Communications to the Editor

Phosphonate Biosynthesis: The Stereochemical Course of Phosphoenolpyruvate Phosphomutase

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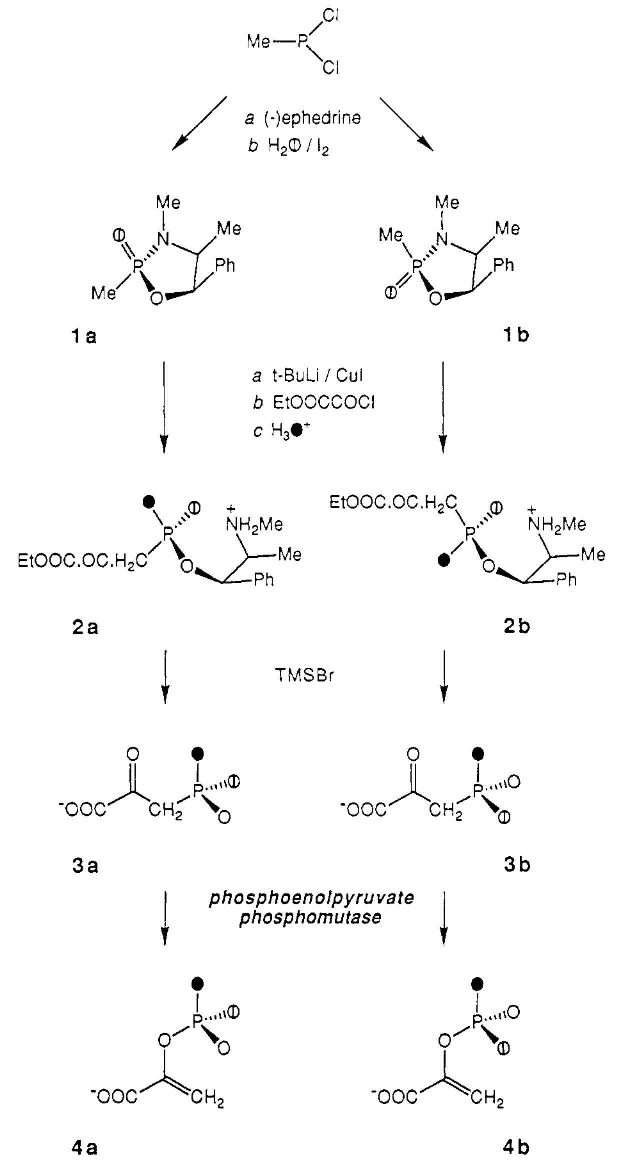
The phosphorus-carbon bond that occurs in a variety of natural products elaborated by several fungi and eucaryotes[1] owes its existence to the enzyme phosphoenolpyruvate phosphomutase,[2] which catalyzes the interconversion of phosphoenolpyruvate and phosphonopyruvate. To narrow the range of mechanistic postulates for this intriguing transformation, we have determined the stereochemical consequence at phosphorus in the enzyme-catalyzed reaction of chiral [^{16}O,^{17}O,^{18}O]phosphonopyruvate. Contrary to a recent report,[3] the reaction proceeds with *overall retention of the configuration at phosphorus.*

[^{16}O,^{17}O,^{18}O]Phosphonopyruvate, of one configuration at phosphorus, was synthesized as illustrated in Scheme I. Methyldichlorophosphine was allowed to react with (−)-ephedrine to produce two oxazaphospholidinones, epimeric at phosphorus.[4] These materials were oxidized in situ with iodine and [^{17}O]H_2O to produce the epimeric cyclic methylphosphonamidates **1a** and **1b**, which were separated chromatographically. Although the identity of these species was reasonably secure from the NMR work of Inch and his colleagues,[4] the structure of **1a** (isotopically unlabeled) was confirmed by crystallographic analysis.[5] The cuprate of **1a** was generated with *tert*-butyllithium followed by addition of CuI[6] and was allowed to react with ethyl oxalyl chloride in tetrahydrofuran. Treatment of the crude product with [^{18}O]H_3O^+ gave **2a**, with its ethyl ester group intact (for the significance of this observation, see below). This ring-opening reaction is known to proceed by an "in-line" pathway,[4,7] with inversion at phosphorus. Deprotection of **2a** with trimethylsilyl bromide gave the desired product [(*R*)-^{16}O,^{17}O,^{18}O]phosphonopyruvate (**3a**) in good yield.

As has been established earlier,[2] the equilibrium of the mutase-catalyzed reaction lies well toward phosphoenolpyruvate, and, in order to proceed with the stereochemical analysis of the product phosphoenolpyruvate (**4a** or **4b**), the mutase reaction was coupled to two further exergonic processes: the pyruvate kinase-catalyzed transfer of the phospho group of phosphoenolpyruvate to ADP, and the hexokinase-catalyzed phosphorylation of glucose by the product ATP. Each of these two kinases is known to proceed with inversion at phosphorus,[8] so that, in one incubation, the phospho group of phosphoenolpyruvate was relocated, with overall retention, on the 6-hydroxyl group of glucose ready for stereochemical analysis. Determination of the absolute configuration at phosphorus now followed established procedures, involving ring closure to the bicyclic 4,6-phosphodiester, methylation, and ^{31}P NMR analysis.[9] The NMR spectra of the bicyclic phosphotriesters (methoxy axial) that derive from the mutase-catalyzed rearrangement of (*R*)- and (*S*)-[^{16}O,^{17}O,^{18}O]phosphonopyruvate (**3a** and **3b**), synthesized independently from **1a** and from **1b**, are shown in Figure 1. It is evident from the middle pair of peaks in each quartet (these are the stereochemically informative resonances) that [(*R*)-^{16}O,^{17}O,^{18}O]phosphonopyruvate produces [(*S*)-^{16}O,^{17}O,^{18}O]phosphoenolpyruvate and, conversely, the *S* isomer produces the *R* isomer. Recognition of the priority rules for *R* and *S* assignment allows the conclusion that the mutase

Scheme I. Synthesis of (*R*)- and (*S*)-[^{16}O,^{17}O,^{18}O]Phosphonopyruvate (**3a** and **3b**, Respectively)



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(1) Hori, T.; Horiguchi, M.; Hayashi, A. *Biochemistry of Natural C-P Compounds*; Maruzen: Tokyo, 1984.
(2) Bowman, E.; McQueney, M.; Barry, R. J.; Dunaway-Mariano, D. *J. Am. Chem. Soc.* **1988**, *110*, 5575. Seidel, H. M.; Freeman, S.; Seto, H.; Knowles, J. R. *Nature* **1988**, *335*, 457. Hidaka, T.; Mori, M.; Imai, S.; Hara, O.; Nagaoka, K.; Seto, H. *J. Antibiot.* **1989**, *42*, 491.
(3) McQueney, M. S.; Lee, S.; Bowman, E.; Mariano, P. S.; Dunaway-Mariano, D. *J. Am. Chem. Soc.* **1989**, *111*, 6885.
(4) Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1969.
(5) Space group, monoclinic, $P2_1$; $a = 6.871$ (3) Å; $b = 7.023$ (1) Å; $c = 12.912$ (5) Å; $\beta = 102.15$ (3)°; $Z = 2$; $R = 0.040$ and $R_w = 0.035$.
(6) Coutrot, P.; Savignac, P.; Mathey, F. *Synthesis* **1978**, 36.
(7) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2558.
(8) Orr, G. A.; Simon, J.; Jones, S. R.; Chin, G. J.; Knowles, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2230. Blättler, W. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 510.
(9) Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3186.

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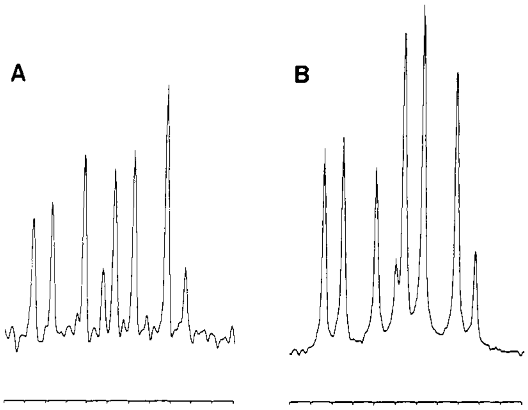
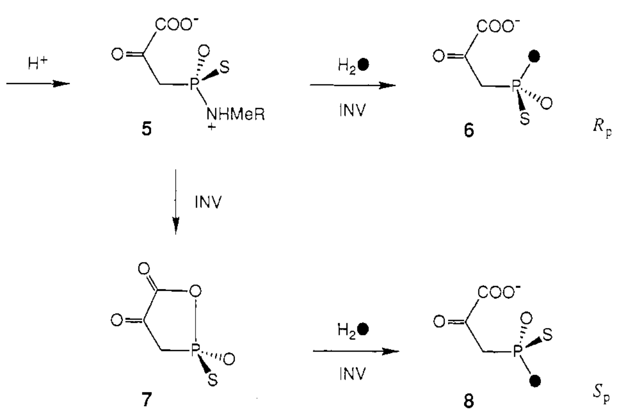


Figure 1. ^{31}P NMR spectra[15] of the axial methyl esters of the α-D-glucopyranoside cyclic 4,6-phosphates derived from stereochemical analysis of the α-D-glucopyranoside 6-[^{16}O,^{17}O,^{18}O]phosphates obtained from the phosphomutase reaction of (*R*)- and (*S*)-[^{16}O,^{17}O,^{18}O]-phosphonopyruvate (A and B, respectively).

Scheme II. Conversion of **5** to (*R*)-Thiophosphonopyruvate **6** with Inversion (as Assumed in Ref 3) or to (*S*)-Thiophosphonopyruvate **8** via **7** with Overall Retention



reaction proceeds with overall *retention* of the configuration at phosphorus.

In contrast to these results, a recent report[3] has suggested that the stereochemical course of the mutase reaction is inversion. This study used ^{18}O and sulfur to create the chirality at phosphorus, and the substrate was therefore the [^{18}O]phosphorothioate of phosphonopyruvate. While there have been occasional concerns that the use of phosphorothioates (as distinct from phosphates) could give misleading stereochemical outcomes, we do not believe this to be the cause of the discrepancy. The fact that phosphorothioate substrates have always been found to follow a stereochemical course identical with that of their all-oxy parents[10] argues against such an explanation.[11] It seems more likely that the synthesis of chiral thiophosphonopyruvate reported in ref 3 included a step in which an unnoticed inversion at phosphorus occurred. Thus the transformation of **5** (Scheme II) was presumed to go with inversion to **6**, yet the free neighboring carboxylate in **5** can displace the ammonium leaving group to give **7**, which then hydrolyzes to **8**. Such a well-precedented[13] double displacement reaction would thus give **8** with overall retention, instead of **6** with inversion, as was assumed by McQueney et al.[3]

We conclude, therefore, that the phosphomutase proceeds with overall retention and follows a mechanistic route that involves either an unremarkable phospho-enzyme intermediate or, conceivably, the intramolecular participation of the substrate's carboxylate group.[14]

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(10) Eckstein, F. *Annu. Rev. Biochem.* **1985**, *54*, 367.
(11) The fact that McQueney et al.[3] have mislabeled the configurations of their chiral thiophosphoenolpyruvate products (according to the Cahn–Ingold–Prelog rules) does not account for the error, provided that the proper course of Frey's analysis (in which, for example, pyruvate kinase catalyzes the phosphorylation of the *pro-S* oxygen of ADPβS[12]) was traced.
(12) Richard, J. P.; Ho, H.-T.; Frey, P. A. *J. Am. Chem. Soc.* **1978**, *100*, 7756. Frey, P. A. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1989**, *62*, 119.
(13) See, e.g.: Clark, V. M.; Kirby, A. J. *J. Am. Chem. Soc.* **1963**, *85*, 3705. Benkovic, S. J.; Schray, K. J. *J. Am. Chem. Soc.* **1969**, *91*, 5653. Simons, S. S. *J. Am. Chem. Soc.* **1974**, *96*, 6492. Steffens, J. J.; Siewers, I. J.; Benkovic, S. J. *Biochemistry* **1975**, *14*, 2431. Species analogous to **7** are known to open by attack at phosphorus.[14]
(14) Schray, K. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1971**, *93*, 2522.
(15) The spectra were run on a Bruker WM-300 instrument at 121.5 MHz with a deuterium field lock and broad-band decoupling: spectral width, 500 Hz; acquisition time, 8.2 s; pulse width, 15.5 μs; number of transients, 4000; Gaussian multiplication and Fourier transform in 8 K (Gaussian broadening, 0.1 Hz; line broadening, −0.4 Hz). The scale used is 2 Hz/division.

Boron-Containing Nucleic Acids: Synthesis of Cyanoborane Adducts of 2′-Deoxynucleosides

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Antiviral[1,2] and antitumor[2] activity associated with a wide variety of structurally divergent modified nucleosides has stimulated a great interest in the synthesis and biological activity of new classes of nucleic acid compounds. We have been interested in the synthesis[3–9] and activity[10–16] of boron-containing antime-

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(1) (a) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911–1915. (b) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Border, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096–7100. (c) Lin, T. S.; Guo, J. Y.; Schinazi, R. F.; Chu, C. K.; Xiang, J. N.; Prusoff, W. H. *J. Med. Chem.* **1988**, *31*, 336–340. (d) Beauchamp, L. M.; Serling, B. L.; Kelsey, J. E.; Biron, K. K.; Collins, P.; Selway, J.; Lin, J. C.; Schaeffer, H. J. *J. Med. Chem.* **1988**, *31*, 144–149. (e) Remy, R. J.; Secrist, J. A., III *Nucleosides Nucleotides* **1985**, *4*, 411–427. (f) Prusoff, W. H.; Ward, D. C. *Biochem. Pharmacol.* **1976**, *25*, 1233–1239.
(2) (a) Marquez, V. E.; Lim, M.-I.; Treanor, S. P.; Plowman, J.; Priest, M. A.; Markovac, A.; Khan, M. S.; Kaskar, B.; Driscoll, J. S. *J. Med. Chem.* **1988**, *31*, 1687–1694. (b) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109–112. (c) Johnson, F.; Pillai, K. M.; Grollman, A. P.; Tseng, L.; Takeshita, M. *J. Med. Chem.* **1984**, *27*, 954–958. (d) Secrist, J. A., III; Shortnacy, A. T.; Montgomery, J. A. *J. Med. Chem.* **1988**, *31*, 405–410.
(3) Spielvogel, B. F.; Das, M. K.; McPhail, A. T.; Onam, K. D.; Hall, I. H. *J. Am. Chem. Soc.* **1980**, *102*, 6343–6344.
(4) Spielvogel, B. F.; Wojnowich, L.; Das, M. K.; McPhail, A. T.; Hargrave, K. D. *J. Am. Chem. Soc.* **1976**, *98*, 5702–5703.
(5) Spielvogel, B. F. *Boron Chemistry-4*; IUPAC, Inorg. Chem. Division; Parry, R. W., Kodama, G., Eds.; Pergamon: New York, 1980; pp 119–129.
(6) Wisian-Neilson, P.; Das, M. K.; Spielvogel, B. F. *Inorg. Chem.* **1978**, *17*, 2327–2329.
(7) Spielvogel, B. F.; Harchelroad, F., Jr.; Wisian-Neilson, P. *J. Inorg. Nucl. Chem.* **1979**, *41*, 1223–1227.
(8) Spielvogel, B. F.; Ahmed, F. V.; Morse, K. W.; McPhail, A. T. *Inorg. Chem.* **1984**, *23*, 1776–1777.
(9) Spielvogel, B. F.; Ahmed, F. V.; Silvey, G. L.; Wisian-Neilson, P.; McPhail, A. T. *Inorg. Chem.* **1984**, *23*, 4322–4324.
(10) Hall, I. H.; Spielvogel, B. F.; McPhail, A. T. *J. Pharm. Sci.* **1984**, *73*, 222–225.
(11) Hall, I. H.; Gilbert, C. J.; McPhail, A. T.; Morse, K. W.; Hasset, K.; Spielvogel, B. F. *J. Pharm. Sci.* **1985**, *74*, 755–758.
(12) Hall, I. H.; Starnes, C. O.; McPhail, A. T.; Wisian-Neilson, P.; Das, M. K.; Harchelroad, F., Jr.; Spielvogel, B. F. *J. Pharm. Sci.* **1980**, *69*, 1025–1029.

0002-7863/89/1511-9234$01.50/0