the cavities and thus forming elevations. There is no evidence whatsoever of spiral patterns on the crystal faces on which these features occur.

Conclusion

Having examined the merits and demerits of the three possible explanations, we feel that the tadpoles and similar features on the rhombohedral faces are best explained by the second of these, while those on the basal planes fit in best with the third.

One of us (M. S. J.) would like to express his sincere gratitude to Prof. S. Tolansky, F.R.S. for his continued guidance and helpful discussions. Crystals from the General Electric Co., London, were studied in his laboratory. We also wish to thank Dr I. Sunagawa, Geological Survey of Japan, and Dr A. F. Seager, Birkbeck College, London for their helpful suggestions. We are thankful to Dr L. A. Thomas and Dr C. S. Brown of G.E.C., London and Dr D. R. Hale of Clevite Corp., Ohio, for an adequate supply of cultured quartz for carrying out the work. Our thanks are also due to Dr A. R. Patel for his interest in our work.

References

Brown, C. S. & Thomas, L. A. (1956). Monograph No. 205R., Inst. Elec. Engrs., 675, 1–11.

Joshi, M. S. & Tolansky, S. (1961). *Proc. Roy. Soc.* A, **260**, 475.

Acta Cryst. (1965). **18**, 525

The Crystal Structure of a Hydrogen Bonded Complex of Adenosine and 5-Bromouridine

By A. E. V. Haschemeyer* and Henry M. Sobell†

Department of Biology, Massachusetts Institute of Technology, Cambridge 39, *Massachusetts, U.S.A.*

(*Received* 4 *May* 1964)

The crystal structure of a 1 : 1 crystalline complex of adenosine and 5-bromouridine has been determined by heavy atom and trial and error methods, and has been refined by the full-matrix least-squares method to a final residual of 13·8% for 1986 measureable reflections. The space group is orthorhombic, $P22_12_1$, with $a = 4{\cdot}82$, $b = 15{\cdot}19$, and $c = 31{\cdot}76$ Å. The bromouracil and adenine rings form a planar complex joined by a N–H...N hydrogen bond (2·80 Å) and a weak N–H...O hydrogen bond (3·10 Å). The overall structure is fully hydrogen bonded with O–H...O contacts of 2·72, 2·79, 2·80 and 2·91 Å and O–H...N contacts of 2·78 and 2·90 Å. Both ribose sugars are puckered with C(3′) lying out of the plane formed by C(1′), C(2′), C(4′), and O(1′), and are oriented *anti* to the bromouracil and adenine planes. The structure was found to contain one water of crystallization per nucleoside pair. Refinement indicated considerable disorder in the water molecule position.

Introduction

The first successful co-crystallization of purine and pyrimidine derivatives was reported by Hoogsteen (1959, 1963). He found that 9-methyladenine and 1-methylthymine form an interlocking structure in which the two bases are hydrogen bonded in a planar complex. Other purine–pyrimidine crystalline complexes which have been studied include 9-ethylguanine and 1-methylcytosine (O'Brien, 1963); 9-ethyladenine and 1-methyluracil (Mathews & Rich, 1964); 9-ethylguanine and 5-bromo-1-methylcytosine (Sobell, Tomita & Rich, 1963). In each case the purine and pyrimidine rings form a highly planar hydrogen bonded complex. These structures are of particular interest because of their relation to the naturally occurring biological polymers, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

This communication describes the structure of a crystalline nucleoside complex containing adenosine and bromouridine. The hydrogen bonding between bases is different from the adenine–thymine pairing found by Hoogsteen (1959, 1963) and from that proposed by Watson & Crick (1953) for DNA. A preliminary report has been published elsewhere (Haschemeyer & Sobell, 1963).

Experimental

Commercial preparations of adenosine and 5-bromouridine were obtained from California Corporation for Biochemical Research, Los Angeles, California. Upon

---

* U.S. Public Health Service Postdoctoral Fellow.

† Helen Hay Whitney Postdoctoral Fellow.

slow evaporation of an aqueous solution containing equimolar quantities of the two nucleosides, the entire mixture crystallized in clusters of thin needles. The presence of both nucleosides in the crystals in approximately equal proportions was confirmed by ultraviolet absorption measurements on aqueous solutions prepared from single crystals and by paper chromatography.

Weissenberg and precession photographs with Ni-filtered Cu $K\alpha$ radiation showed the crystals to be orthorhombic. Systematic absence of $0k0$ reflections for k odd and $00l$ reflections for l odd indicated $P22_12_1$ as the space group. The unit-cell parameters measured on oscillation and Weissenberg photographs were found to be: $a=4{\cdot}82\pm0{\cdot}01$, $b=15{\cdot}19\pm0{\cdot}01$, and $c=31{\cdot}76\pm0{\cdot}03$ Å. The uncertainties given represent estimated standard deviations. A wavelength of 1·5418 Å for the mean of $K\alpha_1$ and $K\alpha_2$ was assumed. The short unit-cell a axis lies along the needle axis of the crystal habit.

The crystal density was determined by the flotation method in a mixture of benzene and methylene iodide. The final value of 1·71 g.cm^{-3} was best fitted by the assumption of four adenosine–bromouridine pairs in the unit cell ($\varrho_c=1{\cdot}686$ g.cm^{-3}). By including one water molecule per nucleoside pair the calculated density is 1·737 g.cm^{-3}.

Equi-inclination Weissenberg photographs using multiple film packets were taken about the a axis at levels out to $h=4$. The intensities were estimated by visual comparison with a standard scale and were corrected with the appropriate Lorentz–polarization factors. No correction was made for absorption effects. A total of 2511 reflections were indexed, representing 94% of the data available within the Cu $K\alpha$ limiting sphere. Measurable intensities were obtained for 1986 reflections; the remaining were taken to be zero. To reduce computing time the unobserved reflections were not included in the calculations.

Determination of the structure

The structure was first solved in projection on the (100) plane by the heavy-atom method. Approximately 450 non-zero $0kl$ reflections were available. The position of the bromine atom was readily obtained from the two-dimensional (100) Patterson function. The first Fourier synthesis based on the bromine phases was not particularly helpful; however, in a second calculation, by eliminating those reflections which showed the poorest agreement between observed and calculated amplitudes, it was possible to obtain a good trial structure for bromouridine. A third Fourier synthesis based on all reflections and phased on the bromouridine molecule confirmed the bromouridine structure but did not yield a recognizable adenosine molecule. Trial structures in which the adenine ring was oriented in order to allow Watson–Crick hydrogen bonding with bromouracil were not successful. Base pairing of the type observed by Hoogsteen was not possible because of packing considerations. A different orientation of the adenine ring was then tried, in which, like the Hoogsteen pairing, hydrogen bonding would occur between N(3) of the uracil ring and N(7) of adenine, but which differed in that the amino group of adenine would bond to O(2) rather than to O(4) of bromouracil. This structure was confirmed by Fourier synthesis. In addition, an extra peak of electron density was located and tentatively identified as a water molecule.

For solution of the structure in three dimensions, the non-zero reflections out to $h=3$ were used. The x coordinate of the bromine atom was given immediately by the three-dimensional Patterson synthesis and a Fourier synthesis was calculated using the bromine phases. With the aid of the y and z coordinates obtained in the (100) projection, most atoms could be located. A second Fourier synthesis based on these atoms established the complete structure with the exception of the hydrogen atoms. All Fourier syntheses were done with the program of Sly, Shoemaker & Van den Hende (1962).

Refinement

The refinement was carried out on an IBM 7094 computer with the full-matrix least-squares program of Busing, Martin & Levy (1962). The atomic scattering factors used were as follows: carbon, nitrogen and oxygen from Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955); bromine from Thomas & Umeda (1957); hydrogen from McWeeny (1951).

The quantity minimized in the least-squares refinement was $\Sigma w[F_o^2-(sF_c)^2]^2$ where w is a weighting factor, F_o is the observed structure factor (on an arbitrary scale), F_c is the calculated structure factor, and s is a scale factor, varied independently for each h level of data. The method used in weighting the data was similar to that of Hughes (1941) and was based upon the reproducibility obtainable in the visual readings of intensities. For readings above 20 on the intensity scale the estimated standard deviation was 10%, giving $\sigma=0{\cdot}1F_o^2$. For the very weak reflections with intensity readings below 20 a constant error of two units seemed more reasonable: this was multiplied by the Lp factor to obtain σ.

The structure was first refined in two dimensions with unweighted non-zero data. Several cycles were carried out on positional parameters with a constant overall temperature factor arbitrarily set at 3 Å^2, then two cycles on positions and individual isotropic temperature factors. The discrepancy index R, based on F, for the projection decreased to 20% in these calculations.

In the first stage of the three-dimensional refinement the 900 most intense reflections were used, and several cycles were calculated varying positions

Table 1. *Positional parameters in fractional coordinates* $\times 10^4$ *and isotropic temperature factors*

	x/a	*y/b*	*z/c*	*B*
Bromouridine				
Br	9229	292	2400	3·20 Å^2
N(1)	14371	−471	3353	2·33
C(2)	13988	146	3661	2·91
O(2)	15372	114	3993	3·81
N(3)	12244	802	3583	2·48
C(4)	10645	896	3221	3·30
O(4)	9034	1521	3174	3·74
C(5)	11166	215	2912	1·95
C(6)	12980	−440	2988	3·23
C(1′)	16457	−1209	3447	2·23
C(2′)	14967	−1941	3717	2·00
C(3′)	14143	−2530	3377	2·46
C(4′)	16507	−2535	3059	2·38
C(5′)	15898	−2772	2611	3·44
O(1′)	17212	−1570	3063	2·37
O(2′)	17215	−2290	3955	3·00
O(3′)	13830	−3441	3513	4·00
O(5′)	13078	−2427	2472	4·59
Adenosine				
N(1)	14022	2299	5273	2·58
C(2)	12375	2984	5368	2·85
N(3)	10506	3352	5125	2·06
C(4)	10322	2976	4738	1·98
C(5)	11815	2251	4618	2·28
C(6)	13793	1944	4902	2·85
N(6)	15495	1228	4810	3·19
N(7)	11111	2032	4215	2·74
C(8)	9213	2590	4106	3·37
N(9)	8635	3183	4418	2·07
C(1′)	6625	3946	4400	2·01
C(2′)	8278	4793	4362	2·48
C(3′)	8478	4887	3892	1·88
C(4′)	5599	4573	3771	2·26
C(5′)	5312	4153	3324	3·40
O(1′)	5032	3877	4064	2·26
O(2′)	6669	5502	4524	3·45
O(3′)	9054	5730	3743	2·56
O(5′)	7571	3622	3245	4·53
Average water position				
0·6 O	19594	556	5441	3·47
Fractional water positions				
0·52 O(1)	19744	441	5390	4·00
0·36 O(2)	19206	728	5525	4·41
Hydrogen atoms				
H(1)	12900	1250	3800	*
H(2)	13000	−1000	2800	
H(3)	12400	3330	5650	
H(4)	15330	1080	4560	
H(5)	16330	1000	5080	

* Hydrogen atom temperature factors were arbitrarily set at 4·0 for the final structure factor calculation.

of the 37 non-hydrogen atoms of the adenosine–bromouridine pair plus the oxygen atom of the water molecule. Individual isotropic temperature factors were then included for several cycles. The temperature factor of the water oxygen atom increased rapidly to a value of 18 Å^2; those for several other atoms fluctuated in the range of 1 to 6 Å^2.

A total of 1460 reflections were used in the next stage of refinement. These represented all but the very weakest reflections. Because of its high temperature factor, the water oxygen atom was omitted from the structure. Several cycles of isotropic refinement then reduced the *R* index to 15%. A three-dimensional Fourier synthesis at this point showed clear resolution of all atoms and again showed a peak of low electron density at the water molecule position. When an oxygen atom at this site was included in the structure factor calculation *R* dropped to 14%, confirming the presence of the water molecule. However, the low peak height on the Fourier map suggested possible incomplete occupancy; therefore an attempt was made to vary the degree of occupancy by refining on the atom multiplier (a factor by which the values in the scattering factor table are multiplied). Refinement on temperature factor and multiplier at the same time again led to very high values of *B*. Therefore, the multiplier was varied first for two cycles (with *B* set at 3·5 Å^2), followed by one cycle on multiplier and temperature factor. Then two cycles were run on positional parameters and isotropic temperature factors for all non-hydrogen atoms of the structure in addition to the multiplier of the water oxygen. This led to a residual of 11·5% and a resultant 0·6 oxygen atom ($B = 3{\cdot}47$ Å^2) at the water site. Omission of the 0·6 oxygen gave $R = 13{\cdot}2\%$. One cycle of anisotropic refinement on the bromine atom made no change in *R* and showed no significant thermal anisotropic motion for the bromine atom.

The final coordinates and isotropic temperature factors based on this refinement are given in Table 1. These values were used to calculate two- and three-dimensional Fourier maps based on the complete structure and on the nucleoside pair alone. Upon omission of the water oxygen atom a very broad region of electron density appeared at the water site showing two maxima separated by a distance of about 0·5 Å. The total electron density corresponded to approximately 0·8 H_2O. When the 0·6 oxygen atom as obtained in the refinement was included in the calculation, the electron density between the two maxima was eliminated in the difference syntheses but significant peaks were left on either side of the oxygen site. This is illustrated in Fig. 1(*a*). These results strongly suggested that the water molecules were randomly distributed among at least two loci. To test this, the water oxygen was divided into four fractional atoms, each with a weight of 0·2, located around the average site. Refinement on the multipliers, followed by two cycles on positions and isotropic temperature factors of the fractional atoms, led to a reduction of *R* to 11·2%. Two of the fractional atoms were eliminated when their multipliers went to zero during the refinement, whereas the two atoms placed closest to the peaks observed in the difference map showed increases in multiplier to 0·52 and 0·36. The distance between their final positions was 0·67 Å. Coordinates and temperature factors are given at the bottom of Table 1. The resultant (100) difference Fourier map is shown in Fig. 1(*b*).

Examination of the (100) and three-dimensional electron density maps led to the location of five of the six hydrogen atoms of the bromouracil and adenine rings. Their coordinates, as obtained directly from the difference maps, are given in Table 1. A final round of structure factors was calculated for all 1986 measureable reflections, based on the parameters for the nucleoside pair, the two fractional oxygen atoms of the water molecule and the five hydrogen atoms. This yielded an overall residual of 13·8% for 1986 reflections, with a value of 12·6% for the 0*kl* data. A table of the final observed and calculated structure factors was submitted with the paper and has been deposited with the American Documentation Institute, Auxiliary Publications Project, Washington 25, D.C.

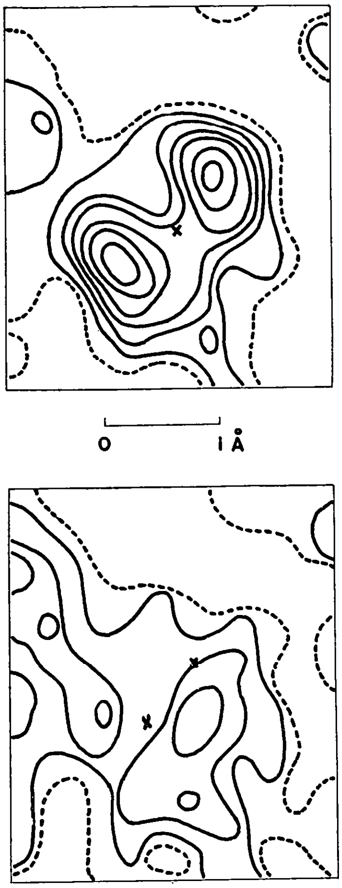

Fig. 1. (100) difference Fourier syntheses at the water molecule site: (*a*) based on an oxygen atom with weight 0·6 after isotropic refinement; (*b*) based on two fractional atoms with weights of 0·52 and 0·36. Contours are drawn at intervals of 0·2 e.Å^{-2}. Broken lines indicate the zero contours. Atom positions are indicated.

Results and discussion

Bond distances and angles and their standard deviations were computed with the crystallographic function-and-error program of Busing & Levy (1959) adapted to the IBM 7094. The standard deviations are based on the standard errors of atomic coordinates calculated from the inverse of the full normal matrix and the estimated standard deviations of the unit cell parameters (Busing, Martin & Levy, 1962). Covalent bond lengths and bond angles for adenosine and bromouridine are shown in the schematic drawing of the base pair in Fig. 2. The standard deviations are as follows: 0·01 Å for the bromine–carbon bond length, 0·03 Å for bond lengths involving light atoms, and 2° for bond angles.

All bond lengths fall within the acceptable range for agreement with expected values (a maximum deviation of 0·07 Å based on the calculated standard deviation of 0·03 Å), except for C(4′)–O(1′) and C(5′)–O(5′) in bromouridine which are rather long and C(1′)–O(1′) in adenosine which is short. It is doubtful that such large deviations are realistic, and, therefore, the calculated standard deviation is probably too low for these bonds. With these exceptions there is good agreement between the present results and those reported for calcium thymidylate (Trueblood, Horn &

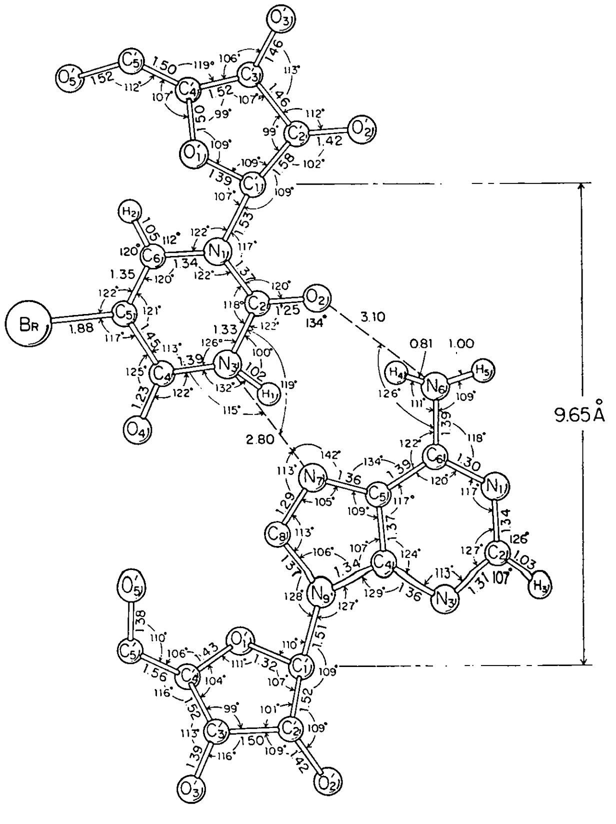

Fig. 2. Molecular dimensions of the nucleoside pair. Approximate positions of five hydrogen atoms located from difference Fourier syntheses are also shown.

Table 2. *Least-squares planes**

Atoms of bromouridine are prefixed *U*; atoms of adenosine are prefixed *A*. Coordinates are expressed in Å

Adenine ring

$$0{\cdot}6964x + 0{\cdot}6205y - 0{\cdot}3606z - 0{\cdot}8268 = 0$$

Atoms comprising the plane	Displacements (Å)	Displacements of other atoms	
*A*N(1)	0·007	*A*N(6)	0·022
*A*C(2)	−0·009	*A*C(1′)	0·076
*A*N(3)	−0·011	*A*O(1′)	−0·138
*A*C(4)	0·016	*U*O(2)	−0·133
*A*C(5)	−0·029	*U*N(3)	−0·065
*A*C(6)	0·021	H(1)	0·329
*A*N(7)	−0·010	H(3)	0·003
*A*C(8)	0·004	H(4)	0·114
*A*N(9)	0·011	H(5)	−0·222

Ribose sugar of adenosine

$$0{\cdot}7348x - 0{\cdot}5264y - 0{\cdot}4278z + 6{\cdot}8126 = 0$$

Atoms comprising the plane	Displacements (Å)	Displacements of other atoms	
*A*C(1′)	0·026	*A*C(3′)	0·620
*A*C(2′)	−0·014	*A*C(5′)	0·857
*A*C(4′)	0·016	*A*O(2′)	−1·371
*A*O(1′)	−0·027	*A*O(3′)	0·352
		*A*O(5′)	2·189

Bromouracil ring

$$0{\cdot}7338x + 0{\cdot}5492y - 0{\cdot}3999z - 0{\cdot}4372 = 0$$

Atoms comprising the plane	Displacements (Å)	Displacements of other atoms	
*U*Br	0·023	*U*C(1′)	−0·003
*U*N(1)	−0·006	*U*O(1′)	0·451
*U*C(2)	−0·018	*A*N(6)	−0·041
*U*O(2)	0·024	*A*N(7)	−0·165
*U*N(3)	0·012	H(1)	0·342
*U*C(4)	−0·016	H(2)	−0·230
*U*C(5)	−0·007	H(4)	0·094
*U*C(6)	−0·008		
*U*O(4)	−0·004		

Ribose sugar of bromouridine†

$$-0{\cdot}8854x + 0{\cdot}2318y - 0{\cdot}4029z + 11{\cdot}8392 = 0$$

Atoms comprising the plane	Displacements (Å)	Displacements of other atoms	
*U*C(1′)	−0·021	*U*C(3′)	0·591
*U*C(2′)	0·012	*U*C(5′)	0·737
*U*C(4′)	−0·012	*U*O(2′)	−1·375
*U*O(1′)	−0·021	*U*O(3′)	0·230
		*U*O(5′)	2·240

* Planes were calculated with a program based on the method of Blow (1960).

† The bromouridine sugar is given as viewed from below the base pair (Fig. 4) in order that the direction of displacements may be more readily compared with the adenosine sugar.

Luzzati, 1961), 5-fluoro-2′-deoxy-β-uridine (Harris & Macintyre, 1964) and adenosine-5′-phosphate (Kraut & Jensen, 1963).

Conformation of adenosine

Least-squares plane calculations show the adenine ring to be planar, within experimental error. Displacements of the nine ring atoms from the least-squares plane are given in Table 2; the r.m.s. deviation is 0·015 Å. Small, but possibly significant, deviations from this plane are indicated for the amino nitrogen N(6) and sugar carbon C(1′). In the latter case, however, the displacement is much less than that observed in adenosine-5′-phosphate (0·21 Å above the plane).

The conformation of the ribose ring is similar to that observed in adenosine-5′-phosphate and in calcium thymidylate. The four ring atoms C(1′), C(2′), C(4′), and O(1′) show a r.m.s. deviation from their least-squares plane of 0·021 Å (Table 2). Atom C(3′) lies 0·62 Å above this plane producing a puckered configuration, as discussed by Spencer (1959).

The dihedral angle between the ribose ring and the adenine ring is about 70°. This may be compared with the values of 76° in adenosine-5′-phosphate and 75° in calcium thymidylate. To facilitate comparison with previous crystal structures and with DNA, the torsion angle introduced by Donohue & Trueblood (1960) was calculated. This quantity refers to rotation of a projection of the sugar bond C(1′)–O(1′) about the axis defined by the glycosidic bond (C(1′)–N(9) in adenosine). In the present case a view down the C(1′)–N(9) bond shows the adenine ring almost end-on. From this vantage point the sugar oxygen is seen to lie below the adenine plane, and the general picture is much like that given in Fig. 2 of Kraut & Jensen (1963). To obtain φ_{CN} the C(1′)–O(1′) bond was projected onto the plane passing through C(1′) and normal to C(1′)–N(9). Because of the deviation of C(1′) from the adenine plane, a plane parallel to the adenine plane and passing through C(1′) was taken to represent zero rotation. From this plane a counterclockwise rotation of 10° about the C(1′)–N(9) axis leads to the C(1′)–O(1′) projection. According to the convention, therefore, φ_{CN} for adenosine in this crystal structure is $-10°$. Values of φ_{CN} given by other workers have included $-18°$ for adenosine-5′-phosphate, $-48°$ for calcium thymidylate, and $-77°$ calculated by Donohue & Trueblood (1960) for the modified Watson–Crick model of DNA (Langridge, Seeds, Wilson, Hooper, Wilkins & Hamilton, 1957). The present result provides further support for the conclusion that the so-called *anti* orientation (with φ_{CN} ranging around $-30°$) is favored over the *syn* orientation (φ_{CN} around $+150°$) in these compounds.

Conformation of 5-bromouridine

Least-squares plane data for the pyrimidine ring and for the sugar residue are given in Table 2. The former shows an excellent fit to the least-squares plane (r.m.s. deviation of 0·013 Å); the sugar carbon atom C(1′) also lies in the plane. The conformation of the ribose ring is similar to that observed in adenosine, with C(3′) lying 0·59 Å above the plane formed by the other four ring atoms. The dihedral angle between

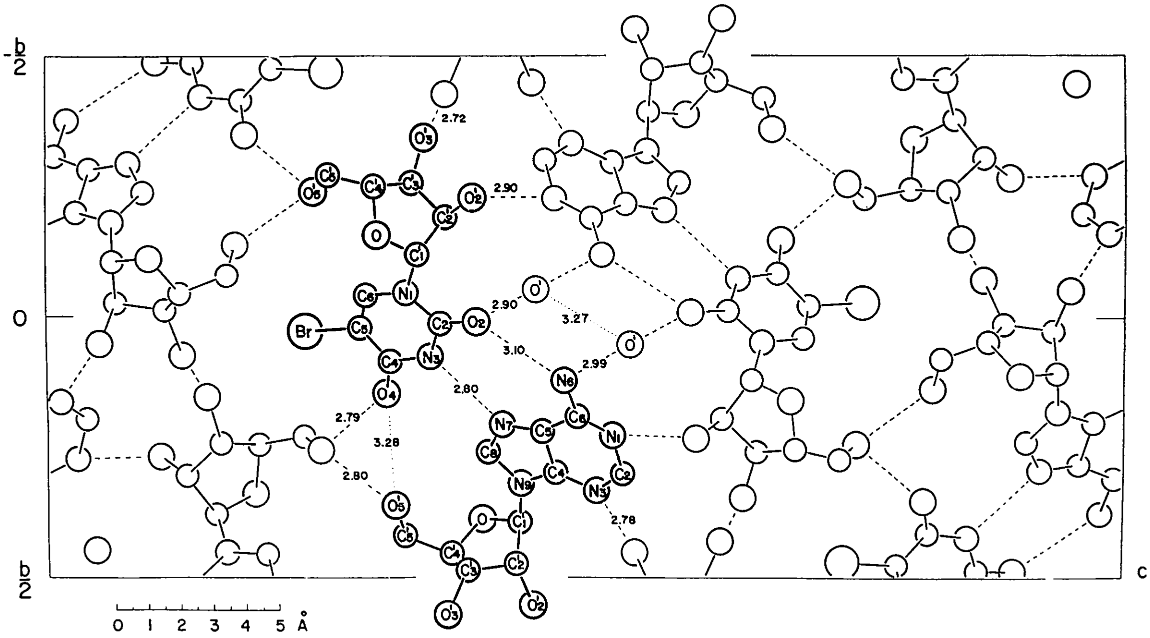

Fig. 3. Packing diagram of the adenosine–bromouridine crystal structure as viewed down the a axis. Probable hydrogen bond contacts are indicated with broken lines. Dotted lines show other contacts of interest. The average water position is denoted by O′.

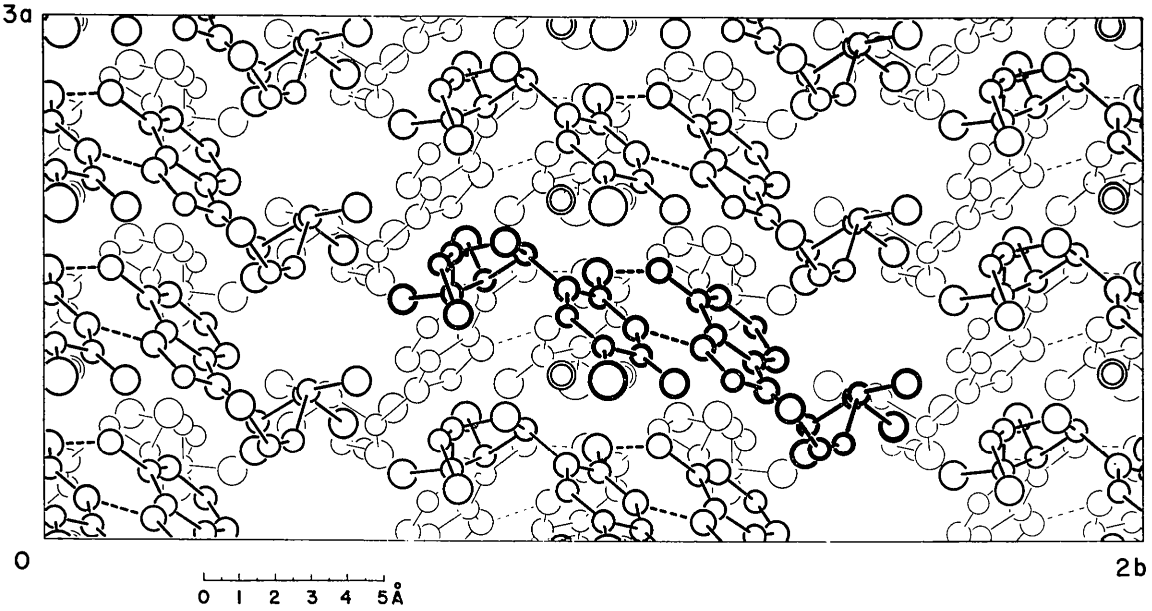

Fig. 4. Packing diagram of the adenosine–bromouridine crystal structure as viewed down the c axis. The base pairs drawn with heavy lines are related to those drawn with light lines by a twofold rotation axis located at $y=0$ and $z=\frac{1}{2}$. Only half the unit cell along the c axis is shown. The average water molecule position is shown by a double circle.

the bromouracil and the ribose planes is 69°. The torsion angle φ_{CN}, calculated as described above, was found to be $-20°$.

Configuration of the base pair

The hydrogen bonding between the purine and pyrimidine rings is shown schematically in Fig. 2. The two ring systems are nearly coplanar; the dihedral angle between their least-squares planes is 5° 8′. A strong hydrogen bond (2·80 Å) is found between N(3) of bromouracil and N(7) of the adenine ring. Although the hydrogen atom positions shown are only approximate, the deviation of H(1) from the UN(3)–AN(7) line is consistent with the shortness of the hydrogen bond. The hydrogen bonding between the carbonyl oxygen O(2) of bromouracil and the amino group of adenine appears to be rather weak; the distance of 3·10 Å is greater than usually observed for this type of bond, although the angles are favorable. The weakening of this bond may be due to the proximity of the two water sites (Fig. 3), related by the twofold axis, or to the effect of other hydrogen bonding in the structure.

In the preliminary report of this structure the base pairing arrangement was compared to that observed by Hoogsteen for the complex of 9-methyladenine and 1-methylthymine and to the Watson–Crick pairing in DNA. It was suggested that the change in pairing may reflect the influence of the bromine atom on the charge distribution over the uracil ring. Further work on similar structures will help to clarify this point.

Overall hydrogen bonding and molecular packing

Fig. 3 shows the crystal packing as viewed down the *a* axis. Suggested hydrogen-bonding contacts are shown with dashed lines. In addition to the hydrogen bonding between the purine and pyrimidine rings, there are probable hydrogen bonds for all of the sugar hydroxyl groups, as well as for the remaining electron donors of the two bases, N(1) and N(3) of adenine and O(4) of bromouracil. O–H · · · O bonding contacts are indicated for *U*O(5′) with an adjacent *U*O(4) (2·79 Å) and with *A*O(5′) (2·80 Å) in the same unit cell. *U*O(3′) shows a probable hydrogen bond with *A*O(3′) in the unit cell above (2·72 Å) and has a close contact with *A*O(3′) (2·91 Å) in the same unit cell. The distances agree well with those observed in other sugar structures, such as β-D-glucose (Ferrier, 1963). The O–H · · · N contacts for the adenine ring nitrogens. *A*N(1) and *A*N(3), are 2·90 and 2·78 Å respectively. The bromine atom is well isolated in the structure and does not participate in hydrogen bonding. The closest approach (3·61 Å) is made by C(5′) and O(5′) of a neighbouring adenosine molecule. A summary of hydrogen bond lengths and angles is presented in Table 3.

Table 3. *Hydrogen bonding distances and angles*

Atoms of bromouridine are prefixed *U*; atoms of adenosine are prefixed *A*. The subscript *s* refers to a symmetry related molecule. The subscript *w* refers to the water molecule

Hydrogen bonded atoms	Distance	Angles	
*U*O(2)–*A*N(6)	3·10 Å	*A*C(6)–*A*N(6)–*U*O(2)	126°
		*U*C(2)–*U*O(2)–*A*N(6)	134
*U*N(3)–*A*N(7)	2·80	*U*C(4)–*U*N(3)–*A*N(7)	115
		*U*C(2)–*U*N(3)–*A*N(7)	119
		*A*C(5)–*A*N(7)–*U*N(3)	142
		*A*C(8)–*A*N(7)–*U*N(3)	113
*A*N(3)–(*A*O(2′))$_s$	2·78	*A*C(2)–*A*N(3)–*A*O(2′)	119
		*A*C(4)–*A*N(3)–*A*O(2′)	126
		*A*C(2′)–*A*O(2′)–*A*N(3)	105
*A*N(1)–(*U*O(2′))$_s$	2·90	*A*C(2)–*A*N(1)–*U*O(2′)	97
		*A*C(6)–*A*N(1)–*U*O(2′)	108
		*U*C(2′)–*U*O(2′)–*A*N(1)	93
*U*O(4)–(*U*O(5′))$_s$	2·79	*U*C(4)–*U*O(4)–*U*O(5′)	139
		*U*O(4)–*U*O(5′)–*U*C(5′)	110
*A*O(5′)–(*U*O(5′))$_s$	2·80	*A*C(5′)–*A*O(5′)–*U*O(5′)	113
		*A*O(5′)–*U*O(5′)–*U*C(5′)	122
*U*O(3′)–(*A*O(3′))$_s$	2·72	*U*C(3′)–*U*O(3′)–*A*O(3′)	127
		*U*O(3′)–*A*O(3′)–*A*C(3′)	133
*U*O(3′)–(*A*O(3′))$_{s'}$	2·91	*U*C(3′)–*U*O(3′)–*A*O(3′)	113
		*U*O(3′)–*A*O(3′)–*A*C(3′)	108
*U*O(2)–O$_w$	2·90	*U*C(2)–*U*O(2)–*O*$_w$	156
*U*O(2)–O(1)$_w$	2·98	*U*C(2)–*U*O(2)–O(1)$_w$	160
*U*O(2)–O(2)$_w$	2·72	*U*C(2)–*U*O(2)–O(2)$_w$	149
*A*N(6)–O$_w$	2·99	*A*C(6)–*A*N(6)–O$_w$	121
*A*N(6)–O(1)$_w$	3·04	*A*C(6)–*A*N(6)–O(1)$_w$	118
*A*N(6)–O(2)$_w$	3·00	*A*C(6)–*A*N(6)–O(2)$_w$	110

Fig. 4 shows the crystal packing as viewed down the *c* axis. The planarity of the base pair is apparent, as well as the relative orientations of the sugar residues. The distance between adenine rings calculated from the least-squares planes is 3·4 Å and between bromouracil rings, 3·5 Å. For clarity, only half of the unit cell along the *c* axis is shown.

The water molecule

Several other cases of structural anomalies associated with water of crystallization have been reported. Some interesting examples are caffeine (Sutor, 1958), thymine monohydrate (Gerdil, 1961) and biuret hydrate (Hughes, Yakel & Freeman, 1961), all of which have shown variable water content and short water–water distances. The present structure bears some resemblance in that the water molecules lie in tunnels (in this case, running parallel to the *a* axis) and probably do not obey the overall space-group symmetry. In addition, the total occupancy is uncertain. On the other hand, it is unlikely that any regular structure like a zigzag chain is formed, since the distances between molecules along the tunnel is too great. Also, there is no indication of a close water–water contact, even thought the water position is close to the twofold rotation axis.

Refinement on a single water position initially suggested incomplete occupancy, and this point is still somewhat in doubt. The finding seemed consistent with the rather long hydrogen-bonding contacts with *U*O(2) (2·90 Å) and *A*N(6) (2·99 Å), as shown in Fig. 3, indicating that the water molecules might not be firmly held in the lattice. However, from the difference Fourier syntheses it appeared that the difficulties of refinement were due to local disorder in the water molecule position. Refinement on fractional atoms then showed that division of the water oxygen between two statistically average sites 0·67 Å apart, with a total occupancy of 88%, gave a better approximation to the structure.

For the two fractional water oxygen atoms O(1) and O(2) at the positions given in Table 1, the distances across the twofold axis are: O(1)–O(1′), 2·76 Å; O(2)–O(2′), 4·00 Å; and O(1)–O(2′), 3·38 Å. The distances from these sites to the nucleoside atoms where hydrogen bonding might occur are: O(1)–*A*N(6), 3·04 Å; O(2)–*A*N(6), 3·00 Å; O(1)–*U*O(2), 2·98 Å; and O(2)–*U*O(2), 2·72 Å. These results possibly explain why the water lattice site may not be uniquely fixed. Position 1 would give favorable O–H · · · O interaction between water molecules (assuming the hydrogen atoms do not satisfy the twofold symmetry), whereas position 2 is more favorable for O–H · · · O hydrogen bonding with the carbonyl oxygen O(2) of bromouridine. For both positions the interaction with the available proton on the amino nitrogen N(6) of the adenine ring is approximately the same.

We thank Prof. Alexander Rich for providing facilities and support for this investigation. We are indebted to Dr F. S. Mathews for his help in computer programming and to Dr Ken-ichi Tomita and Dr Rudy Haschemeyer for their helpful discussions. The work was done in part at the Computation Center at the Massachusetts Institute of Technology.

References

Berghuis, J., Haanappel, IJ. M., Potters, M., Loopstra, B. O., MacGillavry, C. H. & Veenendaal, A. L. (1955). *Acta Cryst.* **8**, 478.

Blow, D. M. (1960). *Acta Cryst.* **13**, 168.

Busing, W. R. & Levy, H. A. (1959). *A Crystallographic Function and Error Program for the IBM* 704. Oak Ridge National Laboratory, Oak Ridge, Tenn.

Busing, W. R., Martin, K. O. & Levy, H. A. (1962). *ORFLS, a Fortran Crystallographic Least-Squares Program.* Oak Ridge National Laboratory, Oak Ridge, Tenn.

Donohue, J. & Trueblood, K. N. (1960). *J. Mol. Biol.* **2**, 363.

Ferrier, W. G. (1963). *Acta Cryst.* **16**, 1023.

Gerdil, R. (1961). *Acta Cryst.* **14**, 333.

Harris, D. R. & Macintyre, W. M. (1964). *Biophys. J.* **4**, 203.

Haschemeyer, A. E. V. & Sobell, H. M. (1963). *Proc. Nat. Acad. Sci., Wash.* **50**, 872.

Hoogsteen, K. (1959). *Acta Cryst.* **12**, 822.

Hoogsteen, K. (1963). *Acta Cryst.* **16**, 907.

Hughes, E. W. (1941). *J. Amer. Chem. Soc.* **63**, 1737.

Hughes, E. W., Yakel, H. L. & Freeman, H. C. (1961). *Acta Cryst.* **14**, 345.

Kraut, J. & Jensen, L. H. (1963). *Acta Cryst.* **16**, 79.

Langridge, R., Seeds, W. E., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. (1957). *J. Biophys. Biochem. Cytol.* **3**, 767.

Mathews, F. S. & Rich. A. (1964). *J. Mol. Biol.* **8**, 89.

McWeeny, R. (1951). *Acta Cryst.* **4**, 513.

O'Brien, E. J. (1963). *J. Mol. Biol.* **7**, 107.

Sly, W. G., Shoemaker, D. P. & Van den Hende, J. H. (1962). *Two and Three-Dimensional Crystallographic Fourier Summation Program for the IBM* 7090 *Computer* Esso Research and Engineering Co. CBRL–22M–62.

Sobell, H. M., Tomita, K. & Rich, A. (1963). *Proc. Nat. Acad. Sci., Wash.* **49**, 885.

Spencer, M. (1959). *Acta Cryst.* **12**, 59.

Sutor, D. J. (1958). *Acta Cryst.* **11**, 453.

Thomas, L. H. & Umeda, K. (1957). *J. Chem. Phys.* **26**, 293.

Trueblood, K. N., Horn, P. & Luzzati, V. (1961). *Acta Cryst.* **14**, 965.

Watson, J. D. & Crick, F. H. C. (1953). *Nature, Lond.* **171**, 737, 964.

Acta Cryst. (1965). **18**, 532

The Epitaxic Growth of Selenium

By O. S. Heavens and C. H. Griffiths

Royal Holloway College, University of London, Englefield Green, Surrey, England

(*Received* 17 *July* 1964)

Films of selenium have been deposited in vacuo on certain faces of NaCl, KBr, BaF_2, CaF_2 and MgO. Under certain conditions, epitaxic growth of the selenium was observed. The orientations obtained are discussed in relation to the substrate configuration and it appears as if the orientation may be governed by misfit considerations. Anomalies were observed in the film structure, which was found to differ slightly from that quoted for bulk selenium.

1. Introduction

An extensive literature exists on the physical properties of films of amorphous selenium and also of films which have been made polycrystalline by heat treatment. These have been generally found to consist of randomly oriented crystallites, although Semiletov (1960) observed fibre orientation, the crystallites lying with their basal faces (0001) parallel to the substrate. Although film growth has been studied on amorphous substrates, few experiments appear to have been carried out with single-crystal substrates at elevated temperatures. Chihaya (1955) condensed selenium on cleavage faces of rocksalt at tempera-