

## The Crystal Structure of Glycylglycine Phosphate Monohydrate

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Crystals of glycylglycine phosphate monohydrate were obtained by evaporating an aqueous solution containing equimolar quantities of phosphoric acid and the dipeptide, glycylglycine. The crystals are orthorhombic, space group  $Pna2_1$ , with  $a = 17.879$  (6),  $b = 12.130$  (4) and  $c = 4.621$  (1) Å. Three-dimensional intensity data were collected using a Picker FACS-1 diffractometer. The structure was solved by the heavy-atom method and was refined by full-matrix least squares to  $R = 0.024$ . The crystal structure features a short hydrogen bond ( $2.503 \pm 0.003$  Å) between the phosphate moiety and the carboxyl group of the dipeptide. Although the least-squares refinement suggested that the hydrogen atom involved in this bond might be positioned symmetrically between the two oxygen atoms, difference Fourier maps and the observed C–O and P–O bond lengths indicate that it is bonded covalently to the carboxyl oxygen atom. Thus, the compound is properly formulated as  $(\text{glycylglycine.H})^+(\text{H}_2\text{PO}_4)^-$ . The crystal structure also involves a short hydrogen bond of  $2.571$  (3) Å between phosphate moieties related by the  $c$  translation. The peptide conformation is not significantly different from that found in other crystal structures.

### Introduction

Interactions of phosphates with proteins are of likely importance in many biological processes, for example in enzyme reactions that require high-energy phosphates, in formation of protein–nucleic acid complexes and in deposition of phosphate minerals in protein matrices. We are at present examining the crystal structures of a series of amino acid and peptide phosphates in order to obtain additional information about the possible factors involved in phosphate–protein interactions, and to determine what effects phosphate interactions have on peptide conformation. In this paper we report the crystal structure of glycylglycine phosphate monohydrate.

### Experimental

Colorless, transparent needles of glycylglycine phosphate monohydrate were obtained by slowly evaporating an aqueous solution containing equimolar quantities of glycylglycine and phosphoric acid. Weissenberg and oscillation photographs showed that the unit cell is orthorhombic, and the systematic absences ( $0kl$ ,  $k+l=2n+1$ ;  $h0l$ ,  $h=2n+1$ ) indicated space groups  $Pnam$  or  $Pna2_1$ . A block with the approximate dimensions  $0.20 \times 0.21 \times 0.23$  mm was cut from a needle crystal and mounted on a Picker FACS-1 diffractometer with the  $c$  axis (the needle axis) slightly inclined to the  $\varphi$  axis of the diffractometer. The  $2\theta$  values for 12 high-angle ( $\text{Cu } K\alpha_1$ ,  $\lambda = 1.54051$  Å) reflections were measured, and the unit-cell parameters were obtained from a least-squares analysis of these measurements. Crystal data are listed in Table 1.

Intensities of the 954 independent reflections with  $2\theta \leq 128^\circ$  were measured with the diffractometer, using

Table 1. *Crystal data*

Stoichiometry	$\text{C}_4\text{H}_8\text{N}_2\text{O}_2 \cdot \text{H}_3\text{PO}_4 \cdot \text{H}_2\text{O}$
$Z$	4
Space group	$Pna2_1$
$a$	17.879 (6) Å
$b$	12.130 (4)
$c$	4.621 (1)
$\rho$ (Calculated)	1.644 g.cm <sup>-3</sup>
$\rho$ (Measured)	1.63 g.cm <sup>-3</sup>
$\mu$	27.9 cm <sup>-1</sup>

The unit cell parameters were measured at  $25 \pm 2^\circ\text{C}$ . The reported standard deviations are ten times greater than those obtained from the least-squares analysis. The density was measured by flotation in a mixture of carbon tetrachloride and ethylene dibromide.

nickel-filtered copper radiation, a  $\theta$ – $2\theta$  scanning technique and a scintillation counter. A scanning speed of  $0.5^\circ/\text{min}$  was employed, and the background was counted for 20 sec at each terminus of the scans.

The intensities were assigned variances,  $\sigma^2(I)$ , based on the counting statistics plus a correctional term  $(0.03S)^2$ ,  $S$  being the scan count. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied using the program *ORABS* (Wehe, Busing & Levy, 1962). The absorption correction factors ranged from 1.58 to 1.73. The first derivatives of the transmission factors with respect to  $\mu$  were also computed for subsequent calculation of extinction corrections. Finally the data were placed on an approximately absolute scale by means of a Wilson (1942) plot.

A Howells, Phillips & Rogers (1950) plot indicated that the crystal structure is noncentrosymmetric. Therefore, we assumed that the space group is  $Pna2_1$ ; this assumption was corroborated by the final structure analysis.

### Determination and refinement of the structure

Trial  $x$  and  $y$  coordinates for the phosphorus atom were obtained from a sharpened three-dimensional Patterson map, and a  $z$  coordinate of 0.7004 was arbitrarily assigned to this atom. A structure factor calculation, including only the phosphorus atom, yielded an  $R$  index ( $=\sum|F_o| - |F_c| / \sum|F_o|$ ) of 0.54. Trial coordinates for the phosphate oxygen atoms were obtained from a Fourier map phased with the phosphorus atom. A structure factor calculation, based on the phosphate moiety, resulted in an  $R$  index of 0.45. At this stage, a difference Fourier map revealed the positions of the peptide heavy atoms; including these atoms, the  $R$  index decreased to 0.33. A difference Fourier calculation then revealed the water oxygen atom. A structure-factor calculation, including all heavy atoms, gave an  $R$  index of 0.28.

The trial structure was refined by full-matrix least squares using a modified version of the program *ORFLS* (Busing, Martin & Levy, 1962). The quantity minimized was  $\sum w[F_o^2 - (1/k^2)F_c^2]^2$  where  $k$  is a scale factor and the weight  $w$  is equal to  $1/\{\sigma^2(F_o^2) + (0.03 \times F_o^2)\}$ . Scattering factors for the heavy atoms were from *International Tables for X-ray Crystallography* (1962). The hydrogen scattering factors were from Stewart, Davidson & Simpson (1965). The heavy-atom positional and isotropic temperature factors were refined to an  $R$  index of 0.11. Additional refinement with anisotropic temperature factors reduced  $R$  to 0.07. At this stage, a difference Fourier map revealed the positions of the thirteen hydrogen atoms. The final cycles of refinement included all positional parameters, along with anisotropic temperature factors for the heavy atoms,

isotropic temperature factors for the hydrogen atoms, and Zachariasen's (1963) isotropic extinction parameter  $g$  (as formulated by Coppens & Hamilton, 1970).

During the refinement, real and imaginary anomalous dispersion corrections from *International Tables for X-ray Crystallography* (1962) were applied to the scattering factors of the phosphorous and oxygen atoms. Two models related to each other by  $\bar{1}$  symmetry were refined to completion. One model refined to  $R=0.026$  and a goodness-of-fit  $[\{\sum w(F_o^2 - F_c^2/k^2)^2 / (m-s)\}^{1/2}]$ , where  $m$  is the number of reflections used and  $s$  is the number of parameters refined] of 1.80. The inverse structure refined to  $R=0.024$  and a goodness-of-fit of 1.66. We have assumed that the latter model is the correct one, and all subsequent discussion of structural properties will be based on the results of that refinement.

During the final cycle of refinement no parameter shift exceeded 0.2 times its estimated standard deviation. At the conclusion of refinement, a three-dimensional electron-density difference map was calculated with only the heavy atom contributions included in the values for the calculated structure factors. Fig. 1 depicts the residual electron density at the hydrogen atom positions. In this map, no other peaks or troughs were noted to exceed  $0.27 \text{ e.}\text{\AA}^{-3}$  in magnitude. A final difference Fourier map calculated with both heavy and hydrogen atom contributions included in the values for the calculated structure factors showed residual troughs and peaks ranging from  $-0.22$  to  $0.18 \text{ e.}\text{\AA}^{-3}$ .

The final heavy-atom parameters and their estimated standard deviations are listed in Table 2; the hydrogen-atom parameters and their estimated devia-

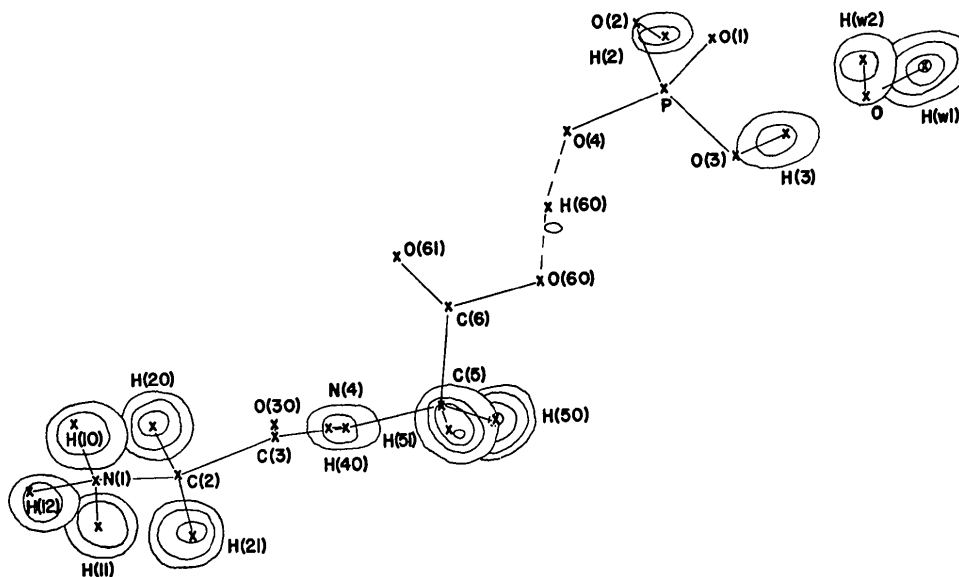


Fig. 1. Three-dimensional difference map in which the hydrogen-atom contributions were omitted from the  $F_c$  values. The view is down the  $c$  axis. The contours are at intervals of  $0.1 \text{ e.}\text{\AA}^{-3}$ , beginning with  $0.2 \text{ e.}\text{\AA}^{-3}$ .

Table 2. The final heavy-atom parameters and their estimated standard deviations

The values have been multiplied by 10<sup>4</sup>. The temperature factors are in the form  $\tau = \exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$ . The final value of the isotropic extinction parameter  $g$  is 0.250 (13).

	<i>x</i>	<i>y</i>	<i>z</i>	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
P	9392 (1)	8514 (1)	7004	14 (1)	29 (1)	205 (4)	0 (1)	-2 (1)	-10 (1)
O(1)	8998 (1)	9170 (1)	9301 (5)	17 (1)	38 (1)	227 (9)	4 (1)	5 (2)	-3 (3)
O(2)	9551 (1)	9318 (2)	4422 (6)	28 (1)	55 (1)	203 (10)	-13 (1)	2 (2)	-1 (3)
O(3)	8881 (1)	7562 (1)	5814 (5)	23 (1)	35 (1)	377 (11)	-4 (1)	-22 (2)	-13 (3)
O(4)	10107 (1)	7992 (1)	8012 (6)	19 (1)	43 (1)	431 (11)	8 (1)	-23 (2)	-34 (3)
N(1)	6151 (1)	6363 (2)	2220 (6)	14 (1)	40 (1)	240 (10)	1 (1)	2 (3)	-5 (4)
C(2)	6787 (1)	6252 (2)	0168 (6)	14 (1)	47 (2)	207 (11)	1 (1)	3 (3)	7 (4)
C(3)	7486 (1)	5878 (2)	1726 (7)	15 (1)	32 (1)	243 (15)	-1 (1)	1 (2)	-4 (4)
O(30)	7490 (1)	5731 (2)	4319 (5)	18 (1)	90 (2)	200 (10)	-1 (1)	-6 (2)	24 (4)
N(4)	8078 (1)	5728 (2)	0019 (6)	17 (1)	47 (1)	221 (10)	6 (1)	4 (2)	11 (3)
C(5)	8809 (1)	5448 (2)	1142 (8)	15 (1)	39 (2)	369 (15)	2 (1)	-2 (3)	-4 (4)
C(6)	8965 (1)	4224 (2)	1210 (7)	15 (1)	43 (2)	328 (14)	2 (1)	-10 (3)	-2 (4)
O(60)	9580 (1)	4012 (1)	2557 (8)	25 (1)	42 (1)	835 (22)	5 (1)	-79 (4)	-15 (4)
O(61)	8571 (1)	3536 (1)	0082 (7)	24 (1)	42 (1)	690 (18)	4 (1)	-57 (3)	-47 (4)
O	7808 (1)	8317 (2)	2512 (6)	20 (1)	58 (1)	481 (15)	1 (1)	18 (3)	36 (4)

Table 3. The final hydrogen-atom parameters and their estimated standard deviations

The positional parameters have been multiplied by 10<sup>3</sup>.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>
H(W1)	739 (2)	866 (3)	315 (11)	4.5 (0.9)
H(W2)	788 (2)	878 (3)	108 (12)	4.6 (0.9)
H(2)	935 (2)	919 (4)	300 (13)	4.9 (1.2)
H(3)	850 (2)	783 (3)	495 (11)	4.7 (0.9)
H(10)	607 (2)	563 (2)	309 (9)	2.6 (0.6)
H(11)	617 (2)	701 (3)	348 (16)	6.7 (1.2)
H(12)	573 (2)	647 (3)	101 (12)	5.0 (1.1)
H(20)	663 (2)	567 (2)	-135 (10)	3.4 (0.7)
H(21)	691 (2)	705 (2)	-068 (8)	2.5 (0.6)
H(40)	798 (2)	570 (3)	-195 (12)	4.2 (0.8)
H(50)	919 (2)	573 (2)	-014 (8)	1.7 (0.5)
H(51)	889 (2)	583 (3)	310 (12)	4.2 (0.9)
H(60)	971 (3)	302 (4)	261 (21)	10.1 (1.6)

ditions are listed in Table 3. Observed and calculated structure factors are given in Table 4.

Fig. 2 depicts the peptide conformation, along with the ellipsoids of thermal vibration (Johnson, 1965). Bond lengths and angles involving only heavy atoms are given in Table 5, and those involving hydrogen atoms are given in Table 6. The average estimated standard deviations (e.s.d.'s) in the positional coordinates of the heavy atoms are 0.001-0.007 Å, and those for the hydrogen atoms are 0.03-0.07 Å. For bond lengths and angles concerning only heavy atoms, the corresponding e.s.d.'s are approximately 0.005 Å and 0.3° respectively. For bond lengths and angles involving hydrogen atoms the e.s.d.'s are approximately 0.07 Å and 6° respectively.

Table 4. Observed and calculated structure factors

From left to right, the columns contain values of *h*, 10*F*<sub>o</sub>, 10|*F*<sub>c</sub>|.

<i>h</i>	10 <i>F</i> <sub>o</sub>	10  <i>F</i> <sub>c</sub>	<i>h</i>	10 <i>F</i> <sub>o</sub>	10  <i>F</i> <sub>c</sub>	<i>h</i>	10 <i>F</i> <sub>o</sub>	10  <i>F</i> <sub>c</sub>	<i>h</i>	10 <i>F</i> <sub>o</sub>	10  <i>F</i> <sub>c</sub>
0	0	0	1	100	100	2	200	200	3	300	300
1	100	100	4	400	400	5	500	500	6	600	600
2	200	200	7	700	700	8	800	800	9	900	900
3	300	300	10	1000	1000	11	1100	1100	12	1200	1200
4	400	400	13	1300	1300	14	1400	1400	15	1500	1500
5	500	500	16	1600	1600	17	1700	1700	18	1800	1800
6	600	600	19	1900	1900	20	2000	2000	21	2100	2100
7	700	700	22	2200	2200	23	2300	2300	24	2400	2400
8	800	800	25	2500	2500	26	2600	2600	27	2700	2700
9	900	900	28	2800	2800	29	2900	2900	30	3000	3000
10	1000	1000	31	3100	3100	32	3200	3200	33	3300	3300
11	1100	1100	34	3400	3400	35	3500	3500	36	3600	3600
12	1200	1200	37	3700	3700	38	3800	3800	39	3900	3900
13	1300	1300	40	4000	4000	41	4100	4100	42	4200	4200
14	1400	1400	43	4300	4300	44	4400	4400	45	4500	4500
15	1500	1500	46	4600	4600	47	4700	4700	48	4800	4800
16	1600	1600	49	4900	4900	50	5000	5000	51	5100	5100
17	1700	1700	52	5200	5200	53	5300	5300	54	5400	5400
18	1800	1800	55	5500	5500	56	5600	5600	57	5700	5700
19	1900	1900	58	5800	5800	59	5900	5900	60	6000	6000
20	2000	2000	61	6100	6100	62	6200	6200	63	6300	6300
21	2100	2100	64	6400	6400	65	6500	6500	66	6600	6600
22	2200	2200	67	6700	6700	68	6800	6800	69	6900	6900
23	2300	2300	70	7000	7000	71	7100	7100	72	7200	7200
24	2400	2400	73	7300	7300	74	7400	7400	75	7500	7500
25	2500	2500	76	7600	7600	77	7700	7700	78	7800	7800
26	2600	2600	79	7900	7900	80	8000	8000	81	8100	8100
27	2700	2700	82	8200	8200	83	8300	8300	84	8400	8400
28	2800	2800	85	8500	8500	86	8600	8600	87	8700	8700
29	2900	2900	88	8800	8800	89	8900	8900	90	9000	9000
30	3000	3000	91	9100	9100	92	9200	9200	93	9300	9300
31	3100	3100	94	9400	9400	95	9500	9500	96	9600	9600
32	3200	3200	97	9700	9700	98	9800	9800	99	9900	9900
33	3300	3300	100	10000	10000	101	10100	10100	102	10200	10200

Table 5. Bond lengths (Å) and angles (°) involving heavy atoms of glycylglycine phosphate monohydrate, glycylglycine hydrochloride (Parthasarathy, 1969),  $\alpha$ -glycylglycine (Biswas, Hughes, Sharma & Wilson 1968) and a weighted average peptide unit (Marsh & Donohue, 1967)

The standard deviations in bond lengths and angles are about 0.006 Å and 0.4° for glycylglycine hydrochloride, and about 0.007 Å and 0.4° for  $\alpha$ -glycylglycine.

	Glycyl- glycine phosphate mono- hydrate	Glycyl- glycine hydro- chloride	$\alpha$ -Glycyl- glycine	Weighted average peptide unit
N(1)—C(2)	1.487	1.478	1.497	
C(2)—C(3)	1.511	1.522	1.528	1.51
C(3)—O(30)	1.211	1.229	1.249	1.24
C(3)—N(4)	1.332	1.325	1.328	1.325
N(4)—C(5)	1.446	1.443	1.462	1.455
C(5)—C(6)	1.512	1.504	1.516	
C(6)—O(60)	1.289	1.320	1.262	
C(6)—O(61)	1.210	1.207	1.239	
P—O(1)	1.502			
P—O(2)	1.567			
P—O(3)	1.571			
P—O(4)	1.501			
N(1)—C(2)—C(3)	110.8	109.6	110.3	
C(2)—C(3)—N(4)	114.5	114.6	116.8	116
C(2)—C(3)—O(30)	121.3	120.0	120.1	120.5
N(4)—C(3)—O(30)	124.1	125.4	123.1	123.5
C(3)—N(4)—C(5)	122.5	123.0	121.6	122
N(4)—C(5)—C(6)	113.9	113.7	112.7	111
C(5)—C(6)—O(60)	111.3	110.3	115.6	
C(5)—C(6)—O(61)	124.1	125.4	117.6	
O(60)—C(6)—O(61)	124.6	124.2	126.7	
O(1)—P—O(4)	113.8			
O(1)—P—O(3)	111.4			
O(1)—P—O(2)	107.1			
O(4)—P—O(2)	110.1			
O(4)—P—O(3)	107.1			
O(2)—P—O(3)	107.2			

Table 6. Bond distances (Å) and angles (°) involving hydrogen atoms

O—H(W1)	0.90	H(W1)—O—H(W2)	94
O—H(W2)	0.88	H(2)—O(2)—P	116
O(2)—H(2)	0.77	H(3)—O(3)—P	111
O(3)—H(3)	0.86	H(60)—O(4)—P	125
N(1)—H(10)	0.99	H(12)—N(1)—H(11)	105
N(1)—H(11)	0.97	H(12)—N(1)—H(10)	105
N(1)—H(12)	0.94	H(12)—N(1)—C(2)	104
C(2)—H(20)	1.04	H(11)—N(1)—H(10)	119
C(2)—H(21)	1.06	H(11)—N(1)—C(2)	115
N(4)—H(40)	0.93	H(10)—N(1)—C(2)	107
C(5)—H(50)	0.96	H(20)—C(2)—H(21)	115
C(5)—H(51)	1.02	H(20)—C(2)—N(1)	106
O(60)—H(60)	1.23	H(20)—C(2)—C(3)	110
O(4)—H(60)	1.28	H(21)—C(2)—N(1)	108
		H(21)—C(2)—C(3)	106
		H(40)—N(4)—C(3)	116
		H(40)—N(4)—C(5)	121
		H(50)—C(5)—H(51)	106
		H(50)—C(5)—N(4)	109
		H(50)—C(5)—C(6)	103
		H(51)—C(5)—N(4)	110
		H(51)—C(5)—C(6)	113
		H(60)—O(60)—C(6)	112
		O(60)—H(60)—O(4)	172

Table 5 compares the bond lengths and angles in the peptide moiety of glycylglycine phosphate monohydrate with those found for glycylglycine hydrochloride (Parthasarathy, 1969) and for the  $\alpha$  crystalline form of glycylglycine (Biswas, Hughes, Sharma & Wilson, 1968). Table 5 also lists the corresponding weighted-average bond lengths and angles of peptide moieties in other crystal structures (Marsh & Donohue, 1967). The dimensions of the dipeptide in this structure are in good agreement with the other glycylglycine structures and with the structures of other peptides. As found in other structural studies of peptides and amino acids having  $-\text{COOH}$  groups

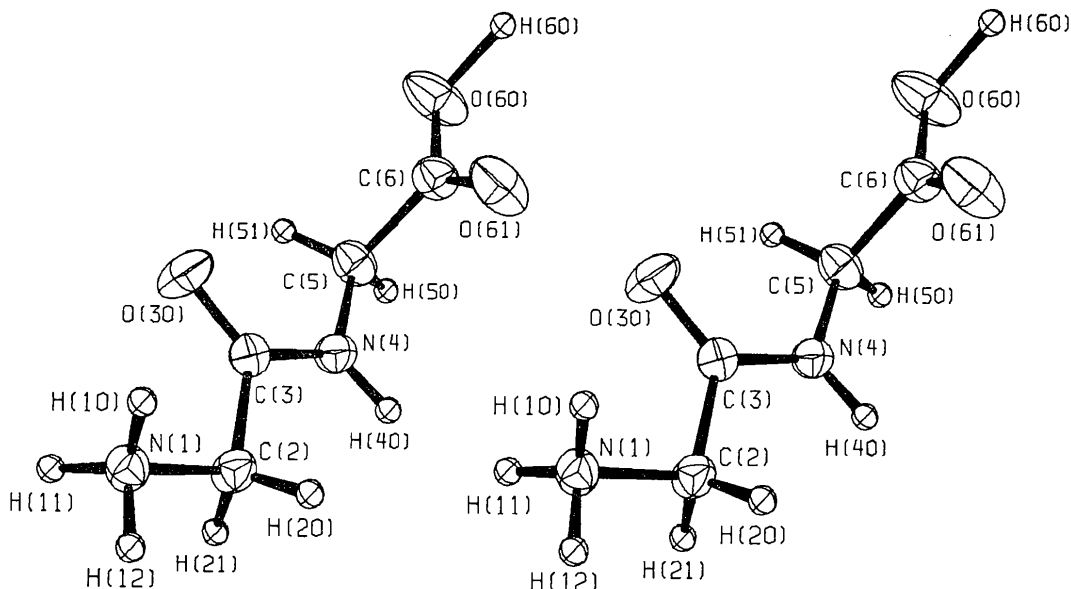


Fig. 2. Stereoscopic view of the dipeptide moiety showing the 50% probability thermal ellipsoids of the nonhydrogen atoms. The hydrogen atoms are represented by spheres of 0.1 Å radius.

(Marsh & Donohue, 1967), the carboxyl angle C(5)–C(6)–O(61) is larger than the angle C(5)–C(6)–O(60) ( $124.1^\circ$  vs.  $111.3^\circ$ ), with N(4) lying closer to O(61) than to O(60). As in other peptides, the terminal nitrogen–carbon bond, N(1)–C(2), is longer than the N(4)–C(5) bond. Deviations from the least-squares planes through the peptide, and carboxyl groups are listed in Table 7. The dihedral angle described by the peptide and carboxyl planes is  $85.2^\circ$ . The carboxyl group is commonly found to be approximately perpendicular to the peptide group.

Table 7. Distances (Å) of atoms from least-squares planes through the peptide and carboxyl groups

In the equations,  $x$ ,  $y$  and  $z$  are fractional coordinates, and the last term is the distance from the origin to the plane.

		Deviation
Peptide	C(2)	0.022
	C(3)	–0.008
	O(30)	–0.005
	N(4)	–0.038
	C(5)	0.028
	*H(40)	–0.25
Carboxyl	C(5)	0.002
	C(6)	–0.008
	O(60)	0.003
	O(61)	0.003
	*H(60)	0.00

Equations for least-squares planes

$$\begin{aligned} \text{Peptide} & 4.339x + 11.638y + 0.662z = 10.210 \text{ \AA} \\ \text{Carboxyl} & 9.183x + 1.039y - 3.945z = 8.202 \text{ \AA} \end{aligned}$$

\* Atoms given zero weight in least-squares calculation.

As expected (Marsh & Donohue, 1967), the conformation around the C(3)–N(4) peptide bond is *trans*. The conformation of the peptide can be conveniently described with the torsion angles as defined by Edsall and co-workers (Edsall, Flory, Kendrew, Liquori, Nemethy & Ramachandran, 1966). Fig. 3 shows projections depicting these torsion angles. The  $\tau$  valence bond angles are  $\tau$  N-residue [N(1)–C(2)–C(3)] =  $110.8^\circ$  and  $\tau$  C-residue [N(4)–C(5)–C(6)] =  $113.9^\circ$ ; these values are within the ranges specified by Ramakrishnan & Ramachandran (1965).

Aoki and co-workers (Aoki, Nagano & Iitake, 1971) have compared and discussed the bond lengths and angles of the phosphate groups in five organic phosphate compounds. It was noted that P–O bonds have rather short lengths of approximately 1.50 Å, while P–OH bonds range from 1.56 to 1.61 Å. The lengths of the valence bonds in the phosphate group of glycylglycine phosphate are in agreement with this finding.

### Hydrogen bonding

Fig. 4 depicts the crystal packing and hydrogen bonding (Johnson, 1965). Table 8 gives the hydrogen-bond distances and angles. All hydrogen atoms that are covalently bonded to nitrogen or oxygen atoms parti-

cipate in hydrogen bonding. Of the atoms participating in hydrogen bonding only the water oxygen atom serves simultaneously as both acceptor and donor. The parallel glycylglycine moieties are joined by N(4)–H(40)···O(30) hydrogen bonds between the nitrogen atoms of the peptide bond and the carbonyl oxygen atoms. The length of these hydrogen bonds is 2.836 Å, which is below the range (2.85 to well over 3.0 Å) reported by Marsh & Donohue (1967) for corresponding hydrogen bonds in other peptide structures.

Table 8. Hydrogen bond distances and angles

Code for symmetry-related atoms				
i	x	y	z	
ii	–x+2	–y+1	z–½	
iii	–x+½	y+½	z+½	
iv	–x+½	y–½	z–½	
v	x–½	–y+½	z–1	
		D···A	H···A	D–H···A
O(2)–H(2)·····O(1 <sup>i</sup> )		2.571 Å	1.82 Å	166°
O(3)–H(3)·····O		2.616	1.77	168
O(60)–H(60)·····O(4 <sup>iii</sup> )		2.503	1.28	172
N(1)–H(10)·····O(1 <sup>iv</sup> )		2.841	1.86	172
N(1)–H(11)·····O(61 <sup>iii</sup> )		2.991	2.05	161
N(1)–H(12)·····O(4 <sup>v</sup> )		2.807	1.90	162
N(4)–H(40)·····O(30 <sup>i</sup> )		2.836	1.94	163
O–H(W1)·····O(61 <sup>iii</sup> )		2.750	1.95	147
O–H(W2)·····O(1 <sup>i</sup> )		2.792	2.21	123

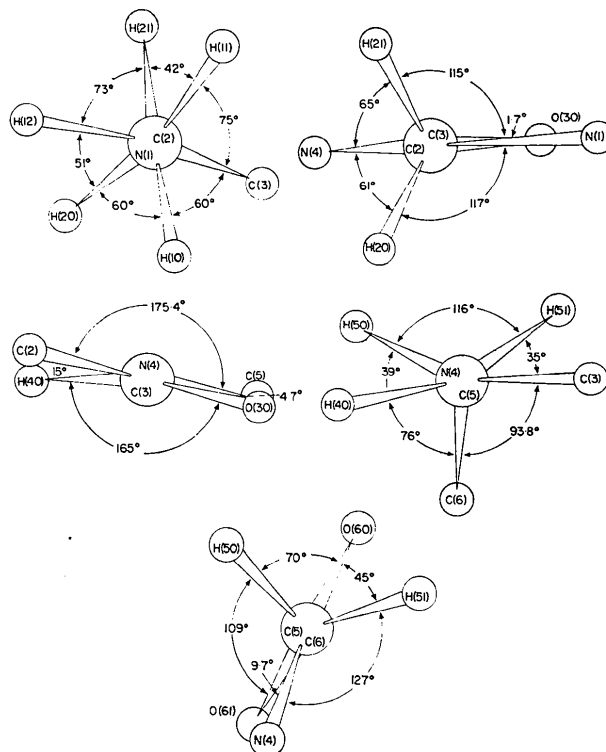


Fig. 3. Conformational projections showing the torsion angles within the dipeptide moiety. The view is down the bond between the atoms shown in the center of each drawing with the atom listed to the left lying above the atom listed to the right.

An outstanding feature of the crystal structure is the unusually short hydrogen bond (2.503 Å) between the phosphate and carboxyl groups. The results of the least-squares refinement indicate that this hydrogen bond may be symmetric, with the hydrogen atom lying 1.23 Å from the carboxyl oxygen atom [O(61)] and 1.28 Å from the phosphate oxygen atom [O(4)] and with an O(60)–H(60)–O(4) angle of 172°. As shown in Fig. 1, atom H(60) is the smallest peak present in the final difference Fourier map calculated with hydrogen atom contributions omitted from the values of the calculated structure factors. However, this map suggests that atom H(60) may be covalently bonded to atom O(60), with a bond distance of about 0.9 Å, and hydrogen bonded to atom O(4). The observed bond lengths within the carboxyl and phosphate groups also indicate that H(60) is covalently bonded to O(60). In an attempt to resolve this discrepancy, we positioned atom H(60) 0.9 Å from atom O(60) along the vector O(60)–O(4), and included its *x*, *y*, *z* and *B* parameters in several additional cycles of least-squares refinement. During the refinement, atom H(60) returned to the position intermediate between atoms O(4) and O(60). Next, H(60) was geometrically placed 0.90 Å from O(4) along the O(4)–O(60) vector, and the refinement was continued. Again the hydrogen atom moved to the position intermediate between the two oxygen atoms. Finally, two half-hydrogen atoms were positioned 0.9 Å from atoms O(4) and O(60)

and were allowed to refine. The two half-atoms shifted to positions 0.67 Å and 0.92 Å from atoms O(4) and O(60) respectively. These results indicate that the O(60)···H(60)···O(4) contact might be a symmetric or a double-minimum hydrogen bond; however, final definition of this feature must await neutron-diffraction studies.

Another unusually short hydrogen bond (2.571 Å) occurs between adjacent phosphate groups related by the *c* unit cell translation. Intimate hydrogen bonding between hydrogen phosphate ions is a common feature of many organic and inorganic phosphate crystal structures (Calleri & Speakman, 1964; Sundaralingam & Putkey, 1970; Philippot & Lindqvist, 1971). A number of other examples have been reported of phosphate–phosphate hydrogen-bonded contacts that are shorter than 2.6 Å, with several examples where the contacts are shorter than 2.5 Å. The phosphate group also forms a relatively short (2.616 Å) hydrogen bond with the water oxygen atom.

We were somewhat surprised to find that no hydrogen bonding existed between the phosphate group and the carbonyl oxygen atom of the peptide bond. Busing & Kostansek (1971) have shown that the crystal structure of a urea–phosphoric acid complex involves a short, symmetric hydrogen bond between phosphoric acid and the carbonyl oxygen of urea. Their results led us to believe that phosphates may bind in a similar manner to the related carbonyl oxygen atoms of

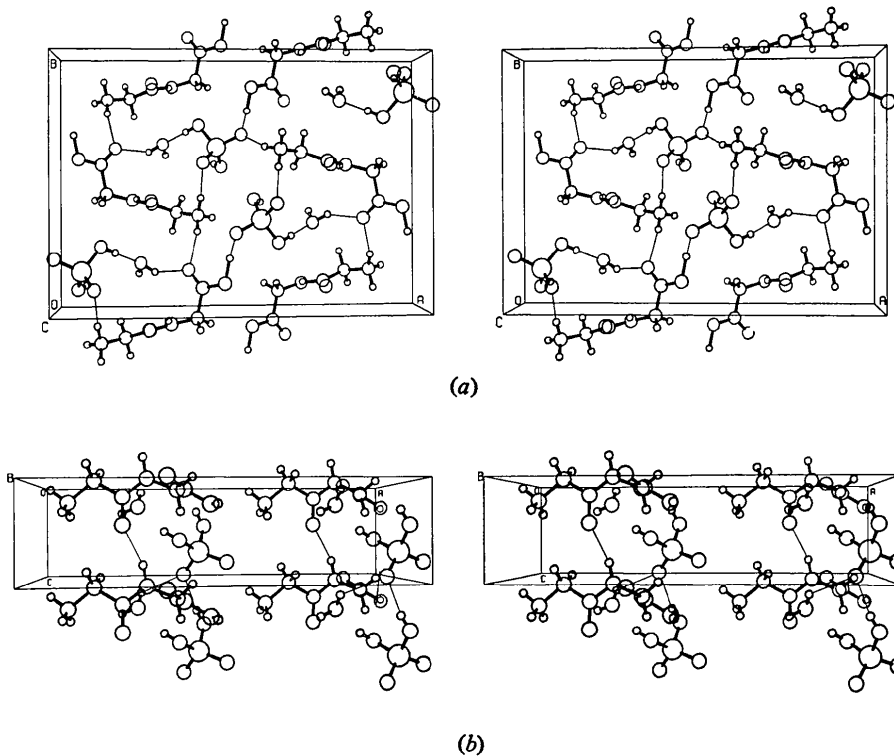


Fig. 4. Stereoscopic views of the crystal structure of glycylglycine phosphate monohydrate: (a) view direction along *c*; (b) view direction along *b*.

peptide bonds. However, the hydrogen bonding scheme in the glycyglycine phosphate structure appears to be largely dominated by the phosphate-carboxyl and phosphate-phosphate interactions. It is possible that, in the absence of a terminal carboxyl group, phosphate-carbonyl hydrogen bonding would be an important mode of phosphate-peptide binding. We are currently investigating this possibility.

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## Crystal Structure Determination of Valinomycin by Direct Methods

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The application to the phase determination for valinomycin of the recently devised method of strong enantiomorph discrimination *via* the calculated values of the cosine invariants is described. The initial 552 term *E* map revealed 74 of the 78 nonhydrogen atoms in the structure.

### 1. Introduction

Crystal structure determination by direct methods is strongly dependent on the tangent formula (Karle & Hauptman, 1956), which in turn requires firstly the evaluation of a more or less broad base of phases. In recent attempts to strengthen the method by solving the latter problem (Hauptman, Fisher, Hancock & Norton, 1969) a least-squares procedure based on an improved method for calculating the values of the cosine invariants was employed. A total of four non-centrosymmetric structures have been solved by this technique. Recent improvements, both in the evaluation of the cosine invariants and in the implementation of the calculated cosines, have led to the solution of some dozen additional noncentrosymmetric crystal structures. However, it has become increasingly apparent that still better procedures for implementing calculated cosine invariants will be needed in order to

tackle successfully non-centrosymmetric structures of great complexity. To this end a method for strong enantiomorph discrimination based on a suitably chosen class of several structure invariants, each approximately equal to  $\pm\pi/2$ , rather than only a single such invariant, has been recently devised (Hauptman & Duax, 1972).

The method is strongly dependent on the calculated values of certain cosine invariants, in particular invariants of special, well defined types, and the further analysis and interpretation of these invariants. It requires the identification of two (orthogonal) Classes, I and II, of phases  $\varphi_{hkl}$ , with fixed *k*, such that: (1) any two phases in Class I differ from each other by 0 or  $\pi$ , approximately; (2) any two phases in Class II differ from each other by 0 or  $\pi$ , approximately; (3) (orthogonality property) any phase in Class I differs from any phase in Class II by  $\pi/2$ , approximately. In addition, the associated values of the normalized structure factor