solvent three times before being washed with ether. The dinucleotide phosphoramidate (slightly brown-colored powder, 164 mg) gave dinucleotide (as triethylammonium salt) pC^{An}pG^{iB}-OiB (3040 OD$_{280}$, 52%) on treatment with isoamyl nitrite.

The Dinucleotide, d-pG^{iB}pT-OAc. An anhydrous pyridine solution (4.0 ml) of TPM-p-G^{iB} (0.25 mmol, 4175 (OD$_{260}$ based on pG^{iB}) and d-pT-OAc (0.5 mmol, 4800 OD$_{267}$) was treated with TPS (303 mg, 1.0 mmol) at room temperature for 3 hr with exclusion of moisture. After usual work-up the aqueous solution (15 ml) was extracted with *n*-butyl alcohol–methylene chloride (three 20-ml portions, 3:7). The organic phase was washed with 0.2 *M* TEAB (two 40-ml portions) and then concentrated (4.0 ml) in the presence of pyridine. The product was precipitated into ethyl acetate–ether (50:50) and washed with the same solvent once more and then with ether. The protected dinucleotide phosphoramidate (300 mg) on treatment with isoamyl nitrite gave the dinucleotide (as triethylammonium salt), d-pG^{iB}pT-OAc (60%, 2750 OD$_{280}$).

The Dinucleotide, d-pG^{iB}pA^{Bz}-OAc. An anhydrous solution of of d-pA^{Bz}-OAc (9150 OD$_{280}$, 0.5 mmol) and TPM-p-G^{iB} (0.25 mmol, 4175 OD$_{260}$ based on pG^{iB}) in pyridine (4.0 ml) was treated with TPS (303 mg, 1.0 mmol) at room temperature for 3 hr. After following the standard work-up procedure,[22] the reaction mixture was concentrated to a thick oil which was treated with *n*-butyl alcohol–methylene chloride (15 ml, 3:7) and 0.2 *M* TEAB (10 ml). The aqueous phase was further extracted with the same organic solvent as described above (two 15-ml portions) and the combined extracts were washed with 0.2 *M* TEAB (three 10-ml portions). Concentration (4 ml) of combined organic extracts was carried out in the presence of pyridine. The concentrated pyridine solution was then precipitated into ethyl acetate–ether (50:50) to give the dinucleotide phosphoramidate (270 mg). This on treatment with isoamyl nitrite yielded the dinucleotide (as triethylammonium salt), d-pG^{iB}pA^{Bz}-OAc (4600 OD$_{280}$, 62%).

The Dinucleotide, d-pG^{iB}pG^{iB}-OiB. TPS (400 mg, 1.3 mmol) was added to an anhydrous pyridine solution (4.5 ml) of TPM-p-G^{iB} (0.25 mmol, 4175 OD$_{260}$ based on pG^{iB}) and d-pG^{iB}-OiB (6850 OD$_{280}$, 0.6 mmol). The total reaction mixture was allowed to stand at room temperature for 3 hr with exclusion of moisture. After the usual work-up,[22] the aqueous solution (15 ml) was extracted with *n*-butyl alcohol–methylene chloride (3:7, three 20-ml portions). The organic phase was further extracted with 0.2 *M* TEAB (three 20-ml portions) and then concentrated in the presence of pyridine to a solution of 3 ml. This was then precipitated into ethyl acetate–ether (50:50) and the precipitate collected by centrifugation. The dinucleotide phosphoramidate (290 mg) was treated with isoamyl nitrite as described in the general method to give the dinucleotide (as triethylammonium salt), d-pG^{iB}pG^{iB}-OAc (58%, 3306 OD$_{280}$).

The Crystal Structure of a Thymine Trimer, $C_{15}H_{20}N_6O_7 \cdot H_2O$, a Photoproduct of Thymine

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Abstract: A hydrated trimer has been isolated by Varghese and Wang from the uv irradiation of a frozen aqueous solution of thymine. The most common products of the irradiation are the cyclobutyl-type dimers. The X-ray crystal-structure analysis has established the stereoconfiguration of the trimer and has shown that it is hydrated (see VII). Similarities to both the *cis-syn*-cyclobutyl dimers and to the thymine–thymine adduct are apparent. The trimer crystallizes in the triclinic space group $P\bar{1}$ with one molecule of H_2O of crystallization per molecule of trimer. Cell parameters are $a = 9.373$, $b = 14.387$, and $c = 7.201$ Å, $\alpha = 103.2$, $\beta = 100.0$, and $\gamma = 91.8°$. The molecules are held together in the crystal by an extensive system of intra- and intermolecular hydrogen bonds. The structure was solved directly by means of the symbolic addition procedure.

Irradiation of frozen aqueous solutions of thymine, uracil, and some of their derivatives has yielded several different kinds of photoproducts such as the cyclobutyl dimers[1,2] and, more recently, the thymine–thymine adduct.[3] For products I,[4–7] II,[8,9] IV,[10,11] and V,[12,13] or simple derivatives thereof, the molecular formulas have been confirmed and the stereoconfigurations have been established by crystal-structure analyses using X-ray diffraction.

Ultraviolet irradiation of [*2*-^{14}*C*]- or [*5-methyl*-^{3}*H*]-thymine in frozen aqueous solution produces photoproducts designated in the literature[14] as PT$_1$ and PT$_2$. The product PT$_2$ has been shown to be a mixture of the *cis-syn*-cyclobutyl dimer and thymine–thymine adduct[3] while PT$_1$ appeared to be a trimer of thymine.[14b] The trimer has the characteristics of the adduct, the photoreversibility of the cyclobutyl dimers, and the instability of the hydration products. In the present study, an X-ray analysis of a single crystal of the trimer has established its molecular formula and stereoconfiguration. A preliminary note on the structure has been reported.[15]

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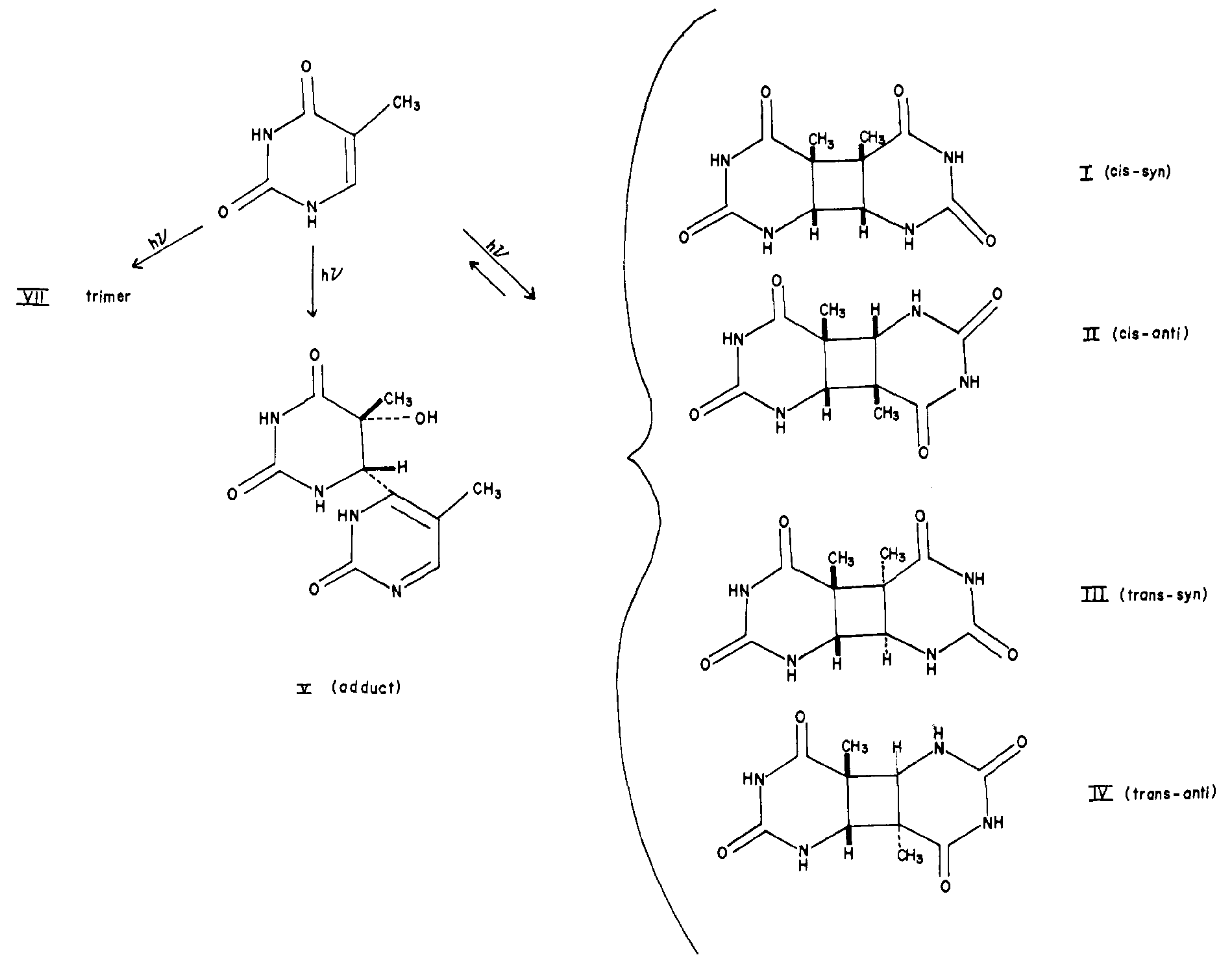

Experimental Section

The crystals used in the X-ray analysis were provided by Professor S. Y. Wang of The Johns Hopkins University. Intensity data were collected on a four-circle automatic diffractometer using the θ-2θ technique with a 2.4° + $2\theta(\alpha_2) - 2\theta(\alpha_1)$ scan over 2θ. Even though a relatively large scan range (2.4°) was needed there was no overlap of neighboring reflections. Due to the small size of the largest available crystal ($\sim 0.06 \times 0.03 \times 0.44$ mm) a slow scan rate (0.5 deg/min) was used and each background was counted for 40 sec. It was possible to collect about 38% of the reflections accessible to the copper sphere (1636 independent reflections out of a possible 4256). The data were collected using Cu Kα radiation (1.5418 Å) with a Ni filter. The colorless acicular crystal was mounted along the c axis. The space group is $P\bar{1}$ with cell dimensions of: $a = 9.373 \pm 0.004$, $b = 14.387 \pm 0.004$, $c = 7.201 \pm 0.003$ Å, $\alpha = 103.2 \pm 0.5°$, $\beta = 100.0 \pm 0.5°$, and $\gamma = 91.8 \pm$ $\pm$ 0.5°. There are 2 molecules per unit cell corresponding to a crystallographic density of 1.48 g/cm^3. The intensity data were corrected for Lorentz and polarization factors, placed on an absolute scale by means of a K curve,[16] and normalized structure factor magnitudes $|E|$ as well as structure factor magnitudes $|F|$ were derived.

Structure Determination

The structure of the thymine trimer was solved by using the symbolic addition procedure[17] for obtaining phases directly from the normalized structure factor magnitudes. Three reflections (1 $\overline{12}$ 3, $\bar{5}$ 0 2, and 3 1 0), having $|E| > 3.0$, were assigned positive signs to specify the origin. Seven additional reflections, having $|E|$ values >2.6, were assigned symbolic phases: $\bar{4}$ 0 1 = a, 1 $\overline{11}$ 3 = b, $\bar{7}$ 4 1 = c, $\bar{7}$ 4 3 = d, $\bar{1}$ 8 0 = e, $\bar{1}$ 11 2 = f, and $\bar{3}$ 9 3 = g. The signs and symbols were used to implement the phase-determining formula, Σ_2.[18] Space group $P\bar{1}$ often requires more symbols than is generally the case for other space groups. Several indications of high probability that b was positive arose early in the phase determination. Therefore, b was assigned positive at this stage and the phase determination was continued with the six remaining symbols. Signs for 100 independent reflections having $|E| \geq 1.6$ were derived in terms of the starting signs and symbols. Relationships among the symbols arose which showed that $c = d = f = g = +$ and $a = e$. To avoid the set of all positive signs, symbols a and e were assigned negative signs. With this assignment of symbols, the phase determination employing the Σ_2 formula was continued and phases for 458 reflections having $|E| \geq 1.0$ were determined. The resulting E map contained 29 peaks of approximately equal weight which corresponded to the complete trimer molecule plus the water of crystallization.

Coordinates and thermal factors were refined in a full-matrix least-squares procedure.[19] Atomic scat-

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Table I. Fractional Coordinates and Thermal Parameters for Thymine Trimer[a]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
N-1	0.3757	0.8709	0.0503	4.83	3.82	0.89	0.94	0.40	0.20
C-2	0.3496	0.9155	0.2306	3.99	3.74	1.07	0.80	0.39	0.57
O-2	0.2897	0.9937	0.2474	6.14	3.27	3.22	2.40	1.92	0.49
N-3	0.3836	0.8761	0.3836	4.70	3.16	1.98	1.28	0.60	0.89
C-4	0.4626	0.7979	0.3870	3.35	3.52	3.35	0.32	0.34	1.56
O-4	0.5014	0.7749	0.5374	3.76	3.85	2.90	1.31	1.22	0.84
C-5	0.4875	0.7361	0.1898	4.38	2.39	3.22	0.74	0.65	0.29
C-6	0.4466	0.7808	0.0223	3.90	2.24	3.58	0.90	0.36	−0.06
C-7	0.4053	0.6384	0.1694	5.75	2.57	6.16	−0.10	1.80	−0.22
N-11	0.6712	0.8882	0.0350	4.42	2.53	1.56	0.29	1.19	0.08
C-12	0.7046	0.9422	0.2198	3.57	3.48	2.37	1.20	1.45	0.41
O-12	0.7117	1.0333	0.2512	4.93	2.28	2.94	0.95	1.17	0.51
N-13	0.7288	0.8965	0.3605	4.53	2.38	2.56	1.13	1.00	1.13
C-14	0.7706	0.7968	0.3230	3.91	2.03	3.51	0.76	0.72	−0.15
O-14	0.8989	0.7907	0.2375	3.80	3.62	4.22	0.90	1.61	0.41
C-15	0.6515	0.7363	0.1626	3.97	1.89	2.37	0.95	0.47	−0.80
C-16	0.6096	0.7905	0.0014	4.73	2.93	2.97	1.11	0.92	0.46
C-17	0.6984	0.6344	0.0827	4.99	2.83	3.99	0.86	0.73	−0.74
N-21	0.7652	0.6765	0.5306	4.08	2.43	3.11	1.42	1.11	0.86
C-22	0.8549	0.6067	0.5178	3.86	3.02	4.86	1.12	1.54	1.62
O-22	0.8158	0.5233	0.5080	4.70	2.55	7.65	1.07	2.05	1.22
N-23	1.0000	0.6302	0.5142	4.46	3.32	6.78	1.63	2.01	1.57
C-24	1.0612	0.7225	0.5531	4.96	2.53	5.63	0.81	1.75	0.77
O-24	1.1885	0.7385	0.5475	3.75	4.02	8.35	0.70	2.69	0.78
C-25	0.9667	0.8010	0.6321	3.36	2.93	4.16	0.96	0.75	1.39
O-25	1.0118	0.8882	0.5877	4.17	2.82	3.58	0.51	1.12	0.63
C-26	0.8059	0.7758	0.5260	3.88	3.17	2.11	1.04	1.06	1.42
C-27	0.9840	0.8115	0.8498	5.27	6.51	2.60	1.59	1.29	0.59
W	0.5431	0.4068	0.3738	8.00	5.20	5.27	−1.00	0.61	−0.27
				Av est stand. dev					
C	0.0010	0.0006	0.0017	0.48	0.39	0.76	0.36	0.41	0.38
N	0.0008	0.0005	0.0012	0.37	0.31	0.55	0.26	0.33	0.31
O	0.0007	0.0004	0.0010	0.34	0.31	0.49	0.26	0.31	0.27

[a] The thermal parameters are of the form $T = \exp[-{}^1/_4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$. The B_{ij} values are in square ångström units.

tering factors used were those listed in the "International Tables for X-Ray Crystallography." During the initial stages of refinement the data were weighted by excluding the unobserved reflections ($|F_o| \leq 5.0$) and the most intense reflections ($|F_o| \geq 70.0$). After one cycle of anisotropic refinement, a difference map was computed and 20 out of the 22 hydrogen atoms were located. Hydrogen atoms were assigned temperature factors equal to those of the atoms to which they were bonded. The hydrogen parameters were then included in the refinement as constants. For the final stages of the refinement all the data were included and a statistical examination of the magnitudes of the remaining discrepancies suggested the weighting function $1/\partial$ where

$$\partial = \left[\left(\frac{|F| - 14}{10}\right)^2 + 1\right]^{1/2}$$

for $|F_o| \leq 14.0$ and

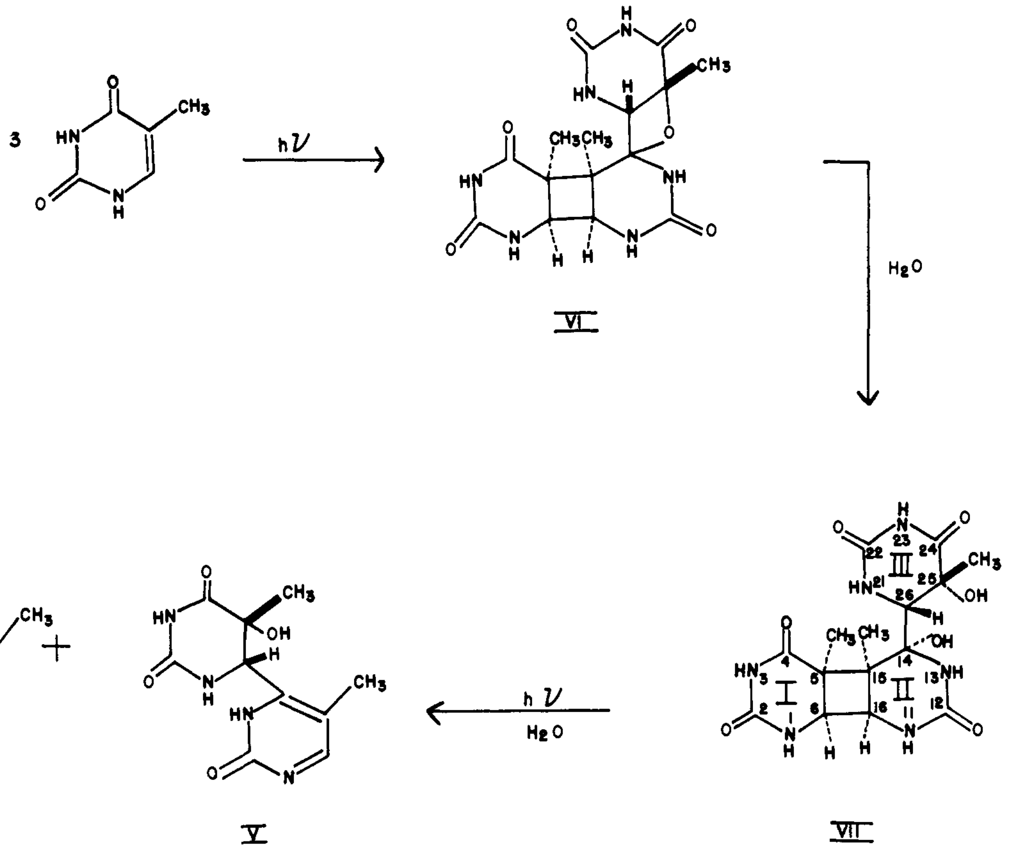
$$\partial = \left[\left(\frac{|F| - 14}{16}\right)^2 + 1\right]^{1/2}$$

for $|F_o| > 14.0$, which gives a distribution of weights consistent with the distribution of errors expected in counter diffractometry. Such errors include instrumental factors which limit the reliability of very intense and also very weak intensity measurements. The function minimized was $\Sigma 1/\partial^2(F_o - F_c)^2$ and the final R factor was 7.9% for the observed data.[19a] Fractional coordinates and thermal parameters for the C, N, and O atoms are listed in Table I. Approximate coordinates for the hydrogen atoms are listed in Table II.

Table II. Approximate Coordinates for Hydrogen Atoms Located in a Difference Map

Atom	x	y	z
H(N-1)	0.364	0.890	−0.084
H(C-6)	0.396	0.735	−0.119
H(C-7A)	0.410	0.610	0.030
H(C-7B)	0.286	0.654	0.174
H(C-7C)	0.444	0.588	0.240
H(N-11)	0.668	0.922	−0.080
H(N-13)	0.228	0.070	0.484
H(O-14)	0.996	0.825	0.310
H(C-16)	0.628	0.759	−0.146
H(C-17A)	0.382	0.386	0.006
H(C-17B)	0.780	0.648	0.060
H(C-17C)	0.680	0.586	0.160
H(N-21)	0.652	0.683	0.514
H(N-23)	1.067	0.583	0.527
H(C-26)	0.252	0.186	0.364
H(C-27A)	0.070	0.142	0.094
H(C-27B)	0.910	0.190	0.090
H(C-27C)	0.056	0.248	0.122
H(O-25)	1.115	0.877	0.662
H(W-1)	0.628	0.466	0.424

(19a) Note Added in Proof. A complete list of observed and calculated structure factors has been deposited as Document No. NAPS-01262 with the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10022. A copy may be secured by citing the document number and by remitting $1.00 for microfiche or $3.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.



Discussion

The molecular formula of the trimer VII, formed from a photoreaction of three molecules of thymine and one molecule of H_2O, shows that the molecule is a combination of the *cis-syn*-cyclobutyl dimer I and the thymine–thymine adduct V with an additional OH and H moiety on ring II. A probable route for the trimer formation is through an intermediate containing an oxetane ring which is ruptured by the addition of a molecule of H_2O. This process is analogous to the probable formation of the T–T adduct V,[3,13] except for the extra molecular equivalent of H_2O which is present in the trimer and absent in the adduct. Additional uv irradiation of the trimer in aqueous solution ruptures the cyclobutyl ring and results in two products, thymine and the thymine–thymine adduct.[14b] An initial product of the decomposition by uv is a T–T adduct which contains the OH group on C-14 but which is readily converted to V.[20] This point had caused some initial difficulties in reconciling the results from the crystal-structure analysis and structural information derived from spectroscopic data.

The stereoconfiguration of the trimer is illustrated in Figure 1, while the bond lengths and angles are shown in Figure 2. The numbering system in rings II and III is the same as the conventional system in ring I with the addition of 10 and 20 in rings II and III, respectively. Ring III of the trimer is quite comparable to the corresponding ring in the T–T adduct V.[13] The bond lengths in the two rings with their estimated standard deviations are shown in Table III. All the bond angles associated with these two rings also have comparable values. In both the trimer and the T–T adduct, the CH_3 group on C-25 is axial to the ring and the OH group on the same C atom is equatorial. This contrasts with dihydrothymine[21] and dihydrothymidine[22] where the CH_3 group on C-5 is equatorial. Ring III assumes the half-chair conformation with atom C-25 0.41 Å above and atom C-26 0.28 Å below the approximate plane (within ±0.04 Å) formed by N-21, C-22, N-23, and C-24, a conformation almost identical with the comparable ring in the T–T adduct.

Table III. Comparison of Bond Lengths for Similar Bonds in Thymine Trimer and T–T Adduct

Trimer, ring III		Adduct[a]	
Bond	Length, Å	Length, Å	Bond
N(21)–C(26)	1.477	1.466	N(1)–C(6)
N(21)–C(22)	1.327	1.302	N(1)–C(2)
C(22)–O(22)	1.225	1.247	C(2)–O(8)
C(22)–N(23)	1.397	1.399	C(2)–N(3)
N(23)–C(24)	1.377	1.393	N(3)–C(4)
C(24)–O(24)	1.217	1.207	C(4)–O(9)
C(24)–C(25)	1.526	1.508	C(4)–C(5)
C(25)–O(25)	1.431	1.421	C(5)–O(10)
C(25)–C(26)	1.557	1.555	C(5)–C(6)
C(25)–C(27)	1.519	1.528	C(5)–C(7)
Std dev	0.015	0.009	

[a] Reference 13.

Ring I, on the other hand, has some significant differences from similar rings in other *cis-syn*-cyclobutyl dimers. A comparison of similar bonds in Table IV shows that the values for the cis-syn dimers of uracil and 6-methyl uracil as well as the internal cis-

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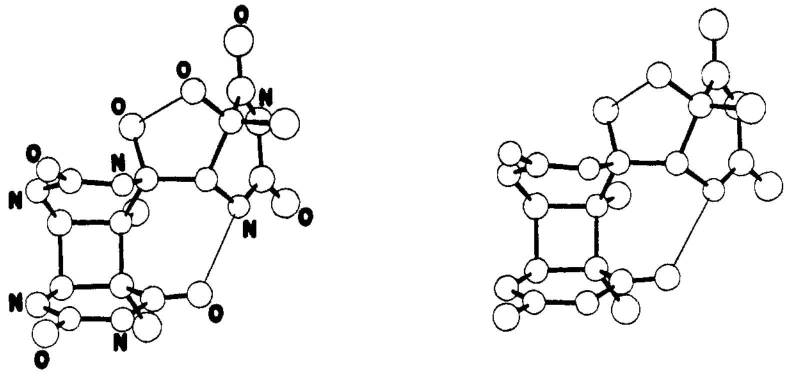


Figure 1. A stereoscopic view of the thymine trimer. The figure was drawn by computer with a program prepared by C. K. Johnson, Oak Ridge National Laboratory, Oak Ridge, Tenn.

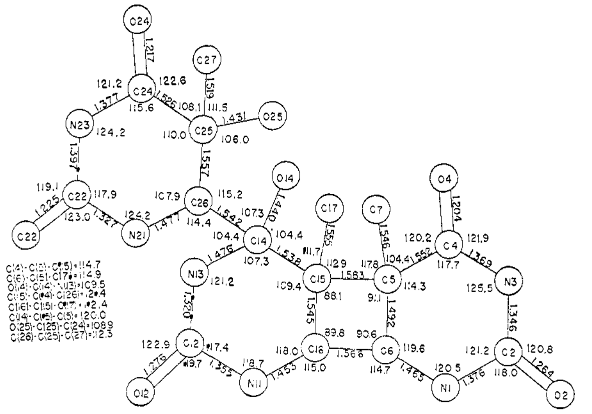


Figure 2. Bond lengths and angles. The standard deviations are of the order of 0.015 Å for the bond lengths and less than 1° for the bond angles.

syn dimer from 1,1′-trimethylenebisthymine are almost identical while five of the bond lengths in ring I of the trimer show fairly large differences from the other three compounds. Even though the standard deviations for the trimer are quite large owing to the limited set of diffraction data, no such large differences occurred in the comparisons of ring III with the T–T adduct. In spite of the presence of two saturated C atoms, ring I is nearly planar. The deviations from a least-squares plane which included the six ring atoms are: N-1, +0.014 Å, C-2, +0.008 Å, N-3, −0.052 Å, C-4, +0.066 Å, C-5, −0.041 Å, and C-6, +0.006 Å.

Table IV. Bond Lengths in *cis-syn*-Cyclobutyl Dimers

Bond	Trimer, ring I, Å	$\widehat{\text{T-C}_3\text{-T}}$,[a,b] Å	Uracil dimer,[a,c] Å	6-Methyl[a,d] uracil dimer, Å
N(1)–C(2)[e]	1.376	1.340	1.334	1.332
C(2)–O(2)[e]	1.264	1.225	1.227	1.226
C(2)–N(3)[e]	1.346	1.392	1.394	1.403
N(3)–C(4)	1.369	1.370	1.362	1.364
C(4)–O(4)	1.204	1.213	1.218	1.209
C(4)–C(5)[e]	1.552	1.510	1.499	1.504
C(5)–C(6)[e]	1.492	1.551	1.540	1.554
N(1)–C(6)	1.465	1.450	1.439	1.445
C(5)–{C(15) C(5′)}	1.583	1.596	1.572	1.546
C(6)–{C(16) C(6′)}	1.566	1.551	1.563	1.586
Std dev	0.015–0.020	0.005–0.009	0.0025	0.008

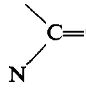
[a] Average value for two similar sections of the molecule. [b] Reference 6. [c] Reference 5. [d] Reference 7. [e] Bond lengths which exhibit the largest difference.

Table V. Torsion Angles[a] about the Bonds in the Three Six-Membered Rings

Ring	Bond	Torsion angle, deg
I	N(1)–C(2)	−2.3
	C(2)–N(3)	+9.2
	N(3)–C(4)	−13.8
	C(4)–C(5)	+11.5
	C(5)–C(6)	−5.8
	C(6)–N(1)	+1.6
II	N(11)–C(12)	+23.4
	C(12)–N(13)	+21.7
	N(13)–C(14)	−56.7
	C(14)–C(15)	+43.7
	C(15)–C(16)	−7.1
	C(16)–N(11)	−29.4
III	N(21)–C(22)	+4.9
	C(22)–N(23)	+10.6
	N(23)–C(24)	+8.9
	C(24)–C(25)	−39.6
	C(25)–C(26)	+49.8
	C(26)–N(21)	−35.3

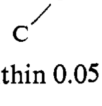
[a] The torsion angle ABCD is labeled positive if, when looking along the B–C bond, atom A has to be rotated clockwise to eclipse atom D.

Ring II is different from any other ring encountered in photoproducts from thymine or uracil in that C-14 as well as C-15 and C-16 is saturated. The C–OH bond, the C–N bond, and the two C–C bonds involving C-14 all have normal single bond values. The OH group on C-14 is axial to ring II while the bond joining ring III is equatorial to ring II. Ring II has assumed an approximate boat conformation with atoms N-11 and C-14, +0.29 and +0.61 Å, respectively, out of the plane formed by C-15, C-16, C-12, and N-13. Torsion angles for rings I, II, and III are shown in Table V. Each of the three



$$\begin{matrix}\text{N}\\ \quad\diagdown\\ \qquad\text{C=O}\\ \quad\diagup\\ \text{N}\end{matrix}$$

groups are planar to within 0.008 Å and each of the two



$$\begin{matrix}\text{N}\\ \quad\diagdown\\ \qquad\text{C=O}\\ \quad\diagup\\ \text{C}\end{matrix}$$

groups are planar to within 0.05 Å.

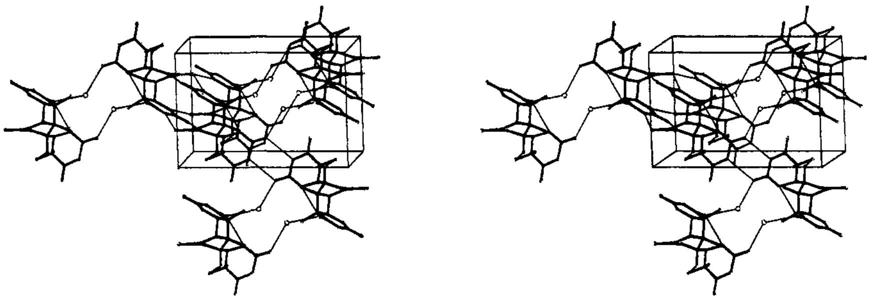
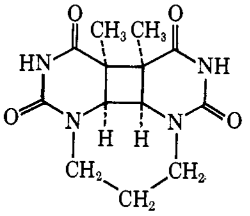
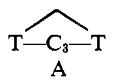


Figure 3. A stereoscopic view of the packing and hydrogen bonding in the thymine trimer. The axial directions are: *a*, ↑ ; *b*, →; and *c*, down into the paper.

The cyclobutyl ring is only slightly puckered with a dihedral angle of 173.5°. It is considerably flatter than the four-membered rings found in unencumbered *cis-syn*-cyclobutyl type dimers. For example, the dihedral angle in the uracil dimer is 155°,[5] in the 6-methyluracil dimer it is ~162°,[7] and it is "markedly puckered" in the dimethylthymine dimer.[4] The only other example of a flat cyclobutane ring in a cis-syn dimer is in the intramolecular dimer



referred to as



A

where the dihedral angle in the four-membered ring is 178°.[6] The flatness of the ring may be caused by the constraints imposed by the formation of the seven-membered ring in A. Similarly, in the thymine trimer, the relatively flat cyclobutyl ring may be a result of steric conditions. Torsion angles about C(5)–C(15) and C(6)–C(16) are only 4.8 and 6.8°, respectively. Consequently, the two CH_3 groups on C-5 and C-15 are nearly eclipsed and the intramolecular C(7)···C(17) distance is only 2.92 Å. If the cyclobutyl ring was puckered to allow a greater separation between C-7 and C-17 then other atoms, already involved in short intramolecular spacings (*i.e.*, N-13···C(4) = 2.89 Å and O(4)···C(26) = 2.87 Å), would come too close together. Even shorter intramolecular distances have been reported such as C···N distances of 2.56 Å in protopine[23] and 2.58 Å in cryptopine.[24] Other features presumably caused by crowding in the molecule are the large departures from tetrahedral values for angles involving C-14 and C-15. The angle C-15,C-14,C-26 is 121.4° and C-14,C-15,C-5 is 120.0°.

The rigidity of the molecule is greatly increased by the formation of two intramolecular hydrogen bonds, indicated by the light lines in Figure 2. The hydrogen bond between O(14)H···O(25)H, 2.61 Å, creates a six-membered ring, and the hydrogen bond between N(21)H···O(12), 2.89 Å, creates an eight-membered ring.

The packing of the molecules is characterized by eight independent intermolecular hydrogen bonds in addition to the two intramolecular hydrogen bonds listed in Table VI. Of the six possible N atoms and the seven possible O atoms available for hydrogen bonding, only O-24 does not participate. Even though O-12 is involved in three independent hydrogen bonds the NH···O distances are within the range normally found for less crowded situations. The 14 intermolecular hydrogen bonds to each trimer molecule account for close intermolecular approaches and, hence, a high value of 1.48 g/cm^3 for the density of the crystal. The arrangement of bonds N(23)H···O(22′) and N(23′)-H···O(22) around a center of symmetry, illustrated at 1, $^1/_2$, $^1/_2$ in Figure 3, is quite similar to that observed in the thymine·H_2O crystal[25] and the N(H)···O lengths at 2.84 Å are identical in the two crystals. The two H_2O molecules near $y = {}^1/_2$ serve to form hydrogen bonds to a pair of trimer molecules. The second hydrogen atom on the water molecule was not located; however, the O(4)···W distance and the H(W1)–W–O(4) angle are consistent with the existence of a hydrogen bond between O-4 and W.

Table VI. Hydrogen Bond Lengths

Donor	Acceptor	Symmetry operation for primed atom	Length, Å
		Intramolecular	
O-14	O-25		2.61
N-21	O-4		2.89
		Intermolecular	
N-1	O-12′	$1 - x, 2 - y, - z$	2.85
N-3	O-12′	$1 - x, 2 - y, 1 - z$	2.96
N-11	O-2′	$1 - x, 2 - y, - z$	2.99
N-13	O-2′	$1 - x, 2 - y, 1 - z$	2.94
N-23	O-22′	$2 - x, 1 - y, 1 - z$	2.84
O-25	O-12′	$2 - x, 2 - y, 1 - z$	2.74
W	O-4		2.86
W	O-22′	$1 - x, 1 - y, 1 - z$	2.90

There are double strands of NH···O bonds near y = 1, again reminiscent of the hydrogen bonding scheme

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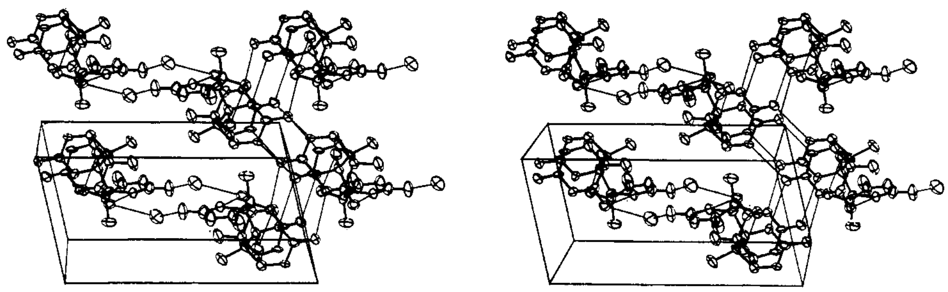

Figure 4. Another view of the packing, viewed at right angles to Figure 4. The axial directions are: *c*, ↑ ; *b*, →; and *a*, out of the paper.

in thymine·H_2O where each C═O is bonded to the NH from two adjacent molecules. However, these double strands occur in two layers, joining ring I to ring II′ and ring II to ring I′ of the molecules related by the centers of symmetry at $^1/_2$, 1, $^1/_2$ and $^1/_2$, 1, 1 as illustrated in Figures 3 and 4. Thus a continuous channel along the *c* axis is enclosed by the four independent bonds, N(1)H···O(12′), O(12′)···HN(3′′), N(11)H···O(2′), O(2′)···HN(13′′) and their symmetry equivalents.

In the trimer crystal there was no evidence of dissociation of the dimer moiety in the X-ray beam during the diffraction experiment. The final difference map was featureless in this area. Dissociation of the cis-syn dimer into monomers in the crystalline state upon X irradiation had been noted for the crystals of A,[6] dimethylthymine dimer,[4] and the Na salt of the *cis-syn*-thymine dimer.[26]

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Thymine Phototrimer[1]

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Abstract: Thymine phototrimer has been isolated from thymine irradiated in a frozen aqueous solution with 254-nm light. The new photoproduct, which presumably forms through the ring opening of an initial oxetane derivative, has been characterized by uv, ir, nmr, and mass spectra. The spectroscopic evidence confirms the results of X-ray diffraction analysis of a single crystal. It contains a 1,3-diol structural element, which is responsible for many of the intriguing reactions observed. One of these reactions is the formation of monomeric thymine from the trimer by acid or base catalysis. The possible importance of the 1,3-diol structure or the reversion reaction in the photochemistry and photobiology of nucleic acids is pointed out.

Observations from this laboratory showed that when a native DNA solution (40 μg/ml of 0.15 *M* NaCl) was irradiated (mainly 254-nm light) at a dose rate of 110 ergs/(mm^2 sec) for 30, 60, 90 sec, etc., there was a gradual decrease in the absorbancy at 260 nm with a simultaneous increase in the absorbancy in the 300–350-nm region (cited in ref 2). Dulbecco[3] reported that the absorption spectrum of phage T_2 particles undergoes complex changes with uv irradiation. He observed a general decrease, rather than an increase, in absorption in wavelengths longer than 320 nm. However, after the first hour of irradiation, a faint band was noticed; it became more evident during the second hour. This band has maximum absorption around 330 nm and extends to about 380 nm. Both experiments showed an apparent increase in absorbancy at wavelengths longer than 320 nm but their characteristics and temporal sequences were quite different. Moreover, the data obtained in this laboratory clearly showed that this spectral increase is the direct result of irradiation of native DNA; it cannot be an artifact resulting from the method of assay. The chemical event associated with such a change must assume some roles in biological systems. Thus, an investigation was undertaken of the chemistry responsible for this change. Earlier reports[4,5] suggested that thymine dimer (T═T) is the sole product of uv irradiation of DNA. Our study revealed that the *above defined* "thymine dimer" is in fact a mixture of cis-syn T═T (P_2A)[6] and the deaminated cytosine–

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