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Enzymatic Synthesis of a Ring-Contracted Analogue of 5-Enolpyruvylshikimate-3-phosphate

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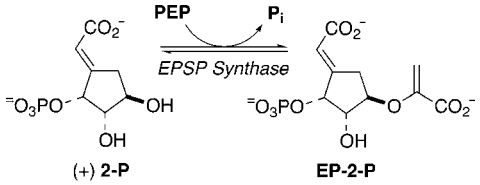
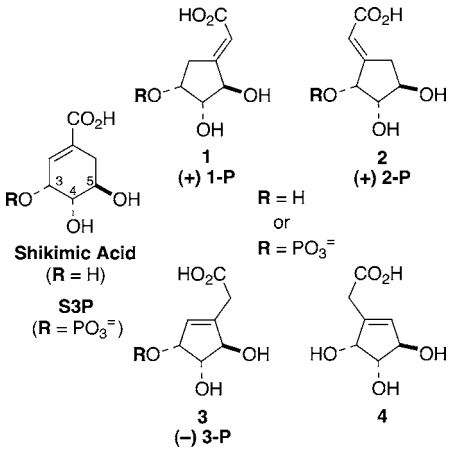
ABSTRACT



Three ring-contracted mimics of shikimate-3-phosphate, formed from the triols by shikimate kinase, were evaluated as substrates of the next enzyme in the pathway, EPSP synthase. The cyclopentylidene analogue (+)-2P was converted enzymatically to the enolpyruvyl derivative, thus demonstrating the second step of an artificial biosynthetic sequence.

The shikimate-chorismate pathway constitutes the gateway through which the biosynthesis of nearly all aromatic compounds in nature must pass,[1] ultimately leading to 35% or more of dry plant matter.[2] The enzymes in this pathway are unique to plants and microorganisms and thus are important targets for herbicide and antibiotic development.[3] However, the development of substrate analogues and inhibitors of these enzymes is challenging because their substrates are small, densely functionalized molecules that seem to offer little opportunity for structural variation. We were thus intrigued by the possibility that isomeric templates with a similar three-dimensional display of functionality could be devised as alternative substrates or inhibitors for the shikimate pathway enzymes.

We reported previously the synthesis and evaluation of the five-membered ring compounds **1**−**4** as novel ring-contracted analogues of shikimate.[4] Molecular modeling suggested that the scaffold represented by the cyclopentylideneacetate isomers **1** and **2** holds the carboxy and three hydroxyl groups in a relationship similar to that of shikimate itself. Indeed, shikimate kinase (E.C.2.7.1.71) phosphorylates cyclopentylidenes **1** and **2**, as well as cyclopentene **3**, to afford the five-membered ring analogues of shikimate-3-phosphate (S3P): **1P**, **2P**, and **3P**.



In view of the fact that three of the four ring-contracted shikimate analogues proved to be alternative substrates for

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(1) Haslam, E. *Shikimic Acid−Metabolism and Metabolites*; Wiley: New York, 1993.

(2) Boudet, A. M.; Graziana, A.; Ranjeva. R. In *The Biochemistry of Plant Phenolics*; Van Sumera, C. F., Lea, P. J., Eds.; Clarendon Press: Oxford, U.K., 1985.

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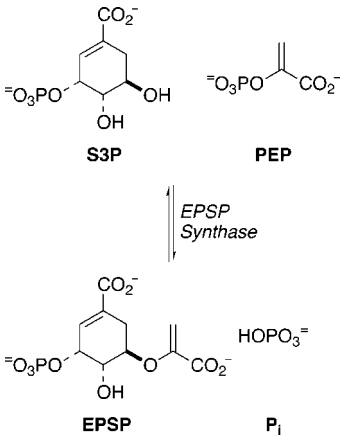
shikimate kinase, we were naturally curious to see if the phosphorylated products **1P**−**3P** can serve as alternative substrates for the subsequent enzyme in the pathway, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (E.C.2.5.1.19). EPSP synthase catalyzes the transfer of a carboxyvinyl group from phosphoenolpyruvate (PEP) to the 5-OH group of S3P (Scheme 1).[5] Such unusual chemistry and the fact that it is

Scheme 1. EPSP Synthase Catalyzes the Transfer of a Carboxyvinyl Group from **PEP** to the 5-OH Group of **S3P**



the target of action for the commercially important herbicide glyphosate[6] have made EPSP synthase a long-standing subject of mechanistic investigations[5,7] and inhibitor design.[3a,b]

When the cyclopentylidene-phosphate derivative **2P** (17 μmol) was treated with PEP (30 μmol) and *E. coli* EPSP synthase (1 unit) in deuterated water, the carboxyvinyl transfer reaction proceeded with a half-life of approximately 28 h to give the ring-contracted EPSP analogue **EP-2-P** as the product (Figure 1).[8] This reaction is ca. 50 times slower than the conversion of S3P to EPSP under similar conditions. However, no equivalent reactions were observed for cyclopentylidene-phosphate **1P** or cyclopentene-phosphate **3P** after 30 days of incubation under similar conditions.

Although only one of the S3P analogues appears to be a substrate for EPSP synthase, all of them catalyze the H/D exchange between methylene protons of PEP and D_2O in

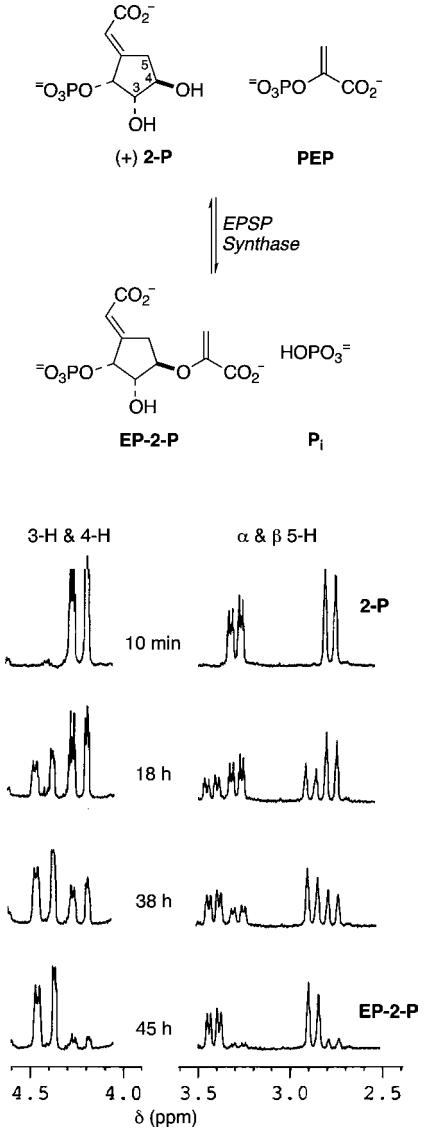


Figure 1. EPSP synthase catalyzes the transfer of a carboxyvinyl group from PEP to the 4-OH group of cyclopentylidene-phosphate **2P** to afford the ring-contracted EPSP analogue **EP-2-P**. The progress of the reaction is readily observed in the ^{1}H NMR spectrum.

the presence of the enzyme. Exchange occurs fastest with the alternative substrate **2P** and slowest with the cyclopentene-phosphate **3P**.[9] In this regard, ring-contracted analogues **1P** and **3P** are similar to 4,5-dideoxy-S3P, which although incapable of reacting with PEP, also catalyzes exchange of the methylene protons.[10] The proton exchange induced by 4,5-dideoxy-S3P was first interpreted as evidence for a protonated PEP−enzyme complex and against direct formation of the tetrahedral S3P−PEP adduct.[11] The unequivocal establishment of the latter mechanism[7] requires another interpretation for the observation that nonreacting substrate analogues can enable the enzyme-catalyzed proton exchange. The catalytic site of EPSP synthase is only assembled as a result of a significant conformational change induced by the binding of S3P.[12] Appropriate positioning of

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(4) An, M.; Toochinda, T.; Bartlett, P. A. *J. Org. Chem.* **2001**, *66*, 1326−1333.

(5) Bondinell, W. E.; Vnek, J.; Knowles, P. F.; Sprecher, M.; Sprinson, D. B. *J. Biol. Chem.* **1971**, *246*, 6191−6196.

(6) (a) Steinrucken, H. C.; Amrhein, N. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207−1212. (b) Steinrucken, H. C.; Amrhein, N. *Eur. J. Biochem.* **1984**, *143*, 351−357.

(7) (a) Anderson, K. S.; Johnson, K. A. *Chem. Rev.* **1990**, *90*, 1131−1149. (b) Mizyed, S.; Wright, J. E. I.; Byczynski, B.; Berti, P. J. *Biochemistry* **2003**, *42*, 6986−6995. (c) Eschenburg, S.; Kabsch, W.; Healy, M. L.; Schonbrunn, E. *J. Biol. Chem.* **2003**, *278*, 49215−49222. (d) An, M.; Maitra, U.; Neidlein, U.; Bartlett, P. A. *J. Am. Chem. Soc.* **2003**, *125*, 12759−12767.

the active site residues may be all that is required to enable reversible protonation and proton exchange of PEP. The unreactive ring-contracted analogues **1P** and **3P** may simply share with 4,5-dideoxy-S3P an ability to induce this conformational change.

The five-membered ring analogues of shikimate, S3P, and EPSP represent the first examples of alternative substrates for the shikimate-chorismate pathway in which the ring size has been altered.[13] Their activities toward shikimate kinase and EPSP synthase establish a two-step unnatural "biosynthetic" sequence in which contraction from a six- to a five-membered ring is tolerated. The next step in the pathway, *anti*-1,4-elimination of phosphate from EPSP catalyzed by chorismate synthase, is not possible starting with the alternative framework of **EP-2-P**. It will be interesting to see if this analogue binds as a competitive inhibitor or if other, more novel transformations result.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of **EP-2-P**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) A solution of 6 mg of phosphate **(+)-2-P** (0.017 mmol) and 6 mg of PEP (0.03 mmol, 1.76 equiv) was incubated at room temperature with 1 unit of *E. coli* EPSP synthase in 1 mL of deuterated $NaHCO_3/Na_2CO_3$ buffer (100 mM, pH = 9.6). Another 0.8 unit of EPSP synthase was added after 25 h, and after an additional 4 d, the mixture was subjected to anion exchange chromatography (DEAE Sephadex A-25, 0 → 1 M $NEt_3H^+HCO_3^-$ buffer, pH = 8.5). After lyophilization and ^{1}H NMR analysis, product-containing fractions were pooled to give **EP-2-P** (3 mg, 55% isolated yield) as the bis-triethylammonium disodium salt. NMR chemical shifts are referenced as follows: ^{1}H, H_2O (4.80 ppm); ^{31}P, internal trimethyl phosphate (3.086 ppm). ^{1}H NMR (D_2O) δ 1.28 (t, 18, J = 7.5) (Et_3NH^+), 2.87 (dm, 1, J = 20), 3.20 (q, 12, J = 7.5) (Et_3NH^+), 3.40 (ddm, 1, J = 20, 6.3), 4.37 (dm, 1, J = 4.5), 4.48 (dm, 1, J = 7.0), 4.68−4.69 (m, 0.3), 5.13 (ddm, 1, J = 10, 4.2), 5.26−5.29 (m, 0.3), 6.08 (m, 1) (Note: low integration for the $=CH_2$ hydrogens reflects H/D exchange during the enzymatic conversion). ^{13}C NMR (D_2O) δ 8.1 (Et_3NH^+), 32.6, 46.6 (Et_3NH^+), 72.7, 77.6 (d), 78.3, 119.3, 153.4, 153.5 (d), 154.2 (d), 170.4, 173.0. ^{31}P NMR (D_2O) δ 0.89.

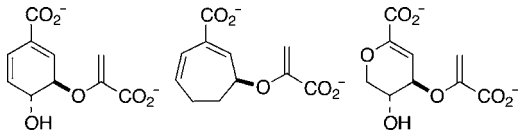
(9) With excess PEP and S3P analogue concentrations of 10−15 mM, the rates of exchange in μmol/h/unit enzyme were approximately 1 (**2P**), 0.3 (**1P**), and 0.15 (**3P**).

(10) Anton, D. L.; Hedstrom, L.; Fish, S. M.; Abeles, R. H. *Biochemistry* **1983**, *22*, 5903−5908.

(11) The distinction Anton et al.[10] made between direct formation of the tetrahedral adduct versus a stepwise process involving a protonated PEP intermediate is subtle, in essence hinging on whether the enzyme provides a negatively charged group capable of stabilizing the cation.

(12) (a) Stallings, W. C.; Abdel-Meguid, S. S.; Lim, L. W.; Shieh, H. S.; Dayringer, H. E.; Leimgruber, N. K.; Stegeman, R. A.; Anderson, K. S.; Sikorski, J. A.; Padgette, S. R.; Kishore, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5046−5050. (b) Schönbrunn, E.; Eschenburg, S.; Shuttleworth, W. A.; Schloss, J. A.; Amrhein, N.; Evans, J. N.; Kabsch, W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1376−1380.

(13) We are aware of only two examples in which the nature of the ring itself has been altered. (a) Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1988**, *53*, 4063−4069. The ring-expanded cycloheptadiene **5** was synthesized as an analogue of chorismate; however, **5** is not processed by chorismate mutase, anthranilate synthase, or *p*-aminobenzoate synthase. (b) Delany, J. J., III; Padykula, R. E.; Berchtold, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 1394−1397. In this case, the cyclohexadiene ring of chorismate was replaced with the dihydropyran of **6**. Although less reactive than chorismate, the Claisen rearrangement of **6** is accelerated by chorismate mutase by almost the same factor as the natural substrate.



Chorismate **5** **6**