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The Structure of a Hydrated 1:2 Complex of Adenylyl(3′-5′)adenosine–Proflavine Hemisulphate

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Abstract

The 1:2 complex (M_r = 1410·28) between adenylyl(3′-5′)adenosine (ApA), $C_{20}H_{25}N_{10}O_{10}P$, and proflavine hemisulphate, $2C_{13}H_{12}N_3^+.SO_4^{2-}$, crystallizes with 16·5 water molecules in the space group $P2_12_12$ and unit-cell parameters a = 32·157 (5), b = 21·450 (4) and c = 10·175 (1) Å; V = 7018·4 Å^3, Z = 4, d_c = 1·33, d_m = 1·32 Mg m^{-3}, μ(Cu $K\alpha$) = 1·32 mm^{-1}, $F(000)$ = 2980. Crystal data were measured up to 2θ = 120° with Cu $K\alpha$ radiation. The structure was determined by a multisolution phase method and refined by a blocked-mode full-matrix least-squares procedure. The final R factor is 0·118 for 3507 observed data. The dinucleoside phosphate, ApA, in this structure has a very unusual conformation which is not found in other oligonucleotide structures. The backbone of ApA is extended and each adenine ring is hydrogen bonded to another symmetry-related one forming an adenine–adenine base pair. Each base pair is sandwiched by proflavine cations which also stack with each other. Solvent molecules lie in the continuous channels between columns of stacked heterocyclic rings.

Introduction

The study of small-molecule–nucleic-acid interactions has been concerned mostly with the intercalative mode of binding of planar chromophores between base pairs in double-helical DNA and RNA (Lerman, 1961; Neidle, 1979). However, these molecules, some of which are drugs, are also known to bind to single-stranded nucleic acids such as synthetic polyribonucleotides (Dourlent & Hélène, 1971) and naturally occurring tRNA (Urbanke, Römer & Maass, 1973). The exact nature of that binding is not well understood at present because of the conformational flexibility of RNA. In this study, we present a high-resolution X-ray crystallographic analysis of the structure of proflavine complexed with the non-self-complementary dinucleoside phosphate, ApA (Neidle, Taylor, Sanderson, Shieh & Berman, 1978). There have been many solution studies on this dinucleoside phosphate (Ts'o, Kondo, Schweizer & Hollis, 1969; Lee, Ezra, Kondo, Sarma & Danyluk, 1976; Evans & Sarma, 1976) in an attempt to determine the extent of its conformational flexibility and whether or not it obeys the 'rigid nucleotide principle' (Yathindra & Sundaralingam, 1973). It has been suggested (Berthod & Pullman, 1973) that this principle pertains only in certain crystalline environments. We show in this study that in the presence of proflavine, the conformation of the dinucleoside phosphate has many unusual features.

Experimental

Deep-red rectangular prismatic crystals were grown from an aqueous solution of ApA and proflavine hemisulphate in a 1:2 ratio. The UV spectrum of a washed crystal clearly indicated complex formation. A crystal of size 0·45 × 0·3 × 0·2 mm was cut from a bigger crystal and sealed inside a capillary tube for all subsequent X-ray measurements.

Reflection data were collected in the θ–2θ scan mode with a variable scan rate using a Syntex $P\bar{1}$ automated diffractometer and graphite-monochromated Cu $K\alpha$ radiation (λ = 1·5418 Å). A total of 5695 unique reflections up to 2θ = 120° were obtained. Intensities were corrected for Lorentz and polarization effects; no absorption correction was applied. The standard deviation (σ_I) of each intensity (I) was calculated based

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on counter statistics and instrumental instability (Stout & Jensen, 1968). 3507 reflections with $I \geq 2{\cdot}33\sigma_I$ were considered to be significant and used for structure refinement. The occurrence of so many unobserved reflections (2188) is probably due to the disordered water network discussed in a subsequent section.

Initial attempts to solve the structure with *MULTAN* (Main, Woolfson & Germain, 1971) were unsuccessful. The structure was solved by an alternative direct-methods approach. The phases of the *hk*0 reflections were obtained by a multisolution method (Sheldrick, 1976) using 12 unknown phases in the starting set. The phases for the best set, as judged by a negative quartet test, were then used as starting points in a tangent-formula phase expansion using the full three-dimensional set of E values above 1·4. The resulting E map showed two full proflavine rings, a phosphate group and most of the atoms comprising the adenine bases. Two consecutive difference Fourier syntheses revealed the rest of the structure. In an independent manner, use of superposition methods on this problem also determined the structure. The P atom was located from the high-resolution Patterson map (using only those reflections with θ greater than 30°). A fourfold superposition of the Patterson functions on the four equivalent positions of the P atom revealed most of the structure.

This is a highly solvated structure. Lack of strong interactions among some of the solvent molecules makes them highly disordered. Therefore, the locations of these solvent molecules were difficult to determine and were sometimes ambiguous. A combination of least-squares refinement and difference Fourier syntheses was carried out step by step to determine unequivocally all 24 positions occupied by 16·5 water molecules and so complete the structure.

At one point, careful examination of the calculated and observed structure factors showed that the major discrepancies lay in the weak reflections and the low-resolution reflections. Therefore, 33 reflections with $F_o < 16{\cdot}5$ and 44 reflections with $\sin\theta/\lambda < 0{\cdot}10$ Å^{-1} were removed from the refinement. The weighting scheme used for the least-squares minimization was: $w = [\sigma^2(F) + 0{\cdot}005F_o^2]^{-1}$. Because of the limitation of the computer-memory storage, the structure was divided into several blocks and all refinements were carried out in a blocked-mode full-matrix least-squares procedure. The atomic scattering factors and dispersion corrections were taken from *International Tables for X-ray Crystallography* (1974).

The positions and anisotropic temperature factors of the ApA and proflavine molecules were varied throughout all refinement cycles. The solvent molecules were divided into four categories:

(1) Sulphate ion: In order to account for the electron density, two idealized tetrahedra of O atoms with occupancy factor of 0·5 on each were placed around the S atom. Their positions were fixed and their isotropic temperature factors were varied in the first few refinements and then fixed. The S atom was refined anisotropically.

(2) Primary water shell: *WP*(01), *WP*(02)... *WP*(11) (where *W* and *P* stand for water and primary shell respectively).

There were 11 water molecules which made direct hydrogen bonds to either ApA or the proflavines. Their positions and anisotropic temperature factors were refined. The occupancy factors (G) were all assigned 1·0 except *WP*(01), which was at a special position with $G = 0{\cdot}5$. Judging from their rather high temperature factors, the water molecules *WP*(07), *WP*(08), *WP*(09), and *WP*(10) may be disordered.

(3) Secondary (S) water shell: *WS*(01), *WS*(02), *WS*(03). Three water molecules hydrogen bond to water in the primary shell. They were given isotropic temperature factors with $G = 0{\cdot}5$. Both their positions and temperature factors were varied.

(4) Disordered (D) water molecules: *WD*(01), *WD*(02), ... *WD*(10). Ten electron density peaks which did not make any unreasonable contacts with all other molecules and some short distances and unusual geometry among themselves were described as disordered water molecules. They are located in the weak polar region of the molecular channel. They were assigned isotropic temperature factors with $G = 0{\cdot}5$ except *WD*(09) and *WD*(10), which are at special positions, having $G = 0{\cdot}25$. Their positions and temperature factors were refined.

No attempt was made to locate H atoms. However, the positions of 30 non-hydrogen-bonded H atoms were calculated and included in the structure factor calculation.

The refinement converged at $R = 0{\cdot}106$ and $R_w = 0{\cdot}139$ for 3431 selected observed data, and $R = 0{\cdot}118$ and $R_w = 0{\cdot}171$ for all 3507 observed data.* The difference Fourier map at this point showed no electron density greater than 0·42 e Å^{-3}.

* List of structure factors and anisotropic temperature parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36329 (27 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

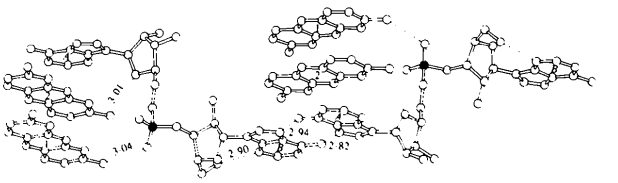

Fig. 1. A perspective view of two ApA–2proflavine complexes to illustrate the base pairing, hydrogen bonding and ring stacking. Dashed lines indicate hydrogen bonds. Distances are in Å.

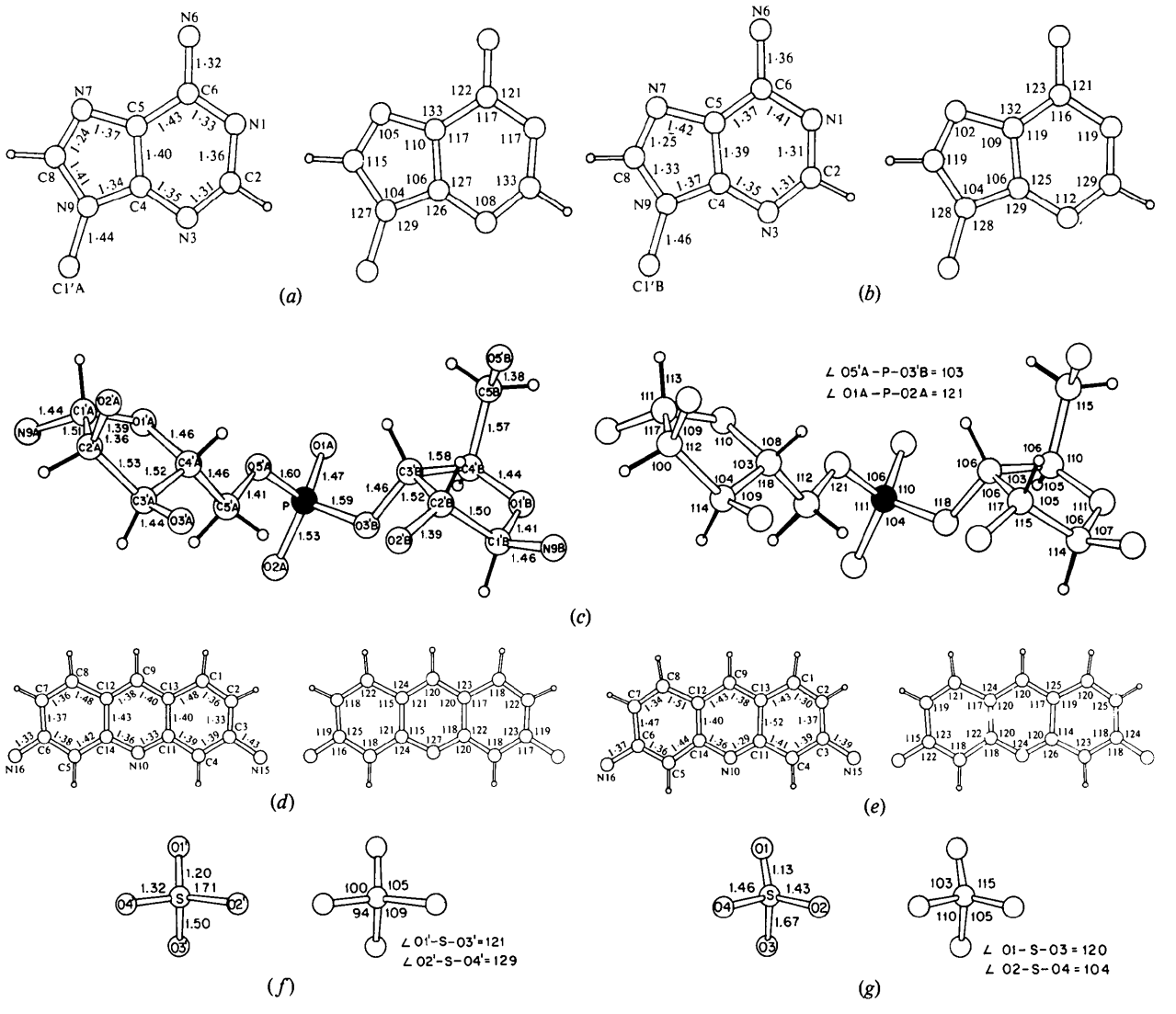

Fig. 2. Numbering system and molecular geometry, showing all covalent bond distances and angles involving non-hydrogen atoms. The 30 H atoms whose positions were calculated and which are included in the structure factor calculations are in the drawing. Those H atoms which participate in the protonation of N(10) positions on proflavine 1 and proflavine 2 and N(1) positions on adenine *A* and adenine *B* are not shown since they were never calculated. The average e.s.d. in bond lengths is 0·01 Å and in angles is 1°. (*a*) Adenine *A*. (*b*) Adenine *B*. (*c*) Ribose phosphate backbone. (*d*) Proflavine 1. (*e*) Proflavine 2. (*f*) Disordered sulphate 1. (*g*) Disordered sulphate 2.

The molecular structure

Each asymmetric unit in the crystal consists of the dinucleoside phosphate ApA in an extended conformation, two proflavine molecules stacked below an adenine, a sulphate anion and several water molecules forming a highly hydrated 1:2 complex. Fig. 1 shows two complexes which interact with each other through base pairing. The atomic parameters are given in Table 1, distances and angles in Fig. 2, molecular planes in Table 2 and torsion angles in Fig. 3 and Table 3.

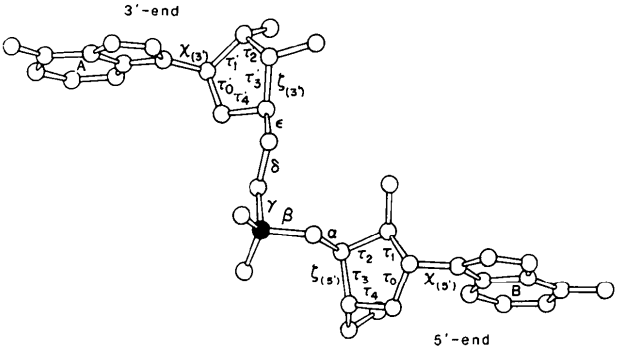

Fig. 3. Molecular configuration of ApA, illustrating the nomenclature of the conformation angles.

Table 1. *Fractional coordinates and equivalent isotropic temperature factors* (Å²) *with e.s.d.'s in parentheses* ($B_{eq} = \langle B_{ii} \rangle$)

	x	y	z	B_{eq} or B_{iso}
(*a*) ApA molecule				
N(1*A*)	0·1237 (3)	0·5063 (6)	1·2993 (11)	7·7 (6)
C(2*A*)	0·1623 (4)	0·5309 (6)	1·3143 (15)	7·7 (8)
N(3*A*)	0·1994 (3)	0·5056 (5)	1·3244 (10)	6·3 (5)
C(4*A*)	0·1952 (3)	0·4434 (6)	1·3139 (11)	5·6 (6)
C(5*A*)	0·1584 (3)	0·4096 (6)	1·2994 (11)	5·8 (5)
C(6*A*)	0·1204 (3)	0·4444 (6)	1·2989 (11)	5·2 (6)
N(6*A*)	0·0839 (3)	0·4169 (6)	1·2878 (11)	7·3 (6)
N(7*A*)	0·1670 (3)	0·3474 (5)	1·2871 (9)	5·9 (5)
C(8*A*)	0·2055 (3)	0·3439 (6)	1·2942 (13)	6·6 (6)
N(9*A*)	0·2261 (3)	0·4015 (5)	1·3103 (9)	5·4 (5)
C(1′*A*)	0·2702 (3)	0·4124 (6)	1·3087 (11)	5·5 (5)
O(1′*A*)	0·2859 (2)	0·4054 (4)	1·1822 (7)	5·4 (4)
C(2′*A*)	0·2961 (4)	0·3741 (6)	1·4030 (12)	6·2 (5)
O(2′*A*)	0·3264 (2)	0·4083 (5)	1·4631 (8)	8·2 (5)
C(3′*A*)	0·3140 (4)	0·3253 (6)	1·3087 (13)	6·2 (5)
O(3′*A*)	0·3531 (3)	0·2990 (5)	1·3510 (9)	8·5 (5)
C(4′*A*)	0·3205 (3)	0·3613 (5)	1·1818 (11)	4·8 (5)
C(5′*A*)	0·3193 (4)	0·3268 (7)	1·0584 (12)	7·3 (7)
O(5′*A*)	0·3191 (2)	0·3671 (4)	0·9493 (8)	5·7 (4)
P	0·3200 (1)	0·3400 (2)	0·8032 (3)	5·6 (1)
O(1*A*)	0·3157 (2)	0·3941 (4)	0·7158 (7)	6·5 (4)
O(2*A*)	0·2909 (2)	0·2837 (5)	0·7903 (9)	8·3 (5)
C(1′*B*)	0·4547 (3)	0·2833 (4)	0·7282 (11)	4·2 (4)
O(1′*B*)	0·4483 (2)	0·3225 (3)	0·6193 (8)	5·3 (4)
C(2′*B*)	0·4375 (3)	0·3178 (5)	0·8439 (10)	4·0 (5)
O(2′*B*)	0·4299 (3)	0·2815 (4)	0·9539 (8)	7·0 (5)
C(3′*B*)	0·4008 (3)	0·3539 (5)	0·7890 (12)	5·4 (5)
O(3′*B*)	0·3655 (2)	0·3113 (3)	0·7916 (8)	5·4 (4)
C(4′*B*)	0·4126 (3)	0·3619 (6)	0·6394 (13)	5·7 (6)
C(5′*B*)	0·4256 (4)	0·4321 (7)	0·6229 (21)	9·7 (9)
O(5′*B*)	0·4557 (3)	0·4523 (4)	0·7103 (15)	10·6 (7)
N(1*B*)	0·6030 (3)	0·3735 (5)	0·7518 (10)	6·5 (5)
C(2*B*)	0·5665 (3)	0·4010 (5)	0·7512 (13)	6·2 (6)
N(3*B*)	0·5293 (3)	0·3759 (4)	0·7429 (10)	5·6 (5)
C(4*B*)	0·5310 (3)	0·3129 (5)	0·7384 (9)	4·4 (5)
C(5*B*)	0·5675 (3)	0·2780 (6)	0·7409 (10)	5·5 (5)
C(6*B*)	0·6049 (3)	0·3081 (5)	0·7351 (11)	5·2 (5)
N(6*B*)	0·6421 (3)	0·2779 (5)	0·7425 (10)	6·3 (5)
N(7*B*)	0·5570 (2)	0·2139 (4)	0·7326 (9)	4·8 (4)
C(8*B*)	0·5181 (3)	0·2155 (5)	0·7298 (10)	4·5 (5)
N(9*B*)	0·4992 (2)	0·2707 (4)	0·7357 (9)	4·1 (4)
(*b*) Proflavine cations				
C(1*P*1)	0·1240 (4)	0·4491 (7)	0·6281 (13)	7·2 (6)
C(2*P*1)	0·1631 (4)	0·4735 (6)	0·6366 (13)	6·0 (6)
C(3*P*1)	0·1966 (3)	0·4375 (6)	0·6405 (10)	5·4 (5)
C(4*P*1)	0·1948 (4)	0·3728 (6)	0·6341 (11)	5·8 (5)
C(5*P*1)	0·1149 (4)	0·1843 (6)	0·5963 (14)	6·6 (6)
C(6*P*1)	0·0764 (4)	0·1566 (5)	0·5862 (12)	6·0 (7)
C(7*P*1)	0·0391 (4)	0·1875 (6)	0·5874 (12)	6·4 (6)
C(8*P*1)	0·0399 (4)	0·2511 (9)	0·5909 (14)	7·6 (8)
C(9*P*1)	0·0809 (3)	0·3510 (7)	0·6077 (11)	6·3 (6)
N(10*P*1)	0·1525 (3)	0·2838 (5)	0·6083 (9)	5·6 (5)
C(11*P*1)	0·1560 (4)	0·3453 (6)	0·6219 (10)	4·9 (5)
C(12*P*1)	0·0792 (4)	0·2869 (8)	0·6017 (11)	6·5 (6)
C(13*P*1)	0·1194 (4)	0·3806 (6)	0·6182 (11)	5·1 (5)
C(14*P*1)	0·1165 (3)	0·2503 (5)	0·6023 (11)	5·1 (5)
N(15*P*1)	0·2367 (3)	0·4655 (5)	0·6518 (10)	6·7 (5)
N(16*P*1)	0·0762 (4)	0·0947 (6)	0·5783 (14)	9·4 (8)
C(1*P*2)	0·1335 (5)	0·4926 (7)	0·9687 (15)	8·0 (8)
C(2*P*2)	0·1719 (5)	0·5110 (6)	0·9732 (13)	6·9 (7)
C(3*P*2)	0·2059 (5)	0·4726 (5)	0·9795 (12)	6·4 (7)
C(4*P*2)	0·1995 (3)	0·4086 (8)	0·9724 (11)	6·7 (6)
C(5*P*2)	0·1093 (3)	0·2304 (5)	0·9358 (11)	5·2 (5)
C(6*P*2)	0·0706 (5)	0·2057 (7)	0·9239 (12)	7·8 (7)
C(7*P*2)	0·0329 (4)	0·2441 (7)	0·9244 (12)	6·9 (7)
C(8*P*2)	0·0363 (4)	0·3060 (9)	0·9385 (13)	8·2 (8)
C(9*P*2)	0·0845 (4)	0·4028 (6)	0·9528 (15)	7·6 (6)
N(10*P*2)	0·1521 (3)	0·3223 (4)	0·9557 (8)	5·1 (4)
C(11*P*2)	0·1598 (3)	0·3812 (6)	0·9635 (11)	5·0 (5)
C(12*P*2)	0·0786 (3)	0·3367 (8)	0·9462 (12)	7·1 (6)
C(13*P*2)	0·1241 (4)	0·4273 (6)	0·9649 (12)	5·4 (5)
C(14*P*2)	0·1132 (3)	0·2973 (6)	0·9464 (11)	5·2 (5)
N(15*P*2)	0·2465 (4)	0·4942 (4)	0·9898 (10)	8·0 (5)
N(16*P*2)	0·0639 (4)	0·1427 (6)	0·9154 (12)	9·4 (7)
(*c*) Solvent molecules (the occupany, G, is ½ unless indicated otherwise)				
WP(01)	0·000	0·000	0·615 (3)	14·7 (13)
WP(02)†	0·223 (1)	0·247 (1)	0·953 (1)	11·7 (4)
WP(03)†	0·433 (1)	0·312 (1)	1·226 (2)	18·3 (10)
WP(04)†	0·396 (1)	0·166 (1)	0·923 (2)	19·7 (6)
WP(05)†	0·230 (1)	0·222 (1)	0·582 (2)	16·4 (12)
WP(06)†	0·314 (1)	0·164 (1)	0·911 (2)	17·6 (7)
WP(07)†	0·343 (1)	0·530 (1)	1·389 (5)	38·9 (25)
WP(08)†	0·162 (1)	0·023 (1)	1·206 (5)	52·6 (19)
WP(09)†	0·266 (1)	0·591 (1)	1·348 (6)	46·5 (36)
WP(10)†	0·133 (2)	0·040 (2)	0·926 (9)	74·3 (44)
WP(11)†	0·337 (1)	0·204 (1)	1·506 (3)	26·9 (14)
WS(01)	0·449 (2)	0·486 (2)	1·035 (5)	22·8 (17)*
WS(02)	0·475 (1)	0·405 (1)	1·197 (3)	15·1 (9)
WS(03)	0·187 (2)	0·116 (3)	0·875 (7)	26·4 (21)
WD(01)	0·367 (2)	0·159 (3)	1·204 (7)	29·4 (24)
WD(02)	0·419 (1)	0·131 (2)	1·251 (4)	16·1 (10)
WD(03)	0·459 (1)	0·073 (1)	1·239 (3)	14·4 (8)
WD(04)	0·482 (1)	0·037 (2)	1·462 (4)	15·4 (10)
WD(05)	0·489 (1)	0·023 (1)	1·224 (3)	14·1 (9)
WD(06)	0·442 (3)	0·093 (5)	1·416 (9)	34·5 (35)
WD(07)	0·063 (1)	0·591 (1)	1·211 (2)	10·1 (5)
WD(08)	0·023 (1)	0·535 (1)	1·183 (1)	4·9 (3)
WD(09)‡	0·000	0·500	1·196 (6)	17·5 (19)
WD(10)‡	0·000	0·500	1·060 (8)	21·6 (23)
S(04)‡	0·249 (1)	0·148 (1)	1·230 (1)	14·3 (14)
O(1SO4′)	0·250	0·104	1·302	14·4
O(2SO4′)	0·300	0·169	1·210	26·5
O(3SO4′)	0·224	0·205	1·263	12·7
O(4SO4′)	0·222	0·128	1·142	34·3
O(1SO4)	0·242	0·196	1·269	16·7
O(2SO4)	0·277	0·112	1·308	20·2
O(3SO4)	0·266	0·139	1·076	25·7
O(4SO4)	0·209	0·116	1·244	16·3

* Values for *WS*(01) to *WD*(10) and O(1SO4′) to O(4SO4) are B_{iso}.
† Occupancy 1.
‡ Occupancy ¼.

The base pairs

There are two adenine rings in the molecule, the 3′ adenine being designated *A* and the 5′ adenine *B*. Because the crystals were obtained at a low pH, the ApA most probably exists as a zwitterion with a

Table 2. *Deviations* (Å) *of atoms from the least-squares planes through the indicated atoms* (*average e.s.d. is* 0·01 Å)

Adenine atoms	Adenine *A*	Adenine *B*	Adenine atoms	Adenine *A*	Adenine *B*
N(1)	−0·05	0·01	N(7)	−0·02	−0·01
C(2)	−0·03	0·01	C(8)	−0·03	−0·00
N(3)	0·03	−0·02	N(9)	−0·01	0·02
C(4)	0·03	−0·01	N(6)	0·07*	−0·04*
C(5)	0·03	0·01	C(1′)	−0·14*	−0·02*
C(6)	0·05	−0·01			
R.m.s. deviation	0·03	0·01		0·03	0·01
Ribose atoms	3′-Ribose	5′-Ribose	Ribose atoms	3′-Ribose	5′-Ribose
C(1′)	−0·02	0·25*	C(3′)	−0·56*	0·0
O(1′)	0·02	0·0	C(4′)	−0·01	0·0
C(2′)	0·01	−0·25*			
R.m.s. deviation	0·01	0·0		0·01	0·0
Proflavine atoms	Proflavine 1	Proflavine 2	Proflavine atoms	Proflavine 1	Proflavine 2
C(1)	0·00	−0·02	C(9)	−0·02	−0·00
C(2)	−0·01	−0·05	N(10)	−0·05	0·02
C(3)	0·02	0·00	C(11)	0·01	0·02
C(4)	0·03	0·01	C(12)	−0·00	0·02
C(5)	0·00	−0·01	C(13)	−0·01	0·03
C(6)	−0·00	−0·04	C(14)	−0·01	0·01
C(7)	0·04	−0·03	N(15)	0·03*	0·02*
C(8)	−0·00	0·04	N(16)	−0·01*	−0·05*
R.m.s. deviation	0·02	−0·03		0·02	−0·03

* Atoms not involved in least-squares-plane calculations.

Table 3. *Conformation angles* (°) (*e.s.d.'s* 1–2°)

Backbone and glycosidic bonds		
$\chi(5')$	C(8*B*)–N(9*B*)–C(1′*B*)–O(1′*B*)	240°
$\psi(5')$	C(5′*B*)–C(4′*B*)–C(3′*B*)–O(3′*B*)	143
α	C(4′*B*)–C(3′*B*)–O(3′*B*)–P	262
β	C(3′*B*)–O(3′*B*)–P–O(5′*A*)	291
γ	O(3′*B*)–P–O(5′*A*)–C(5′*A*)	291
δ	P–O(5′*A*)–C(5′*A*)–C(4′*A*)	177
ε	O(5′*A*)–C(5′*A*)–C(4′*A*)–C(3′*A*)	171
$\psi(3')$	C(5′*A*)–C(4′*A*)–C(3′*A*)–O(3′*A*)	85
$\chi(3')$	O(1′*A*)–C(1′*A*)–N(9*A*)–C(8*A*)	71
Ribose, endocyclic bonds		
τ_0	C(4′*B*)–O(1′*B*)–C(1′*B*)–C(2′*B*)	−28
τ_1	O(1′*B*)–C(1′*B*)–C(2′*B*)–C(3′*B*)	34
τ_2	C(1′*B*)–C(2′*B*)–C(3′*B*)–C(4′*B*)	−26
τ_3	C(2′*B*)–C(3′*B*)–C(4′*B*)–O(1′*B*)	10
τ_4	C(3′*B*)–C(4′*B*)–O(1′*B*)–C(1′*B*)	11
$\tau_{0'}$	C(4′*A*)–O(1′*A*)–C(1′*A*)–C(2′*A*)	3
$\tau_{1'}$	O(1′*A*)–C(1′*A*)–C(2′*A*)–C(3′*A*)	−24
$\tau_{2'}$	C(1′*A*)–C(2′*A*)–C(3′*A*)–C(4′*A*)	35
$\tau_{3'}$	C(2′*A*)–C(3′*A*)–C(4′*A*)–O(1′*A*)	−34
$\tau_{4'}$	C(3′*A*)–C(4′*A*)–O(1′*A*)–C(1′*A*)	20

negative phosphate group and a protonated adenine much as was found in the structures of ApA^+pA^+ (Suck, Manor & Saenger, 1976) and UpA^+ (Sussman, Seeman, Kim & Berman, 1972). Since the distances and angles (Fig. 2) have average estimated standard deviations of 0·01 Å and 1° respectively, it is not possible to utilize them to assign definitely the protonation site. Consideration of the hydrogen-bonding environment (see discussion later) suggests that it is the N(1) of adenine *B* which bears the positive charge. The distances and angles in both adenine rings are unremarkable with the exception of the C(8)–N(7) distances in both rings which are shorter and the N(9)–C(8)–N(7) angle in ring *B* which is greater than the corresponding values found in other adenine structures.

The adenine rings are each planar (Table 2) within ±0·05 Å. Adenine *A* forms hydrogen bonds to adenine *B* of a symmetry-related molecule *via* N(6) and N(7). This hydrogen-bonding pattern was first proposed for acid poly(A^+) (Rich, Davis, Crick & Watson, 1961) and occurs in a variety of adenine-containing crystal structures including UpA^+ (Sussman *et al.*, 1972) and ApA^+pA^+ (Suck *et al.*, 1976). The average deviation of the plane of the base pairs is 0·2 Å and the tilt between bases 5°.

The conformation of ApA

The dinucleoside phosphate adopts an extended conformation (Fig. 3) which results from some unusual values in the torsion angles of the nucleoside segments; this is in contrast to the situation observed in other extended dinucleoside phosphates where only the torsion angles of phosphodiester linkage differ from the normal helical conformations (Sussman *et al.*, 1972).

The two sugars differ from one another. The 5′ ribose has a C(1′)-*exo*, C(2′)-*endo* conformation with a pseudorotation parameter (P_s) (Altona & Sundaralingam, 1972) of 142°. The glycosidic torsion angle (χ) is −120° and is thus *syn*; the free 5′-hydroxyl forms a hydrogen bond with N(3) of the adenine perhaps stabilizing this unusual conformation. While the *syn* conformation is not theoretically disallowed as has been shown by energy calculations (Pullman & Saran, 1976), it has been observed in very few crystals of nucleosides (Suck, Saenger & Vorbrüggen, 1972; Tavale & Sobell, 1970; Bugg & Thewalt, 1969; Subramanian, Madden & Bugg, 1973). The only *syn*-containing oligonucleotide structures are alternating guanosine–cytidine ones such as the recently reported left-handed helical hexamer α(CpGpCpGpCpG) (Wang *et al.*, 1979). The other ribose in the complex is in the C(3′)-*endo* conformation (P_s = 14°). χ is in the high-*anti* (71°) conformation as has been observed in the structures of other drug–dinucleoside phosphate complexes (Neidle

et al., 1977; Berman *et al.*, 1979; Tsai, Jain & Sobell, 1977; Jain, Tsai & Sobell, 1977). The C(2′)-*endo* (3′ → 5′) C(3′)-*endo* ordering of the mixed sugar pucker occurs in the crystal structure of the complex between ApU and 9-aminoacridine (Seeman, Day & Rich, 1975), the only other non-intercalated complex where structure has been reported. In the self-complementary ribodinucleoside intercalative complexes with mixed puckering the order is C(3′)-*endo*–C(2′)-*endo*.

Some significant conformational angles are presented in Table 3. The α angle of the molecule is 262°. As has been observed in the structures of pTpT (Camerman, Fawcett & Camerman, 1976) and tRNA (Jack, Ladner & Klug, 1976; Holbrook, Sussman, Warrant & Kim, 1978) this high value can be correlated with C(2′)-*endo* sugar puckering. When sugars are C(3′)-*endo* the value of α has always been found to be about 210° (Arnott & Hukins, 1969). The value of ε is 171° and together with the increased α is responsible for stretching apart the planes of the bases from the usual 3·4 Å to 6·3 Å. The *trans* value for this ε angle is not usually found in 5′ nucleotides although there is no *a priori* theoretical basis for this angle to be always *gauche* (Pullman & Saran, 1976). The *trans* value has been observed in dGMP (Young, Tollin & Wilson, 1974), the modified nucleotide azaUMP (Saenger & Suck, 1973), and in a few residues of tRNA. Curiously, the otherwise variable β and γ angles have almost precisely the values found in RNA 11 (Arnott, Hukins & Dover, 1972), as does δ.

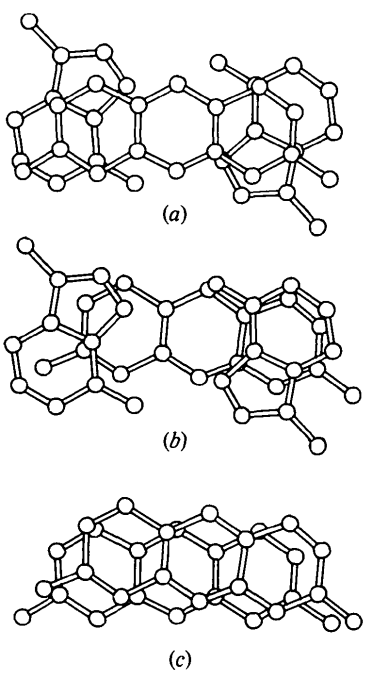

Fig. 4. The stacking patterns of the molecular complex. (*a*) Proflavine 1/base pair. (*b*) Base pair/proflavine 2. (*c*) Proflavine 2/proflavine 1.

Table 4. *Hydrogen bonds and short intermolecular contacts* (Å)

Positional parameters of the O atoms of the SO_4^{2-} ion were not refined. Distances involving these atoms are marked with an asterisk.

(*a*) Intramolecular hydrogen bonds

N(3*B*)···O(5′*B*)	2·90 (1)		

(*b*) ApA → ApA

N(6*A*)···N(7*B*^{i})	2·94 (2)	N(7*A*)···N(6*B*^{i})	2·82 (2)
O(2′*A*)···O(1*A*^{ii})	2·61 (1)		

(*c*) ApA · proflavine, sulphate and water molecules

N(1*A*)···*WD*(07)	2·81 (3)	O(2*A*)···*WP*(02)	2·85 (1)
N(3*A*)···*WP*(09)	2·83 (3)	O(2*A*)···*WP*(05)	3·17 (2)
C(8*A*)···O(3SO4′)	3·05 (1)*	O(2*A*)···*WP*(06)	2·94 (2)
O(1′*A*)···N(15*P*2)	3·01 (1)	O(2′*B*)···*WP*(03)	2·85 (2)
O(2′*A*)···*WP*(07)	2·77 (3)	O(2′*B*)···*WP*(04)	2·72 (3)
O(3′*A*)···*WP*(03)	2·88 (2)	N(1*B*)···*WP*(08^{iv})	2·95 (3)
O(3′*A*)···*WP*(11)	2·63 (3)	N(6*B*)···O(3SO4^{iv})	2·66 (1)*
O(1*A*)···N(15*P*1)	3·04 (1)	N(6*B*)···O(4SO4^{iv})	3·13 (1)*
O(1*A*)···*WP*(08^{iii})	2·97 (3)		

(*d*) Proflavine → sulphate and water molecules

N(10*P*1)···*WP*(05)	2·84 (2)	N(16*P*1)···*WP*(07^{v})	2·96 (3)
N(15*P*1)···O(1SO4′^{iii})	3·04 (1)*	N(10*P*2)···*WP*(02)	2·79 (1)
N(15*P*1)···O(2SO4^{iii})	3·20 (1)*	N(15*P*2)···O(3SO4^{iii})	3·20 (1)*
N(16*P*1)···*WP*(01)	3·20 (1)	N(16*P*2)···*WP*(10)	3·13 (6)

(*e*) Sulphate → water molecules

O(2SO4′)···*WP*(06)	3·08 (2)*	O(3SO4)···*WP*(02)	2·97 (1)*
O(2SO4′)···*WD*(01)	2·17 (6)*	O(3SO4)···*WP*(06)	2·34 (2)*
O(4SO4′)···*WP*(02)	3·20 (1)*	O(4SO4)···*WP*(08)	2·53 (3)*
O(4SO4′)···*WP*(08)	3·04 (3)*		
O(4SO4′)···*WS*(03)	2·95 (7)*		

(*f*) Normal water → water molecules

WP(01)···*WS*(02^{i})	2·91 (3)	*WP*(07)···*WP*(09)	2·83 (4)
WP(02)···*WS*(03)	3·14 (7)	*WP*(08)···*WP*(10)	3·02 (10)
WP(03)···*WS*(02)	2·43 (3)	*WP*(09)···*WS*(03^{iii})	2·78 (9)
WP(04)···*WS*(06)	2·64 (3)	*WP*(10)···*WS*(01^{v})	2·91 (9)
WP(04)···*WD*(01)	3·01 (7)	*WP*(10)···*WS*(03)	2·44 (9)
WP(04)···*WD*(07^{v})	2·49 (3)	*WS*(01)···*WS*(02)	2·54 (6)
WP(05)···*WP*(09^{v})	2·90 (3)		

(*g*) Disordered water → disordered water molecules

WD(03)···*WD*(04)	2·51 (5)	*WD*(05)···*WD*(06)	2·89 (10)
WD(03)···*WD*(05^{vi})	2·66 (4)	*WD*(05)···*WD*(10^{iv})	2·95 (9)
WD(04)···*WD*(05)	2·45 (5)	*WD*(07)···*WD*(09)	2·82 (3)
WD(04)···*WD*(05^{vi})	2·90 (5)		

(*h*) Short contacts (≤2·2 Å) in group 1 disordered water molecules [*WD*(01) to *WD*(06)]

WD(01)···*WD*(02)	1·84 (7)	*WD*(03)···*WD*(06)	1·93 (10)
WD(02)···*WD*(03)	1·79 (5)	*WD*(04)···*WD*(04^{vi})	1·96 (6)
WD(02)···*WD*(06)	2·01 (10)	*WD*(04)···*WD*(06)	1·82 (11)
WD(03)···*WD*(05)	1·45 (4)	*WD*(05)···*WD*(05^{vi})	1·21 (4)

(*i*) Short contacts (≤2·2 Å) in group 2 disordered water molecules [*WD*(07) to *WD*(10)]

WD(07)···*WD*(08)	1·78 (3)	*WD*(08)···*WD*(10)	1·64 (6)
WD(08)···*WD*(08^{vii})	2·11 (3)	*WD*(09)···*WD*(10)	1·38 (10)
WD(08)···*WD*(09)	1·06 (2)		

(*j*) Short stacking contacts (≤3·40 Å)

N(1*A*)···C(1*P*2)	3·39 (2)	C(2*B*)···N(16*P*1^{viii})	3·37 (2)
C(4*A*)···C(3*P*1^{ii})	3·33 (2)	C(4*B*)···C(7*P*1^{viii})	3·33 (2)
C(6*A*)···C(1*P*1^{ii})	3·35 (2)	C(6*B*)···C(5*P*2^{iv})	3·35 (2)

Symmetry code

None x,y,z
(i) $-0·5+x, 0·5-y, 2-z$
(ii) $x,y,1+z$
(iii) $0·5-x, 0·5+y, 2-z$
(iv) $0·5+x, 0·5-y, 2-z$
(v) $0·5-x, -0·5+y, 2-z$
(vi) $1-x, -y, z$
(vii) $-x, 1-y, z$
(viii) $0·5+x, 0·5-y, 1-z$

The proflavine cations

Both proflavine cations are essentially planar and are protonated at N(10), as found in the crystal structure of proflavine hemisulphate (Jones & Neidle, 1975). They are stacked almost directly on top of one another, 3·4 Å apart. Unlike the proflavine molecules in the complex with CpG (Neidle *et al.*, 1977; Berman *et al.*, 1979) which are at an angle of 97° to each other the angle between the stacked proflavines in this crystal is 0° (Fig. 4*c*). The proflavines are symmetrically stacked above and below an adenine–adenine base pair (Fig. 4*a*,*b*). The central rings of the proflavines are not involved in this stacking. In this sense the stacking is similar to that found in the intercalated portion of CpG–proflavine (Berman *et al.*, 1979).

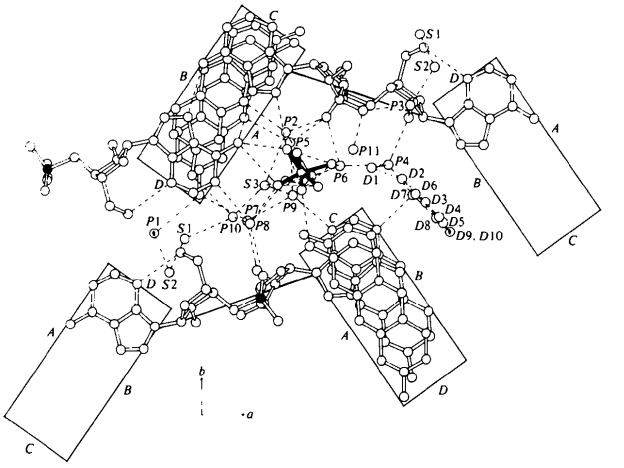

Fig. 5. A view of the structure perpendicular to the *ab* plane. Dashed lines indicate hydrogen bonds. The outlines of molecular stacking columns, chain connections, and water channels are shown. Two 2_z axes which are perpendicular to the paper are denoted by ♦. The origin of the unit cell is on the 2_z axis coinciding with water molecule *P*1. Water molecules are named as in Table 1 except that the prefix *W* is omitted.

The crystal structure

Proflavine–ApA associations

As shown in Fig. 1 each adenine *A* base-pairs to adenine *B* in a symmetrically related molecule to form an extended chain of dinucleoside molecules. The proflavine cations stack above and below each base pair. The sequence of stacked rings is thus proflavine, proflavine, base pair, proflavine, proflavine, ... in the **c** direction (Fig. 4). N(15) of proflavine 1 hydrogen bonds to O(1*A*) of a phosphate group and N(15) of proflavine 2 hydrogen bonds to the ribosyl O(1′) (Table 4). Amino–phosphate oxygen hydrogen bonds were also observed in CpG–proflavine (Berman *et al.*, 1979). The crystal structure consists of infinite columns of heterocyclic stacked planes extending along **c** linked by hydrophilic ribose–phosphate groups, forming zigzag chains along **a** (Fig. 5). Along **c**, translationally-related dinucleosides are connected by a hydrogen bond, O(2′*A*) of the 5′ ribose to the O(1) phosphate O atom (Table 4).

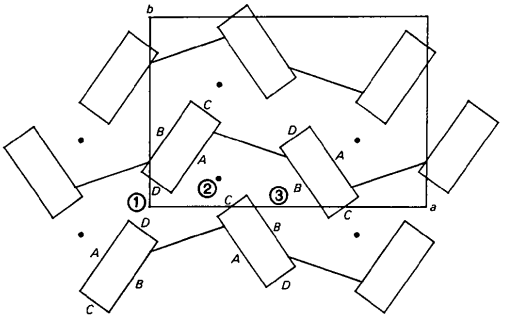

Fig. 6. A schematic representation of the crystal structure. This is an expansion of Fig. 5. The unit-cell outline and the nomenclature of stacking-block faces are shown. The three distinct channel regions are marked (1), (2) and (3). The lines connecting the blocks represent the sugar phosphate chain as shown in Fig. 5. The filled circles represent the sulphate ions.

The solvent structure

In addition to the 1:2 ApA–proflavine complex the asymmetric unit contains a disordered sulphate ion and 24 water molecules. A view of the structure projected onto the *ab* plane is shown in Fig. 5. Fig. 6 presents a simple extended schematic representation of this projection. All the solvent molecules reside in channels between infinite chains of stacked molecules. The major role of solvent in this structure is to hold molecules together between the chains, and to provide additional interactions which aid in maintaining the layers of stacked molecules.

The molecular stacking block, shown in Fig. 6, approximates a rectangular column. Since the proflavines are stacked parallel to each other and the sugar puckering is different in base *A* and base *B*, the distribution of polarity on the faces of the rectangular columns is not homogeneous. The four boundaries of the stacking column are designated as faces *A*, *B*, *C*, and *D* (Fig. 6). *A* and *B* are wide faces. Face *A* is much more polar than face *B* as all the polar atoms N(10), N(15), and N(16) of the proflavines lie on face *A*. The exposure of N(3) of adenine *A* on face *C* makes it more polar than face *D*, where N(3) of adenine *B* is essentially buried in the sugar domain.

The unique part of the channel structure in the crystal is confined by two diad axes parallel to the *z* axis (2_z) as shown in Fig. 5. It consists of three distinct regions. The first one (1) is delimited by two weak polar faces *D* and *D*′, which are related to one another by the first twofold axis. The space related in this area is not

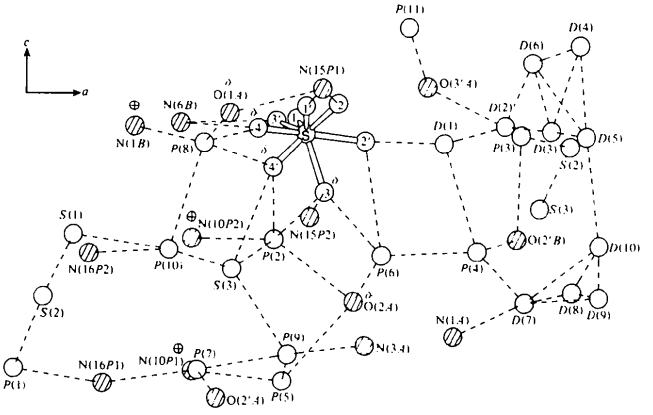

Fig. 7. The solvent network in the crystal structure. This view is obtained by rotating 90° along the horizontal axis of Fig. 5. The hydrogen donors or acceptors of the ApA or proflavine molecules are shown as shaded atoms. The symbol + denotes positive charge, with $\delta-$ for partial negative charge.

large. Three water molecules, *WP*(01), *WS*(01), and *WS*(02) occupy the unique portion of this region. They loosely interact with each other and with N(16) of proflavine 1. The next region (2) has both strong polar faces *A* and *C* forming a highly polar channel side. The majority of solvent molecules are in this region. The sulphate ion is in the middle of the channel with its disordered O atoms fully utilized to bind to ApA, the proflavine and other solvent molecules. *WS*(03) also occupies a pivotal position. Although it does not interact with ApA or proflavines, it connects the sulphate ion, *WP*(02), *WP*(10) and *WP*(09) together and also weakly binds to *WP*(07). The third region (3) of the channel is confined by two weakly polar and long faces, *B* and *B*′, related to one another by the second twofold axis. Due to its weak polar surrounding and ample space, all water molecules in this region are very disordered. *WD*(01), *WD*(02), *WD*(03), *WD*(04), *WD*(05), and *WD*(06) form one group, while *WD*(07), *WD*(08), *WD*(09), and *WD*(10) forms the other. These two groups are connected to each other through the interaction *WD*(05)···*WD*(10) (2·95 Å), and through the bridge molecule *WP*(04) [*WD*(01)···*WP*(04) 3·01 Å; *WD*(07)···*WP*(04), 2·49 Å]. The attachments of *WD*(01) to the sulphate ion and *WD*(07) to N(1) of base *A* hold these two groups in place. The continuity of a channel is constructed through the 2_z axes as described above. There are no direct contacts among the solvent molecules in different channels. However, as seen on the top right of Fig. 5, one channel interacts with another *via* hydrogen bonding among water molecules with O(3′*A*) and O(2′*B*).

The solvent network is shown in Fig. 7. All the solvent molecules, except *WP*(11), which had to go through O(2′*A*) to interact with other solvent molecules, are hydrogen-bonded to each other. Fig. 7 also shows the particular pattern of the charge balance in this structure. Positively charged N(1*B*) interacts with negatively charged O(4) and O(4′) of the sulphate ion and O(1*A*) of the phosphate group via *WP*(08). N(10)$^+$ of proflavine 1 interacts with O(2*A*)$^-$ *via* *WP*(05) while N(10)$^+$ of proflavine 2 interacts with O(4′) and O(3) of the sulphate ion and O(2*A*)$^-$ *via* *WP*(02). Thus, all the charged species in this structure interact with one another *via* bridges of neutral water molecules.

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Conformational Analysis of Synthetic Androgens. VI. Structure and Crystal Packing of 17β-Hydroxy-7β-methyl-4,14-androstadien-3-one Monohydrate

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Abstract

The X-ray crystal structure of 17β-hydroxy-7β-methyl-4,14-androstadien-3-one monohydrate was investigated to determine the influence of the 7β-methyl substituent on the overall conformation. The steroid hydrate ($C_{20}H_{28}O_2.H_2O$) crystallizes in the monoclinic space group $P2_1$ with $Z = 2$, $a = 10{\cdot}868$ (6), $b = 8{\cdot}472$ (4), $c = 9{\cdot}838$ (4) Å, $\beta = 98{\cdot}29$ (5)°, $\lambda = 1{\cdot}5418$ Å, $T = 291$ K, $V = 896{\cdot}3$ Å^3, $\rho_x = 1{\cdot}18$ Mg m^{-3}. $R = 4{\cdot}3\%$ for 1972 reflections. Subtle conformational differences between 17β-hydroxy-7β-methyl-4,14-androstadien-3-one, 17β-hydroxy-4,14-androstadien-3-one and 17β-hydroxy-7β-methyl-4,14-estradien-3-one are attributable to differences in methyl substitution. While the overall shapes of these molecules are very similar, the molecular packings in the crystals of these steroids are entirely different. In contrast to this, the crystal structure of 17β-hydroxy-7β-methyl-4,14-androstadien-3-one is isomorphous with those of the monohydrates of the most active endogenous androgens, testosterone and dihydrotestosterone. The order of hydrogen-bond lengths and their orientations are remarkably similar in these three structures. Since the hydrophobic surfaces of the molecule are significantly different, the crystal packing in these isomorphs appears to be a function of hydrate formation and the directionally specific hydrogen bonding mediated by the water molecules in the crystals.

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Introduction

The structure determination was undertaken as part of a study of substituent influence on the conformations of modified androgenic steroids. The title compound has approximately 10% of the androgenicity of testosterone when administered parenterally in the castrate