

The Structure of 'High-Energy' Phosphate Compounds. II.* An X-ray Analysis of Cyclohexylammonium Phosphoenolpyruvate

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Cyclohexylammonium phosphoenolpyruvate is monoclinic, $P2_1/c$, with $a = 13.218$ (5), $b = 8.117$ (3), $c = 12.977$ (5) Å, $\beta = 109^\circ 54'$, $Z = 4$. The structure was solved by Patterson methods and refined to an R of 7.9%. The length of the phosphate ester bond is 1.625 (5) Å. The geometry of the phosphate group is compared with other phosphate monoester monoanions.

Introduction

The investigation presented in this paper is part of a systematic survey of organic phosphates aimed at a better understanding of the nature of compounds containing the 'high-energy' phosphate bond. The free-energy changes associated with the hydrolysis of the P–O bond in compounds like adenosine triphosphate are fundamental to a variety of biochemical mechanisms and it is hoped that details of the molecular structure and conformation of these molecules will help to elucidate their mode of action. As one aspect of this work we are examining the system RHPO_4^- to study the effect of changing R so as to alter the steric environment of the HPO_4^- residue and the degree of conjugation between R and HPO_4^- .

The X-ray analysis of disodium adenosine triphosphate trihydrate has been reported elsewhere (Kennard *et al.*, 1971). This paper presents results on a salt of another 'high energy' compound, phosphoenolpyruvic acid, whose free energy of hydrolysis is one of the highest of all known naturally occurring phosphate compounds. The formula of the cyclohexylammonium salt used for the analysis and the numbering system adopted throughout this paper are shown in Fig. 1.

Experimental

Dr A. J. Kirby of this laboratory kindly provided us with crystals.

The unit-cell parameters were determined by measurement of the 2θ values of axial reflexions with a Picker automatic 4-circle diffractometer.

Reflexions within the range of Cu $K\alpha$ radiation were recorded on equi-inclination Weissenberg photographs with the multiple-film technique. Integrated hnl reflexions ($n = 0-5$) were recorded with a Nonius camera and the intensities measured with a Joyce-Loebl microdensitometer. The hkn reflexions ($n = 0-9$) were not in-

tegrated and the intensities were estimated visually by comparison with a standard scale. 1824 reflexions were recorded, 1300 being of measurable intensity. After correction for Lorentz and polarization effects the data were correlated and put on a single scale. No corrections were applied for absorption.

Crystal data

Cyclohexylammonium phosphoenolpyruvate.

$\text{C}_6\text{H}_{14}\text{N}^+ \text{C}_3\text{H}_4\text{O}_6\text{P}^-$; F.W. 267.2.

λ (Cu $K\alpha$) = 1.5418 Å.

Monoclinic, $a = 13.218$ (5) Å

$b = 8.117$ (3)

$c = 12.977$ (5)

$\beta = 109^\circ 54' (1)'$.

$U = 1309.2 \text{ \AA}^3$

$D_x = 1.355 \text{ g cm}^{-3}$, $Z = 4$

μ (Cu $K\alpha$) = 20.3 cm^{-1}

$F(000) = 568$

Space group $P2_1/c$, from systematic absences.

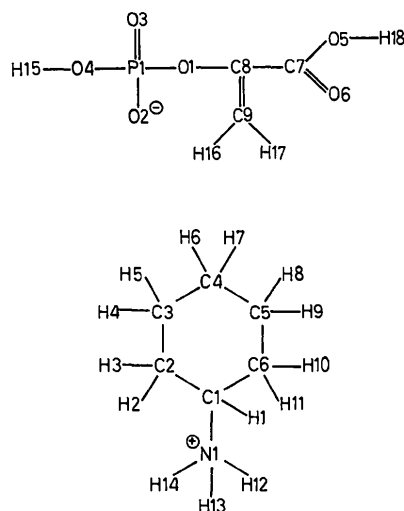


Fig. 1. Chemical formula and numbering scheme.

* Part I: *The Crystal and Molecular Structure of Adenosine Triphosphate*, Proc. Roy. Soc. (1971). A325, 401–436.

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Determination and refinement of the structure

All computations were carried out on the Cambridge TITAN computer with programs written by Dr J. Wheeler, or on the IBM 7090 computer at Imperial College, London, with the X-RAY 63 system of programs devised by Professor J. M. Stewart and Dr D. High.

A three-dimensional, origin-removed, sharpened Patterson function clearly indicated the position of the phosphorus atom. Three successive Fourier summations introducing more atoms at each stage were sufficient to establish the coordinates of all atoms other than hydrogen. *R* at this stage computed over some 900 terms was 31% (these 900 terms represent the *hkl*

reflexions together with the *hk0* and *hk1* layers used for cross-correlation purposes).

The structure was refined by least-squares analysis with the Busing, Martin & Levy (1962) full-matrix Fortran program as adapted by Stewart & High. The function minimized was $\sum w(F_o - F_c)^2$ and unit weights were applied. At an intermediate stage a three-dimensional difference synthesis was computed to determine the positions of the hydrogen atoms. The following atomic scattering factors were used: for phosphorus the values listed in *International Tables for X-ray Crystallography* (1962); for oxygen, nitrogen and carbon, those given by Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955); for hydrogen, the values calculated by McWeeny (1951).

The full set of experimental data was used in the refinement. Unobserved reflexions were included with intensity equal to half the minimum intensity observed for the film in question. At the correlation stage more weight was attached to visual intensities of weak reflexions than to the values measured photometrically.

Three cycles of isotropic refinement followed by two cycles of anisotropic least squares reduced *R* to 9.8%. At this stage a three-dimensional difference synthesis was computed and positions for the hydrogen atoms were chosen. The data were edited and two further cycles computed, refining only the scale factor and the hydrogen positional parameters. A final cycle of refinement of the heavy-atom parameters resulted in an *R* of 7.9% (if unobserved reflexions are included *R* = 9.3%).* In the last cycle of refinement the largest shift in any heavy-atom parameter was 1.7σ and in a hydrogen-atom coordinate 2.3σ .

The positional parameters for the heavy atoms are given in Table 1 and the anisotropic thermal parameters in Table 2. Interatomic distances and bond angles (not involving hydrogen atoms) are shown in Figs. 2 and 3. Their mean standard deviations are:

| | |
|---------------|--------------|
| P-O = 0.005 Å | O-P-O = 0.3° |
| C-O = 0.009 | C-O-P = 0.5 |
| C-N = 0.009 | O-C-O = 1.0 |
| C-C = 0.014 | O-C-C = 0.8 |
| | C-C-N = 0.7 |
| | C-C-C = 0.7. |

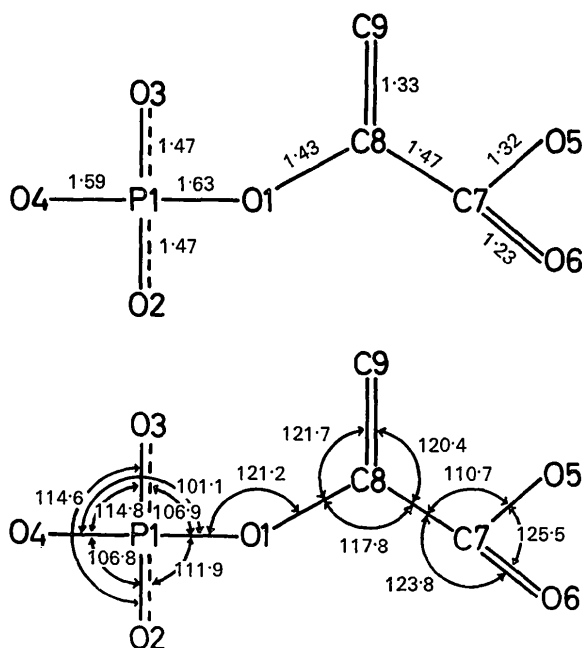


Fig. 2. Bond lengths and angles for the phosphoenolpyruvate ion.

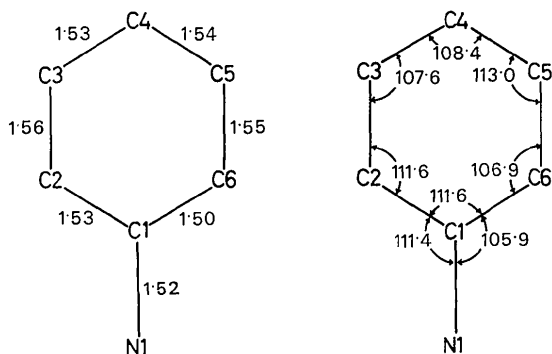


Fig. 3. Bond lengths and angles for the cyclohexylammonium ion.

The location of the hydrogen atoms from the difference synthesis was quite satisfactory in the sense that peaks occurred where hydrogen atoms were expected either from stereochemical considerations or from the hydrogen-bonding scheme. These peaks varied in height from 0.2 to 0.6 $e \text{ \AA}^{-3}$ with a mean of 0.4 $e \text{ \AA}^{-3}$. The hydrogen fractional coordinates and isotropic

* A list of structure factors is available from the authors and has also been deposited with the National Lending Library, England, as Supplementary Publication No. SUP 30153 (10 pp.). Copies may be obtained from the Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. Final fractional coordinates for the non-hydrogen atoms ($\times 10^4$)

| | <i>x</i> | <i>y</i> | <i>z</i> |
|------|-----------|-----------|-----------|
| P(1) | 682 (2) | 1742 (2) | 2112 (2) |
| O(1) | 1685 (4) | 2914 (6) | 2800 (4) |
| O(2) | 966 (4) | 656 (6) | 1342 (4) |
| O(3) | 322 (4) | 874 (6) | 2919 (4) |
| O(4) | -140 (4) | 3068 (6) | 1395 (4) |
| O(5) | 1423 (4) | 3644 (7) | 4644 (4) |
| O(6) | 2858 (5) | 2209 (12) | 5724 (6) |
| N(1) | -971 (4) | 2300 (7) | 3937 (5) |
| C(1) | -2107 (6) | 1641 (10) | 3418 (6) |
| C(2) | -2540 (6) | 1959 (13) | 2179 (7) |
| C(3) | -3724 (8) | 1340 (17) | 1649 (9) |
| C(4) | -4410 (7) | 2251 (17) | 2209 (10) |
| C(5) | -3949 (7) | 1889 (17) | 3448 (10) |
| C(6) | -2773 (7) | 2503 (14) | 3991 (8) |
| C(7) | 2281 (6) | 2715 (11) | 4825 (7) |
| C(8) | 2470 (6) | 2361 (11) | 3798 (6) |
| C(9) | 3363 (7) | 1603 (14) | 3811 (9) |

Table 3. Final fractional coordinates ($\times 10^3$) and isotropic thermal parameters (\AA^2) for the hydrogen atoms

| | <i>x</i> | <i>y</i> | <i>z</i> | <i>B</i> |
|-------|----------|----------|----------|----------|
| H(1) | -212 (6) | 29 (11) | 349 (6) | 5.1 |
| H(2) | -252 (7) | 328 (12) | 214 (7) | 6.1 |
| H(3) | -218 (7) | 136 (12) | 175 (7) | 6.1 |
| H(4) | -406 (8) | 177 (14) | 80 (8) | 8.7 |
| H(5) | -365 (8) | -10 (14) | 168 (8) | 8.7 |
| H(6) | -429 (8) | 350 (14) | 194 (8) | 8.6 |
| H(7) | -526 (8) | 174 (14) | 193 (8) | 8.6 |
| H(8) | -445 (8) | 245 (14) | 372 (8) | 8.8 |
| H(9) | -384 (8) | 47 (14) | 361 (8) | 8.8 |
| H(10) | -280 (7) | 405 (12) | 381 (8) | 6.9 |
| H(11) | -250 (7) | 234 (12) | 483 (7) | 6.9 |
| H(12) | -72 (6) | 216 (10) | 478 (6) | 4.4 |
| H(13) | -46 (6) | 173 (10) | 346 (6) | 4.4 |
| H(14) | -100 (6) | 352 (10) | 364 (6) | 4.4 |
| H(15) | -11 (6) | 427 (11) | 173 (7) | 5.3 |
| H(16) | 339 (7) | 154 (13) | 295 (8) | 7.4 |
| H(17) | 394 (7) | 146 (13) | 476 (8) | 7.4 |
| H(18) | 132 (7) | 354 (11) | 540 (7) | 5.5 |

temperature factors are listed in Table 3. Random background fluctuations of $\pm 0.4 \text{ e \AA}^{-3}$ were also observed on the difference map.

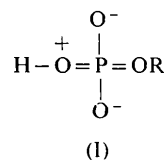
Molecular dimensions

The phosphate group

To facilitate comparison with other phosphate structures of comparable precision containing monoester monoanions RHPO_4^- , relevant molecular dimensions have been summarized in Tables 4 and 5.

O(4) is established as the oxygen atom of the un-ionized hydroxyl function by location of hydrogen atom H(15) in the difference synthesis. The value of 1.586 \AA for the P-OH bond, although slightly longer than the average, is still markedly shorter than the P-O single-bond length of 1.71 \AA calculated by the Schomaker & Stevenson (1941) rule. Pauling (1952) has in fact amended this latter value to 1.55 \AA by making a correction for the degree of π -bond character. As a general rule, it has been observed (Bacon &

Pease, 1955; Cruickshank, 1961) that the P-OH bond length is a mean of the P---O and P-OR bond lengths. This generalization is well exemplified in Table 4. The P-OH bond lengths in un-ionized orthophosphoric acid have been determined (Furberg, 1955) as 1.57, 1.57 and 1.58 \AA . For the HPO_4^{2-} ion present in the spermine phosphate hexahydrate structure (Iitaka & Huse, 1965) the P-OH distance is 1.589 \AA . As suggested by Sundaralingam & Jensen (1965*b*) the partial double-bond character of the P-OH bond may be due to the stabilization of resonance form (I) by hydrogen bonding.

Table 2. Anisotropic thermal parameters in the form $T = \exp [-(\beta_{11}h^2 + \dots + \beta_{12}hk + \dots)]$

Values are $\times 10^5$.

| | β_{11} | β_{22} | β_{33} | β_{12} | β_{13} | β_{23} |
|------|--------------|--------------|--------------|--------------|--------------|--------------|
| P(1) | 656 (16) | 912 (27) | 547 (16) | -42 (17) | 281 (12) | -43 (19) |
| O(1) | 823 (47) | 1351 (86) | 661 (47) | -237 (51) | 362 (38) | -47 (52) |
| O(2) | 959 (52) | 1240 (82) | 777 (49) | -38 (53) | 551 (41) | -83 (55) |
| O(3) | 748 (44) | 1192 (78) | 682 (45) | -145 (46) | 450 (35) | -12 (50) |
| O(4) | 978 (54) | 1252 (87) | 684 (49) | 171 (54) | 287 (43) | -6 (53) |
| O(5) | 808 (49) | 2192 (118) | 701 (50) | 93 (64) | 370 (40) | -130 (64) |
| O(6) | 1044 (67) | 4563 (243) | 881 (64) | 571 (109) | 204 (55) | 547 (108) |
| N(1) | 684 (49) | 1398 (103) | 644 (53) | -57 (61) | 325 (42) | 121 (65) |
| C(1) | 643 (62) | 1774 (150) | 829 (81) | -45 (83) | 369 (58) | -135 (95) |
| C(2) | 755 (74) | 2675 (217) | 925 (90) | 35 (99) | 246 (67) | -21 (115) |
| C(3) | 834 (93) | 3930 (313) | 1044 (110) | -93 (145) | 14 (82) | -131 (160) |
| C(4) | 711 (80) | 3493 (300) | 1607 (148) | 134 (132) | 211 (91) | -131 (173) |
| C(5) | 732 (78) | 3614 (300) | 1971 (161) | -244 (128) | 722 (93) | -288 (181) |
| C(6) | 789 (77) | 3162 (258) | 1083 (102) | -221 (124) | 543 (75) | -244 (141) |
| C(7) | 643 (63) | 1988 (163) | 908 (89) | -110 (90) | 337 (64) | -52 (105) |
| C(8) | 675 (62) | 1694 (142) | 819 (82) | -159 (85) | 315 (61) | -142 (96) |
| C(9) | 713 (83) | 3316 (261) | 1401 (127) | -18 (117) | 451 (82) | -248 (148) |

The enolpyruvate system

In Table 4 the C(8)–O(1) bond length of 1.428 Å is compared with values found in other monoester monoanions. These values appear to cluster around 1.43 and 1.47 Å compared with the standard C(sp³)–O bond length of 1.428 (3) Å found in methanol (Kimura & Kubo, 1959). The rather large values of the angle

P–O–C listed in Table 5 might suggest that the oxygen atom is partially re-hybridized towards the sp² state, in which case we could expect the C–O bond to be even shorter than 1.43 Å. Such an electron flow, however, does not appear to be dominant in the phosphoenolpyruvate ion. In the case of adenylic acid where the C–O bond is 1.475 Å the angle P–O–C is indeed

Table 4. Comparison of bond lengths (Å) in organic phosphates (monoester monoanions)

| | P–OR | P–OH | P–O–O | | C–OP |
|--------------------------------------------------------------------|-------|-------|-------|-------|-------|
| Phosphoenolpyruvate (cyclohexylammonium salt) | 1.625 | 1.585 | 1.470 | 1.472 | 1.428 |
| Cytidine 3'-phosphate ¹ (orthorhombic form) | 1.611 | 1.551 | 1.483 | 1.504 | 1.431 |
| Cytidine 3'-phosphate ² (monoclinic form) | 1.610 | 1.588 | 1.480 | 1.498 | 1.435 |
| Adenosine 3'-phosphate ³ (dihydrate) | 1.612 | 1.579 | 1.477 | 1.486 | 1.440 |
| Adenosine 5'-phosphate ⁴ (monohydrate) | 1.610 | 1.566 | 1.495 | 1.514 | 1.475 |
| Guanosine 5'-phosphate ⁵ (trihydrate) | 1.608 | 1.569 | 1.499 | 1.501 | 1.447 |
| L-Serine phosphate ⁶ (monohydrate) | 1.590 | 1.544 | 1.500 | 1.491 | 1.433 |
| Galactosamine 1-phosphate ⁶ | 1.606 | 1.553 | 1.510 | 1.499 | 1.434 |
| Hydrolysed cocarboxylase ⁷ | 1.607 | 1.570 | 1.498 | 1.519 | 1.466 |
| 2-Aminoethanol phosphate ⁸ | 1.591 | 1.557 | 1.493 | 1.503 | 1.429 |
| Pyridoxamine 5'-phosphate ⁹ (hydrochloride) | 1.608 | 1.563 | 1.494 | 1.515 | 1.440 |
| Monosodium inosine 5'-phosphate ¹⁰ (octahydrate) | 1.62 | 1.53 | 1.52 | 1.53 | 1.45 |
| Deoxycytidine 5'-phosphate ¹¹ (monohydrate) | 1.597 | 1.572 | 1.490 | 1.508 | 1.449 |
| 2,4-Dinitrophenyl hydrogen phosphate ¹² (lutidine salt) | 1.643 | 1.530 | 1.444 | 1.463 | |
| Phenyl hydrogen phosphate ¹² (cyclohexylammonium salt) | 1.602 | 1.556 | 1.488 | 1.491 | 1.383 |
| Ethyl hydrogen phosphate ¹² (cyclohexylammonium salt) | 1.596 | 1.563 | 1.522 | 1.535 | 1.472 |

References

1. Sundaralingam & Jensen (1965a).
2. Bugg & Marsh (1967).
3. Sundaralingam (1966).
4. Kraut & Jensen (1963).
5. Murayama, Nagashima & Shimizu (1969).
6. Sundaralingam & Putkey (1970).
7. Karle & Britts (1966).
8. Kraut (1961).
9. Giordano & Mazzarella (1971).
10. Rao & Sundaralingam (1969).
11. Viswamitra, Reddy, Hung-Yin Lin & Sundaralingam (1971).
12. Kennard, Kerr, Fawcett & Watson, unpublished data.

Table 5. Comparison of bond angles in organic phosphates (monoester monoanions)

| | For references, see Table 4. | | | | | | |
|-----------------------------------------------------------------------|------------------------------|-------|---------|-------|--------|-------|-------|
| | HO–P–O | | HO–P–OR | O–P–O | O–P–OR | | P–O–C |
| Phosphoenolpyruvate (cyclohexylammonium salt) | 106.9 | 114.8 | 101.1 | 114.5 | 106.9 | 111.9 | 121.2 |
| Cytidine 3'-phosphate ¹ (orthorhombic form) | 105.4 | 112.6 | 104.3 | 117.5 | 105.5 | 110.9 | 118.5 |
| Cytidine 3'-phosphate ² (monoclinic form) | 106.1 | 113.1 | 101.5 | 116.0 | 108.5 | 110.1 | 121.2 |
| Adenosine 3'-phosphate ³ (dihydrate) | 107.1 | 112.3 | 105.5 | 117.2 | 103.4 | 110.7 | 119.1 |
| Adenosine 5'-phosphate ⁴ (monohydrate) | 106.9 | 110.2 | 106.4 | 118.2 | 105.7 | 108.7 | 114.7 |
| Guanosine 5'-phosphate ⁵ (trihydrate) | 107.7 | 111.1 | 104.9 | 117.0 | 104.0 | 111.3 | 120.1 |
| L-Serine phosphate ⁶ (monohydrate) | 109.6 | 112.2 | 101.5 | 114.8 | 108.8 | 109.0 | 121.5 |
| Galactosamine 1-phosphate ⁶ | 109.6 | 110.8 | 101.3 | 117.2 | 107.3 | 109.4 | 122.1 |
| Hydrolysed cocarboxylase ⁷ | 107.5 | 111.8 | 105.6 | 116.9 | 104.8 | 109.6 | 119.7 |
| 2-Aminoethanol phosphate ⁸ | 109.3 | 109.8 | 106.2 | 117.4 | 103.9 | 109.6 | 118.7 |
| Pyridoxamine 5'-phosphate ⁹ (hydrochloride) | 107.5 | 110.0 | 105.9 | 118.7 | 105.3 | 107.7 | 117.3 |
| Monosodium inosine 5'-phosphate ¹⁰ (octahydrate) | — | — | — | — | — | — | — |
| Deoxycytidine 5'-phosphate ¹¹ (monohydrate) | 105.4 | 113.0 | 105.5 | 117.3 | 105.9 | 109.1 | 118.6 |
| 2,4-Dinitrophenyl hydrogen phosphate ¹² (lutidine salt) | 106.9 | 108.0 | 100.4 | 118.2 | 107.8 | 113.8 | 126.5 |
| Phenyl hydrogen phosphate ¹² (cyclohexylammonium salt) | 103.4 | 109.4 | 106.4 | 118.4 | 107.0 | 111.7 | 125.4 |
| Ethyl hydrogen phosphate ¹² (cyclohexylammonium salt) | 108.2 | 112.9 | 107.7 | 115.5 | 102.8 | 108.4 | 117.2 |

smaller, *viz.* 114.7°. In hydrolysed cocarboxylase, on the other hand, we find the combination of a long bond and a large angle.

The C(8)=C(9) double bond, 1.346 Å, compares favourably with 1.36 Å in both acrylic acid (Ukaji, 1959*a*) and α -methacrylic acid (Ukaji, 1959*b*). C(8)–C(7), of length 1.456 Å, is reasonable for a C(sp²)–C(sp²) single bond (Dewar & Schmeising, 1959). The geometry of the carboxyl group is normal.

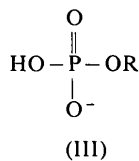
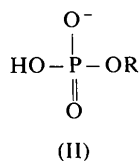
The atoms C(8), C(7), O(5) and O(6) form a plane which is inclined at 86° to the plane P(1)–O(1)–C(8). The angle between planes O(4)–P(1)–O(1) and O(2)–P(1)–O(3) is 85°.

The phosphate hydroxyl function O(4) and also O(5) of the carboxyl group form O–H...O hydrogen-bonds thus:

O(4)–H...O(3) ($-x, \frac{1}{2} + y, \frac{1}{2} - z$), of length 2.487 Å, with angle O–H...O = 167.0°;

O(5)–H...O(2) ($x, \frac{1}{2} - y, \frac{1}{2} + z$), of length 2.540 Å, with angle O–H...O = 149.1°.

Despite the availability of some careful structure analyses of the mononucleotides it is probably unwise to attempt to distinguish between P=O and P–O[−] on the basis of bond lengths. Thus we shall consider contributions equally from resonance forms (II) and (III):



Electron-diffraction studies of gaseous phosphoric oxide P₄O₁₀ (Akishin, Rambidi & Zasorin, 1959) indicate a value of 1.40 Å for the 'pure' double bond P=O. The distances 1.471 and 1.480 Å found in the phosphoenolpyruvate ion are probably not significantly different from the values 1.496 and 1.513 Å in the H₂PO₄[−] ion of the hydrolysed cocarboxylase structure and some of the values listed in Table 4. For the diester, dibenzylphosphoric acid (Dunitz & Rollett, 1956), the P=O distance is 1.469 Å but for the triesters, methyl ethylene phosphate (Steitz & Lipscomb, 1965), triphenyl phosphate (Svetich & Caughlan, 1965), and tri-*p*-nitrophenyl phosphate (Mazhar-ul-Haque & Caughlan, 1970), the values are 1.44, 1.43 and 1.44 Å respectively. Thus in the case of the triesters the P=O bond appears to be more nearly a 'pure' double bond with a smaller contribution from the charged species. A value of 1.40 Å for the P=O bond length has been reported for acetoinediol cyclic phosphate (Swank, Caughlan, Ramirez, Madan & Smith, 1967).

Examination of Table 4 shows that in each case the P–O ester linkage is close to the mean value of 1.610 Å. The distance 1.628 Å found in the phosphoenolpyruvate ion and 1.632 Å found in the 2,4-dinitrophenyl

hydrogen phosphate ion (Kennard, Watson, Fawcett, Kerr & Coppola, 1967), another 'high-energy' phosphate, are the longest so far recorded for an organic phosphate and the possible significance of this lengthening will be discussed later in connection with the 'high-energy bond' concept.

Comparison of bond angles is always complicated by environmental factors such as hydrogen bonding and non-bonded interactions, but Table 5 shows that, by dividing the results into five groups, reasonable trends become apparent.

The angles HO–P---O appear to assume values on either side of the tetrahedral value, the spread being greatest for the phosphoenolpyruvate ion, *viz.* 7.9°. Angles HO–P–OC have values falling into two groups, both significantly smaller than 109°, one group with a value of about 101° and the other 106°. The angle subtended at the phosphorus by the unshared oxygen atoms, O---P---O, is in all cases markedly larger than the tetrahedral value, which is reasonable since the shorter the phosphorus–oxygen bonds the stronger their mutual repulsion. Angles O---P–OC form two groups, the first rather smaller than 109° and the other roughly tetrahedral.

The cyclohexylammonium cation

The geometry of the cation is fairly regular with the possible exception that C(2)–C(3) is rather longer and C(6)–C(1) shorter than the accepted C–C single bond of 1.533 Å (Bartell, 1959). Similarly, the deviations of angles C(4)–C(5)–C(6) and C(6)–C(1)–N(1) from the tetrahedral value may possibly be significant, C(1)–N(1), with a length of 1.519 Å, is 0.04 Å longer than the average value, 1.479 Å, quoted in *Interatomic Distances Supplement* (1965) for the bond linking a carbon atom to a 4-covalent nitrogen atom. In terms of the standard deviation of this bond, ($\sigma = 0.009$ Å), such a large difference is almost certainly significant.

Hydrogen bonding

The hydrogen-bonding scheme established for this structure and illustrated in Fig. 4 involves all atoms which would normally be used for hydrogen-bond formation.

The nitrogen atom forms three N–H...O bonds:

- (a) of length 2.748 Å, with O(2) at $-x, \frac{1}{2} + y, \frac{1}{2} - z$;
- (b) of length 2.746 Å, with O(3) at x, y, z ;
- (c) of length 3.014 Å, with O(4) at $x, \frac{1}{2} - y, \frac{1}{2} + z$.

The N–H...O angles are 159.2, 174.1 and 175.4°, respectively. The short contact N(1)...O(5), 3.18 Å, was rejected as a possible weak hydrogen bond on the basis of Fuller's (1959) criterion, *viz.* if X–Y...Z is a potential hydrogen-bond system with Y as donor and Z as acceptor, then for Y = NH₃⁺ we should expect the angle X–Y...Z to be approximately tetrahedral. The angle C(1)–N(1)...O(5) is *ca.* 160°, whereas the corresponding angles for the three hydrogen bonds listed above are 109.7, 108.8 and 112.6° respectively.

Table 6. Comparison of hydrogen bond lengths in organic phosphates

| | P-O-H...O-P |
|---------------------------------------------------------|-----------------------------|
| Phosphoenolpyruvate (cyclohexylammonium salt) | 2.487 |
| Cytidine 3'-phosphate (orthorhombic form) | 2.532 |
| Adenosine 5'-phosphate (monohydrate) | 2.525 |
| L-Serine phosphate (monohydrate) | 2.558 |
| 2-Aminoethanol phosphate | 2.557 |
| Pyridoxamine 5'-phosphate (hydrochloride) | 2.54 |
| 2,4-Dinitrophenyl hydrogen phosphate (lutidine salt) | 2.38 |
| Phenyl hydrogen phosphate (cyclohexylammonium salt) | 2.54 |
| Ethyl hydrogen phosphate | 2.59 |
| | P-O-H...O(H ₂ O) |
| Adenosine 3'-phosphate (dihydrate) | 2.61 |
| Guanosine 5'-phosphate (trihydrate) | 2.59 |
| | P-O-H...O(5') |
| Cytidine 3'-phosphate (monoclinic form) | 2.783 |

Table 6 shows values for hydrogen-bonds found in various phosphate structures. Bonds formed between two phosphate oxygens are particularly short, as for example in the lutidine salt of 2,4-dinitrophenyl hydrogen phosphate (2.38 Å). In general all hydrogen bonds involving phosphate oxygen atoms are markedly shorter than the corresponding COH...OC bonds. As suggested by Calleri & Speakman (1964) π -bonding within the PO₄ group and its potential acidity result in an effective reduction in the radius of the oxygen atoms which would be reflected in the shortening of the hydrogen bonds.

High-energy phosphate bond

For some considerable time phosphates which occur in biological systems have been divided into two groups, the high-energy and low-energy phosphates. This classification is based principally on the free energy of hydrolysis of the phosphate ester. It is generally held that when a high-energy phosphate is hydrolysed the energy associated with the high-energy P-O bond is made available to drive a coupled endergonic reaction.

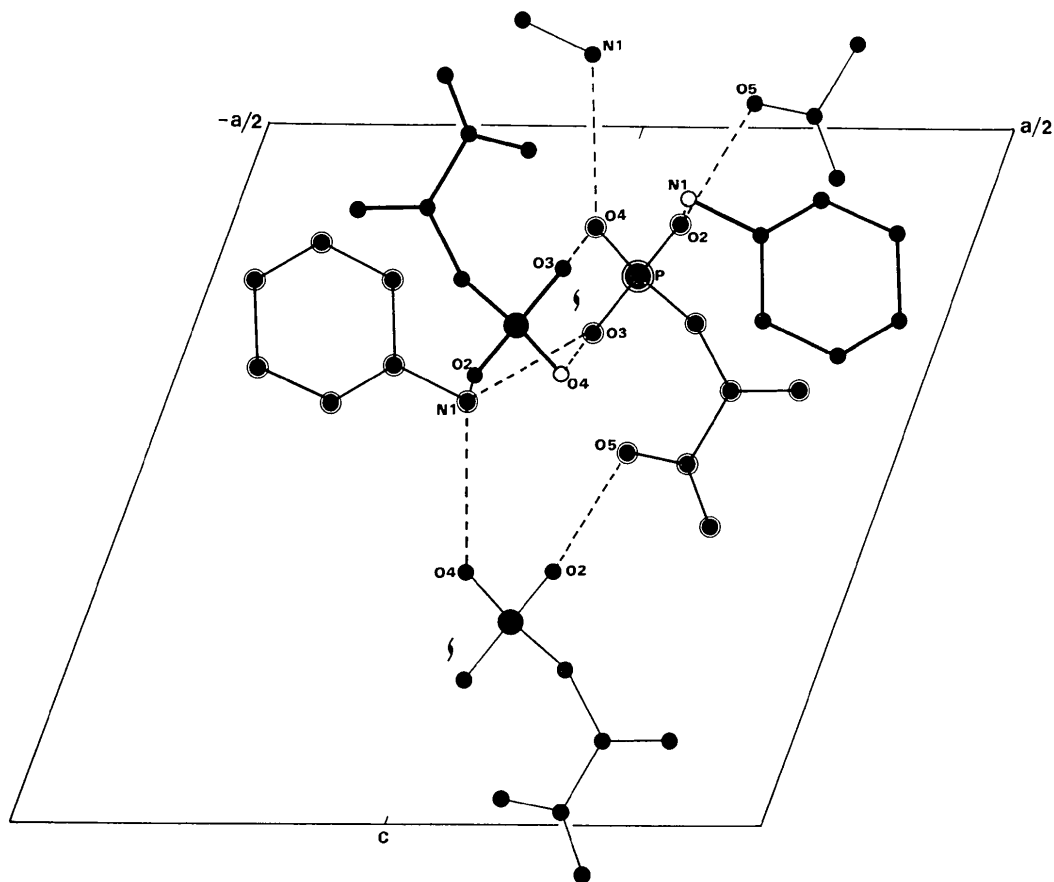


Fig. 4. Hydrogen bonding in cyclohexylammonium phosphoenolpyruvate.

To date no precise X-ray measurements have been available on the lengths of P–O ester linkages in high-energy phosphates. The phosphoenolpyruvate ion can be classed as a high-energy system and we find that the P–O ester bond is 1.628 Å, slightly longer than the mean value in Table 4. The P–O ester bond in the 2,4-dinitrophenyl hydrogen phosphate ion (DNP), also classed as a high-energy compound is even longer (1.632 Å). The average value of the P–O ester bond in the two independent molecules of ATP is 1.65 Å but the estimated standard deviation is 0.05 Å because of the limited data set on which the analysis was based. It would not be prudent to attach too much significance to these rather greater bond lengths until more accurate information is available on other 'high-energy' compounds, especially in view of the fact that a similar long bond of 1.637 (7) Å was found in disodium DL-glycerol-3-phosphate hexahydrate (Fenn & Marshall, 1971) which is not a 'high-energy' compound.

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