The Crystal Structure of the Tri(cyclohexylammonium) Salt of Phosphonopyruvate; Theoretical Calculations on the Equilibrium Constant for the Rearrangement of Phosphonopyruvate to Phosphoenolpyruvate

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The crystal structure of phosphonopyruvate is reported and the co-ordinates are used to calculate that the theoretical equilibrium constant for its rearrangement to phosphoenolpyruvate (PEP) lies substantially towards PEP.

The enzyme phosphoenolpyruvate phosphomutase, which catalyses the primary formation of the carbon–phosphorus bond in the biosynthetic path to naturally occurring phosphonates,[1] has recently been reported by three groups.[2—4] The enzyme facilitates a 1,3-phospho group transfer from phosphonopyruvate (1) to phosphoenolpyruvate (PEP) (2). The equilibrium constant for this rearrangement is greater than 100, in favour of PEP. This result was not expected, and all early efforts to isolate the enzyme were unsuccessful because they searched for the catalysed formation of phosphono-pyruvate from PEP. It had seemed plausible that phosphono-pyruvate would be more stable than PEP for two reasons.

Table 1. Energy differences, ΔE (kcal mol^{-1}) between conformers of (1) and (2) with coplanar (c) and perpendicular (p) carboxylate groups.[a]

Method	(1),c→p	(2),c→p	c(1)→c(2)	p(1)→p(2)	x(1)→p(2)	Ratio[b](1):(2)
MNDO	−5.4	−6.3	−5.7	−6.6	−6.7	6.9×10^4
STO-3G	−0.4	−2.2	−4.5	−6.3	−4.6	2.5×10^3
3-21G	+2.4	+4.7	−11.4	−9.2	−9.9	2.3×10^8

[a] Coplanar and perpendicular orientations of the CO_2^- group are defined by periplanar and clinal O–C–C–R torsion angles respectively. Other torsion angles in the O–C–C–R–P–O chain (R=CH$_2$ or O) are antiperiplanar, except in the optimised crystal structure (x) of (1). [b] Based on the forms of (1) and (2), calculated to be most stable, and assuming identical solvation and entropy effects.
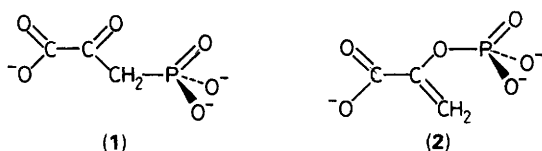
First, PEP is one of the 'highest energy' phosphates known, and second, despite the relative instability of the C–P bond compared to the O–P bond, it was expected that the ketonisation energy of enolpyruvate would dominate the overall ΔG°.

In an attempt to rationalise the experimental observation, we have determined the crystal structure of the trianion of (1), the dominant form at physiological pH.[5] No comparable crystal structures exist for the trianion of (2),[6] but the structures of several salts of the monoanion are known.[7—10] From the crystal data we have constructed several optimised models of the trianions of (1) and (2), thus enabling a comparison of their energy by various methods.

The tri(cyclohexylammonium) salt of phosphonopyruvate was prepared in 56% yield from ethyl diethylphosphono-pyruvate[11] by treatment with trimethylsilylbromide,[12] followed by hydrolysis in the presence of cyclohexylamine. Recrystallisation from acetone–water (3:1, v/v) gave an analytically pure sample of needle shaped crystals as the dihydrate.

A PLUTO[13] drawing of the crystal structure of (1) is shown in Figure 1.† The expected tautomeric form is confirmed by bond distances and thermal parameters. The pyruvate fragment is relatively flat, with only a 5° angle between the carboxyl and the C(3)–C=O planes. The C–C–C–P torsion angle of 106° about C(2)–C(3) elevates the phosphonate out of the plane of the –O₂C–CO–CH₂– group. Optimisation using MNDO calculations‡ twists the $PO_3{}^{2-}$ unit 35° towards the antiperiplanar disposition which is expected to minimise like-charge interaction with carboxylate, and the carboxylate group becomes nearly perpendicular to the molecular plane. A starting conformation with the carboxylate group only 1° out of coplanarity remains nearly coplanar under optimisation, but with higher energy.

A similar result is obtained when the known PEP monoanion crystal structures,[7—10] which all have carboxylate groups within 7° of coplanarity with C=C–O, are converted to the trianion‡ and optimised analogously. Both perpendicular and coplanar carboxylate orientations are found, but the former
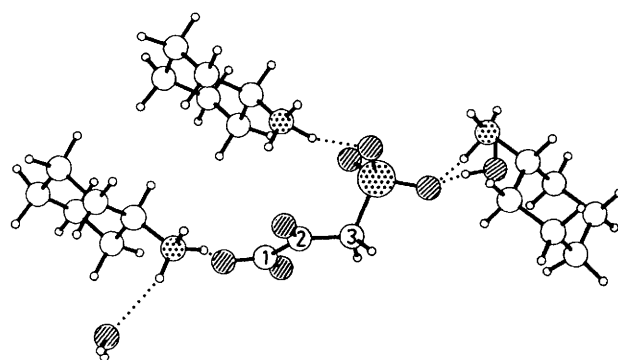
Figure 1. View of the tri(cyclohexylammonium) salt dihydrate of (1) projected onto its least-squares plane. Nitrogen and phosphorus atoms are stippled, and oxygen atoms hatched. Of the thirteen unique hydrogen bonds in the structure, the five visible in this projection are shown by dotted lines.

are more stable. Energies of the optimised species were checked by *ab initio* calculation‡ with STO-3G and 3-21G basis sets; representative results appear in Table 1. The STO-3G calculations indicate a preference for perpendicular forms. Thus, within this formalism there exists a slightly more stable conformation of the PEP trianion than the planar one assumed by Hayes, Kenyon, and Kollman[14] in their STO-3G study. However, this order is reversed in the 3-21G basis, planar forms now being favoured.

In aqueous solution the energies calculated for the isolated ions would be drastically modified by extensive solvation. Attempts were made to establish the relative magnitudes of the solvation energies by a reaction field treatment previously used for PEP.[14] Considering the bridge methylene carbon or oxygen atom to be at the centre of a cavity within the solvent delineated by the terminal oxygen atoms, the cavity radius is ~0.2 Å larger with phosphonopyruvate than with PEP. This difference is attributable to the greater length of C–C and P–C bonds in phosphonopyruvate, compared with the C–O and P–O bonds in PEP, and would imply less effective solvation of phosphonopyruvate by ~15 kcal mol⁻¹ (1 cal = 4.184 J). However, the greater length of phosphonopyruvate is balanced by its reduced width. The van der Waals volumes calculated‡ on a 0.1 Å mesh are 105 Å³ for several conformations of phosphonopyruvate, compared with 106 Å³ for PEP. It therefore appears that solvation energies are very similar for the two species. This conclusion is reinforced by the similarity of the charges on the solvent-accessible oxygen atoms calculated with the 3-21G basis set. Averaged over chemically equivalent O atoms in planar and perpendicular conformations, the charges on $PO_3{}^{2-}$ are −1.054 each in (1) and −1.059 in (2); for $CO_2{}^-$ they are −0.820 and −0.824, respectively.

Assuming that the Gibbs free energies in solution follow the trend of gas-phase energies calculated above, ΔG° should be at least as negative as −4.6 kcal mol⁻¹, which implies an equilibrium constant of at least 2500 in favour of PEP, at 298 K. Our ΔE values agree in sign but not in magnitude with the −20 kcal mol⁻¹ cited in a recent report.[15] The miniscule amount of phosphonopyruvate that is predicted by these calculations to be present at equilibrium, is entirely consistent with the limits on the equilibrium constant that have been found experimentally.

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