Biosynthesis of Natural Products with a P—C Bond, X[1]

Incorporation of D-[1-$^2H_1$]Glucose into 2-Aminoethylphosphonic Acid in *Tetrahymena thermophila* and into Fosfomycin in *Streptomyces fradiae*. — The Stereochemical Course of a Phosphoenolpyruvate Mutase-Catalyzed Reaction

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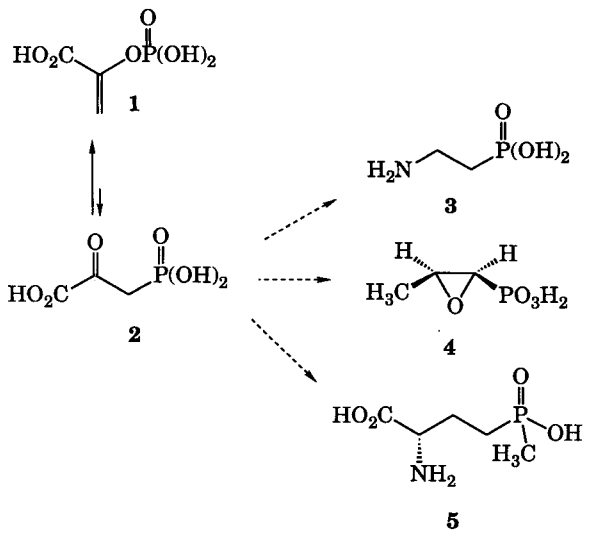
2-Aminoethylphosphonic acid and fosfomycin produced on feeding D-[1-$^2H_1$]glucose to *Tetrahymena thermophila* and *Streptomyces fradiae*, respectively, indicate that the phosphoenolpyruvate mutase catalyzes the stereospecific transfer of the phospho group of (*Z*)-phosphoenol-[3-$^2H_1$]pyruvate from oxygen to carbon from the *si* face. ^{2}H-NMR spectroscopy was used to determine the configuration at C-1 of biosynthetically formed 2-amino-[1-$^2H_1$]ethylphosphonic acid after derivatization with (−)-camphanoyl chloride and diazomethane.

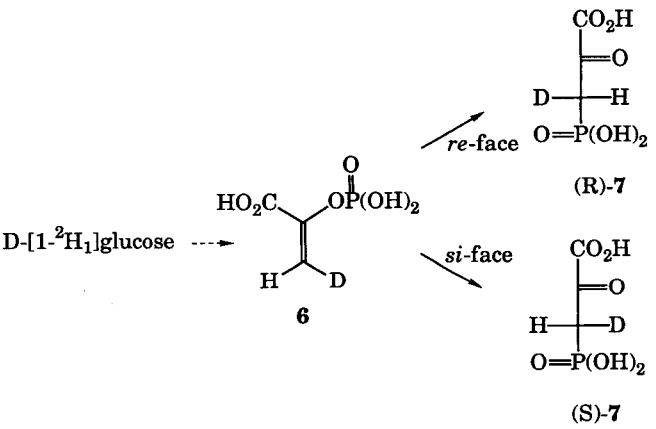
The discovery of 2-aminoethylphosphonic acid (AEP) by Horiguchi and Kandatsu[2] marks the beginning of the isolation of natural products with a P—C bond. They display interesting properties and are produced by organisms ranging from protozoa to *Streptomyces*[3]. The biosynthesis is only partly known[3,4].

Scheme 1



The P—C bond is biosynthetically formed by the rearrangement of phosphoenolpyruvate (PEP) (**1**) to phosphonopyruvate (**2**), catalyzed by the PEP mutase (Scheme 1)[5]. This enzyme has been isolated and purified from *Tetrahymena*[6] producing AEP, and from *Streptomyces hygroscopicus*[7] producing bialaphos. Knowles et al.[8] have demonstrated that the migration of the phospho group from oxygen to carbon of enolpyruvate occurs with overall retention of configuration at phosphorus. Dunaway-Mariano and Mariano et al.[9] have proved the intramolecular nature of the process. Phosphonopyruvic acid (**2**) is transformed into AEP (**3**), fosfomycin (**4**), and bialaphos (**5**).

Scheme 2



To probe the stereochemistry at carbon, that is whether the phospho group is transferred to C-3 of enolpyruvate from the *re* or from the *si* face of the methylene group, the easily available D-[1-$^2H_1$]glucose has been used as a precursor in feeding experiments. It is prepared by reduction of D-glucono-δ-lactone with NaB^2H_4[10]. D-[1-$^2H_1$]Glucose is transformed via the glycolytic pathway into unlabelled PEP and into (*Z*)-[3-$^2H_1$]PEP (**6**) (Scheme 2)[11,12]. This compound is isomerized by the PEP mutase either to (*R*)- or (*S*)-3-phosphono-[3-$^2H_1$]pyruvic acid (**7**), depending on the specificity (*re* or *si*) of the enzyme. Two feeding experiments have been carried out. First, D-[1-$^2H_1$]glucose is fed to *Tetrahymena thermophila* and the AEP formed is isolated as described in ref.[13]. It contains 18% deuterium at C-1 as determined indirectly by ^{1}H-NMR spectroscopy (400 MHz, D_2O). The AEP isolated is derivatized with (−)-

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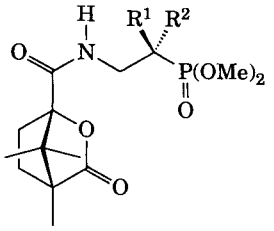
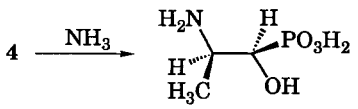
camphanoyl chloride[14] and esterified with diazomethane to give the amide **8a**. Reference samples **8b**, **8c**, and **8d** of unlabelled AEP, of racemic [1-$^2H_1$]AEP, and of (*S*)-[1-$^2H_1$]AEP[14] with an enantiomeric excess of 76%, respectively, have been obtained similarly. The sections of the signals for the hydrogens at C-1 from the ^{1}H-NMR spectra with simultaneous decoupling of ^{31}P and hydrogens at C-2 of four samples have been compared. The spectrum for derivative **8b** of the unlabelled AEP shows for the two hydrogens at C-1 an AB system (δ_A = 1.89, δ_B = 1.815, J_{AB} = 15 Hz). The spectrum of a mixture of **8b** (65%) and **8c** (35%) reveals additionally two shoulders at δ = 1.875 and 1.795, corresponding to two singlets broadened by coupling to deuterium for the two diastereomeric derivatives **8c** of racemic [1-$^2H_1$]AEP, shifted upfield[15] by 0.015 and 0.02 ppm relative to the resonances for hydrogens A and B, respectively. The same two broadened singlets (integration ratio 12:88) are present in the spectrum of the derivative **8d** of (*S*)-[1-$^2H_1$]AEP with an enantiomeric excess of 76%. The spectrum of the biosynthetic sample **8a** shows besides the AB system for **8b** a diagnostic shoulder at δ = 1.795, corresponding to the presence of 18% of the derivative of (*S*)-[1-$^2H_1$]AEP.



8a AEP isolated
8b $R^1 = R^2 = H$
8c $R^1 = H$, $R^2 = {}^2H$ and $R^1 = {}^2H$, $R^2 = H$
8d $R^1 = H$, $R^2 = {}^2H$, d.e. = 76%

Because of overlapping signals observed in the ^{1}H-NMR spectra, ^{2}H-NMR spectra (61.4 MHz) with simultaneous decoupling of ^{31}P and hydrogens have been recorded as well. The ^{2}H-NMR spectrum of a mixture of **8b** (93%) and **8c** (7%) shows two broad singlets at δ = 1.82 and 1.75 for the two diastereomeric amides obtained from racemic [1-$^2H_1$]AEP, the spectrum of **8d** reveals a singlet for the derivative of the (*S*) enantiomer of [1-$^2H_1$]AEP and a shoulder at higher field for the (*R*) enantiomer. The ^{2}H-NMR spectrum of **8a** of the biosynthetic sample shows only one resonance, and additionally with a shoulder at higher field after doping the NMR probe with derivative **8c**. The signal from sample **8a** corresponds to the derivative of (*S*)-[1-$^2H_1$]AEP. This result indicates that (*Z*)-[3-$^2H_1$]PEP is stereospecifically rearranged by attack from the *si* face into (*S*)-phosphono-[3-$^2H_1$]pyruvic acid [(*S*)-**7**] and further metabolized without affecting the stereochemistry at C-1 to (*S*)-[1-$^2H_1$]AEP.



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Second, D-[1-H_1]glucose is fed to *Streptomyces fradiae* as described in ref.[16]. The fosfomycin (10 μg cm^{-3}) formed is transformed into (1*R*,2*R*)-(2-amino-1-hydroxypropyl)phosphonic acid[16] (**9**) to yield 7 mg of crystalline material. The sample contains 15% deuterium as determined indirectly by ^{1}H-NMR spectroscopy. The proton noice-decoupled ^{13}C-NMR spectrum (100.6 MHz, D_2O) shows besides the signals for the unlabelled compound deuterium-induced satellite doublets for C-2 and C-3 which are in agreement with the values reported in ref.[17]. It has been demonstrated that the deuterium of (1*S*)-2-hydroxy-[1-$^2H_1$]ethylphosphonic acid[17] is retained on incorporation into fosfomycin via the putative intermediate (1*S*)-phosphono-[1-$^2H_1$]acetaldehyde. Consequently, phosphono-[3-$^2H_1$]pyruvate formed in *Streptomyces fradiae* from (*Z*)-[3-$^2H_1$]PEP must have (*S*) configuration, since decarboxylation leads to the corresponding aldehyde with the same absolute configuration.

It is tempting to conclude on the basis of the results reported above that the PEP mutase(s) catalyze(s) the stereospecific transfer of the phospho group of [3-$^2H_1$]PEP from oxygen to carbon from the *si* face (from the *re* face of PEP[18]). Experiments are under way to prepare (6*R*)- and (6*S*)-D-[6-$^2H_1$]glucose suitable as precursors for both (*E*)- and (*Z*)-[3-$^2H_1$]PEP for feeding experiments.

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Experimental

^{1}H-NMR spectra (for samples **8**: C_6D_6, 42 °C, concentration about 100 mg/ml; the resonances of 1-H are very concentration-dependent) were recorded at 400.1 MHz, ^{13}C-NMR spectra at 100.6 MHz, and ^{2}H-NMR spectra [C_6H_6 and C_6D_6 (10%), 42 °C, concentration about 100 mg/ml; δ relative to $\delta(C_6D_6)$ = 7.16; 12228 scans for sample **8a**] at 61.4 MHz with a Bruker AM 400 WB instrument.

D-[1-$^2H_1$]Glucose was prepared according to the procedure used for the reduction of D-glucono-δ-lactone with $NaBH_4$. Thus, 34.0 g (191 mmol) of lactone was added in one portion to a mixture of 200 ml of ice-cold water and 40 g of ice and dissolved by stirring for 20 s. A freshly prepared, ice-cold solution of 2.0 g (47.8 mmol) of NaB^2H_4 (98% ^{2}H) in 60 ml of water was added. Stirring was continued for 20 min, keeping the temp. by cooling at 0 °C. 2 ml of acetic acid was added. The reaction solution was passed through a column of Dowex 50W-X4, H^+ (100 ml), and the eluate was dropped directly onto a column of Dowex MWA 1 (200 ml, washed with 2 N NaOH and water until neutral). 1 l of water was used for elution. The combined eluates were concentrated in a rotary evaporator at a water bath temp. of 40 °C. Three times 200 ml of methanol was added to the residue and evaporated. The residue was dissolved in methanol, filtered, concentrated, dissolved in 40 ml of hot methanol, and allowed to cool after adding a seeding crystal of D-glucose. The crystals were collected. The mother liquor was concentrated and treated as before to give a second crop; yield 28.2 g (82%) of D-[1-$^2H_1$]glucose, m.p. 143–150 °C (mixture of α and β anomer) [ref.[20]: m.p. 146–147 °C (for α anomer)], $[\alpha]_D^{20}$ = +52.45 (*c* = 4 in water, equilibrium mixture) {ref.[20]: $[\alpha]_D^{23}$ = +52.65 (equilibrium mixture)}. Its ^{1}H-NMR spectrum (400 MHz, D_2O) was identical with that of the unlabelled material[21] except for the changes caused by replacing hydrogen by deuterium at C-1.

Feeding D-[1-$^2H_1$]Glucose to Tetrahymena thermophila and Isolation of AEP[13]: Six 1000-ml Erlenmeyer flasks each containing 250 ml of medium with 0.8% of D-[1-$^2H_1$]glucose were inoculated and cultured with shaking for 48 h at 37 °C to give 80 g of wet cell mass. Part of the crude AEP (30 mg) isolated was crystallized from water/ethanol; m.p. 269–274 °C (ref.[22] m.p. from 250 °C to 296–299 °C) for determination of deuterium by ^{1}H-NMR spectroscopy. All the AEP isolated was transformed into 59 mg of **8a**[14].

Feeding D-[1-$^2H_1$]glucose to Streptomyces fradiae and Isolation of Aminophosphonic Acid **9** *as Described in Ref.*[16]: The bacteria were grown in six 1000-ml Erlenmeyer flasks each containing 220 ml of medium, 1.4 g of corn starch, and 3.0 g of D-[1-$^2H_1$]glucose. The aminophosphonic acid **9** was isolated by four-fold ion-exchange chromatography (Dowex 50, H^+; Dowex 50; H^+;

Dowex 1, AcO^-; Dowex 50, H^+, 1 N HCO_2H) and crystallized; yield: 7 mg.

[1] Part IX: F. Hammerschmidt, *Liebigs Ann. Chem.* **1992**, 553.
[2] M. Horiguchi, M. Kandatsu, *Nature (London)* **1959**, *184*, 901.
[3] For a review see: T. Hori, M. Horiguchi, A. Hayashi, *Biochemistry of Natural C-P Compounds*; Maruzen: Kyoto Branch Publishing Service, **1984**.
[4] K. W. Shimotohno, M. Seto, N. Otake, *J. Antibiot.* **1988**, *41*, 1057, and references cited therein.
[5] W. A. Warren, *Biochim. Biophys. Acta* **1968**, *156*, 340.
[6] E. Bowman, M. McQueney, R. J. Barry, D. Dunaway-Mariano, *J. Am. Chem. Soc.* **1988**, *110*, 5575; H. M. Seidel, S. Freeman, H. Seto, J. R. Knowles, *Nature (London)* **1988**, *335*, 457.
[7] T. Hidaka, M. Mori, S. Imai, O. Hara, K. Nagaoka, H. Seto, *J. Antibiot.* **1989**, *42*, 491.
[8] H. M. Seidel, S. Freeman, C. H. Schwalbe, J. R. Knowles, *J. Am. Chem. Soc.* **1990**, *112*, 8149.
[9] M. S. McQueney, S. Lee, W. H. Swartz, H. L. Ammon, P. S. Mariano, D. Dunaway-Mariano, *J. Org. Chem.* **1991**, *56*, 7121.
[10] M. Urquiza, N. N. Lichtin, *Tappi* **1961**, *44*, 221; A. Hajos, *Methoden Org. Chem. (Houben-Weyl), 4th Ed.* **1981**, *IV/1d*, 222.
[11] I. A. Rose, E. L. O'Connell, *Biochim. Biophy. Acta* **1960**, *42*, 159.
[12] M. Cohn, J. E. Pearson, E. L. O'Connell, I. A. Rose, *J. Am. Chem. Soc.* **1970**, *92*, 4095.
[13] F. Hammerschmidt, *Liebigs Ann. Chem.* **1988**, 531.
[14] F. Hammerschmidt, *Liebigs Ann. Chem.* **1988**, 955.
[15] P. E. Hansen, *Annu. Rep. NMR Spectrosc.* **1983**, *15*, 105.
[16] F. Hammerschmidt, G. Bovermann, K. Bayer, *Liebigs Ann. Chem.* **1990**, 1055.
[17] F. Hammerschmidt, H. Kählig, *J. Org. Chem.* **1991**, *56*, 2364.
[18] The change from *si* to *re* is a consequence of the nomenclature for faces of trigonal carbons as proposed by Hanson[19], caused by replacing ^2H by H at C-3 of enol-[3-$^2H_1$]pyruvate.
[19] K. R. Hanson, *J. Am. Chem. Soc.* **1966**, *88*, 2731.
[20] R. Bentley, D. S. Bhate, *J. Biolog. Chem.* **1960**, *235*, 1225.
[21] K. Kakinuma, *Tetrahedron* **1984**, *40*, 2089.
[22] A. F. Isbell, J. P. Berry, L. W. Tansey, *J. Org. Chem.* **1972**, *37*, 4399.

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CAS Registry Numbers

1: 138-08-9 / **2**: 5824-58-8 / AEP: 2041-14-7 / fosfomycin: 23155-02-4 / phosphoenolpyruvate mutase: 115756-49-5