Acta Cryst. (1969). B**25**, 498

The Crystal Structure of Spermidine Phosphate Trihydrate

BY YUKIKO HUSE
The Institute of Food Microbiology, University of Chiba, Izumi-cho, Narashino, Japan

AND YOICHI IITAKA
Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo, Japan

(*Received* 27 *March* 1968)

Spermidine phosphate trihydrate, $C_7H_{19}N_3.\frac{3}{2}H_3PO_4.3H_2O$, is found to have a monoclinic unit cell with dimensions, $a=9{\cdot}22$, $b=26{\cdot}03$, $c=6{\cdot}58$ Å, $\beta=96{\cdot}0°$. The space group is $P2_1$ and the asymmetric unit contains two formula units. The structure was solved by a sharpened Patterson function and refined by the block-matrix least-squares method with anisotropic temperature parameters to an R value of 0·09 for 2868 observed reflexions.

The molecules take a normal extended zigzag chain conformation. The chemical formula of this compound has been shown to be $[NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_3^+]_2.3[HPO_4]^{2-}.6H_2O$. The structure consists of two kinds of bands, the molecular band and the phosphate–water band. They are extended along the a axis and joined together alternately side by side in the **b** direction to form a sheet structure with stripes of the bands. Within the molecular band, the spermidine molecules are arranged in parallel along the b axis as is generally seen in aliphatic long chain compounds. The angle of tilt is 90°. The phosphate–water band consists of dimer and monomer ions of monohydrogen phosphate which are separated by water molecules, forming a network of hydrogen bonds. The striped sheets are piled up alternately along the c axis through N–H···O hydrogen bonds in such a way that the phosphate–water band comes next to the molecular band of spermidine. The structure is discussed in comparison with that of spermine phosphate hexahydrate.

Introduction

Spermidine is an aliphatic polyamine and is widely distributed in biological materials. It is almost always accompanied by spermine and is found at higher concentrations in tissues of the pancreas, spleen, liver and prostate, but not in human semen, the molar concentration of spermidine being in general higher than that of spermine. Recently, a close correlation has been found between the concentration of ribonucleic acid (RNA) and polyamines, and the stimulation of biosynthesis of these compounds. It is now well known that spermine, spermidine and putrescine play a role in the stability of cellular organelles, membranes, nucleic acids and ribosomes, and much stimulating work has been reported concerning the biological activities of these polyamines. Some of these studies suggested that spermine and spermidine directly affect the rate and extent of nucleic acid biosynthesis; they stimulate the synthesis of RNA (catalysed by RNA polymerase) when the reaction is primed by native deoxyribonucleic acid (DNA) (Krakow, 1963). It is also found that amino acid incorporation in yeast cell-free protein synthetic systems is stimulated by spermine and spermidine (Bretthauer, Marcus, Chaloupka, Halvorson & Bock, 1963); the site of action of spermidine has been shown to be the ribosome (Tanner, 1967). It has been shown, on the other hand, that these polyamines have a strong affinity for nucleic acids. A stabilizing effect on DNA has been demonstrated by Mahler, Mehrotra & Shays (1961) and Tabor (1962). In order to obtain information on the binding relation, we performed the crystal structure analysis of spermine phosphate hexahydrate, and the framework features of the structure, as well as the binding relations, were discussed in comparison with those of DNA (Iitaka & Huse, 1965; refinement of the structure will be reported, Huse & Iitaka, 1969). The study of the crystal structure of spermidine phosphate trihydrate has now been carried out but it took rather a long time owing to the complexity of the structure.

Experimental

Commercial spermidine phosphate (Nutritional Biochemicals Co.) was recrystallized as flat plate crystals from a hot aqueous solution by slow cooling. The crystals are elongated along the c axis, flattened on the (010) face and often twinned on the (100) face. The lattice constants were determined from b and c axis equatorial precession photographs taken with Cu $K\alpha$ radiation. The density was measured by flotation in a mixture of ethanol and chloroform solutions.

Crystal data

Spermidine phosphate trihydrate, $C_7H_{19}N_3.\frac{3}{2}H_3PO_4.3H_2O$, M.W. 346·3.

Monoclinic,

$a=9{\cdot}22\pm0{\cdot}02$, $b=26{\cdot}03\pm0{\cdot}05$, $c=6{\cdot}58\pm0{\cdot}01$ Å,
$\beta=96{\cdot}0°\pm0{\cdot}2°$.

$U=1571{\cdot}1$ Å^3.

$D_x=1{\cdot}469$ g.cm^{-3}, $D_m=1{\cdot}465$ g.cm^{-3}.

$Z=4$ of $C_7H_{19}N_3.\frac{3}{2}H_3PO_4.3H_2O$.
$F(000)=748$.
$\mu_{Cu\ K\alpha}=25{\cdot}0\ cm^{-1}$.
Absent spectra: $0k0$ when k is odd.
Space group: $P2_1$.

The space group was at first assumed to be $P2_1/m$. However, an attempted structure determination with this space group was not a success. A test for piezoelectricity by the method of a vibrating electrode (Iitaka, 1953) showed the piezoelectric response clearly. The space group was, therefore, assumed to be $P2_1$.

Three-dimensional intensity data for Cu $K\alpha$ radiation were obtained from equi-inclination Weissenberg photographs taken about the b axis (up to the 2nd layer) and the c axis (up to the 5th layer). The multiple-film technique was employed and the relative intensities were estimated visually with the calibrated intensity scales prepared for each axis. The X-ray specimen for the c axis was a plate with approximate cross-section $0{\cdot}8\times0{\cdot}08$ mm and that for the b axis was about $0{\cdot}8\times0{\cdot}3$ mm. Intensities were corrected for Lorentz and polarization factors, but no absorption correction was applied. A total of 2868 independent observed structure factors were derived on a common scale by comparing equivalent structure factors recorded on various layers.

The atomic scattering factors used for oxygen and carbon atoms were those given by Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955) and for the phosphorus atom those given by Viervoll & Øgrim (1949).

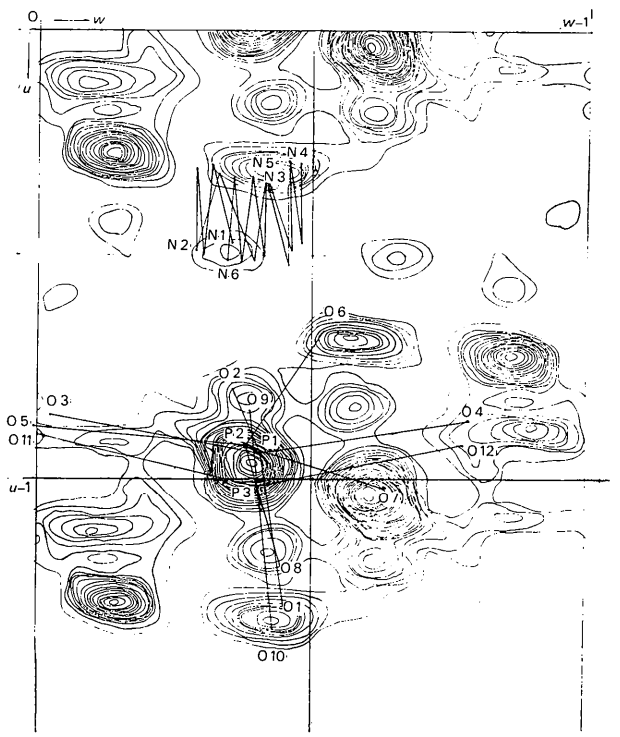

Fig. 1. Harker section at $v=\frac{1}{2}$. Superposed are the spermidine molecules and phosphate groups found in the final structure.

Determination and refinement of the structure

In the light of the result of the piezoelectric test, a sharpened Patterson map was reinterpreted assuming the space group to be $P2_1$. It was, however, very difficult to resolve any features of the structure on the Patterson map because there were so many atoms (two spermidine molecules, three phosphate groups and six water molecules in the asymmetric unit). Fig. 1 shows the Harker section, indicating the interatomic Harker vectors between symmetry related molecules. It is seen that the spermidine molecules lie along the b axis and their Harker peaks are crowded around the restricted positions. The determination of the positions of the three phosphorus atoms was a really puzzling problem. However, plausible coordinates of the three phosphorus atoms were finally determined from the Patterson map by taking into account the images of the phosphate groups buried in the map around each P–P peak The phases of the structure factors based on the three phosphorus atoms were calculated. The R value was 0·52. In the subsequent Fourier synthesis, although it contained many spurious peaks, the atoms of the spermidine molecules could be located at reasonable positions. Several repeated calculations of Fourier and difference Fourier syntheses revealed the whole structure and the R value was reduced to 0·36.

Refinement of the structure was carried out by three cycles of isotropic, and four cycles of anisotropic, least-squares calculations by a block-matrix approximation (program by Okaya & Ashida, 1967). The final R value was 0·09 for 2868 observed reflexions. The weighting system was:

$$\sqrt{w}=40/F_o\ ,\quad \text{when}\quad F_o>40\ ,$$
$$\sqrt{w}=1\ ,\quad \text{when}\quad F_o\leq 40\ .$$

The final parameters and their standard deviations are listed in Tables 1 and 2. A comparison of the observed and calculated structure factors is given in Table 3.

Discussion of the structure

The spermidine molecule

The crystal contains two crystallographically independent molecules, I and II. The bond lengths and angles are shown in Fig. 2 and listed in Table 4, where the values are compared with those found in analogous compounds such as spermine phosphate hexahydrate (Iitaka & Huse, 1965; Huse & Iitaka, 1969), spermine tetrahydrochloride (Giglio, Liquori, Puliti & Ripamonti, 1966*a*) and spermidine trihydrochloride (Giglio, Liquori, Puliti & Ripamonti, 1966*b*).

In the present structure, both molecules I and II take a normal extended zigzag chain conformation with the full lengths for N(1)–N(3) of 11·12 Å and N(4)–N(6) of

Table 1. *Final positional parameters x,y,z in fractional coordinates and anisotropic thermal parameters β_{ij}*

The thermal parameters are of the form:

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

Estimated standard deviations of β_{ij} are given in parentheses.

	x	y	z	β_{11} $10^4\sigma$	β_{22} $10^4\sigma$	β_{33} $10^4\sigma$	β_{12} $10^4\sigma$	β_{13} $10^4\sigma$	β_{23} $10^4\sigma$
P(1)	0·9832	−0·0005	0·2032	0·0049 (3)	0·0006 (0)	0·0104 (7)	0·0001 (1)	0·0019 (3)	0·0001 (1)
O(1)	1·1456	0·0087	0·2258	0·0059 (8)	0·0007 (1)	0·0184 (22)	−0·0000 (2)	0·0029 (10)	0·0002 (4)
O(2)	0·9027	0·0523	0·1772	0·0082 (10)	0·0006 (1)	0·0242 (26)	0·0002 (3)	0·0036 (13)	−0·0002 (4)
O(3)	0·9312	−0·0311	0·0117	0·0100 (11)	0·0009 (1)	0·0139 (21)	−0·0006 (3)	0·0038 (12)	−0·0005 (4)
O(4)	0·9332	−0·0262	0·3939	0·0065 (9)	0·0009 (1)	0·0148 (21)	−0·0005 (3)	0·0042 (10)	0·0003 (4)
P(2)	0·9632	0·1849	0·1899	0·0053 (3)	0·0006 (0)	0·0106 (7)	0·0002 (1)	0·0018 (3)	−0·0000 (1)
O(5)	0·9443	0·1712	−0·0329	0·0078 (9)	0·0009 (1)	0·0153 (21)	0·0004 (3)	0·0012 (10)	−0·0000 (4)
O(6)	0·8386	0·2158	0·2599	0·0111 (12)	0·0013 (1)	0·0237 (28)	0·0011 (3)	0·0038 (14)	0·0004 (4)
O(7)	1·0002	0·1388	0·3273	0·0127 (12)	0·0010 (1)	0·0116 (21)	−0·0005 (3)	−0·0020 (12)	0·0004 (5)
O(8)	1·0986	0·2236	0·2219	0·0114 (12)	0·0010 (1)	0·0203 (26)	−0·0009 (3)	0·0018 (12)	−0·0002 (4)
P(3)	0·5078	0·3975	0·2004	0·0050 (3)	0·0006 (0)	0·0112 (27)	0·0001 (1)	0·0019 (3)	−0·0001 (1)
O(9)	0·4279	0·3428	0·1939	0·0083 (11)	0·0009 (1)	0·0253 (29)	−0·0004 (3)	0·0057 (14)	−0·0005 (5)
O(10)	0·6693	0·3857	0·2159	0·0052 (8)	0·0010 (1)	0·0212 (24)	0·0004 (3)	0·0021 (11)	−0·0003 (4)
O(11)	0·4534	0·4253	0·0047	0·0085 (10)	0·0011 (1)	0·0140 (21)	0·0005 (3)	0·0009 (11)	0·0003 (4)
O(12)	0·4638	0·4255	0·3891	0·0055 (8)	0·0009 (1)	0·0165 (21)	0·0006 (3)	0·0019 (10)	0·0002 (4)
O(13) *W*(1)	0·3551	0·1781	0·2323	0·0116 (13)	0·0016 (2)	0·0534 (44)	−0·0002 (4)	0·0059 (20)	0·0015 (8)
O(14) *W*(2)	0·9019	0·3245	0·2211	0·0079 (10)	0·0009 (1)	0·0410 (35)	0·0006 (3)	0·0033 (15)	0·0008 (5)
O(15) *W*(3)	0·1521	0·3769	0·1831	0·0084 (11)	0·0014 (1)	0·0264 (28)	−0·0004 (3)	0·0001 (14)	0·0010 (5)
O(16) *W*(4)	0·3676	0·0766	0·2574	0·0113 (12)	0·0013 (2)	0·0293 (32)	−0·0006 (4)	0·0030 (15)	−0·0010 (5)
O(17) *W*(5)	0·5994	0·0198	0·1529	0·0103 (12)	0·0018 (2)	0·0359 (35)	0·0001 (4)	0·0070 (15)	−0·0023 (6)
O(18) *W*(6)	0·5633	0·2434	0·1340	0·0105 (12)	0·0016 (2)	0·0269 (29)	−0·0006 (4)	0·0039 (15)	0·0001 (6)
N(1)	0·7379	0·0004	0·6769	0·0067 (10)	0·0007 (1)	0·0170 (26)	−0·0002 (3)	0·0014 (12)	−0·0005 (5)
N(2)	0·7463	0·1899	0·6462	0·0068 (11)	0·0008 (1)	0·0185 (28)	−0·0001 (3)	0·0001 (13)	−0·0007 (5)
N(3)	0·6699	0·4264	0·7276	0·0075 (11)	0·0009 (1)	0·0159 (27)	0·0005 (3)	0·0023 (13)	−0·0001 (5)
C(1)	0·6506	0·0481	0·6614	0·0077 (14)	0·0008 (2)	0·0287 (41)	0·0000 (3)	0·0046 (19)	−0·0002 (4)
C(2)	0·7479	0·0954	0·6533	0·0077 (13)	0·0006 (1)	0·0239 (36)	−0·0003 (3)	0·0023 (16)	−0·0009 (5)
C(3)	0·6541	0·1436	0·6441	0·0090 (15)	0·0008 (2)	0·0235 (37)	−0·0002 (3)	−0·0006 (19)	0·0001 (5)
C(4)	0·6601	0·2379	0·6650	0·0072 (14)	0·0010 (2)	0·0167 (34)	0·0001 (4)	0·0030 (17)	−0·0001 (7)
C(5)	0·7572	0·2842	0·6969	0·0097 (15)	0·0008 (2)	0·0197 (38)	−0·0002 (4)	0·0058 (19)	−0·0006 (6)
C(6)	0·6645	0·3316	0·7092	0·0087 (15)	0·0009 (2)	0·0218 (37)	0·0005 (4)	0·0066 (19)	−0·0002 (6)
C(7)	0·7610	0·3809	0·7272	0·0077 (14)	0·0008 (2)	0·0233 (37)	0·0001 (4)	0·0054 (18)	−0·0000 (6)
N(4)	0·1425	−0·0333	0·7304	0·0078 (11)	0·0006 (1)	0·0129 (25)	0·0002 (4)	0·0026 (13)	0·0003 (4)
N(5)	0·1535	0·1559	0·7065	0·0063 (11)	0·0007 (1)	0·0168 (26)	−0·0000 (4)	0·0018 (14)	−0·0007 (6)
N(6)	0·2539	0·3929	0·6743	0·0061 (11)	0·0007 (1)	0·0164 (27)	0·0001 (4)	0·0012 (13)	−0·0000 (6)
C(8)	0·2363	0·0137	0·7429	0·0076 (13)	0·0008 (1)	0·0176 (33)	0·0006 (3)	0·0033 (16)	0·0000 (6)
C(9)	0·1435	0·0619	0·7319	0·0104 (15)	0·0005 (1)	0·0217 (38)	−0·0002 (4)	0·0033 (19)	0·0001 (6)
C(10)	0·2433	0·1085	0·7317	0·0078 (12)	0·0008 (1)	0·0150 (31)	0·0002 (3)	0·0011 (15)	0·0002 (5)
C(11)	0·2519	0·2029	0·7066	0·0083 (13)	0·0007 (1)	0·0230 (37)	−0·0001 (3)	0·0022 (17)	0·0009 (6)
C(12)	0·1581	0·2509	0·7082	0·0078 (12)	0·0008 (1)	0·0128 (30)	0·0001 (3)	0·0027 (15)	−0·0009 (5)
C(13)	0·2574	0·2977	0·6881	0·0092 (15)	0·0008 (2)	0·0239 (39)	−0·0000 (3)	0·0033 (19)	−0·0001 (6)
C(14)	0·1645	0·3454	0·6782	0·0086 (13)	0·0007 (1)	0·0227 (37)	−0·0003 (3)	0·0028 (17)	−0·0003 (6)

Table 2. *Standard deviations of x,y,z (in Å)*

	σ(ax)	σ(by)	σ(cz)
P(1)	0·002 Å	0·000 Å	0·003 Å
O(1)	0·007	0·007	0·008
O(2)	0·008	0·007	0·009
O(3)	0·008	0·008	0·008
O(4)	0·007	0·008	0·008
P(2)	0·002	0·003	0·003
O(5)	0·007	0·008	0·008
O(6)	0·009	0·009	0·009
O(7)	0·009	0·008	0·008
O(8)	0·009	0·009	0·009
P(3)	0·003	0·003	0·003
O(9)	0·008	0·008	0·009
O(10)	0·007	0·008	0·008
O(11)	0·007	0·008	0·008
O(12)	0·007	0·008	0·008
O(13) *W*(1)	0·010	0·011	0·013
O(14) *W*(2)	0·008	0·009	0·011
O(15) *W*(3)	0·008	0·009	0·009
O(16) *W*(4)	0·009	0·009	0·010
O(17) *W*(5)	0·009	0·010	0·011
O(18) *W*(6)	0·009	0·009	0·010
N(1)	0·008	0·009	0·009
N(2)	0·009	0·009	0·009
N(3)	0·009	0·009	0·009
C(1)	0·012	0·011	0·014
C(2)	0·011	0·011	0·013
C(3)	0·012	0·011	0·013
C(4)	0·011	0·012	0·013
C(5)	0·012	0·011	0·014
C(6)	0·012	0·012	0·013
C(7)	0·011	0·011	0·013
N(4)	0·009	0·008	0·009
N(5)	0·009	0·009	0·009
N(6)	0·008	0·009	0·009
C(8)	0·011	0·011	0·012
C(9)	0·012	0·010	0·013
C(10)	0·011	0·010	0·012
C(11)	0·012	0·010	0·013
C(12)	0·011	0·010	0·011
C(13)	0·012	0·011	0·014
C(14)	0·011	0·010	0·013

11·15 Å. The deviations of the atoms from the best plane formed by the atoms of each molecule are shown in Table 5. It is seen that the molecule I takes an almost planar conformation whereas the molecule II is slightly waved with a maximum deviation of about 0·2 Å. As will be discussed later, the hydrogen bond directions from the imino and amino nitrogen atoms suggest that the configuration of the bonds about each nitrogen atom is tetrahedral. All of the nitrogen atoms are, therefore, protonated and exist as $-NH_2^+-$ or $-NH_3^+$ ions.

The phosphate groups

Bond lengths and angles of the three crystallographically independent phosphate groups are listed in Table 6 together with the values observed in spermine phosphate hexahydrate. It will be seen that the main feature of the structures is the strong similarity between them. As in the case of spermine phosphate hexahydrate, the chemical structure of the crystal can be written as:

$$2[NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_3^+].3[HPO_4]^{2-}.6H_2O\,.$$

It is clear that each phosphate group has two formal negative charges distributed mainly among three oxygen atoms.

The P–O bond lengths for the ionized oxygen atoms range from $1{\cdot}50_2$ Å in P(2)–O(5) to $1{\cdot}53_4$ Å in P(1)–O(4), and the P–OH bond lengths are $1{\cdot}56_4$ Å, $1{\cdot}59_9$ Å and $1{\cdot}60_3$ Å for phosphate groups I, II and III respectively, which should be compared with $1{\cdot}59_5$ Å, found for P–O(4)H of spermine phosphate hexahydrate.

The hydrogen atoms of the three P–OH groups are involved either in the hydrogen bonding between the phosphate groups [P(1)–O(2)H···O(7)–P(2) as in the

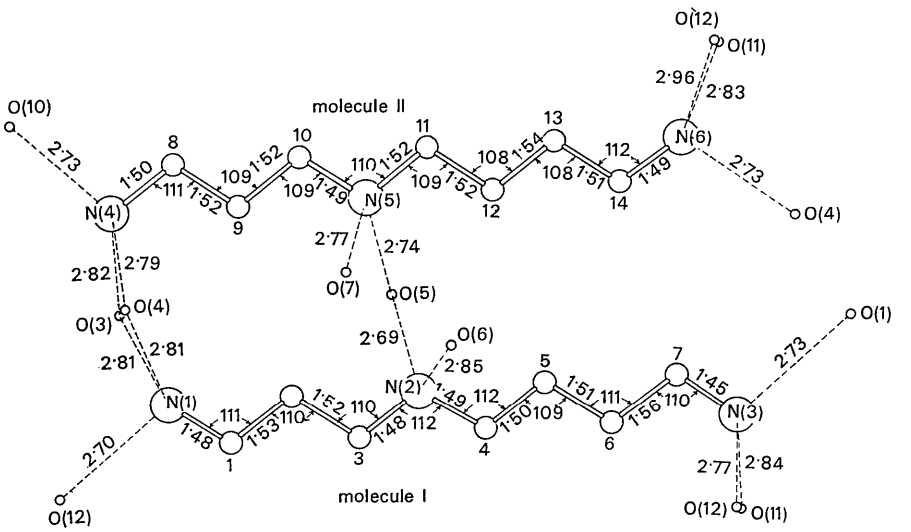

Fig. 2. Bond lengths and angles found in spermidine molecules I and II. Hydrogen bonds involving amino and imino nitrogen atoms are also shown.

Table 3. *Observed and calculated structure factors*

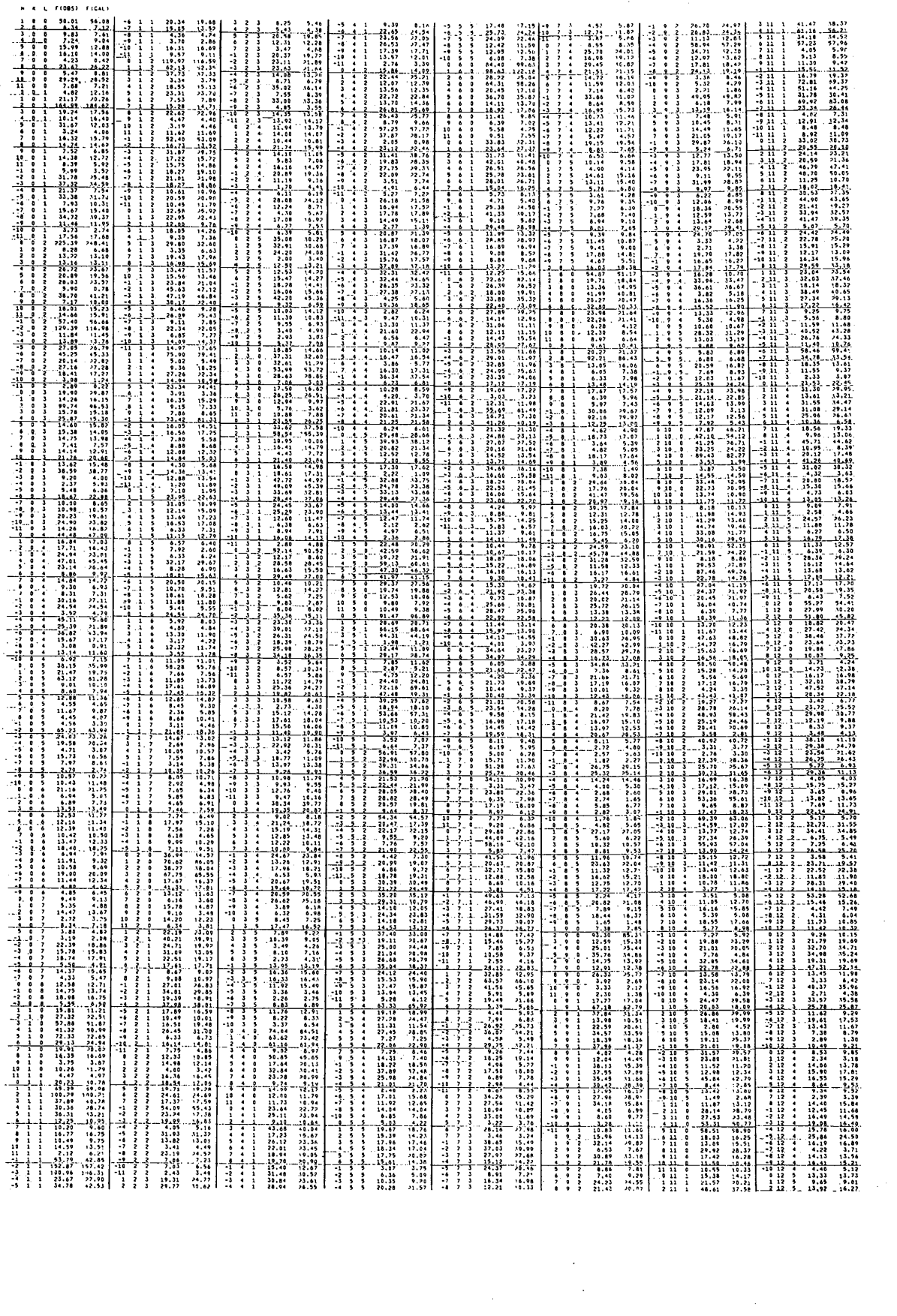

Table 3 (*cont.*)

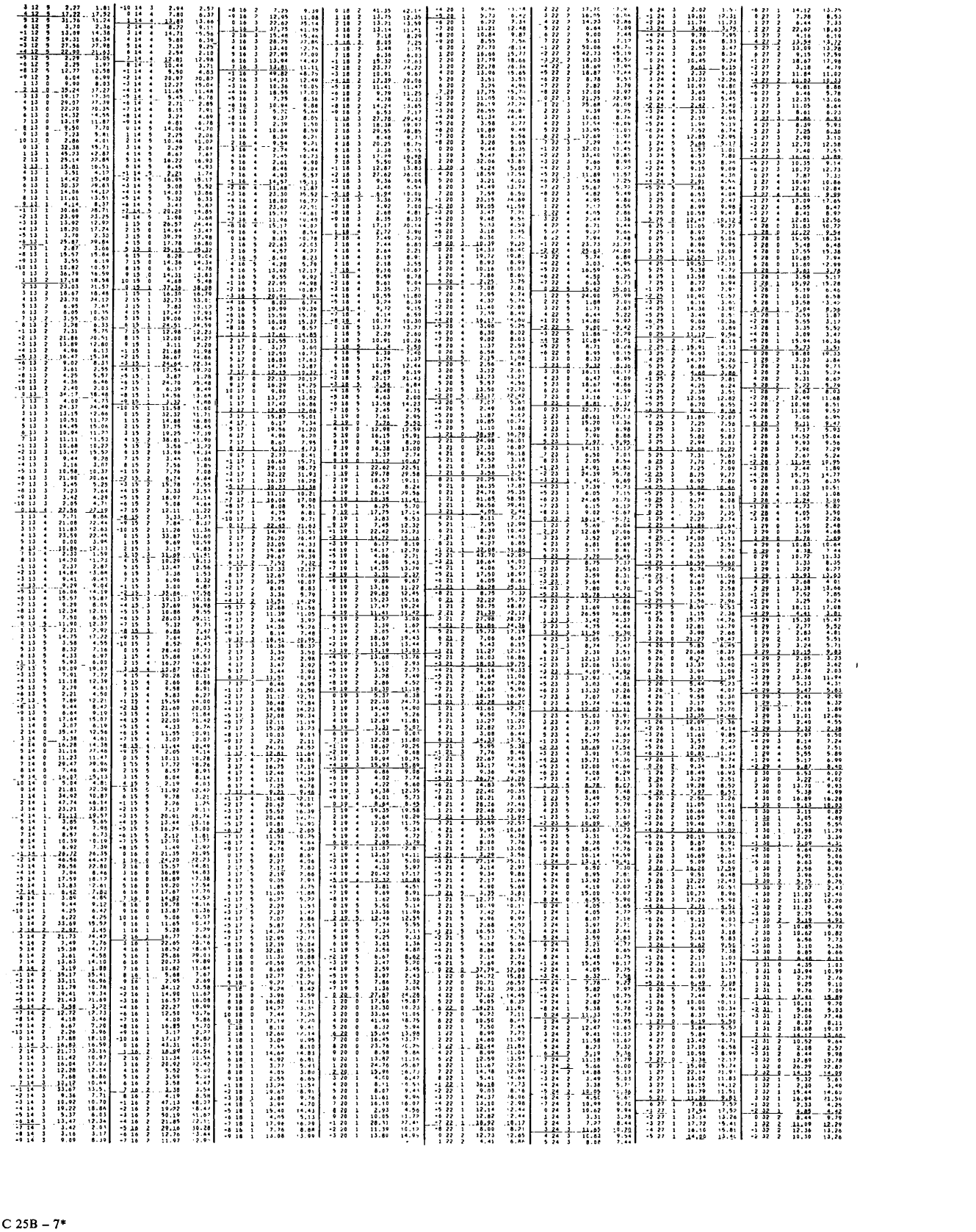

Table 4. *Bond lengths and angles found in spermidine and spermine molecules*

Bond†	Spermidine phosphate trihydrate (present study) Molecule I	Molecule II	Spermidine trihydrochloride (Giglio *et al.* 1966*b*)	Spermine phosphate hexahydrate (Huse & Iitaka, 1969)	Spermine tetrahydrochloride (Giglio *et al.* 1966*a*)
N(1)–C(1)	1·48 ± 0·015 Å	1·50 ± 0·015	1·49 ± 0·013	1·503 ± 0·010	1·49 ± 0·009
C(1)–C(2)	1·53 ± 0·017	1·52 ± 0·017	1·50 ± 0·013	1·516 ± 0·012	1·51 ± 0·009
C(2)–C(3)	1·52 ± 0·017	1·52 ± 0·017	1·51 ± 0·012	1·531 ± 0·012	1·52 ± 0·009
C(3)–N(2)	1·48 ± 0·015	1·49 ± 0·015	1·47 ± 0·012	1·496 ± 0·010	1·50 ± 0·008
N(2)–C(4)	1·49 ± 0·015	1·52 ± 0·015	1·47 ± 0·011	1·494 ± 0·010	1·50 ± 0·010
C(4)–C(5)	1·50 ± 0·017	1·52 ± 0·017	1·52 ± 0·013	1·510 ± 0·012	1·53 ± 0·010
C(5)–C(6)	1·51 ± 0·017	1·54 ± 0·017	1·50 ± 0·012	1·546 ± 0·017	1·53 ± 0·012
C(6)–C(7)	1·56 ± 0·017	1·51 ± 0·017	1·53 ± 0·013		
C(7)–N(3)	1·45 ± 0·015	1·49 ± 0·015	1·49 ± 0·012		
N(1)–C(1)–C(2)	111·3 ± 0·7°	110·8 ± 0·7	110·5 ± 0·9	111·8 ± 0·6	111·2 ± 0·5
C(1)–C(2)–C(3)	109·5 ± 0·7	108·8 ± 0·7	112·7 ± 0·8	107·7 ± 0·6	109·2 ± 0·5
C(2)–C(3)–N(2)	110·4 ± 0·7	109·4 ± 0·7	112·6 ± 0·9	111·6 ± 0·6	111·1 ± 0·5
C(3)–N(2)–C(4)	111·9 ± 0·7	110·0 ± 0·7	111·8 ± 0·9	111·0 ± 0·6	114·6 ± 0·5
N(2)–C(4)–C(5)	111·6 ± 0·7	108·7 ± 0·7	112·9 ± 0·9	112·6 ± 0·6	113·4 ± 0·6
C(4)–C(5)–C(6)	109·3 ± 0·7	107·8 ± 0·7	113·5 ± 0·9	110·1 ± 0·8	110·5 ± 0·5
C(5)–C(6)–C(7)	110·8 ± 0·7	108·4 ± 0·7	108·6 ± 0·8		
C(6)–C(7)–N(3)	110·3 ± 0·7	111·9 ± 0·7	109·5 ± 0·9		

† Numbering system adopted in the Table is:

for spermidine N(1)–C(1)–C(2)–C(3)–N(2)–C(4)–C(5)–C(6)–C(7)–N(3)
for spermine N(1)–C(1)–C(2)–C(3)–N(2)–C(4)–C(5)*C(6) ·····(centre of symmetry at *)

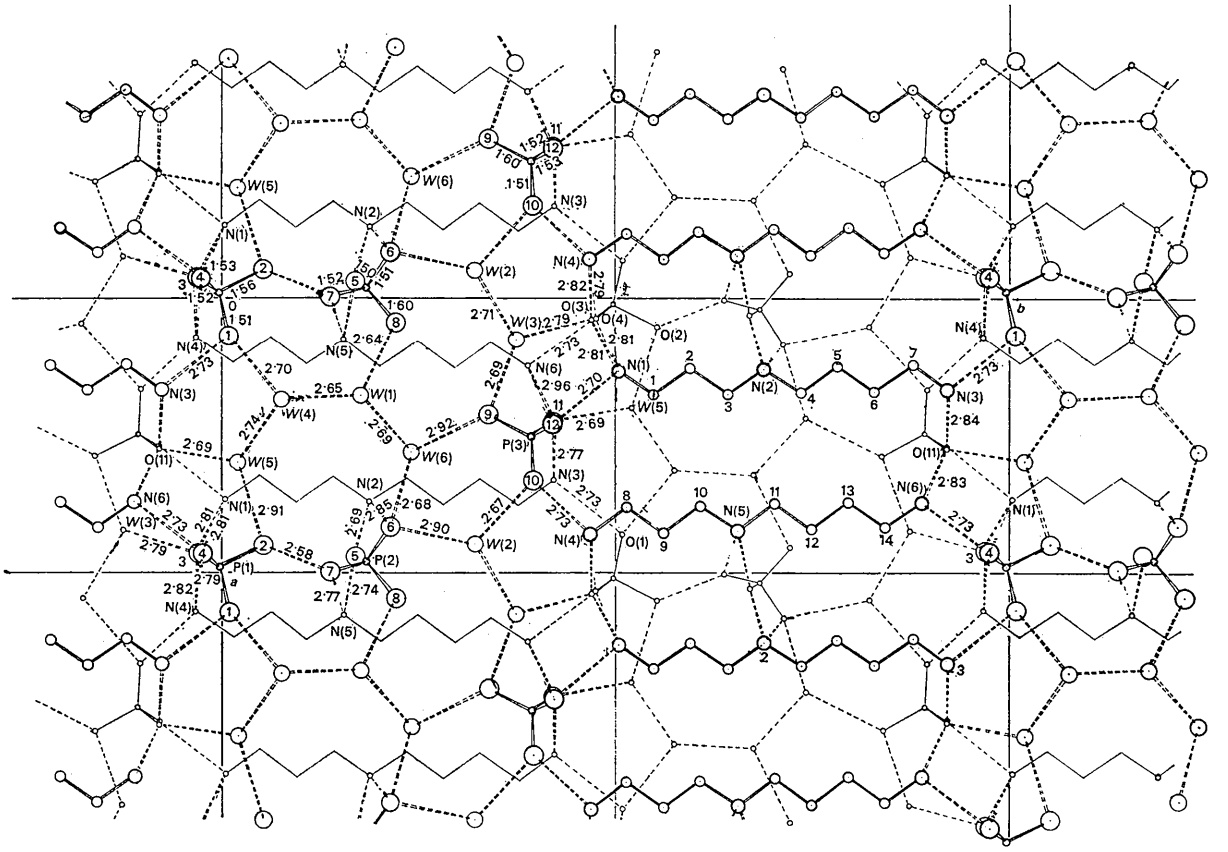

Fig. 3. Projection of the structure along the *c* axis. Broken lines indicate hydrogen bonds. Single lines show the structure of the lower sheet. The right-handed coordinate system is assumed.

Table 5. *Perpendicular distances of atoms from the best plane formed by each molecule*

Molecule I		Molecule II	
N(1)	0·21 Å	N(4)	−0·08 Å
C(1)	0·09	C(8)	0·02
C(2)	−0·06	C(9)	0·04
C(3)	−0·13	C(10)	0·05
N(2)	−0·22	N(5)	−0·03
C(4)	−0·11	C(11)	−0·01
C(5)	0·00	C(12)	0·08
C(6)	0·07	C(13)	−0·03
C(7)	0·08	C(14)	−0·01
N(3)	0·08	N(6)	−0·02

present crystal, and P–O(4)H···O(2)–P as in spermine phosphate hexahydrate] or in the hydrogen bondings between phosphate groups and water molecules [P(2)–O(8)H···*W*(1) and P(3)–O(9)H···*W*(3)]. The two phosphate groups I and II form a dimer ion through the hydrogen bond, P(1)–O(2)H···O(7)–P(2), as mentioned above.

Each of the ionized phosphate oxygen atoms accepts two or three hydrogen bonds from water molecules or protonated nitrogen atoms. O(6) accepts three hydrogen bonds in approximately tetrahedral directions, whereas the other oxygen atoms accept two hydrogen bonds in a roughly planar trigonal arrangement.

Hydrogen bonds and framework features

Two projections of the crystal structure of spermidine phosphate trihydrate viewed along the *c* and the *b* axes are shown in Figs. 3 and 4 respectively. As seen in Fig. 3, the structure consists of bands of spermidine molecules separated by regions of phosphate ions and water. The inorganic region has a chemical composition $HPO_4^{2-}.2H_2O$ and also forms a band structure through O–H···O hydrogen bonds. The two kinds of

Table 6. *Bond lengths and angles found in monohydrogen phosphate groups*

Spermidine phosphate trihydrate						Spermine phosphate hexahydrate (Huse & Iitaka, 1969)	
I		II		III			
P(1)–O(1)	1·508 Å	P(2)–O(5)	1·502	P(3)–O(9)H	1·603	P–O(1)	1·505
P(1)–O(2)H	1·564	P(2)–O(6)	1·513	P(3)–O(10)	1·514	P–O(2)	1·523
P(1)–O(3)	1·525	P(2)–O(7)	1·522	P(3)–O(11)	1·516	P–O(3)	1·527
P(1)–O(4)	1·534	P(2)–O(8)H	1·599	P(3)–O(12)	1·531	P–O(4)	1·595
	(±0·009)		(±0·009)		(±0·009)		(±0·006)
O(1)–P(1)–O(2)	109·1°	O(5)–P(2)–O(6)	114·2	O(9)—P(3)–O(10)	105·5	O(1)–P–O(2)	113·6
O(1)–P(1)–O(3)	112·8	O(5)–P(2)–O(7)	112·7	O(9)—P(3)–O(11)	107·0	O(1)–P–O(3)	109·5
O(1)–P(1)–O(4)	111·6	O(5)–P(2)–O(8)	106·8	O(9)—P(3)–O(12)	106·6	O(1)–P–O(4)	108·1
O(2)–P(1)–O(3)	105·3	O(6)–P(2)–O(7)	111·6	C(10)–P(3)–O(11)	112·9	O(2)–P–O(3)	112·3
O(2)–P(1)–O(4)	107·0	O(6)–P(2)–O(8)	103·5	C(10)–P(3)–O(12)	112·6	O(2)–P–O(4)	108·2
O(3)–P(1)–O(4)	110·7	O(7)–P(2)–O(8)	107·1	O(11)–P(3)–O(12)	111·7	O(3)–P–O(4)	104·7
	(±0·5°)		(±0·5°)		(±0·5°)		(±0·3°)

Table 7. *Hydrogen bond distances*

O–H···O hydrogen bond

Phosphate···phosphate		Water···water	
O(2)–H···O(7)	2·58±0·011 Å	*W*(1)–H···*W*(4)	2·65±0·014
Phosphate···water		*W*(1)–H···*W*(6)	2·69
O(1)···H–*W*(4)	2·70±0·013	*W*(3)–H···*W*(2)	2·71
O(2)···H–*W*(5)	2·91	*W*(4)–H···*W*(5)	2·74
O(3)···H–*W*(3)	2·79		
O(6)···H–*W*(2)	2·90		
O(6)···H–*W*(6)	2·69		
O(8)–H···*W*(1)	2·64		
O(9)–H···*W*(3)	2·69		
O(9)···H–*W*(6)	2·92		
O(10)··H–*W*(2)	2·67		
O(11)··H–*W*(5)	2·70		

N–H··· hydrogen bond

Molecule I		Molecule II	
N(1)–H···O(3)	2·81±0·012	N(4)–H···(O3)	2·82±0·012
N(1)–H···O(4)	2·81	N(4)–H···O(4)	2·79
N(1)–H···O(12)	2·70	N(4)–H···O(10)	2·73
N(2)–H···O(5)	2·69	N(5)–H···O(5)	2·74
N(2)–H···O(6)	2·85	N(5)–H···O(7)	2·77
N(3)–H···O(1)	2·73	N(6)–H···(O4)	2·73
N(3)–H···O(11)	2·84	N(6)–H···O(11)	2·83
N(3)–H···O(12)	2·77	N(6)–H···O(12)	2·96

band are extended along the *a* axis and joined together side by side through N–H···O hydrogen bonds, forming a striped sheet parallel to the (001) plane. These striped sheets are piled up alternately along the *c* axis in such a way that the phosphate–water band comes immediately next to the molecular band of spermidine. Within the phosphate–water band, the dimer ions and the isolated single ions of monohydrogen phosphate are separated by the water molecules, forming a network of hydrogen bonds. The structure of the phosphate–water band is somewhat similar to that of the phosphate–water sheet found in the crystals of spermine phosphate hexahydrate. In the latter structure, the monohydrogenphosphate ions are hydrogen bonded to form an infinite chain, and the chains are bound together through the water molecules, forming a hexagonal planar network of hydrogen bonds. The lengths of hydrogen bonds found in the present structure are summarized in Table 7.

The lengths of the O–H···O bonds are within the range $2{\cdot}64_0$ Å ~ $2{\cdot}91_7$ Å; the only appreciably short hydrogen bond, $2{\cdot}58_2$ Å, is found between the phos-

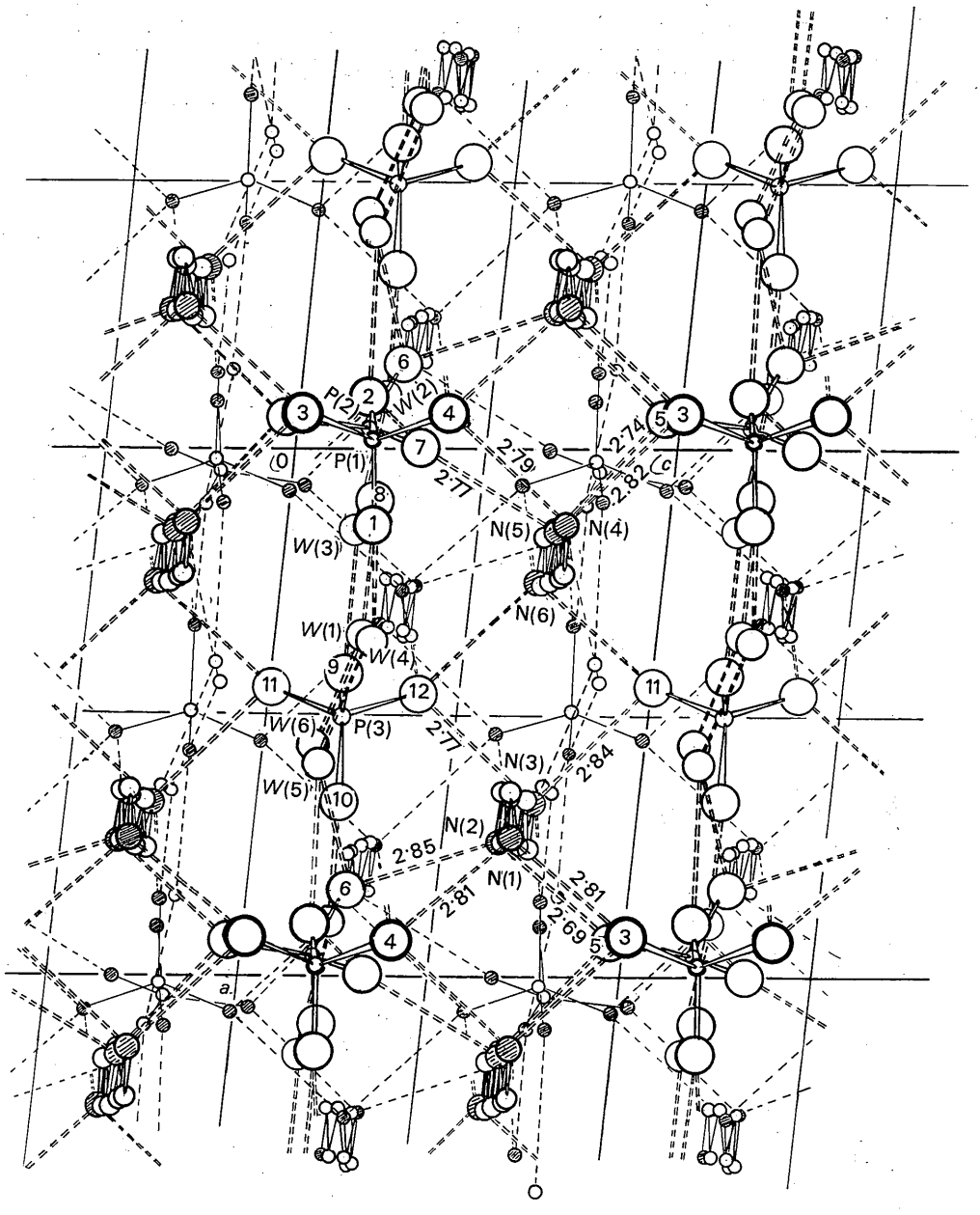

Fig. 4. Projection of the structure along the *b* axis. The right-handed coordinate system is assumed.

phate ions I and II, which links the ions to form a dimer. It is clearly seen that each water oxygen atom forms hydrogen bonds approximately in three of the four tetrahedral directions. The fourth direction turns to the adjoining layer of spermidine molecules. The partners of these hydrogen bonds are the oxygen atoms of either water or phosphate groups and not the nitrogen atoms of the spermidine molecule. A similar feature of the hydrogen bond system was found in spermine phosphate hexahydrate, in which, however, the $W(3)$ atom accepts a hydrogen bond from the NH_2^+ group, thereby completing a tetrahedral arrangement. As to the location of the hydrogen atoms, a disordered arrangement among the chains of water molecules was found in spermine phosphate hexahydrate. In the present structure, however, no such chains of hydrogen bonds are observed.

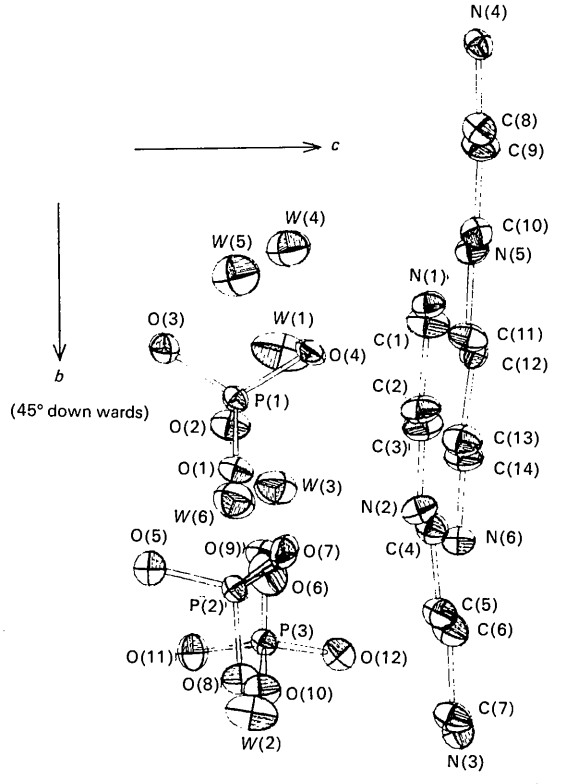

Fig. 5. Projection of the structure showing the ellipsoids of thermal vibrations drawn for atoms in the asymmetric unit. The Figure is drawn by rotating the structure shown in Fig. 4 by 45° about the c axis.

As is generally observed in long chain aliphatic compounds, the zigzag chains of spermidine molecules are arranged parallel to each other along the b axis keeping a distance of about 4·5 Å from adjacent molecules. However, the arrangement of molecules is alternating, crystallographically different kinds of molecules being always immediate neighbours. The angle of tilt defined in the layer structure of long chain compounds may also be applied to this sort of structure. In the present case, as is evident from Fig. 3, the tilt angle is 90°, whereas in spermine phosphate hexahydrate, it was 33°. As shown in Fig. 3, the zigzag planes of the molecules are all parallel and coplanar with the molecular layer. The closest approaches of the carbon and nitrogen atoms of neighbouring molecules I and II are as follows:

C(2)–C(9′)	$3{\cdot}73_4$ Å,
C(2)–N(5′)	$4{\cdot}04_0$ Å,
N(1)–N(4′)	$3{\cdot}81_2$ Å.

The hydrogen bonds from the amino and imino nitrogen to the phosphate oxygen atoms are listed in Table 7. These hydrogen bonds are arranged about each nitrogen atom in tetrahedral directions as shown in Table 8. The root mean square displacements of thermal vibrations of atoms along the principal axes of the ellipsoids, and their direction cosines referred to the orthogonal axes ($x||a$, $y||b$, z in a–c plane) are listed in Table 9.

Table 8. *Arrangement of hydrogen bonds about amino and imino nitrogen atoms*

Molecule I		Molecule II	
C(1)—N(1)···O(3)	126·6 ± 0·5°	C(8)—N(4)···O(10)	105·6 ± 0·5°
C(1)—N(1)···O(4)	122·9	C(8)—N(4)···O(3)	112·4
C(1)—N(1)···O(12)	103·6	C(8)—N(4)···O(4)	109·7
O(3)···N(1)···O(4)	92·9 ± 0·3	O(4)···N(4)···O(10)	122·1 ± 0·3
O(3)···N(1)···O(12)	106·7	O(3)···N(4)···O(4)	93·0
O(4)···N(1)···O(12)	101·2	O(3)···N(4)···O(10)	113·8
C(3)—N(2)···O(6)	114·3 ± 0·5	C(10)—N(5)···O(5)	118·1 ± 0·5
C(3)—N(2)···O(5)	101·7	C(10)—N(5)···O(7)	101·1
C(4)—N(2)···O(6)	95·0	C(11)—N(5)···O(7)	112·2
C(4)—N(2)···O(5)	114·3	C(11)—N(5)···O(5)	110·0
C(3)—N(2)—C(4)	111·9	C(10)—N(5)—C(11)	110·0
O(6)···N(2)···O(5)	120·2 ± 0·3	O(5)···N(5)···O(7)	105·1 ± 0·3
C(7)—N(3)···O(1)	106·7 ± 0·5	C(14)—N(6)···O(4)	107·6 ± 0·5
C(7)—N(3)···O(11)	116·0	C(14)—N(6)···O(11)	123·5
C(7)—N(3)···O(12)	109·9	C(14)—N(6)···O(12)	130·6
O(1)···N(3)···O(11)	114·0 ± 0·3	O(4)···N(6)···O(11)	104·2 ± 0·3
O(1)···N(3)···O(12)	117·6	O(4)···N(6)···O(12)	96·8
O(11)··N(3)···O(12)	92·7	O(11)··N(6)···O(12)	89·2

Table 9. *Principal axes of thermal ellipsoids*

The root mean square displacement $(\text{r.m.s.})_i$ (Å) corresponds to the ith principal axis of the ellipsoid, and q_{ix}, q_{iy}, q_{iz} are the angles between the ith axis and the orthogonal axes x, y, z respectively. The orthogonal axes are chosen in such a way that x is coincident with a, y with b, and z with c^*.

	i	$(\text{r.m.s.})_i$	q_{ix}	q_{iy}	q_{iz}
P(1)	1	0·134	0·8105	−0·2049	−0·5485
	2	0·143	−0·0040	0·9347	−0·3551
	3	0·159	0·5856	0·2901	0·7568
O(1)	1	0·148	0·8806	0·3812	−0·2812
	2	0·158	−0·3960	0·9181	0·0043
	3	0·203	0·2598	0·1075	0·9596
O(2)	1	0·143	−0·2275	0·9686	0·0995
	2	0·180	0·9406	0·2451	−0·2346
	3	0·232	0·2517	−0·0402	0·9669
O(3)	1	0·160	−0·3887	0·2174	0·8952
	2	0·169	0·4626	0·8864	−0·0144
	3	0·221	−0·7967	0·4086	−0·4452
O(4)	1	0·124	−0·7527	−0·4311	0·4975
	2	0·183	0·1693	−0·8571	−0·4864
	3	0·198	0·6361	−0·2818	0·7181
P(2)	1	0·132	0·7113	−0·5598	−0·4248
	2	0·149	−0·1858	−0·7328	0·6545
	3	0·161	−0·6777	−0·3866	−0·6253
O(5)	1	0·170	0·7384	−0·6711	−0·0652
	2	0·182	−0·0312	0·0625	−0·9975
	3	0·195	−0·6735	−0·7386	−0·0252
O(6)	1	0·177	0·6831	−0·7235	−0·0988
	2	0·217	−0·3377	−0·4331	0·8356
	3	0·248	−0·6474	−0·5375	−0·5402
O(7)	1	0·150	−0·2295	0·1383	−0·9634
	2	0·181	−0·2707	−0·9598	−0·0733
	3	0·245	−0·9348	0·2440	0·2577
O(8)	1	0·169	0·4970	0·8641	0·0786
	2	0·209	−0·1770	0·0122	0·9841
	3	0·235	−0·8494	0·5030	−0·1590
P(3)	1	0·133	0·7501	−0·4812	−0·4535
	2	0·145	−0·4044	−0·8765	0·2609
	3	0·162	−0·5231	−0·0123	−0·8521
O(9)	1	0·163	−0·8640	−0·4077	0·2952
	2	0·177	0·3102	−0·8931	−0·3255
	3	0·246	−0·3964	0·1896	−0·8982
O(10)	1	0·140	−0·9416	0·3234	0·0928
	2	0·190	−0·3362	−0·9151	−0·2222
	3	0·216	−0·0131	0·2405	−0·9705
O(11)	1	0·169	−0·4165	0·4940	−0·7631
	2	0·179	−0·6307	0·4474	0·6339
	3	0·207	0·6546	0·7454	0·1252
O(12)	1	0·139	0·8942	−0·4409	−0·0767
	2	0·182	−0·2697	−0·6677	0·6937
	3	0·195	−0·3571	−0·5997	−0·7160
O(13)	1	0·211	0·8573	0·4886	−0·1615
W(1)	2	0·234	−0·5097	0·8494	−0·1359
	3	0·344	0·0708	0·1989	0·9774
O(14)	1	0·159	0·6717	−0·7368	0·0759
W(2)	2	0·197	−0·7402	−0·6638	0·1067
	3	0·299	−0·0282	−0·1279	−0·9913
O(15)	1	0·184	0·9400	0·3247	0·1044
W(3)	2	0·208	−0·2083	0·7890	−0·5779
	3	0·254	−0·2701	0·5215	0·8093
O(16)	1	0·197	−0·5421	−0·8060	−0·2373
W(4)	2	0·222	0·8077	−0·4221	−0·4115
	3	0·261	0·2315	−0·4148	0·8799
O(17)	1	0·180	0·8248	−0·3876	−0·4115
W(5)	2	0·232	−0·5340	−0·7732	−0·3418
	3	0·304	0·1856	−0·5017	0·8448
O(18)	1	0·196	0·8792	0·3982	−0·2612
W(6)	2	0·238	−0·2357	0·8404	0·4879
	3	0·247	−0·4138	0·3674	−0·8328

Table 9 (*cont.*)

	i	$(\text{r.m.s.})_i$	q_{ix}	q_{iy}	q_{iz}
N(1)	1	0·145	0·2188	0·9443	0·2455
	2	0·169	0·9710	−0·1860	−0·1500
	3	0·195	−0·0959	0·2713	−0·9576
N(2)	1	0·156	0·4121	0·8063	0·4242
	2	0·172	−0·8783	0·4753	−0·0501
	3	0·208	0·2420	0·3519	−0·9041
N(3)	1	0·153	0·7148	−0·6319	−0·2994
	2	0·186	−0·0574	−0·4798	0·8754
	3	0·197	−0·6969	−0·6086	−0·3792
C(1)	1	0·163	0·4036	−0·9059	−0·1282
	2	0·172	−0·8887	−0·4214	0·1802
	3	0·253	−0·2172	0·0412	−0·9752
C(2)	1	0·139	0·1346	0·9628	0·2340
	2	0·179	0·9858	−0·1063	−0·1297
	3	0·232	−0·1000	0·2482	−0·9635
C(3)	1	0·159	0·2149	0·9758	0·0396
	2	0·191	0·8693	−0·2096	0·4475
	3	0·233	−0·4450	0·0617	0·8933
C(4)	1	0·160	−0·8569	0·2110	0·4702
	2	0·189	−0·1954	−0·9772	0·0823
	3	0·197	−0·4769	0·0213	−0·8786
C(5)	1	0·158	−0·3933	0·7526	0·5279
	2	0·169	0·6712	0·6275	−0·3944
	3	0·235	0·6282	−0·1992	0·7520
C(6)	1	0·144	−0·7622	0·4526	0·4627
	2	0·190	0·3374	0·8879	−0·3126
	3	0·239	0·5523	0·0821	0·8295
C(7)	1	0·157	−0·8832	0·2699	0·3834
	2	0·170	−0·2417	−0·9627	0·1210
	3	0·235	−0·4018	−0·0142	−0·9155
N(4)	1	0·144	−0·0323	−0·9245	0·3795
	2	0·158	0·6110	−0·3188	−0·7245
	3	0·190	−0·7909	−0·2085	−0·5753
N(5)	1	0·148	−0·2593	0·8852	0·3860
	2	0·163	0·9600	0·2798	0·0031
	3	0·197	−0·1052	0·3714	−0·9224
N(6)	1	0·155	−0·5227	0·8524	0·0017
	2	0·162	0·8524	0·5227	0·0054
	3	0·188	−0·0037	−0·0043	0·9999
C(8)	1	0·143	0·6263	−0·7538	−0·1985
	2	0·179	−0·5414	−0·6039	0·5849
	3	0·206	−0·5608	−0·2588	−0·7864
C(9)	1	0·134	0·0954	0·9937	−0·0573
	2	0·200	0·8131	−0·1110	−0·5713
	3	0·224	0·5741	−0·0079	0·8186
C(10)	1	0·156	−0·2847	0·9301	−0·2319
	2	0·181	−0·4601	0·0796	0·8842
	3	0·184	0·8409	0·3584	0·4052
C(11)	1	0·146	0·1749	0·9489	−0·2623
	2	0·187	0·9835	−0·1805	0·0027
	3	0·228	0·0447	0·2584	0·9649
C(12)	1	0·131	−0·3312	0·6705	0·6637
	2	0·177	0·7072	0·6421	−0·2957
	3	0·191	−0·6245	0·3714	−0·6869
C(13)	1	0·164	0·0125	−0·9996	−0·0247
	2	0·191	−0·9547	−0·0193	0·2968
	3	0·230	−0·2972	0·0199	−0·9545
C(14)	1	0·150	−0·2505	−0·9660	−0·0630
	2	0·189	−0·9342	0·2241	0·2772
	3	0·224	−0·2537	0·1283	−0·9587

Fig. 5 shows the ellipsoids of thermal vibrations drawn with the use of the *ORTEP* program (Johnson, 1965), the surfaces of which enclose 50% of normally distributed displacements. For the sake of simplicity, the Figure was drawn only for independent atoms; the c axis is horizontal and the positive direction of the b axis is tilted at an angle of 45° downwards from

the surface of the paper. It is seen that the maximum vibrations of the spermidine and water molecules occur mostly in a direction perpendicular to the sheet [parallel to (001)] whereas in the phosphate groups the oscillatory motions seem to be predominant.

The authors wish to express their gratitude to Professor M. Tsuboi of the University of Tokyo and Professor K. Miyaki of the University of Chiba, for their helpful advice and constant interest in this work. We are also grateful to C. Itoh Electronic Computing Service for the use of a CDC 3600 computer. This investigation was supported by the Research Grants from the Ministry of Education and Hohansha.

References

BERGHUIS, J., HAANAPPEL, IJ. M., POTTERS, M., LOOPSTRA, B. O., MACGILLAVRY, C. H. & VEENENDAAL, A. L. (1955). *Acta Cryst.* **8**, 478.

BRETTHAUER, R. H., MARCUS, L., CHALOUPKA, J., HALVORSON, H. O. & BOCK, R. M. (1963). *Biochemistry*, **2**, 1079.

GIGLIO, E., LIQUORI, A. M., PULITI, R. & RIPAMONTI, A. (1966*a*). *Acta Cryst.* **20**, 652.

GIGLIO, E., LIQUORI, A. M., PULITI, R. & RIPAMONTI, A. (1966*b*). *Acta Cryst.* **20**, 683.

HUSE, Y. & IITAKA, Y. (1969). To be published.

IITAKA, Y. (1953). *Acta Cryst.* **6**, 663.

IITAKA, Y. & HUSE, Y. (1965). *Acta Cryst.* **18**, 110.

JOHNSON, C. K. (1965). *ORTEP*. Oak Ridge National Laboratory, Oak Ridge, Tennessee,. U.S.A.

KRAKOW, J. S. (1963). *Biochem. biophys. Acta*, **72**, 566.

MAHLER, H. R., MEHROTRA, B. D. & SHAYS, C. W. (1961). *Biochem. biophys. Res. Commun.* **4**, 79.

OKAYA, Y. & ASHIDA, T. (1967). *HBLS* IV, *The Universal Crystallographic Computing System* (I), p. 65. Japanese Crystallographic Association.

TABOR, H. (1962). *Biochemistry*, **1**, 496.

TANNER, M. J. A. (1967). *Biochemistry*, **6**, 2686.

VIERVOLL, H. & ØGRIM, O. (1949). *Acta Cryst.* **2**, 277.

Acta Cryst. (1969). B**25**, 509

Crystal Structure of Glycylglycine Hydrochloride

BY R. PARTHASARATHY

Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, New York 14203, *U.S.A. and Biophysics Department, State University of New York at Buffalo, Buffalo, New York, U.S.A.*

(*Received* 13 *December* 1967 *and in revised form* 27 *April* 1968)

Crystals of glycylglycine hydrochloride ($C_4N_2O_3H_9.Cl.H_2O$) are monoclinic, space group $P2_1/c$ with four molecules in the unit cell. The cell parameters at $22°\pm3°C$ are: $a=8{\cdot}813\pm0{\cdot}003$, $b=9{\cdot}755\pm0{\cdot}005$, $c=9{\cdot}788\pm0{\cdot}003$ Å, $\beta=104{\cdot}10°\pm0{\cdot}02°$ (Cu $K\alpha_1=1{\cdot}54051$ Å). Using a G.E. XRD-3 diffractometer, equipped with goniostat, three-dimensional intensity data were collected by the stationary-crystal stationary-counter method, to the limit $2\theta=140°$. The structure has been refined by Fourier and least-squares method to a final R value of 0·065. The crystal structure is stabilized by a network of hydrogen bonds. An interesting structural feature is the occurrence of apparently bifurcated hydrogen bonds, involving each of the three hydrogen atoms on the terminal nitrogen atom. It is found that in all these bifurcated hydrogen bonds, the hydrogen atom lies close to the plane through the donor and the two acceptor atoms. The planarity of the peptide and the conformation of the molecule are discussed in detail.

Introduction

Accurate crystal structure determinations of amino acids and simple peptides are of fundamental importance in arriving at the configurations of polypeptides and proteins. Such studies have greatly aided the establishment of stereochemical criteria for polypeptide and protein chain conformations (for a review, see Schellman & Schellman, 1964). The present article describes an accurate three-dimensional structure analysis of a dipeptide, glycylglycine hydrochloride. A thorough analysis of the hydrogen bonding and conformation angles is presented.

Glycylglycine hydrochloride was selected for analysis for several reasons. Excellent crystals of glycylglycine can be grown from aqueous solutions by slow evaporation. In this laboratory, many glycine peptides are being studied by paramagnetic resonance techniques (Box, Freund & Lilga, 1961) and detailed X-ray studies on these peptides are in progress.

Experimental

Single crystals of glycylglycine hydrochloride were grown by slow evaporation from aqueous solutions. Excellent crystals in the form of fine needles elongated