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Redetermination of Disodium DL-α-Glycerophosphate Hexahydrate

By J. McAlister* and M. Sundaralingam

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706, *USA*

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Abstract. $Na_2[C_3H_7O_6P].6H_2O$, monoclinic, $C2/c$, $a = 24{\cdot}099\,(5)$, $b = 8{\cdot}353\,(2)$, $c = 13{\cdot}802\,(3)$ Å, $\beta = 105{\cdot}38\,(4)^\circ$, $V = 2678$ Å^3, $Z = 8$, $D_m = 1{\cdot}59$, $D_c = 1{\cdot}61$ Mg m^{-3}, $F(000) = 1344$. Diffractometer data have been collected to $\sin\theta/\lambda = 0{\cdot}580$ Å^{-1} with Cu $K\alpha$ radiation, and the structure originally determined by Fenn & Marshall [*Biochem. J.* (1972), **130**, 1–10] has been refined to yield $R = 0{\cdot}044$ for 1985 observed reflections. The two sodium ions are distributed over three sites; one in a general position, one on a dyad, and one on a center of symmetry. None of the sodium ions are directly coordinated to the phosphate group, but are linked through water bridges. The intramolecular hydrogen bond between the β-hydroxyl group and an anionic phosphate O atom produces a near *gauche* conformation for the C–O bond linking the phosphate group.

Introduction. α-Glycerophosphate is a biologically important molecule. The L isomer is a constituent of the membrane phospholipids while the D isomer is both a substrate and inhibitor of enzymes involved in the metabolic pathway. We collected a set of three-dimensional X-ray intensities for this structure in 1971 using an automated diffractometer. Shortly thereafter, a preliminary crystal structure report on this compound was published by Fenn & Marshall (1971) which was followed by a full structural paper (Fenn & Marshall, 1972). Their work was based on visually estimated film data and did not reveal all of the H atoms. We decided to refine this structure as part of a general program of research in this laboratory on the structures and conformations of the sugar phosphates, phospholipids and their constituents. The present refinement has yielded results which are in good agreement with those of the original work but has provided more accurate parameters for the structure and has provided the positions for all of the H atoms.

Crystals of disodium DL-α-glycerophosphate hexahydrate were grown by slow evaporation of an aqueous ethanol solution. Weissenberg and precession photographs showed systematic absences consistent with space group $C2/c$ (hkl, $h + k = 2n + 1$; $h0l$, $l = 2n + 1$; $0k0$, $k = 2n + 1$). Unit-cell parameters were determined and refined on a Picker FACS I diffractometer and are reported in the *Abstract*. 2204 reflections were collected on this instrument, of which 2008 had $I > 1{\cdot}5\sigma(I)$ and were used in the initial refinement starting with the published coordinates of Fenn & Marshall (1972) for the nonhydrogen atoms. This gave an initial agreement index R [$= \sum(||F_o| - |F_c||)/\sum F_o$] of 0·128. Two cycles of isotropic refinement using block-diagonal least squares reduced the agreement index to 0·087. A difference Fourier synthesis calculated at this stage revealed all 19 of the H atoms in the structure. Three further cycles of refinement with anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the H atoms and including the secondary-extinction parameter g (Larson, 1967) reduced the R factor to 0·055. At this stage, 23 strong reflections which showed a large difference between F_o and F_c were removed and three additional cycles of refinement were performed, yielding improved geometry for the molecule and smaller standard errors on the atomic parameters and producing a final agreement index of 0·044.† The maximum shift/σ ratios for the nonhydrogen and H atoms were 0·07 and 0·06 respectively. The final value of the extinction coefficient g was $0{\cdot}428 \times 10^{-5}$ and the estimated standard deviation for an observation of unit weight was 1·27. The weights used for the reflections in the refinement were derived from counting statistics and are given by $w = 1/[\sigma^2_{F_o} + (0{\cdot}04F_o)^2]$. The residual peaks in the final difference Fourier map were confined to the molecular bonds; the higher peaks, 0·6 e Å^{-3}, were on the P–O bonds, and the lower peaks, 0·4 e Å^{-3}, were on the other bonds.

Discussion. The atomic coordinates and their associated e.s.d.'s are given in Table 1. Fig. 1 is an

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* Present address: Computer Systems Laboratory, Washington University, 724 S. Euclid Avenue, St Louis, Missouri 63110, USA.

† Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35189 (9 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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ORTEP diagram (Johnson, 1965) of the L isomer of α-glycerophosphate showing the bond lengths and angles for the nonhydrogen atoms. The e.s.d.'s for these bond distances (av. 0·003 Å) and bond angles (av. 0·2°) are better than those of the previous determination by a factor of 4. The present refinement confirms the presence of a slightly elongated ester P—O bond [1·633 (1) Å] and the intramolecular hydrogen bond (Fenn & Marshall, 1972), and provides more accurate values for the other molecular dimensions. The C—H bonds have a range of 0·97 (3) to 1·00 (3) Å with an average of 0·98 Å; the O—H distances of the two hydroxyl groups are 0·87 (4) and 0·83 (3) Å, and those of the water molecules range from 0·75 (4) to 0·88 (4) Å with an average of 0·82 Å.

The conformational angles for the DL-α-glycerophosphate are given in Table 2. These values are in the range observed in the other structures containing the glycerophosphate moiety (Sundaralingam, 1972), with the exception of the torsion angle C(2)—C(3)—O(31)—P [±90·0 (2°)] which has rotated

Table 1. *Fractional coordinates and isotropic thermal parameters* (Å^2) *with e.s.d.'s in parentheses*

The thermal parameters for the nonhydrogen atoms are equivalent *B*'s calculated from the expression $B_{eq} = \frac{4}{3}\sum_i\sum_j (\mathbf{a}_i.\mathbf{a}_j)\beta_{ij}$ and have e.s.d.'s in the range 0·04 to 0·10 Å^2.

	x	*y*	*z*	*B*
Na(1)	0·25000 (0)	0·25000 (0)	0·50000 (0)	2·1
Na(2)	0·00000 (0)	0·43841 (15)	0·25000 (0)	2·1
Na(3)	0·13682 (4)	0·45013 (11)	0·32221 (7)	2·1
P	0·12668 (2)	−0·14469 (7)	0·51121 (4)	1·2
C(1)	0·0787 (1)	0·0986 (3)	0·2529 (2)	2·1
C(2)	0·1421 (1)	0·0772 (3)	0·3077 (2)	1·8
C(3)	0·1563 (1)	−0·0938 (3)	0·3406 (2)	2·0
O(11)	0·0673 (1)	0·2578 (2)	0·2157 (1)	2·2
O(21)	0·1582 (1)	0·1867 (2)	0·3906 (1)	2·1
O(31)	0·1162 (1)	−0·1600 (2)	0·3897 (1)	2·0
O(32)	0·1847 (1)	−0·2181 (2)	0·5611 (1)	2·1
O(33)	0·0768 (1)	−0·2387 (2)	0·5281 (1)	2·1
O(34)	0·1248 (1)	0·0325 (2)	0·5344 (1)	2·2
O(*W*1)	0·2125 (1)	0·2288 (2)	0·6436 (1)	2·7
O(*W*2)	0·0661 (1)	−0·3686 (2)	0·2292 (1)	2·9
O(*W*3)	0·0660 (1)	−0·5304 (2)	0·4193 (1)	2·8
O(*W*4)	0·1993 (1)	−0·4418 (2)	0·7124 (1)	2·9
O(*W*5)	0·2183 (1)	−0·4623 (2)	0·4497 (1)	2·4
O(*W*6)	0·0400 (1)	0·2325 (2)	0·5433 (1)	2·7
H(11)	0·066 (1)	0·021 (3)	0·199 (2)	2·3 (6)
H(12)	0·055 (1)	0·081 (3)	0·300 (2)	1·5 (5)
H(21)	0·167 (1)	0·105 (3)	0·263 (2)	1·6 (5)
H(31)	0·156 (1)	−0·163 (3)	0·282 (2)	1·1 (5)
H(32)	0·196 (1)	−0·100 (4)	0·383 (2)	2·7 (6)
H(O11)	0·073 (1)	0·254 (4)	0·156 (3)	3·9 (7)
H(O21)	0·143 (1)	0·154 (4)	0·434 (2)	3·0 (7)
H(1*W*1)	0·237 (2)	0·182 (5)	0·683 (3)	5·3 (9)
H(2*W*1)	0·187 (1)	0·166 (4)	0·621 (2)	2·6 (6)
H(1*W*2)	0·068 (1)	−0·293 (5)	0·272 (3)	4·6 (8)
H(2*W*2)	0·060 (1)	−0·322 (4)	0·178 (2)	3·8 (7)
H(1*W*3)	0·068 (1)	−0·447 (4)	0·442 (3)	3·5 (7)
H(2*W*3)	0·066 (1)	−0·608 (4)	0·462 (2)	3·3 (7)
H(1*W*4)	0·200 (2)	−0·537 (5)	0·690 (3)	5·6 (10)
H(2*W*4)	0·193 (1)	−0·378 (4)	0·665 (3)	4·8 (8)
H(1*W*5)	0·207 (1)	−0·398 (3)	0·483 (2)	1·9 (5)
H(2*W*5)	0·244 (1)	−0·403 (5)	0·432 (3)	4·6 (8)
H(1*W*6)	0·009 (2)	0·216 (5)	0·519 (3)	5·6 (9)
H(2*W*6)	0·061 (2)	0·152 (5)	0·535 (3)	5·3 (9)

Table 2. *Conformation angles*

O(11)–C(1)–C(2)–C(3)	±175·4 (2)°
O(11)–C(1)–C(2)–O(21)	∓59·4 (2)
C(1)–C(2)–C(3)–O(31)	±47·6 (3)
O(21)–C(2)–C(3)–O(31)	∓76·6 (2)
C(2)–C(3)–O(31)–P	±90·0 (2)
C(3)–O(31)–P–O(32)	±56·9 (2)
C(3)–O(31)–P–O(33)	±176·5 (2)
C(3)–O(31)–P–O(34)	∓63·7 (2)

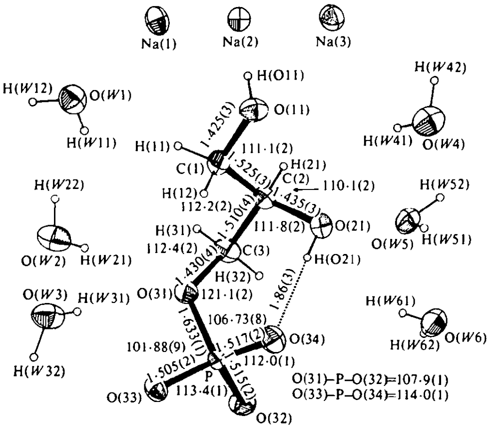

Fig. 1. An *ORTEP* diagram (Johnson, 1965) of the atoms in disodium DL-α-glycerophosphate hexahydrate showing the bond distances (Å) and angles (°) and their associated e.s.d.'s. The water molecules and sodium ions are positioned arbitrarily, but have the same orientation parameters as the α-glycerophosphate molecule.

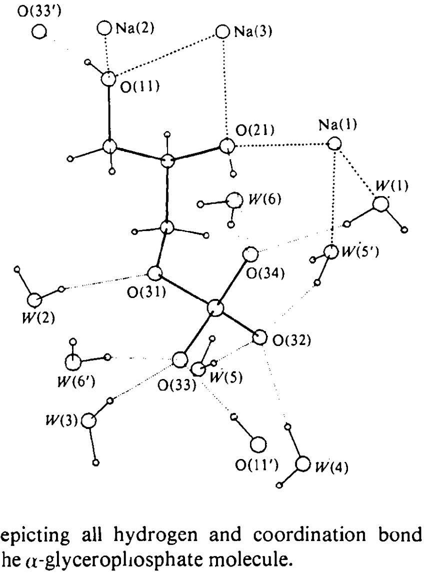

Fig. 2. Diagram depicting all hydrogen and coordination bonds for the α-glycerophosphate molecule.

Table 3. *Hydrogen-bond parameters*

A–H···B	Symmetry code*	d_{HB} (Å)	d_{AB} (Å)	θ_{AHB} (°)
O(21)–H(O21)···O(34)	I	1·86 (3)	2·663 (3)	163 (3)
O(11)–H(O11)···O(33)	II	1·80 (4)	2·664 (2)	173 (2)
O(*W*1)–H(1*W*1)···O(*W*4)	III	2·08 (4)	2·873 (2)	179 (4)
O(*W*1)–H(2*W*1)···O(34)	I	1·99 (3)	2·781 (3)	166 (3)
O(*W*2)–H(1*W*2)···O(31)	I	2·05 (4)	2·825 (2)	149 (2)
O(*W*2)–H(2*W*2)···O(*W*6)	II	1·94 (3)	2·723 (2)	172 (3)
O(*W*3)–H(1*W*3)···O(33)	I	2·09 (4)	2·837 (3)	170 (4)
O(*W*3)–H(2*W*3)···O(*W*6)	IV	1·95 (3)	2·794 (3)	162 (2)
O(*W*4)–H(1*W*4)···O(*W*1)	IV	2·10 (4)	2·954 (2)	173 (4)
O(*W*4)–H(2*W*4)···O(32)	I	1·93 (4)	2·754 (2)	175 (3)
O(*W*5)–H(1*W*5)···O(32)	I	2·00 (3)	2·799 (3)	173 (2)
O(*W*5)–H(2*W*5)···O(32)	V	1·97 (3)	2·818 (3)	161 (4)
O(*W*6)–H(1*W*6)···O(33)	VI	2·01 (5)	2·725 (3)	162 (4)
O(*W*6)–H(2*W*6)···O(34)	I	1·84 (5)	2·667 (3)	160 (4)

* Symmetry operations required to bring acceptor atom within hydrogen-bond distance of donor are defined as follows: (I) x,y,z; (II) $x, \bar{y}, -\frac{1}{2} + z$; (III) $\frac{1}{2} - x, \frac{1}{2} + y, \frac{3}{2} - z$; (IV) $x, -1 + y, z$; (V) $\frac{1}{2} - x, -\frac{1}{2} - y, 1 - z$; (VI) $\bar{x}, \bar{y}, 1 - z$.

Table 4. Na—O *coordination distances*

Ion	Ligand	Symmetry code*	$d_{\text{Na-O}}$ (Å)
Na(1)	O(21)	I	2·384 (2)
	O(*W*1)	I	2·393 (2)
	O(*W*5)	II	2·560 (2)
Na(2)	O(11)	I	2·353 (2)
	O(*W*2)	II	2·336 (2)
	O(*W*3)	II	2·467 (1)
Na(3)	O(11)	I	2·498 (2)
	O(21)	I	2·395 (2)
	O(*W*2)	II	2·384 (2)
	O(*W*3)	II	2·439 (3)
	O(*W*4)	III	2·404 (2)
	O(*W*5)	II	2·378 (2)

* Symmetry operations required to bring O atom within coordination distance of the sodium ion are defined as follows: (I) x,y,z; (II) $x, 1 + y, z$; (II) $x, \bar{y}, -\frac{1}{2} + z$.

from the preferred *trans* conformation apparently to accommodate the intramolecular hydrogen bond between the β hydroxyl group and an anionic phosphate O atom. It should be noted that the Newman projection given in Fig. 3 of Fenn & Marshall (1972) corresponds to the L isomer.

There is an extensive hydrogen-bond network throughout the structure due to the large amount of water in the asymmetric unit. All hydroxyl H atoms and water H atoms are involved in hydrogen bonds. The distances and angles for the hydrogen bonds are given in Table 3 and those directly involving the α-glycerophosphate molecule are shown in Fig. 2. It is interesting that the ester oxygen atom O(31) is also engaged in a hydrogen bond to a water molecule, *W*(2).

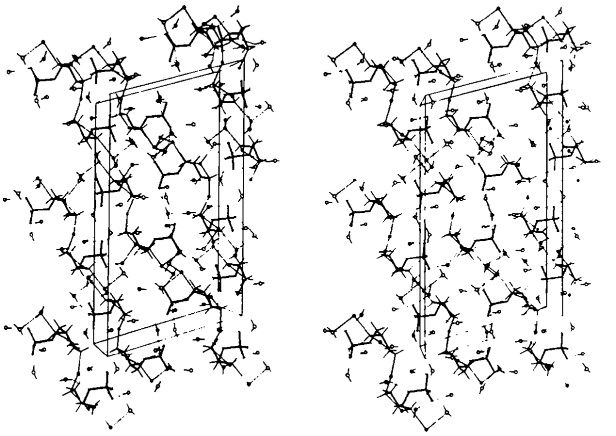

Fig. 3. A stereo packing diagram for the molecules in the structure of disodium DL-α-glycerophosphate hexahydrate viewed down the *b* axis. The α-glycerophosphate molecules are shown in heavy lines.

The two sodium ions are distributed over three sites: Na(1) and Na(2) are on symmetry elements, and Na(3) is in a general position. All three sodium sites are octahedrally coordinated by water molecules and the hydroxyl groups of the glycerophosphate molecule. Thus, the sodium ions are not directly linked to the phosphate O atoms, rather they are linked through water molecules. Similar modes of interaction between hydrated metal ions and anionic phosphate O atoms have been observed in a number of crystal structures (Swaminathan & Sundaralingam, 1979). The sodium ions share atoms in their coordination spheres. The octahedra for Na(1) and Na(3) share an edge formed by O(21) and O(*W*5) while those for Na(2) and Na(3) share a face formed by oxygens O(11), O(*W*2) and O(*W*3). The Na–O distances are in the range 2·336 (2) to 2·560 (2) Å (Table 4). Fig. 3 is a stereo packing diagram of the structure.

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