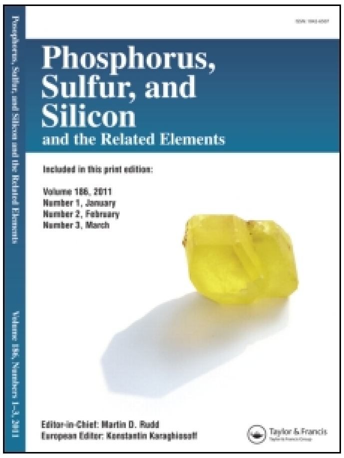

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BIOSYNTHESIS OF NATURAL PRODUCTS WITH A P-C BOND: INCORPORATION OF *D*-[1-$^2H_1$]GLUCOSE INTO 2-AMINOETHYLPHOSPHONIC ACID IN *TETRAHYMENA THERMOPHILA* AND OF *D*-[1-$^2H_1$]GLUCOSE AND *L*-[METHYL-$^2H_3$]METHIONINE INTO FOSFOMYCIN IN *STREPTOMYCES FRADIAE*

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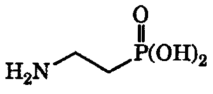
Abstract *D*-[1-$^2H_1$]Glucose was fed to *Tetrahymena thermophila* and to *Streptomyces fradiae* producing 2-aminoethylphosphonic acid and fosfomycin, respectively. The labelled products isolated indicate that the phosphoenol pyruvate mutase catalyzes the stereospecific transfer of the phospho group of (*Z*)-phosphoenol [3-$^2H_1$]pyruvate from oxygen to carbon from the *si*-face. The methyl group of *L*-[methyl-$^2H_3$]-methionine is transferred intact to a precursor for fosfomycin.

INTRODUCTION

2-Aminoethylphosphonic acid (AEP) **1** was the first natural product isolated to contain a P-C bond. The number of known compounds with a P-C bond is steadily increasing.[1] The most important representatives are fosfomycin **2** used clinically as antibiotic and phosphinothricin **3** used as herbicide. The biosynthesis of these compounds is only partly known.



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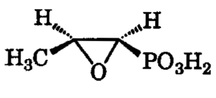

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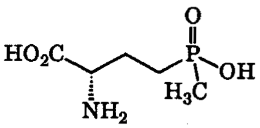

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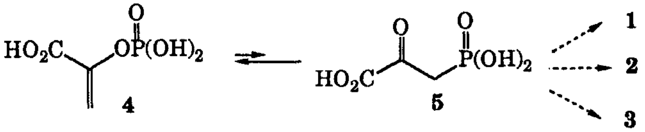
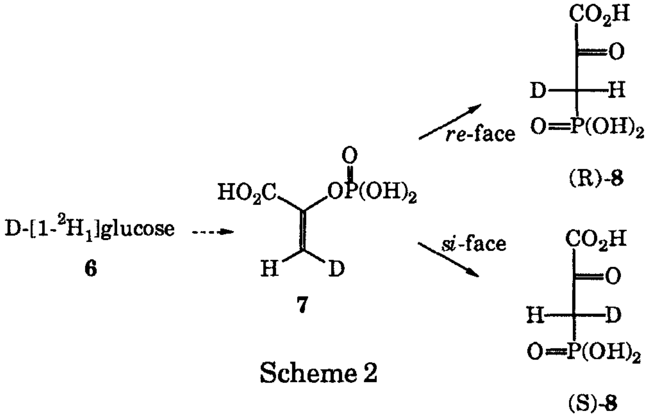
The P-C bond is biosynthetically formed by rearrangement of phosphoenol pyruvate (PEP) **4** to phosphonopyruvate **5**, catalyzed by the PEP mutase (Scheme 1).[2,3] It was demonstrated that the transfer of the phospho group from oxygen to carbon of enol pyruvate is an intramolecular process[4] occuring with overall retention[5] of configuration at phosphorus. Phosphonopyruvate is transformed into AEP and fosfomycin. To probe the stereochemistry at carbon, that is whether the phospho group is transferred to C-3 of enol pyruvate from the *re*- or from the *si*-face of the methylene group, the easily available *D*-[1-$^2H_1$]glucose was used as precursor in feeding experiments.



Scheme 1

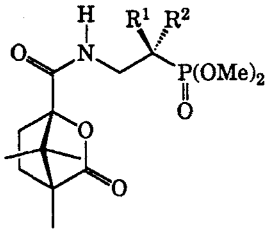
INCORPORATION OF *D*-[1-$^2H_1$]GLUCOSE INTO AEP IN *TETRAHYMENA THERMOPHILA*

D-[1-$^2H_1$]glucose **6** is taken up by the microorganisms and transformed[6,7] via the enzymes of the glycolytic pathway into unlabelled PEP **4** and (*Z*)-[3-$^2H_1$]PEP **7**, which is isomerised to (*R*)- or (*S*)-**8** (Scheme 2). First,



Scheme 2

D-[1-$^2H_1$]glucose was added to the growth medium for *Tetrahymena* *thermophila*. The AEP formed was isolated.[8] It was shown indirectly by ^{1}H NMR spectroscopy to contain 18 % monodeuterated spezies at C-1. The AEP was then derivatized with (-)-camphanoyl chloride and esterified with diazomethane to give the amide **9a** .[9] Reference samples **9b**, **9c**, and **9d** of unlabelled AEP, of racemic [1-$^{2}H_{1}$]AEP, and of (S)-[1-$^{2}H_{1}$]AEP[9] with an enatiomeric excess of 76%, respectively, were



9a AEP isolated
9b $R^1 = R^2 = H$
9c $R^1 = H, R^2 = {}^{2}H$ and
$R^1 = {}^{2}H, R^2 = H$
9d $R^1 = H, R^2 = {}^{2}H$, d.e. = 76%

obtained similarily. The ^{2}H NMR spectra (61.4 MHz, C_6H_6/C_6D_6, decoupling ^{31}P and ^{1}H) of compounds **9** are given in Figure 1. The amide

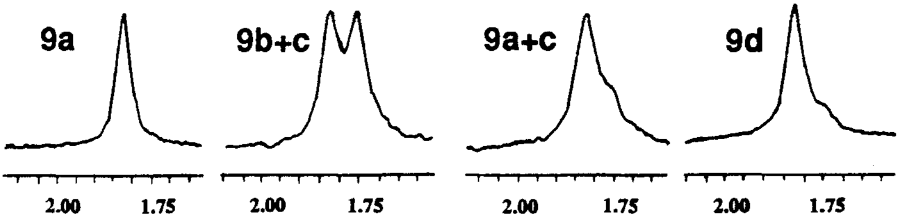
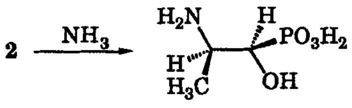


Figure 1

9a of the biosynthetic sample is labelled at only one site of the two prochiral positions at C-1 of AEP and has (*S*)-configuration. This result is indicating that (*Z*)-[3-$^{2}H_{1}$]PEP is stereospecifically rearranged by attack from the *si*-face into (*S*)-phosphono-[3-$^{2}H_{1}$]pyruvate and further metabolized to (*S*)-[1-$^{2}H_{1}$]AEP without affecting the stereochemistry at C-1.

INCORPORATION OF *D*-[1-$^{2}H_{1}$]GLUCOSE INTO FOSFOMYCIN IN *STRETPOMYCES FRADIAE*

D-[1-$^{2}H_{1}$]glucose was also fed to *Streptomyces fradiae*.[10] The fosfomycin formed was transformed with ammonia at 60 ^{0}C into the 2-aminopropylphosphonic acid **10** and isolated by ion exchange chromatography.



10

This sample contains 15 % deuterium at C-1 (^{1}H NMR). The ^{13}C NMR spectrum (100.6 MHz) shows beside the signals for the unlabelled compound deuterium induced satellite doublets for C-2 and C-3. From the proposed biosynthesis of fosfomycin and the presence of deuterium it is concluded that the phosphono-[3-$^2H_1$]pyruvate formed in *Streptomyces fradiae* must have (*S*)-configuration.

On the basis of the above results it is concluded 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.

L-[methyl-$^2H_3$]methionine was fed to *Streptomyces fradiae*. The aminophosphonic acid **10** isolated was a mixture of triply deuterated (at C-3, 81%) and nondeuterated material (19 %).

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