Molecular structure of deoxyadenylyl-3'-methylphosphonate-5'-thymidine dihydrate, (d-ApT•2H$_2$O), a dinucleoside monophosphate with neutral phosphodiester backbone. An X-ray crystal study*

K.K.Chacko§, K.Lindner and W.Saenger+
Abteilung Chemie, Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-Str. 3, D-3400 Göttingen, FRG, and

P.S.Miller
Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, USA

Received 24 March 1983; Accepted 12 April 1983

ABSTRACT

dApT, a modified deoxyribose dinucleoside phosphate with an uncharged methylphosphonate group, crystallizes as dihydrate in space group $P2_12_12$, a = 9.629(3), b = 20.884(6) and c = 14.173(4)Å, Z = 4. The structure has been determined using 2176 X-ray diffractometer reflections and refined to a final R of 0.105. Torsion angles about P-O(5') and P-O(3') bonds are -91.8° and 117.8°. The former is in the normal (-)gauche range while the latter is eclipsed. Bases are oriented anti, the sugar of adenosine is puckered $^2T_3$ (C(2')endo) whereas that of thymidine displays puckering disorder with major and minor occupancy sites. Major site is a half-chair $^2T_1$ (C(2')endo-C(1')exo) and minor site an envelope $^3T_2$ (C(3')endo). Adenine and thymine bases of symmetry related molecules form reversed Hoogsteen type base pairs, water molecules are disordered in the crystal lattice.

INTRODUCTION

Using single crystal X-ray diffraction methods, structural details for several di-ribonucleoside monophosphates (1-4), for one tri-ribonucleoside diphosphate (5), for several oligo deoxyribonucleotides (6) and for one oligoribo-oligodeoxyribo-nucleotide hybrid (7) have been reported. All these oligonucleotides contain normal nucleoside and phosphate moieties and are directly related to polymeric RNA and DNA.

Recently, a series of dideoxyribonucleoside methyl phosphonates have been synthesized (8). Owing to their non-ionic phosphonate group, they can readily diffuse into culture cells and may display promising biological effects because they are not degraded by nucleases. Structurally, the methyl phosphonates are of interest because (a) from the chemical

synthesis, two diastereomers with phosphorous atom in S and R configuration are obtained, (b) the electronic distribution in this moiety is different from that in normal phosphodiester and (c) the negative charge on the latter is no longer present. Spectroscopic studies (CD, UV hypochromicity, NMR) showed that dideoxy ribonucleoside methyl phosphonates have similar overall conformation as their parent analogues (9). The stacking of bases, however, is reduced and different for isomers R and S. On the other hand, the thermal stability of complexes between deoxyadenylyl-3'-methylphosphonate-5'-adenosine (d-ApA) and Poly(U) or Poly(T) is enhanced relative to normal d-ApA, probably because there is no charge-charge repulsion between negative phosphates of the complexing molecules (9). The present X-ray study describes the structural properties of deoxyadenylyl-3'-methylphosphonate-5'-thymidine (d-ApT) and gives a comparison with those obtained from unmodified nucleotides.

EXPERIMENTAL

The dihydrate of (d-ApT) (see Fig. 1 for molecular formula and atomic numbering scheme) crystallizes from ethanol/water in space group $P2_12_12$. Unit cell dimensions are a = 9.629(3), b = 20.884(6) and c = 14.173(4) Å. With four formula units of $C_{20}H_{28}N_7O_9P \cdot 2H_2O$ in the unit cell, the calculated density (ρc) is 1.42 g cm^{-3}. The three dimensional X-ray intensity data were collected up to a limiting value of 2θ of 120° using a Stoe automatic diffractometer equipped with Cu-tube. The θ-2θ scan mode was employed with stationary background measurements on both sides of each scan. Out of a total of 2353 reflections, 176 had intensities below the 3σ level of significance calculated from counting statistics (10). The structure was solved by MULTAN (11) and initially refined with isotropic temperature factors to an R value of 0.16. This refinement showed large temperature factors for C(2')T and O(3')T atoms of the thymidyl-deoxyribose as well as for the two water molecules, suggesting possible disorder. A difference Fourier map computed with these atoms omitted from structure factor calculations

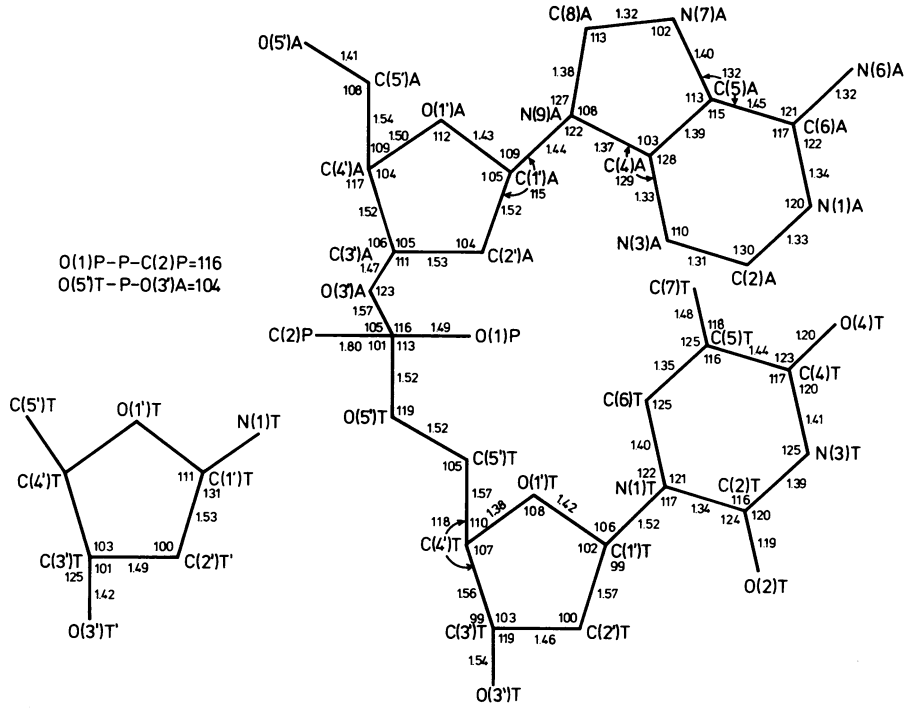

Fig. 1. Bond lengths, angles and numbering scheme of d-ApT

showed elongated, broad and only partially resolved peaks for atoms C(2')T and O(3')T corresponding to twofold occupancy with unequal statistical distribution (Fig. 2). The

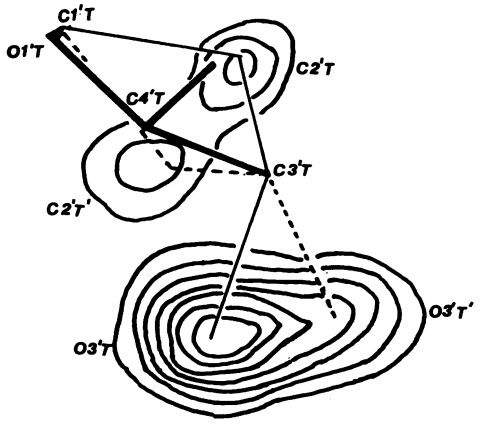

Fig. 2. Difference Fourier with the relevant sections picturing the disorder of the thymidyl sugar involving C2'T and O3'T atoms. Contours are drawn at intervals of 1e/A³. Starting contour is at 2e/A³.

Table 1 Final positional parameters of dApT

Atom	X	Y	Z
P	.2817(4)	.8136(2)	.2227(3)
O1P	.2708(13)	.8807(5)	.1885(8)
C2P	.1256(15)	.7801(8)	.2723(10)
C(1')A	.4783(18)	.8467(7)	.4939(10)
O(1')A	.5968(12)	.8088(5)	.4689(6)
C(2')A	.4498(17)	.8881(7)	.4076(11)
C(3')A	.5026(17)	.8478(7)	.3251(9)
O(3')A	.3988(10)	.7999(4)	.2973(6)
C(4')A	.6246(16)	.8103(8)	.3650(10)
C(5')A	.7698(19)	.8383(9)	.3477(12)
O(5')A	.7669(11)	.9038(6)	.3713(9)
N1A	.4035(14)	.8492(6)	.8464(8)
C2A	.3409(14)	.8178(7)	.7772(11)
N3A	.3626(12)	.8197(6)	.6862(8)
C4A	.4599(15)	.8629(7)	.6655(10)
C5A	.5349(15)	.9013(7)	.7272(10)
C6A	.4985(16)	.8936(7)	.8258(10)
N6A	.5597(14)	.9283(6)	.8912(8)
N7A	.6292(12)	.9418(6)	.6815(8)
C8A	.6124(16)	.9261(7)	.5922(11)
N9A	.5075(13)	.8819(5)	.5786(7)
C(1')T	.5432(18)	.6280(8)	.0727(13)
O(1')T	.5577(10)	.6954(5)	.0794(7)
C(3')T	.3543(17)	.6695(9)	-.0069(11)
C(4')T	.4617(18)	.7228(7)	.0193(10)
C(5')T	.4049(21)	.7868(9)	.0624(12)
O(5')T	.3122(11)	.7663(5)	.1441(6)
N1T	.5616(13)	.6026(6)	.1724(9)
C2T	.6481(16)	.5526(7)	.1833(13)
O2T	.7007(11)	.5246(5)	.1197(7)
N3T	.6734(12)	.5342(5)	.2760(9)
C4T	.6154(18)	.5636(9)	.3567(14)
O4T	.6540(13)	.5486(5)	.4338(8)
C5T	.5198(16)	.6157(8)	.3403(11)
C6T	.4992(14)	.6323(6)	.2497(10)
C7T	.4606(17)	.6491(7)	.4230(10)
O3'T (b)	.3995(18)	.6580(8)	-.1094(12)
O3'T'(b)	.2871(30)	.6638(12)	-.0954(18)
C2'T (a)(b)	.3820	.6200	.0630
C2'T'(a)(b)	.4400	.6100	-.0050
W1 (b)	.0501(17)	.9306(7)	.3882(11)
W2 (b)	.3426(19)	.9623(8)	.0592(12)
W' (b)	.7022(37)	.9337(16)	.1508(23)
W'' (b)	.5896(36)	.9234(15)	.1108(22)
W''' (b)	.0972(66)	.9853(29)	.1894(38)

a) Positional parameters from difference Fourier, not refined.

b) Occupancy factors of disordered sites are O(3')T, 0.65; O(3')T', 0.35; C(2')T, 0.65; C(2')T', 0.35; W1, 0.6; W2, 0.6; W', 0.3; W'', 0.3; W''', 0.2.

Table 2 Hydrogen atom positions

Atom	X	Y	Z
HC1'A1	.3848	.8158	.5007
HC2'A1	.5165	.9352	.4115
HC2'A2	.3437	.9040	.3964
HC3'A1	.5357	.8789	.2653
HC4'A1	.6222	.7622	.3363
HC5'A1	.8491	.8165	.3938
Hc5'A2	.8030	.8340	.2747
HO5'A1	.8850	.9100	.3600
HC2A1	.2551	.7861	.8039
HN6A1	.6373	.9681	.8897
HN6A2	.5630	.9279	.9638
HC8A1	.6746	.9452	.5346
HC1'T1	.6060	.6010	.0179
HC2'T1	.3183	.6379	.1178
HC2'T2	.3371	.5829	.0218
HC3'T1	.2482	.6898	-.0019
HC4'T1	.5146	.7384	-.0487
HC5'T1	.4859	.8191	.0831
HC5'T2	.3384	.8131	.0066
HN3T1	.7458	.5009	.2904
HC6T1	.4275	.6740	.2342

difference Fourier map displayed three other weak peaks which could be assigned to three minor occupancy sites of the two water molecules. Further refinement was carried out, incorporating the disordered positions of the water molecules as well as the two alternate sites of the C(2')T and O(3')T atoms of the thymidine sugar. Because coordinate refinement of the two positions of C(2')T showed wide oscillations after each cycle, leading to unreasonable bond lengths and angles involving C(2')T, the two sites of this atom were held fixed in further refinement cycles. Anisotropic temperature factors were applied to all the non-hydrogen atoms and the disordered atoms were treated isotropically. C-H and N-H hydrogen positions were calculated and included in the structure factor calculations. The hydrogen attached to N(6)A, O(5')A and N(3)T could be obtained from a difference Fourier map which also showed peaks corresponding to most of the calculated hydrogen positions. However, hydrogens of the thymine and phosphonate methyl groups, of the disordered O(3')T and those corresponding to the disordered water molecules could not be located, probably due

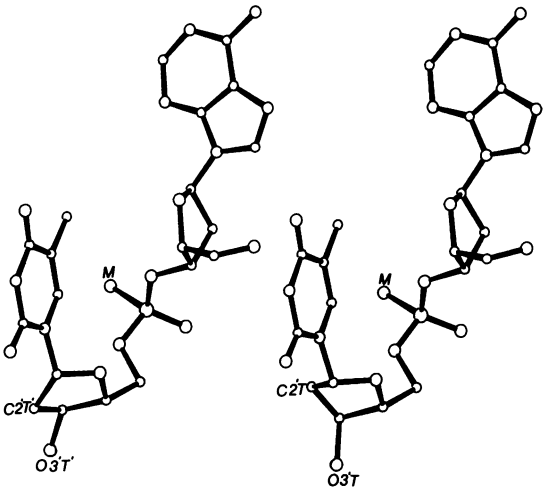

Fig. 3. A view of d-ApT with respect to the major (C(2')T and O(3')T) and minor (C(2')T' and O(3')T') conformation of the disordered thymidyl sugar. The methyl group C(2)P is marked "M" in order to distinguish it from O(1)P

to rotational as well as statistical disorder. The structure was refined to a final R value of 0.105 using weights obtained from counting statistics for the 2176 observed reflections with intensities greater than 3σ. The atomic scattering factors were those listed in the International Tables for X-ray Crystallography (12). The final coordinates of the non-hydrogen atoms of the molecule are presented in Table 1. Table 2 gives the hydrogen atom parameters included in the structure factor calculations.

RESULTS AND DISCUSSION

Geometrical details for d-ApT are given in Fig. 1 which is drawn with respect to the major site of C(2')T of the thymidyl sugar ring. Those data involving the minor site (C(2')T) are also indicated in the figure. The average standard deviations in bond lengths are about 0.02 Å and the average deviations of bond angles are 1°.

The bond lengths and angles of the purine and pyrimidine base are close to the average values reported by Voet and Rich (13) and are within the limits of accuracy of the structure. Data for the adenosyl sugar ring have normal values except for O(1')A-C(4')A, 1.50(2) Å, which is longer than the normally observed value near 1.41 Å. Some of the

Table 3 Torsion Angles

	Adenyl sugar	Thymidyl sugar with resp. to C(2')T	C(2')T'
a) Deoxyribose rings:			
C(1')-C(2')-C(3')-C(4')	-32.4°	-34.9°	32.3°
C(2')-C(3')-C(4')-O(1')	23.4	14.6	-37.8
C(3')-C(4')-O(1')-C(1')	-5.5	15.6	15.6
C(4')-O(1')-C(1')-C(2')	-14.8	-37.8	6.2
O(1')-C(1')-C(2')-C(3')	28.9	45.5	-25.5
b) Glycosyl bond (χ_{C-N})			
O(1')A-C(1')A-N(9)A-C(8)A	59.8°		
O(1')A-C(1')A-N(9)A-C(4)A	-100.8		
O(1')T-C(1')T-N(1)T-C(6)T	43.3		
O(1')T-C(1')T-N(1)T-C(2)T	-132.4		
c) Phosphate sugar backbone			
C(5')A-C(4')A-C(3')A-O(3')A	146.1°	ϕ'	
C(4')A-C(3')A-O(3')A-P	-158.1	Φ'	
C(3')A-O(3')A-P-O(5')T	117.8	ω'	
O(3')A-P-O(5')T-C(5')T	-91.8	ω	
P-O(5')T-C(5')T-C(4')T	158.0	Φ	
O(5')T-C(5')T-C(4')T-C(3')T	51.6	ϕ	
C(5')T-C(4')T-C(3')T-O(3')T	127.4	ϕ'	
C(5')T-C(4')T-C(3')T-O(3')T'	89.2	ϕ'	
C(3')A-O(3')A-P-O(1)P	-6.6		
C(3')A-O(3')A-P-C(2)P	-136.3		
C(5')T-O(5')T-P-O(1)P	34.7		
C(5')T-O(5')T-P-C(2)P	159.2		
d) Conformation about the C(4')-C(5') bonds			
O(1')A-C(4')A-C(5')A-O(5')A	-69.2°		
C(3')A-C(4')A-C(5')A-O(5')A	48.0		
O(1')T-C(4')T-C(5')T-O(5')T	-71.7		
O(5')T-C(5')T-C(4')T-C(3')T	51.6		

bond lengths and angles involving the thymidyl sugar have fairly large deviations from standard values due to the conformational disorder of the deoxyribose involving C(2')T and O(3')T atoms. The disorder might also influence the N(1')T-C(1')T bond length, 1.52(2) Å, which is larger than the mean value of 1.468 Å (13).

The configuration at the methylphosphonate group is S (Fig. 3)(8). The uncharged methyl phosphonate group linking the adenosyl and thymidyl moieties displays some unusual structural features. The P-O distances involving the esteri-

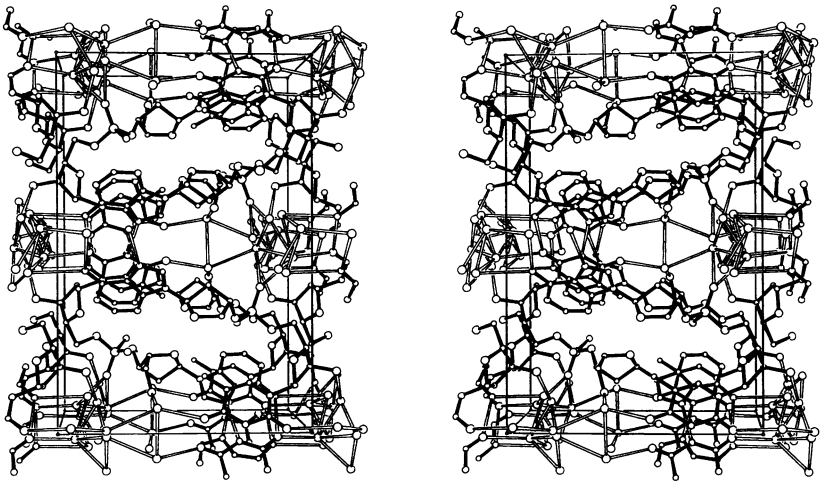

Fig. 4. Molecular packing viewed down the a axis, drawn with respect to the major conformation of the thymidyl sugar. Hydrogen bonding contacts indicated by open lines. At phosphonate groups, methyls are not involved in H-bonding to water but the free oxygen is.

fied oxygens observed in nucleotide structures, around 1.6Å, are generally longer compared to non-esterified P-O distances of 1.5 Å. However, for this molecule only the P-O(3')A distance of 1.57(1) Å is close to the value usually observed in dinucleotide structures whereas the P-O(5')T distance of 1.52(1) Å is significantly shorter. The non-esterified P-O(1)P of 1.49 Å is longer than expected for a double bonded P-O, around 1.40 Å yet in the range found for non-esterified P-O bonds. In the comparable methyl-phosphonate crystal structures of 1'-hydroxyl-1-cyclodecyl- and 1'-hydroxyl-1-cyclotridecyl phosphonate dimethylate and 1'-hydroxyl-1-cyclononyl phosphonate dimethylate (14-16), the P-O bonds have slightly shorter values of 1.476(6), 1.485(5) and 1.473(6) Å. The P-C(2)P bond distance involving the methyl group, 1.80(2) Å, compares with the P-C distances of 1.779(3) and 1.789(3) Å observed for adenosine-5'-methyl phosphonate hemihydrate (17). However, for the cyclodecyl, cyclotridecyl, and cyclononyl phosphonate structures this distance has larger values, 1.848(15), 1.826(30) and

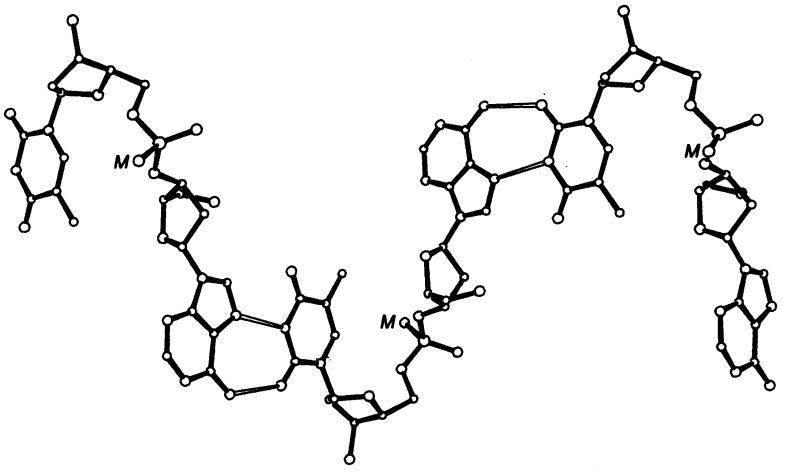

Fig. 5. The reversed Hoogsteen base pairing between adenine and thymine bases

1.818(15) Å. In conclusion, the distribution of P-O distances suggests that in the phosphonates the $P\text{-}O_{ester}$ bonds have slightly more double bond character compared to normal phosphodiesters. The P = O bond, on the other hand, displays as much single bond character as the unesterified P-O bonds in the diesters. This involves charge separation and a partial negative charge on the P = O oxygen is indicated by the short hydrogen bonding contacts to water molecules, Table 4.

The bond angles around the P-atoms have fairly large, yet systematic deviations from tetrahedral values. The C-P-O bond angles involving the methyl carbon atom and the esterified oxygens O(3')A and O(5')T, 105(1)° and 101(1)° are distinctly lower than tetrahedral while the O(1)P-P-O(5')A, O(1)P-P-O(3')T and O(1)P-P-C(2)P angles are significantly more obtuse with values 113(1), 116(1) and 116(1)° respectively. Those bond angle differences are also reflected in the cyclodecyl, cyclotridecyl and cyclononyl phosphonate crystal structures (14-16).

The orientations of the heterocycles with respect to the deoxy ribose sugars both at the deoxyadenosyl and thymidyl

Table 4 Hydrogen bonded contacts A...B of dApT

Atom A	Atom B	A...B distance (Å)
N3T	N7A(f)	2.78 Å
N6A	O2T(e)	3.06
	W''(c)	3.13
O(5')A	W1(b)	2.79
O(3')T	W'(d)	2.76
O(3')T'	W''(d)	2.64
	W'(d)	2.33
W1	O4T(h)	2.75
	W'''(a)	3.07
W2	O1P(a)	2.60
	O2T(g)	2.89
	W''(a)	2.62
	W'''(a)	3.04
W'''	O1P(a)	2.75
Symmetry code:	a) x, y, z	
	b) 1+x, y, z	
	c) x, y, 1+z	
	d) -1/2+x, 3/2-y, z	
	e) 3/2-x, 1/2+y, -z+1	
	f) 3/2-x, -1/2+y, -z+1	
	g) 1/2+x, 3/2-y, -z	
	h) -1/2+x, 3/2-y, -z+1	

part of the molecule are anti, with torsion angles of χ = 59.8° for the adenosyl and χ = 43.3° for the thymidine moieties (Fig. 3). Least squares plane calculations show that the adenosyl sugar exists in C(2')endo-C(3')exo conformation $^{2}T_{3}$ with C(2') and C(3') atoms deviating by 0.38 and -0.14 Å respectively from the plane defined by C(1'), O(1') and

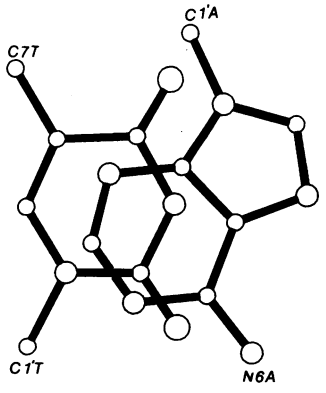

Fig. 6. Base stacking of d-ApT between adenine and thymine bases

C(4') atoms and the pseudorotational angle at 172° (18). The conformation with respect to the major site (C(2')T) of the thymidyl sugar is C(2')endo-C(1')exo $^{2}_{1}$T with C(2')T and C(1')T deviating equally by 0.36 and -0.36 Å from the plane defined by C(3'), C(4') and O(1') atoms, and pseudorotational phase angle at 143°. The conformation with respect to the minor occupancy site C(2')T' is C(3')endo-C(2')exo with C(3')T and C(2')T' deviating by 0.40 and -0.15 Å from the plane defined by atoms C(1')T, O(1')T, C(4')T and the pseudorotational phase angle, P = 8°, corresponds to $^{3}T_{2}$.

Thus the puckering of the major site of the thymidyl sugar belongs to the S-type and that of the minor site is N (18), i.e. both furanose puckering modes are displayed by this moiety. As the energy barrier between these two states is rather shallow (19), it is not surprising that this disorder is observed. Sometimes, within the same asymmetric unit of a nucleoside or nucleotide crystal structure, two molecules with different puckering modes corresponding to the N and S states, are found. A statistical disorder of unit, as in the dApT crystal structure, must be due to special packing circumstances in this case.

The conformational angles ω' and ω (20) of the methyl phosphonate linkage have values 117.8 and -91.8° respectively (Table 3c), the former occurring in an eclipsed range. In the comparable cyclodecyl, cyclotridecyl and cyclononyl phosphonate structures, however, the conformational angles about the esterified P-O bonds exist in gauche and trans forms (14-16). The unusual ω' angle (= 117.8°) for dApT leads to eclipsed atoms C(3')A and O(1)P, i.e. the torsion angle C(3')A-O(3')A-P-O(1)P is only -6.6°. This conformation is probably due to the uncharged phosphonate group of the molecule in contrast to the negatively charged phosphate in normal nucleotide phosphates. As found in other comparable phosphonate structures (14-16), there is a widening of the O-P-O angle involving esterified oxygens and O(1)P. In particular, the angle O(1)P-P-O(3')A is significantly obtuse (116°) and thus relieves non-bonded interactions between C(3')A and O(1)P, thereby facilitating the eclipsed confor-

mation. All the other sugar-phosphate chain torsion angles are in the normal range. Thus, the conformational angles about C(3')A-O(3')A and C(5')T-O(5')T bonds, φ' and φ, are trans with values -158.1 and 158.0° respectively and the conformations about the C(4')A-C(5')A bond of the adenosyl sugar and about the C(4')T-C(5')T bond of the thymidyl sugar are both in the preferred gauche-gauche range.

MOLECULAR PACKING AND HYDROGEN BONDING

The most interesting feature of the molecular packing of d-ApT is a reversed Hoogsteen base pairing (21-23) between symmetry related adenine and thymine bases to yield an infinite hydrogen bonded network linking the molecules in the lattice, Figs. 4 and 5. The N(6)A...O(2)T and N(3)T... N(7)A hydrogen bonding distances are 3.07 and 2.78 Å respectively, Table 4. The paired adenine and thymine bases are nearly coplanar to each other with an angle of 5.8° between the plane normals. Adenine and thymine bases of adjacent nucleotides are stacked, Fig. 6.

The two water molecules contained in the crystal lattice are disordered with two major occupancy sites (W1 und W2) and three minor sites (w', w'' and w''') which add up to a total of two water molecules. They serve as hydrogen bond donors and acceptors with respect to the d-ApT molecule and also have hydrogen bonded interactions between each other. Table 4 displays the hydrogen bonded distances within this crystal structure.

ACKNOWLEDGEMENT

K.K. Chacko thanks the Alexander von Humboldt Stiftung for the award of a fellowship. All computations were carried out using the UNIVAC 11/82 of the Gesellschaft für Wissenschaftliche Datenverarbeitung, Göttingen. This work was supported in part by a grant from the National Institutes of Health, GM 16066, to Professor Paul O.P. Ts'o whose interest and encouragement is gratefully acknowledged.

§Present address: Department of Crystallography and Biophysics, University of Madras, Madras 600 025, India

+Present address: Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, D-1000 Berlin 33, FRG

*Dedicated to Professor Dr.Friedrich Cramer on the occasion of his 60th birthday

REFERENCES

1 Hingerty, B., Subramanian, E., Stellman, S.D., Sato, T. Broyde, S.D. and Langridge, R. (1976) Acta Cryst. B32, 2998-3013
2 Rosenberg, J.M., Seeman, N.C., Day, R.O. and Rich, A. (1976) J. Mol. Biol. 104, 145-167
3 Day, R.O., Seeman, N.C., Rosenberg, J.M. and Rich, A. (1973) Proc. Natl. Acad. Sci., USA, 70, 849-853
4 Seeman, N.C., Rosenberg, J.M., Suddath, F.L., Kim, J.J.P. and Rich, A. (1976) J. Mol. Biol. 104, 100-144
5 Suck, D., Manor, P.C. and Saenger, W. (1976) Acta Cryst. B32, 1727-1737
6 Dickerson, R.E., Drew, H.R., Conner, B.N., Wing, R.M. Fratini, A.V. and Kopka, M.L. (1982) Science 216, 475-485; Dickerson, R.E., Kopka, M.L. and Drew, H.R., in Srinivasan, R. and Sarma, R.H., Eds. Conformation in Biology (1983) pp. 227-257. Adenine Press, Guilderland, New York
7 Wang, A.H.-J., Fujii, S., van Boom, J.H., van der Marel, G.A. van Boeckel, S.A.A. and Rich A. (1982) Nature 299, 601-604
8 Miller, P.S., Yano, Y., Yano, E., Carrol, C., Jayaraman, K. and Ts'o, P.O.P. (1979) Biochemistry 18, 5134-5143
9 Kan, L.S., Cheng, D.M., Miller, P.S., Yano, Y. and Ts'o, P.O.P. (1980) Biochemistry 19, 2122-2132
10 Stout, G.H. and Jensen, L.H. (1968) X-ray structure determination, p. 457. Macmillan, New York
11 Main, P., Lessinger, L., Woolfson, M.M., Germain, G. and Declerq, J.P. (1977) MULTAN: A system of computer programs for the automatic solution of non-centrosymmetric crystal structures, Univs. of York, England and Louvain, Belgium
12 International Tables for X-ray Crystallography, Vol. III (1962) Kynoch Press, Birmingham
13 Voet, D. and Rich A. (1970) Prog. Nucl. Acid Res. Mol. Biol. 10, 183-265
14 Samuel, G. and Weiss, R. (1969) Tetrahedron Lett. 33, 2803-2806
15 Samuel, G. and Weiss, R. (1969) Tetrahedron Lett. 33, 3529-3532
16 Samuel, G. Ph. (1969) D. Dissertation, Univ. of Strasbourg, France
17 Barnes, C.L. and Hawkinson, S.W. (1979) Acta Cryst. B35, 1724-1727
18 Altona, C. and Sundaralingam, M. (1972) J. Amer. Chem. Soc. 94, 8205-8212

19 Levitt, M. and Warshel, A. (1978) J. Amer. Chem. Soc. 100, 2607-2613
20 Pullman, B., Saenger, W., Sasisekharan, V., Sundaralingam, M. and Wilson, H.R. (1973) The Jerusalem Symposia on Quantum Chemistry and Biochemistry, V, eds. Bergman, E.D. and Pullman, B., pp. 815-820
21 Hoogsteen, K. (1963) Acta Cryst. 16, 907-912
22 Haschemeyer, A.E.V. and Sobell, H.M. (1963) Proc. Natl. Acad. Sci . USA 50, 872-877
23 Katz, L., Tomita, K. and Rich, A. (1965) J. Mol. Biol. 13, 340-350