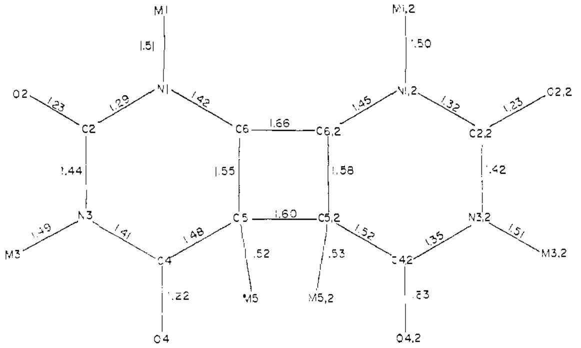

Figure 2. Bond distances (Å) in dimethylthymine photodimer A. Standard deviations of bond lengths are 0.025–0.035 Å.

Bond N(1)–C(2) is somewhat shorter than in the other photodimers, and bond C(2)–N(3) somewhat longer, which may indicate some incorrect positioning of the C(2) atoms, or that these atoms change position relative to the other atoms of the molecule during splitting of the dimers by X-rays. The rest of the bond distances agree quite well with the other quoted values except for the cyclobutane ring bonds, especially the *inter*thymine bonds, which is highly indicative of the dimer-splitting effect of the X-rays.

It appears to us that the relative stabilities of the pyrimidine dimers to X-radiation, and possibly to acid–base hydrolysis, can be explained by their stereochemistries. As was indicated by change of cell dimensions and variation in intensities with time, and proved by the presence of monomer peaks in the uv spectra of X-irradiated crystals, photodimer A of dimethylthymine is monomerized by splitting of the 5–5 and 6–6 bonds by X-rays. (A study initiated on the basis of these results has shown that the same effect is achieved by irradiation with γ-radiation.[13]) This behavior has also been corroborated in an X-ray study of the sodium and potassium salts of the same isomer of the photodimers of thymine,[14] while the *cis-syn* isomer of uracil photodimer shows no such behavior and is stable to X-rays. Thus it appears that the presence of the C(5) methyl groups is a contributing factor to the radiation-instabilities. Dimer B, the *trans-syn* isomer, of 1-methylthymine and of dimethylthymine, also undergoes monomerization when irradiated by X-rays[15] and γ radiation,[13] though not as rapidly as dimer A, while the *cis-anti* and *trans-anti* isomers (isomers C and D) of thymine and methylated-thymine photodimers are stable to X-rays. Isomers A and B differ from C and D in that the former are head-to-head dimers, and hence have their C(5) methyl groups *on the same side* of the cyclobutane ring (*i.e.*, across the C(5)–C(5) bond), the two methyl groups being *cis* to each other in the case of dimer A, and *trans* in dimer B. This could lead to a large amount of steric overcrowding across the C(5)–C(5) bond in both isomers: in dimer A by conflict between the two methyl groups *and* between the two C(4)'s, and in dimer B by two sets of C(4)–methyl group interactions. (It is important to note that highly localized multiple steric interference is possible *only* in thymine dimers A and B; in C and D the transannular separation of the C(5) methyl groups removes any possibility of *two* steric conflicts between the halves of the dimer across any one bond.) If such overcrowding does exist it would certainly make dimers A and B of thymine much more susceptible to monomerization by high-energy radiation than dimers C and D. We have calculated the interatomic distances between the C(5) methyl groups and between the C(4) atoms in photodimer A of dimethylthymine, and we have indeed found that they are very significantly short; the C(5) methyl–

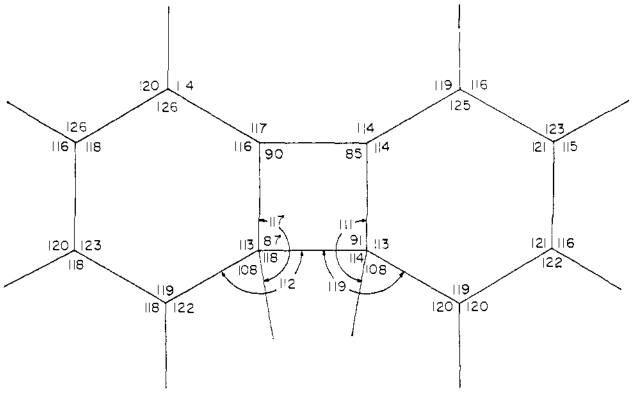

Figure 3. Bond angles (degrees) in dimethylthymine photodimer A. Standard deviations are 1–2°.

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(13) D. Weinblum, *Radiat. Res.*, **39**, 731 (1969).

(14) C. H. Wei and J. R. Einstein, *Amer. Cryst. Assoc.*, Buffalo Meeting, Abstracts, 102 (1968).

(15) N. Camerman, unpublished observations.

C(5) methyl distance is 3.01 Å, and the C(4)–C(4) distance is 2.94 Å. For comparison the normal van der Waals separation between methyl groups of neighboring molecules is 4.0 Å,[16] and between carbon atoms about 3.5 Å. Thus overcrowding and steric strain across the C(5)–C(5) cyclobutane bond are almost certainly present in photodimer A, and, although the crystal structure of dimer B has not been elucidated, one suspects that steric interference is also present there.

This stereochemical explanation of the relative radiation sensitivities of the pyrimidine photodimers may also shed some light on their relative sensitivities to acid-base hydrolysis. Thymine photodimers C and D are much more sensitive to hydrolysis than are dimers A and B (principal product is thymine in each case),[17] and it may be that the steric overcrowding in the latter isomers obstructs attack by hydrolyzing agents, while the openness in the cyclobutane region in dimers C and D facilitates it.

Acknowledgments. The authors are indebted to Professors L. H. Jensen and R. F. Stewart for very kindly making available the photographic intensity data which were used in the refinement of the structure. We also thank Dr. D. Weinblum for supplying the material and Professor S. C. Nyburg for the use of facilities in his laboratory. Support at Toronto was from the Medical Research Council of Canada and at Seattle from National Institutes of Health Grant No. GM-13366.

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(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 260. The use of van der Waal's separation for methyl groups has been criticized by a referee who feels that in view of the fact that coordinates have been determined for the hydrogen atoms, the H...H distances should be quoted. Since it is our purpose to show qualitatively that steric crowding exists between the C(5) methyl groups, we felt nothing is gained by this distinction. Nonetheless, we have calculated the H...H separations involved and find there are two short H...H contacts of 2.08 and 2.24 Å (vdw distance = 2.40 Å) between the two methyl groups and two short C...H contacts of 2.48 and 2.71 Å (vdw distance = 2.80 Å).

(17) M. A. Herbert, J. C. LeBlanc, D. Weinblum, and H. E. Johns, *Photochem. Photobiol.*, **9**, 33 (1969).

Chemical Evolution. III. The Photochemical Conversion of Enaminonitriles to Imidazoles[1,2a]

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Abstract: A mechanistic investigation of the photocyclization of enaminonitriles to imidazoles is reported utilizing diaminomaleonitrile (HCN tetramer) (I) and β-aminocrotononitrile (ACN; V). The reaction proceeds from a π,π^* excited state which has some charge transfer character. The photocyclization must proceed *via* a singlet since the reaction is not sensitized by benzophenone or triphenylene and is not quenched by cyclohexadiene or piperylene. However, the *cis–trans* isomerization of ACN involves a triplet since it is sensitized by benzophenone and triphenylene. In addition, I quenches the phosphorescence of biacetyl. No luminescence was observed from the compounds studied. Flash photolysis studies revealed the presence of a long-lived transient ($t^{1/2}$ = 0.5 msec); however, since the transient was quenched by dienes, it cannot be associated with the photocyclization. When the photolysis of ACN is carried out in D_2O solution, deuterium was not photolytically incorporated into the 5 position of 4-methylimidazole. Furthermore, only 4-methylimidazole and none of the 2-methyl isomer is the reaction product. These data show that the reaction proceeds by way of an intermediate in which the C–CN bond is broken by a process which does not involve a ketimine intermediate. An azetine intermediate, which undergoes further rearrangement to the imidazole, is suggested by the photochemical conversion of the vinylogous enaminonitrile XIII to the vinylogous azetine (pyridine) (XIV).

Hydrogen cyanide has been proposed as the starting point in the chemical evolution of purines and amino acids. Hydrolysis of a polymer formed from HCN yields fourteen amino acids.[3] An intermediate in the polymerization is diaminomaleonitrile (HCN tetramer) (I), an essential compound in the proposed prebiotic synthesis of purines.[4] The tetramer, which exists in the *cis* form in the ground state, is photochemically converted to the *trans* isomer. The tetramer also undergoes a photochemical rearrangement to 4-aminoimidazole-5-carbonitrile[5] (II) which, in a dark reaction with hydrogen cyanide, forms adenine (III). In addition, II or its hydrolysis product IV readily affords guanine, isoguanine, diaminopurine, hypoxanthine, and xanthine.[6]

The photochemical step is the only efficient pathway from hydrogen cyanide to the purine precursor II which is feasible in dilute aqueous solution. Because of its central role in the proposed prebiotic synthesis sequence, it was decided to investigate the photolysis of enaminonitriles further, with the aim of determining:

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(1) For the previous paper in this series see: J. P. Ferris, J. E. Kuder and A. Catalano, *Science*, **166**, 765 (1969). For the previous paper in a related series see ref 6.

(2) (a) Supported by Grant No. GM 15915 from the National Institutes of Health; (b) USPHS Research Career Development Awardee (GM 6380) of the National Institute of General Medical Sciences.

(3) C. N. Mathews and R. E. Moser, *Nature*, **215**, 1230 (1967).

(4) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **30**, 223 (1967).

(5) J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.*, **88**, 1074 (1966).

(6) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **38**, 121 (1968).