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Supplementary Material Available: Tables of positional and thermal parameters and bond length and angles for $(Ph_4As)_2I$ (10 pages); table of observed and calculated structure factors (36 pages). Ordering information is given on any current masthead page.

Catalysis and Thermodynamics of the Phosphoenolpyruvate/Phosphonopyruvate Rearrangement. Entry into the Phosphonate Class of Naturally Occurring Organophosphorus Compounds

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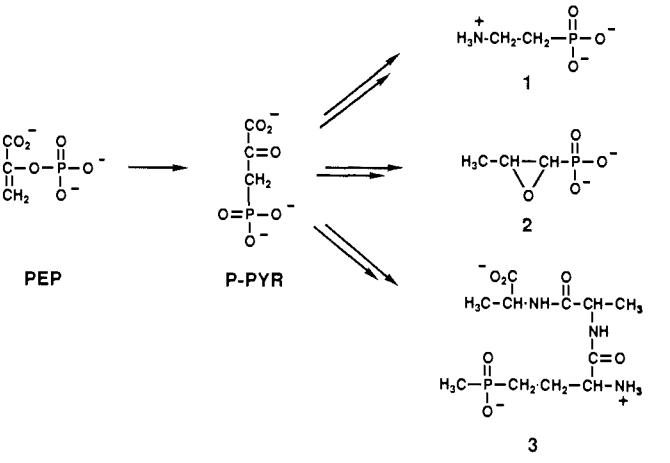
Organophosphates are most commonly found in nature as oxygen esters, diesters, and anhydrides of phosphoric acid. Phosphonates, which contain a P–C linkage, by comparison are more sparse in the natural environment. Nonetheless, they have been discovered in a variety of organisms (ranging from bacteria to man) and have been shown to occur in a number of structural forms.[1]

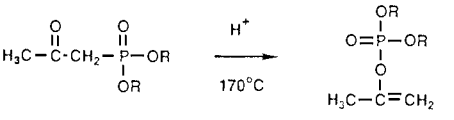
Studies of the biosynthetic pathways leading to the phosphonolipid precursor, 2-aminoethylphosphonate (AEP)[2,6] (**1**) in *Tetrahymena pyriformis*, and to the antibiotics, fosfomycin[7] (**2**), and bialaphos[8] (**3**) in certain strains of *Streptomyces* have suggested that phosphoenolpyruvate (PEP)[9] is the key precursor (see Scheme I) of naturally occurring phosphonates. Twenty years ago Warren postulated that the P–C bond in **1** is formed from PEP by its isomerization to phosphonopyruvate (P-pyr).[4] In this communication we wish to report the discovery of the reversible rearrangement reaction of PEP and P-pyr which takes place in the active site of a Mg^{2+} activated phosphomutase found in *T. pyriformis*.

The phosphomutase was isolated from *T. pyriformis* cells that were cultivated and harvested according to published procedures.[6] Cell lysis was carried out in buffered solutions at pH 8.2 (4 °C) with a French Pressure Cell Press (8000 psi).[10] The cell debris was removed at 17 000 g (10 min), and *Streptomycin* sulfate (2% wt/v) was added to the supernatant to precipitate the nucleic acids.

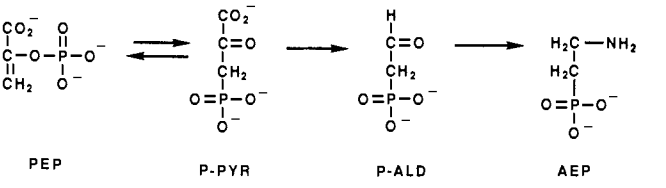
Scheme I



Scheme II



Scheme III



The P-pyr ⇌ PEP phosphomutase activity (measured with synthetic P-pyr[11] and with the ADP/pyruvate kinase–NADH/lactate dehydrogenase coupled spectrophotometric assay at pH 7.8, 25 °C) was found in the 60–70% $(NH_4)_2SO_4$ protein fraction. The phosphomutase was purified as a ~40 000 D protein having a specific activity of 30 unit/mg (5 mM $MgCl_2$, 50 mM K^+Hepes, pH 7.5, 25 °C) by using the following sequence of column chromatographies at 4 °C: DEAE-Sephadex (0.1 M NaCl to 0.4 M NaCl in 10 mM $MgCl_2$ and 20 mM triethanolamine pH 7.5), Sephadex G-100 (10 mM $MgCl_2$, 20 mM triethanolamine pH 7.5), and Superose-12 (Pharmacia) FPLC (10 mM $MgCl_2$, 20 mM triethanolamine pH 7.5). In the absence of Mg^{2+} ion the phosphomutase was found to be catalytically inactive.

The thermodynamically favored direction of the phosphomutase-catalyzed PEP/P-pyr isomerization reaction (20 mM PEP or P-pyr, 10 mM $MgCl_2$, 50 mM K^+Hepes, pH 8.0, 25 °C) was determined by using ^{31}P NMR (Figure 1) and HPLC (Altex C-18 reversed phase analytical column, 3% triethylamine, 25 mM P_i, 1.5% methanol, pH 6.5 as isocratic eluent, A^{231nm}) techniques. The PEP/P-pyr ratio at equilibrium was found to be ≥500. Because PEP is the highest energy naturally occurring organophosphate, this finding was not anticipated. Specifically, compared to a simple phosphate ester (for which the standard free energy of hydrolysis is 2–3 kcal/mol) PEP releases ca. 10 kcal/mol more standard free energy upon hydrolysis.[12] This energy release is brought about largely as a result of the conversion of the phosphoenol moiety in PEP to the ketone moiety in pyruvate and the relief of the repulsive interaction which takes place between the charged carboxylate and phosphate moieties present in the PEP.[13] Both of these factors are expected to also contribute to the en-

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(9) Abbreviations used: phosphoenolpyruvate (PEP), phosphonopyruvate (P-pyr), (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (HEPES), ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), adenosine 5'-diphosphate (ADP), orthophosphate (P_i), 2-aminoethylphosphonate (AEP), dihydronicotinamide adenine dinucleotide (NADH), high pressure liquid chromatography (HPLC), nuclear magnetic resonance (NMR).

(10) Cell lysis was carried out with 25 mL of packed cells in 100 mL of suspension buffer (pH 8.2) containing 50 mM K^+HEPES, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.8% BSA, 1 mM 1,10-phenanthroline, 1 mM benzamidine·HCl, 50 μM phenylmethylsulfonyl fluoride, and 50 μg/mL trypsin inhibitor.

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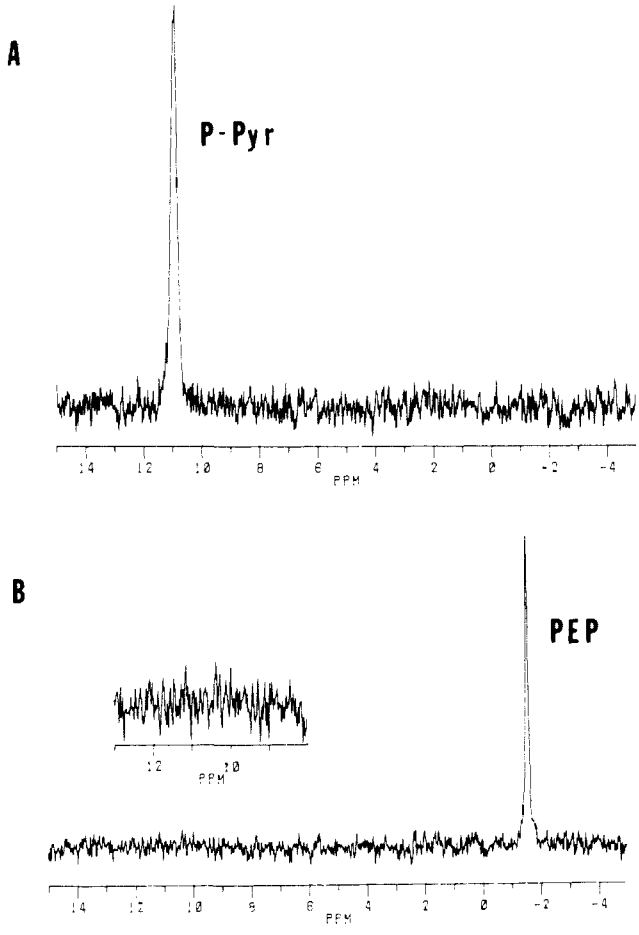

Figure 1. ^{31}P NMR spectra (162.04 MHz, 0.1 M D_3PO_4 external standard, 25 °C, 50% D_2O) of a solution initially containing 20 mM phosphonopyruvate, 10 mM $MgCl_2$, and 50 mM K^+Hepes (pH 8.0) before (A) and after (B) incubation with 0.3 unit/mL of phosphomutase for 1 h.

ergetics of the PEP ⇒ P-pyr conversion. However, opposing the PEP ⇒ P-pyr conversion is the difference in the P–O and P–C bond energies. Although the exact difference in the P–O and P–C bond energies of PEP and P-pyr has not been reported, published values for other systems range from 10–17 kcal/mol in favor of the P–O bond.[14] Relevant to the PEP ⇔ P-pyr rearrangement reaction are the reported thermal rearrangements of diesters of α-ketophosphonates in acid solution to the corresponding vinyl phosphates[15] (Scheme II). Thus, the equilibrium position of the vinyl phosphate ⇔ ketophosphonate interconversion in this system as well as in the PEP ⇔ P-pyr system appears to be dominated by the comparatively higher energy of the P–C bond of the ketophosphonate.

The unexpected equilibrium position for the catalyzed PEP ⇔ P-pyr isomerization reaction has no doubt contributed to the failure of previous attempts[1,8,16] to observe P-pyr formation from PEP in whole cell or cell free systems. Nevertheless, the current results along with those from recent studies of the PEP to AEP pathway[18] (Scheme III) in *T. pyriformis* leave little doubt that the PEP to P-pyr conversion is the key P–C bond-forming step of phosphonate biosynthesis in this organism. It should be recognized, however, that because of the unfavorable position of the PEP ⇔ P-pyr equilibrium that in order for the phosphomutase-catalyzed reaction to serve as the source of P-pyr it must be coupled to a thermodynamically favorable ensuing step in the pathway. In the case of the AEP[6,18] and bialaphos[8] biosynthetic pathways, P-pyr formation is followed by its decarboxylation to produce phosphonoacetaldehyde. Studies carried out in our laboratory on the competing reaction pathways of PEP and P-pyr in *T pyriformis* cellular homogenates suggest that the phosphomutase and α-ketodecarboxylase reactions are coupled in such a way that the P-pyr formed at the phosphomutase active site (where the PEP ⇔ P-pyr equilibrium seems to be more favorable) is shuttled directly into the active site of the decarboxylase.[19]

Ongoing studies in our laboratory are focussed on distinguishing a concerted vs stepwise mechanism for the PEP ⇔ P-pyr rearrangement by determining the stereochemical course of the phosphomutase catalyzed reaction of a chiral P-pyr derivative.

Acknowledgment. This work was supported by a National Institutes of Health Grant GM-28688 and by a Fellowship Award from the Alfred P. Sloan Foundation. We thank Patrick S. Mariano for helpful discussion of this work.

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(16) Horiguchi,[17] however, reported that P-pyr was isolated from a *T. pyriformis* cellular homogenate–PEP mixture as a hydrazone derivative by reacting the mixture with 2,4-dinitrophenylhydrazine under acidic conditions. Several attempts to reproduce this result failed. Because appreciable amounts of PEP-derived P-pyr could not possibly be present in the reaction mixture failure to trap the P-pyr under enzyme denaturing conditions is understandable.

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(19) We have shown[18] (1) that the conversion of PEP to AEP in the homogenate is significantly more efficient than the conversion of the pathway intermediate, P-pyr to AEP and (2) that the reason for this is that the P-pyr added to the homogenate is rapidly consumed by a competing hydrolysis reaction, while the added PEP is not. The P-pyr generated from PEP via the phosphomutase (as opposed to the "added" P-pyr) is therefore protected from the reaction catalyzed by the crude homogenate. Such protection would be afforded by transfer of the P-pyr formed in the active site of the phosphomutase directly into the active site of the decarboxylase.

First Experimental Evaluation of Hypervalent N–S–N Bond Energy from the Restricted Rotation of Pyrimidine Ring in 10-S-3 Sulfuranes

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Since the discovery of the unusual structure in trithiapentalenes,[1] i.e., sometimes referred to as "no bond resonance compounds", many examples of the 10-S-3 species have been prepared.[1,2] In these species,[2,3] the bond distances in the three-center four-electron bond (X-S-Y) vary according to the electronegativity of the two ligands.[3b] In connection with these studies, we have shown the presence of ring transformation equilibrium ("bond switching") in sulfur-containing heterocyclic systems via such 10-S-3 sulfuranes, which show susceptibility of the hypervalent bond to the ligand stability.[5]

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