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Disodium 4-nitrophenylphosphate hexahydrate

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Two formula units of disodium 4-nitrophenylphosphate hexahydrate, $2Na^+ \cdot C_6H_4NO_6P^{2-} \cdot 6H_2O$, are present in the asymmetric unit. The 4-nitrophenyl groups of the two dianions are essentially identical in structure, while the phosphate groups differ in conformation.

Comment

4-Nitrophenylphosphate has been used for decades as a marker of phosphatase activity. The anion is colorless, but the 4-nitrophenyl product liberated after cleavage of the phosphate is yellow, and thus progress of the reaction may be monitored by measuring absorption at 410 nm. It was originally employed clinically in the detection of alkaline phosphatase activity in blood serum (Bessey *et al.*, 1946) and is used today as a general indicator of phosphohydrolase activity. The anion is a component of a set of general enzymatic screens against libraries of proteins of unknown function (Kuznetsova *et al.*, 2005).

(I)
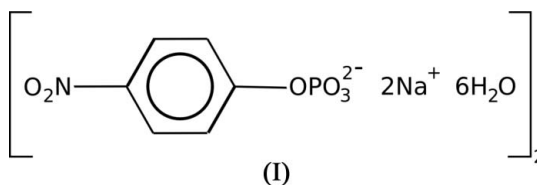
The anion is commercially available in the dianion form as a hexahydrate disodium salt. Jones *et al.* (1984*b*) reported the structure using bis(cyclohexylammonium) as the countercation, but were unable to crystallize the disodium salt satisfactorily. We report the structure of the disodium 4-nitrophenylphosphate hexahydrate salt, (I), solved at 103 K (Fig. 1). There are two 4-nitrophenylphosphate dianions in the asymmetric unit of (I). While the 4-nitrophenyl groups of both anions are nearly identical in structure, the phosphate groups differ in conformation, in both the C2—C1—O3—P1 torsion angle [anion *A*: 137.96 (9)°; anion *B*: 158.52 (9)°] and the C1—O3—P1—O4 torsion angle [for *A* −49.26 (10)°; for *B* −43.42 (10)°].

In the crystal structure of (I), alternating non-polar and hydrophilic layers are observed (Fig. 2). The non-polar regions consist of tightly packed nitrophenyl groups, while the hydrophilic layers contain the phosphate ions, sodium ions and their bound water molecules. The crystal packing for (I) differs from that of the bis(cyclohexylammonium) salt (Jones *et al.*, 1984*b*). In the latter, the cyclohexylammonium cations interdigitate between the nitrophenyl groups, while in the former the aromatic rings stack directly.
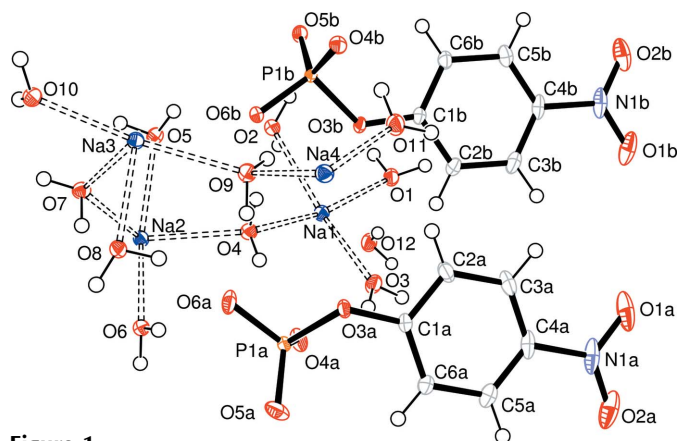
Figure 1
The asymmetric unit of (I). Displacement ellipsoids are drawn at the 50% probability level. H atoms are drawn as spheres of arbitrary radii. Na$\cdots$O contacts are shown by dashed lines.

Figure 2
Crystal packing of (I) projected along the b axis. Na$\cdots$O contacts are shown by dashed lines. H atoms have been omitted for clarity.

Jones *et al.* (1984*b*) report that the length of the CO—P bond in their structure of the 4-nitrophenylphosphate dianion is significantly longer [1.664 (5) Å] than those of other dianion alkylphosphates, with a mean of 1.614 (4) Å (Jones *et al.*, 1984*a*). The measured CO—P distances in (I) [P1A—O3A = 1.6461 (8) Å and P1B—O3B = 1.6519 (8) Å] are not significantly different from that found in the bis(cyclohexylammonium) salt. Our measure of the mean CO—P—O$^-$ angle of 105.2° in (I) is significantly smaller than both the mean of 114.0° for the bis(cyclohexylammonium) salt and the reported mean of 112.8° for dianion alkylphosphates (Jones *et al.*, 1984*b*). Finally, the bis(cyclohexylammonium) salt shows significantly shorter lengths for the remaining three P—O bonds compared with the means (1.514, 1.519 and 1.510 Å) for 22 other dianion alkylphosphates (Jones *et al.*, 1984*a*). In contrast, our values for these bonds in (I) do not differ significantly from the reported mean values. While (I) follows the trend for CO—P bond lengths as a function of R—OH pK_a, as explored by Jones and co-workers, we find that the pK_a of the 4-nitrophenyl group alone is not sufficient to explain the differences in P—O$^-$ bond length or CO—P—O$^-$ bond angles. Neighboring cations and water molecules also influence the geometry of the phosphate group.

Experimental

Crystal data

2Na$^+$·C$_6$H$_4$NO$_6$P^{2-}·6H$_2$O	D_x = 1.652 Mg m^{-3}
M_r = 742.30	Mo $K\alpha$ radiation
Monoclinic, $P2_1/c$	Cell parameters from 130337
a = 19.007 (1) Å	reflections
b = 11.982 (1) Å	θ = 2.0–40.3°
c = 13.492 (1) Å	μ = 0.30 mm^{-1}
β = 103.708 (1)°	T = 103 (2) K
V = 2985.2 (4) Å^3	Block, colorless
Z = 4	0.20 × 0.20 × 0.15 mm

Data collection

Rigaku R-AXIS RAPID diffractometer	18300 independent reflections
ω scans with χ offset	12628 reflections with $I > 2\sigma(I)$
Absorption correction: multi-scan (Otwinowski *et al.*, 2003)	θ_{max} = 40.3°
T_{min} = 0.941, T_{max} = 0.946	$h = -34 \rightarrow 33$
130337 measured reflections	$k = 0 \rightarrow 21$
	$l = 0 \rightarrow 24$

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0727P)^2$
$R[F^2 > 2\sigma(F^2)] = 0.048$	$+ 0.9368P]$
$wR(F^2) = 0.146$	where $P = (F_o^2 + 2F_c^2)/3$
S = 1.10	$(\Delta/\sigma)_{max}$ = 0.001
18300 reflections	$\Delta\rho_{max}$ = 0.97 e Å^{-3}
397 parameters	$\Delta\rho_{min}$ = −0.88 e Å^{-3}
H-atom parameters constrained	

Table 1
Hydrogen-bond geometry (Å, °).

D—H$\cdots A$	D—H	H$\cdots A$	$D\cdots A$	D—H$\cdots A$
O1—H1O$A\cdots$O4B^{i}	0.87	1.93	2.7985 (13)	172
O1—H2O$A\cdots$O2A^{ii}	0.87	2.12	2.9725 (15)	166
O2—H1O$B\cdots$O5B	0.85	2.49	2.9903 (12)	119
O2—H2O$B\cdots$O4B^{i}	0.85	2.23	2.7396 (12)	119
O3—H1O$C\cdots$O2B^{iii}	0.85	2.33	3.0285 (14)	139
O3—H2O$C\cdots$O4A	0.85	2.06	2.7653 (12)	140
O4—H1O$D\cdots$O6B	0.85	1.96	2.7941 (12)	164
O4—H2O$D\cdots$O4A	0.85	2.09	2.9204 (14)	163
O5—H1O$E\cdots$O5A^{iv}	0.85	2.37	3.1163 (14)	147
O5—H1O$E\cdots$O6A^{iv}	0.85	2.63	3.1780 (15)	123
O5—H2O$E\cdots$O6B	0.85	2.23	2.8902 (12)	135
O6—H2O$F\cdots$O6A	0.85	2.04	2.8153 (12)	150
O7—H1O$G\cdots$O5A^{iv}	0.85	2.21	2.7170 (13)	118
O7—H2O$G\cdots$O5B^{v}	0.85	2.54	3.1354 (13)	127
O8—H1O$H\cdots$O5B^{v}	0.85	2.28	2.7582 (12)	116
O8—H2O$H\cdots$O6A	0.85	2.10	2.8631 (12)	149
O9—H1O$I\cdots$O6B	0.85	2.04	2.7693 (12)	144
O9—H2O$I\cdots$O6A	0.85	1.92	2.7689 (14)	170
O10—H1O$J\cdots$O6A^{vi}	0.85	2.52	2.9701 (13)	114
O10—H2O$J\cdots$O4A^{iv}	0.85	2.04	2.7252 (12)	136
O11—H1O$K\cdots$O4B	0.85	2.01	2.7878 (13)	151
O11—H2O$K\cdots$O1A^{vii}	0.85	2.17	2.9369 (15)	150
O12—H2O$L\cdots$O1B^{viii}	0.85	2.15	2.9633 (16)	160

Symmetry codes: (i) $x, -y + \frac{3}{2}, z + \frac{1}{2}$; (ii) $-x, y + \frac{1}{2}, -z + \frac{1}{2}$; (iii) $-x, y - \frac{1}{2}, -z + \frac{1}{2}$; (iv) $-x + 1, y + \frac{1}{2}, -z + \frac{1}{2}$; (v) $-x + 1, y - \frac{1}{2}, -z + \frac{1}{2}$; (vi) $-x + 1, -y + 1, -z$; (vii) $-x, -y + 1, -z$; (viii) $-x, -y + 1, -z + 1$.

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All H atoms were positioned geometrically. The aromatic C—H atoms were allowed to ride on their parent C atoms, with the C—H bond distance fixed at 0.93 Å and $U_{iso}(H) = 1.2U_{eq}(C)$. The water molecule H-atom positions were calculated using *CALCOH* (Nardelli, 1999) and they were also treated as riding, with $U_{iso}(H) = 0.03$ Å^2.

Data collection: *HKL-2000* (Otwinowski & Minor, 1997); cell refinement: *HKL-2000*; data reduction: *HKL-2000*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 1990) and *HKL2000*; program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997) and *HKL-2000*; molecular graphics: *HKL-2000*, *ORTEPIII* (Burnett & Johnson, 1996) and *ORTEP-3* (Farrugia, 1997); software used to prepare material for publication: *HKL2000*.

References

Bessey, O. A., Lowry, O. H. & Brock, M. J. (1946). *J. Biol. Chem.* **164**, 321–329.
Burnett, M. N. & Johnson, C. K. (1996). *ORTEPIII*. Report ORNL-6895. Oak Ridge National Laboratory, Tennessee, USA.
Farrugia, L. J. (1997). *J. Appl. Cryst.* **30**, 565.
Jones, P. G., Sheldrick, G. M., Kirby, A. J. & Abell, K. W. Y. (1984*a*). *Acta Cryst.* C**40**, 547–549.
Jones, P. G., Sheldrick, G. M., Kirby, A. J. & Abell, K. W. Y. (1984*b*). *Acta Cryst.* C**40**, 550–552.
Kuznetsova, E., Proudfoot, M., Sanders, S. A., Reinking, J., Savchenko, A., Arrowsmith, C. H., Edwards, A. E. & Yakunin, A. F. (2005). *FEMS Microbiol. Rev.* **29**, 263–279.
Nardelli, M. (1999). *J. Appl. Cryst.* **32**, 563–571.
Otwinowski, Z., Borek, D., Majewski, W. & Minor, W. (2003). *Acta Cryst.* A**59**, 228–234.
Otwinowski, Z. & Minor, W. (1997). *Methods in Enzymology*, Vol. 276, *Macromolecular Crystallography*, Part A, edited by C. W. Carter Jr & R. M. Sweet, pp. 307–326. New York: Academic Press.
Sheldrick, G. M. (1990). *Acta Cryst.* A**46**, 467–473.
Sheldrick, G. M. (1997). *SHELXL97*. University of Göttingen, Germany.