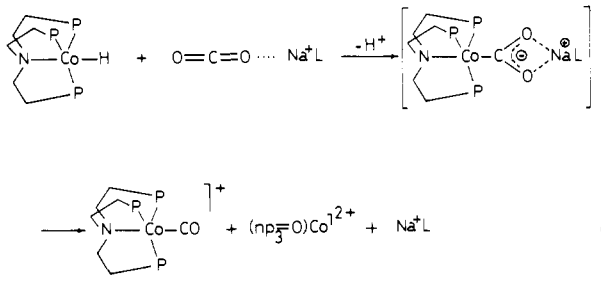

(2)

saturated by its coligands and the fragment has a free coordination site.[12] Nonetheless the initial approach of the metal fragment and CO_2 may be difficult and the reaction may be greatly facilitated if CO_2 is kept in place by a second function having ionic character.

Unusual features of the reactions reported in this paper are the mild conditions required to activate CO_2 and the good yields and rates. Furthermore the present experimental results suggest that a new and perhaps general strategy for CO_2 activation could be pursued in coordination and organometallic chemistry. So far, in fact, the major part of the chemical speculation has been focused mainly on basic systems, thus neglecting the role that acidic species could have. The acidic center in bifunctional systems could be indeed responsible of the initial promotion of CO_2. Its contribution to stabilize the eventual CO_2 adduct or to function as oxygen acceptor will depend then on the particular chemical system. A reconsideration in this light of many metal–CO_2 reactions could reveal how often the presence of Lewis acids in the reaction mixture has been misunderstood.

Current studies are under way to investigate the reactivity of CO_2 toward nucleophilic complexes in the presence of different types of acidic species.

Acknowledgment. Thanks are due to Dr. C. Mealli for helpful discussion.

(12) (a) For a comprehensive description and reference list of reactions of CO_2 with transition-metal complexes, see: Sneeden, R. P. A. "Comprehensive Organometallic Chemistry"; Wilkinson, G., Stone, F. G. A., Abel, E. W., Eds.; Pergamon Press: Oxford, 1982; Vol. 8, p 255. (b) Mealli, C.; Hoffmann, R.; Stockis, A. *Inorg. Chem.* **1984**, *23*, 56. Calabrese, J. C.; Herskovitz, T.; Kinney, J. B. *J. Am. Chem. Soc.* **1983**, *105*, 5914.

Synthesis of Stereoselectively Labeled [9-^{2}H,^{3}H]Chorismate and the Stereochemical Course of 5-Enolpyruvoylshikimate-3-phosphate Synthetase

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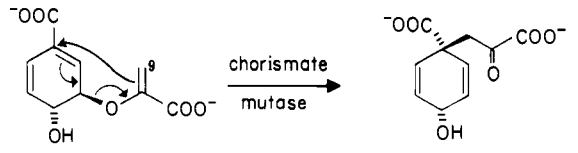
The 3,3-sigmatropic shift of chorismate (**1**) to prephenate (**2**)



chorismate **1** prephenate **2**

is perhaps the only example of a pericyclic reaction in primary metabolism. The nonenzymic reaction occurs smoothly at 60 °C in neutral aqueous solution, and the enzyme chorismate mutase effects a rate acceleration of (2×10^6)-fold at 37 °C.[2] One of the most basic questions concerning this enzyme-catalyzed Claisen rearrangement is whether the reaction involves a chair or a boat transition state, and to answer this question we required isotopically labeled chorismate in which the *E* and *Z* hydrogens at carbon 9 of **1** were stereochemically distinguished. We report here the synthesis of [9-^{2}H,^{3}H]chorismic acid in which the tritium label is stereoselectively located, and we report the independent stereochemical determination of the tritium position. This work not only yields labeled chorismate suitable for the evaluation of the stereochemical course of the chorismate mutase reaction[3] but also provides information about the stereochemical events in the enzymic reaction used to generate the labeled chorismate: that catalyzed by 5-enolpyruvoylshikimate-3-phosphate synthetase.

Specifically labeled chorismate was synthesized by the condensation of shikimate 3-phosphate (**3**) with specifically labeled phosphoenolypyruvate (**4**) catalyzed by 5-enolpyruvoylshikimate-3-phosphate synthetase. The accepted mechanism for this reaction[4–6] involves the addition-elimination sequence shown in Scheme I. Since carbon 3 of the enolpyruvate moiety transiently becomes a methyl group and methyl group rotation is fast with respect to the chemical steps leading to and from this intermediate, use of specifically monodeuterated **4** leads to a sample of 5-enolpyruvoylshikimate-3-phosphate (**5**) having deuterium equally at *both* the *E* and *Z* positions. We have shown, however, that the synthetase reaction is subject to a kinetic isotope effect in both addition and elimination steps,[6] so if a stereospecific *doubly labeled* sample of phospho[3-^{2}H,^{3}H]enolpyruvate (**6**) is used as substrate,[7] the kinetic isotope effect should result in preferential retention of the heavy isotopic labels. Since, however, tritium is used as a *trace* label whereas deuterium is used *stoichiometrically*, the product **5** that derives from **6** will contain (in the bulk) deuterium randomly in both *E* and *Z* positions yet (for those few molecules that contain tritium) tritium will be preferentially *E* or *Z*.[12] This consequence is illustrated in Scheme II.

To determine whether the tritium label at carbon 9 of chorismate (derived from the doubly labeled sample of **5**) is mainly *E* or *Z*, the stereoanalytical sequence shown in Scheme III was followed. Doubly labeled chorismic acid (prepared[13] from a stereospecific doubly labeled sample of **6**) was dissolved in di-

(1) National Institutes of Health Postdoctoral Fellow.

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

(3) Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* following in this issue.

(4) Levin, J. G.; Sprinson, D. B. *J. Biol. Chem.* **1964**, *239*, 1142.

(5) Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1776.

(6) Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596.

(7) The doubly labeled samples of phosphoenolpyruvate were made either from [1-^{3}H]glucose (using phosphoglucose isomerase–D_2O) or from [1-^{3}H]-mannose (using phosphomannose isomerase–D_2O), by modification of the method of Cohn et al.[8] The configuration and stereochemical integrity of these samples were confirmed by conversion to lactate using pyruvate kinase–ADP[9] plus lactate dehydrogenase–NADH. The resulting lactate samples were subjected to Kuhn–Roth oxidation to acetate, followed by chiral methyl analysis.[10] The *F* values[11] for the acetate samples so derived from (*Z*)- and from (*E*)-[3-^{2}H,^{3}H]phosphoenolpyruvate were 0.30 (sample from [1-^{3}H]-glucose) and 0.66 (sample from [1-^{3}H]mannose), respectively.

(8) Cohn, M.; Pearson, J. E.; O'Connell, E. L.; Rose, I. A. *J. Am. Chem. Soc.* **1970**, *92*, 4095.

(9) Rose, I. A. *J. Biol. Chem.* **1970**, *245*, 6052.

(10) Luthy, J.; Rétey, J.; Arigoni, D. *Nature (London)* **1969**, *221*, 1213. Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutschow, C. *Eur. J. Biochem.* **1970**, *14*, 1; *Nature (London)* **1969**, *221*, 1212.

(11) Arigoni, D., referred to in: Floss, H. G.; Tsai, M-D. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1979**, *50*, 243.

(12) The deuterium kinetic isotope effect in the synthetase reaction is about 2 (at pH 6.25) to nearly 3 (at pH 10), so the tritium in the enolpyruvoylshikimate phosphate will be located *E* (or *Z*) in 2:1–3:1 ratio.

(13) A partially purified preparation of 5-enolpyruvoylshikimate-3-phosphate synthetase[6] was used at pH 7.4. To avoid scrambling and loss of the isotopic labels[4–6] the reaction was stopped after <15% of the doubly labeled phospho[3-^{2}H,^{3}H]enolpyruvate had been converted into product. After purification by ion-exchange chromatography, the [9-^{2}H,^{3}H]-5-enolpyruvoylshikimate-3-phosphate was converted into [9-^{2}H,^{3}H]chorismate using chorismate synthetase.[14]

(14) Floss, H. G.; Onderka, D. K.; Carroll, M. *J. Biol. Chem.* **1972**, *247*, 736.

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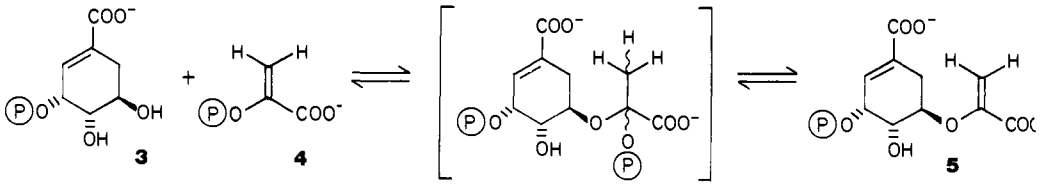
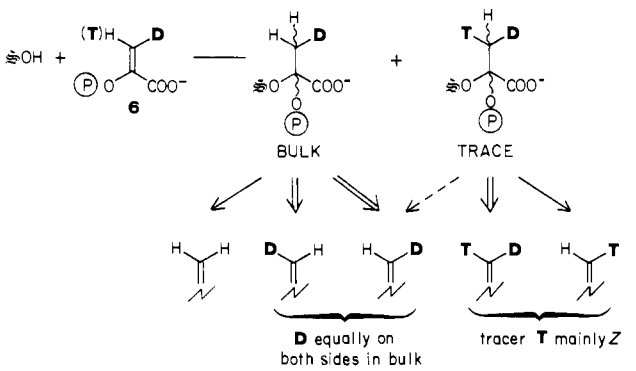
Scheme I. Probable Mechanism of the Reaction Catalyzed by 5-Enolpyruvoylshikimate-3-phosphate Synthetase

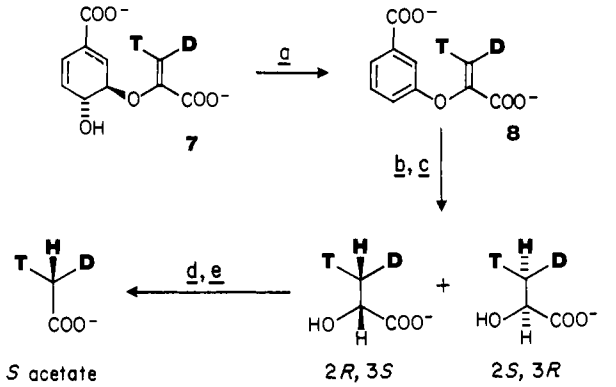


Scheme II. Predicted Fate of the Stoichiometric Deuterium and Tracer Tritium Labels in the Enzyme-Catalyzed Condensation of Phosphoenolpyruvate with Shikimate 3-Phosphate[a]



[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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