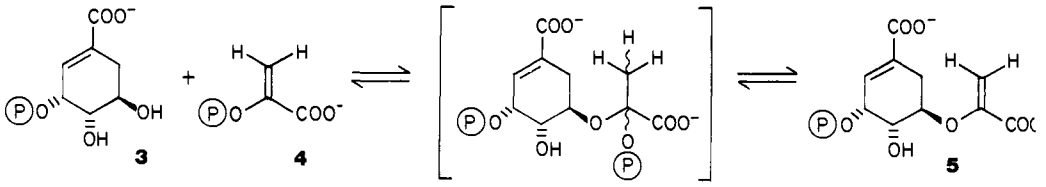
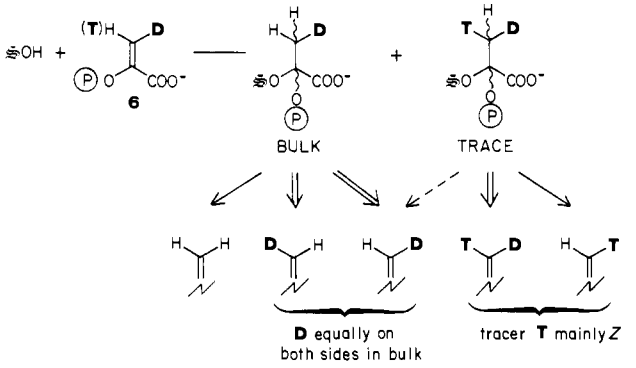
Scheme I. Probable Mechanism of the Reaction Catalyzed by 5-Enolpyruvoylshikimate-3-phosphate Synthetase

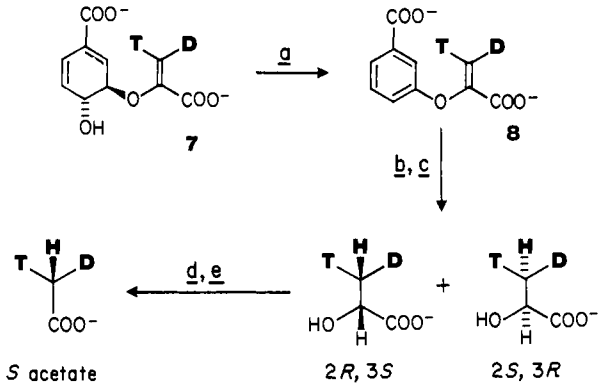


Scheme II. Predicted Fate of the Stoichiometric Deuterium and Tracer Tritium Labels in the Enzyme-Catalyzed Condensation of Phosphoenolpyruvate with Shikimate 3-Phosphate[a]



[a] It is assumed for illustration only that the addition step is anti and the elimination step is syn; ⬬OH is the shikimic acid 5-hydroxyl function.

Scheme III. Stereoanalytical Scheme for Determining if the Tritium Label in [9-^{2}H,^{3}H]Chorismate is *E* or *Z*[a]



[a] The products from (*Z*)-chorismate (**7**) are illustrated. (a) Dimethyl sulfoxide, room temperature, 12 h; (b) Wilkinson's catalyst/H_2; (c) Birch reduction; (d) (*S*)-lactate dehydrogenase/3-acetylpyridine adenine dinucleotide; (e) Kuhn-Roth oxidation.

methyl-d_6 sulfoxide and left at room temperature for 12-24 h, until the ^{1}H NMR showed the presence only of the aryl enol ether **8**.[15] This material was then reduced with cis stereochemistry using Wilkinson's catalyst[17] to yield the two diastereoisomeric aryl lactyl ethers. Birch reduction[18] afforded (*R,S*)-lactate in which the configuration at carbon 2 is related to that at carbon 3 (Scheme III). The (*S*)-lactate was removed by oxidation with (*S*)-lactate dehydrogenase[19] and the remaining (*R*)-lactate subjected to Kuhn-Roth oxidation[20] to yield chiral [^{2}H,^{3}H]acetic acid. The configuration of the acetic acid was then analyzed.[10] It was found that when (*Z*)-phospho[3-^{2}H,^{3}H]enolpyruvate was the substrate in the reaction with shikimate-3-phosphate (**3**) to make **5**, the acetate configuration after conversion to chorismate and stereoanalysis as outlined in Scheme III was *S* ("*F*" value[11] 0.39). When (*E*)-phospho[3-^{2}H,^{3}H]enolpyruvate was the substrate, the final acetate was *R* ("*F*" value[11] 0.62).[21] These data show that (*Z*)-phosphoenolpyruvate results in (*Z*)-chorismate (the isotopic labels in the enolpyruvoyl moiety remain "in place"), which requires that in the 5-enolpyruvoylshikimate-3-phosphate synthetase reaction, the addition step (Scheme I) has an opposite stereochemical course from the elimination step. If the addition is anti the elimination is syn, or conversely. The implications of this finding in terms of the mechanism of the synthetase reaction and its evident preference for a minimal motion pathway will be discussed elsewhere.

Acknowledgment. We are grateful to S. Benner and J. D. Rozzell for the malate synthetase required in the chiral methyl analysis, to D. Arigoni and G. Berchtold for helpful discussions, to John Frost, Daniel Brenner, and Jean-Paul Lavalleye for assistance with the reduction step, and to the National Institutes of Health and Merck Sharp and Dohme for support.

Chemical Synthesis of Stereoselectively Labeled [9-^{2}H,^{3}H]Chorismate

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Chorismate (**1**) is the last common intermediate in the biosynthesis of aromatic substances through the shikimate pathway in bacteria, fungi, and higher plants.[1] Of the enzyme-catalyzed reactions available to chorismate, that which has received the most attention is the [3,3]-sigmatropic rearrangement to prephenate (**2**). This transformation, catalyzed by chorismate mutase, is cited

1 **2**

(15) The usual method of aromatization using acetic anhydride/pyridine[5] was found, using a stereospecifically deuterated sample of [9-^{2}H]-chorismate,[16] to result in the complete stereochemical randomization of the isotopic label at carbon 9. This problem is happily avoided by the simple method reported here.

(16) Hoare, J. H.; Berchtold, G. A. *J. Am. Chem. Soc.*, following paper in this issue.

(17) In methanol/benzene (50:50, v/v), H_2 at 4 atm, room temperature, 15 h. Yield ~80%. The product was purified by ion-exchange chromatography.

(18) Lithium in ammonia, followed by mild acid hydrolysis. Yield 75-80%.

(19) Using 3-acetylpyridine adenine dinucleotide as cofactor at pH 9.5, to drive the reaction in the direction of lactate oxidation. The (*R*)-lactate was purified by ion-exchange chromatography.

(20) Control experiments using an unlabeled sample of lactic acid in D_2O showed that the product acetate was <10% d_1.

(21) It should be noted that these *F* values[11] are close to those predicted. On the basis of a deuterium isotope effect of 2 (at pH 7.4) in the 5-enolpyruvoylshikimate-3-phosphate synthetase reaction, we may predict that one-third of the tritium label will be in the "wrong" position in the product, compared with the phosphoenolpyruvate substrate. From the *F* values[11] for the substrate,[7,22] the predicted *F* values of the acetate samples deriving from chorismate are 0.37 and 0.61. The observed values of 0.39 and 0.62 show that little if any loss of stereochemical integrity has occurred in the preparation or stereochemical analysis of the doubly labeled chorismate.

(22) Though these are probably minimum values.[9]

(1) For detailed reviews, see: (a) Weiss, U.; Edwards, J. M. "The Biosynthesis of Aromatic Compounds"; Wiley: New York, 1980. (b) Haslam, E. "The Shikimate Pathway"; Halstead Press, Wiley: New York, 1974. (c) Ganem, B. *Tetrahedron* **1978**, *34*, 3353-3383.

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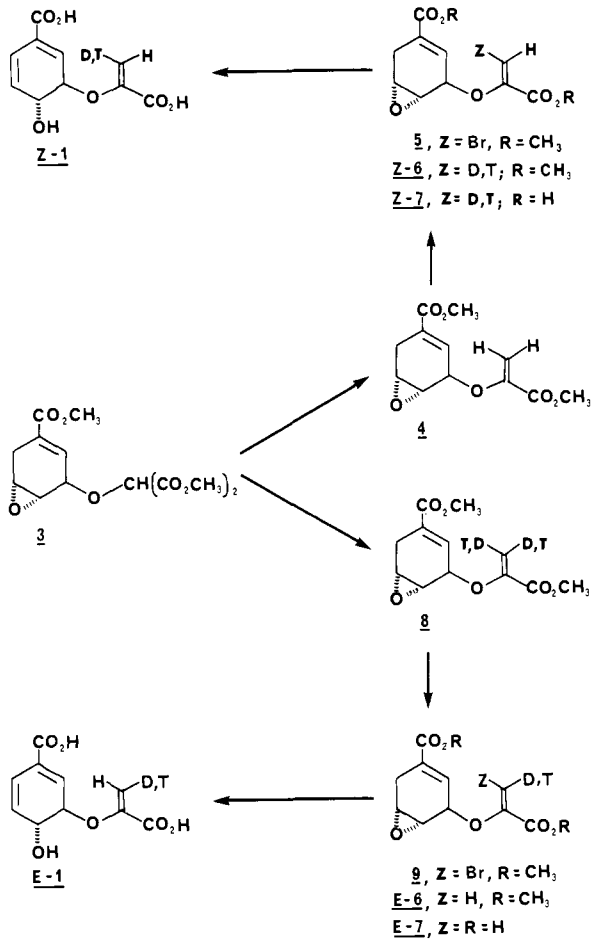
Scheme I



as the only established enzyme-catalyzed pericyclic reaction in primary metabolism.[2] In view of the rarity of [3,3]-sigmatropic rearrangements in primary metabolism, whether the enzyme-catalyzed Claisen rearrangement of **1** to **2** proceeds by a chair- or boat-like transition state is of considerable interest.

In order to establish the transition-state geometry of the enzyme-catalyzed rearrangement of **1** of **2**, it is necessary to have selective hydrogen isotope labeling of the *E* and *Z* hydrogen atoms at C-9 of **1**. Described below is our stereoselective synthesis of racemic (*E*)- and (*Z*)-[9-^{2}H,^{3}H]chorismic acid.[3]

Malonate **3** (Scheme I) was converted to **4** as described previously.[5] Bromination of **4** (Br_2, CH_2Cl_2) followed by elimination of HBr (DBN,CH_2Cl_2) gave only *Z* bromo derivative **5** (73% yield).[6] Reductive cleavage of **5** with Zn/Ag couple in THF/D_2O doped with TOH resulted in stereoselective formation of (*Z*)-**6** (93% yield; 90% (*Z*)-**6**/10% (*E*)-**6**).[7,8] The product contains ~5% unlabeled material (**4**) due to H_2O adsorbed on the catalyst. Saponification of (*Z*)-**6** (NaOH, THF/H_2O) and acidification provided the diacid ((*Z*)-**7**) with no change in distribution of the D,T label. Oxirane ring opening at C-5 with $PhSe^-$ in $CH_3OH/H_2O/NaHCO_3$[9] and subsequent selenoxide elimination with 3,5-dimethoxyaniline as scavanger as described previously for the synthesis of **1**[5] provided stereoselectively labeled (*Z*)-**1** (75% (*Z*)-**1**/25% (*E*)-**1**) and 5% unlabeled **1**.[8,10]

In order to prepare **1** with the D,T label predominantly in the *E* C-9 position, malonate **3** was subjected to Mannich reaction with $(CH_3)_2NH$ and D_2CO doped with THCO. Quaternization of the Mannich product (CH_3I, CH_2Cl_2) followed by fragmentation (NaOH, THF/H_2O) provided **8** (23% yield from **3**). Bromination of **8** and elimination of DBr(TBr) gave **9** (22% yield).[11] Reductive cleavage of **9** (Zn/Ag, THF/H_2O) produced stereoselectively labeled (*E*)-**6** (94% yield; 94% (*E*)-**6**/6% (*Z*)-**6**).[8] Treatment of (*E*)-**6** as described above gave (*E*)-**7** and, subsequently, stereoselectively labeled (*E*)-**1** (90% (*E*)-**1**/10% (*Z*)-**1**).[10]

Acknowledgment. We are grateful to J. R. Knowles and T. S. Widlanski for helpful discussion and assistance with tritium-labeling experiments and to the National Institutes of Health, Grant GM 31958, for financial support.

(2) Rétey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag Chemie: Weinheim, 1982; p 47–48.

(3) The rearrangement of (+)-**1** (unnatural enantiomer) to **2** is not catalyzed by chorismate mutase–prephenate dehydrogenase from *E. coli*, and (+)-**1** does not inhibit the enzyme-catalyzed rearrangement of (−)-**1** to **2**.[4] Consequently, labeled (±)-**1** may be used in the enzymatic investigations.

(4) Hoare, J. H.; Berchtold, G. A. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 660–662.

(5) Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105*, 6264–6267.

(6) 250-MHz ^{1}H NMR: δ 7.13 (s, 1 H); 6.87 (m, 1 H); 3.84 (s, 3 H); 3.77 (s, 3 H); 3.49 (m, 2 H); 2.88 (AB q, 2 H, J_{AB} = 20 Hz).

(7) Modified procedure of: Fryzuk, M. D.; Bosnick, B. *J. Am. Chem. Soc.* **1979**, *101*, 3043–3049 and references cited therein.

(8) The ratio was established from the (9)-**E** and (9)-**Z** proton signals in the ^{1}H NMR spectrum.

(9) When the oxirane ring of (*Z*)-**6** is cleaved with $PhSe^-$, complete scrambling of D,T label at C-9 is observed. The scrambling presumably is due to reversible Michael addition of $PhSe^-$ at C-9 of the enolpyruvate ester.

(10) We believe that the scrambling of label that occurs during (*Z*)-**7** → (*Z*)-**1** was due to the presence of unreacted 3,5-dimethoxyaniline, which underwent some reversible Michael addition during workup of the selenoxide elimination reaction. This situation was improved in the preparation of (*E*)-**1**, and the scrambling was not observed.

(11) The rate of the elimination reaction shows a significant isotope effect, and 50% of the T label is lost in the elimination reaction. The absence of an isotope discrimination in the elimination reaction suggests that the *Z* bromide is the sole product; i.e., there is no formation of *E* bromide followed by isomerization to *Z* bromide.

Stereochemistry of the Rearrangement of Chorismate to Prephenate: Chorismate Mutase Involves a Chair Transition State

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The Claisen rearrangement of chorismate (**1**) is catalyzed in bacteria and in plants by the enzyme chorismate mutase and provides the common percursor prephenate (**2**) for the essential amino acids tyrosine and phenylalanine.[1] While it is believed that in unrestricted nonenzymic systems the chair transition state for such rearrangements is favored over the boat by at least 5 kcal/mol[2] and although it has been calculated that for the chorismate–prephenate transformation a chair transition state should be favored by about 2 kcal/mol,[3,4] these energies are obviously too small to be decisive for an enzymic process the stereochemical course of which could be dominated by catalytic imperatives of much larger free energy. We have therefore de-

[†] Harvard University.
[‡] Massachusetts Institute of Technology.

(1) Haslam, E. "The Shikimate Pathway"; Wiley: New York, 1974.

(2) Doering, W. v. E.; Roth, W. R. *Tetrahedron* **1962**, *18*, 67. Brown, A.; Dewar, M. J. S.; Schoeller, W. W. *J. Am. Chem. Soc.* **1970**, *92*, 5516. Dewar, M. J. S.; Ford, G. P.; McKee, M. L.; Rzepa, H. S.; Wade, L. E. *Ibid.* **1977**, *99*, 5069.

(3) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.

(4) Andrews, P. R.; Haddon, R. C. *Aust. J. Chem.* **1979**, *32*, 1921.

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