Implications for the Mechanism of the Enzyme-Catalyzed Reaction. The relatively small difference in the free energies of the two conformers of chorismate in neutral aqueous solution has implications for the mechanism of the enzyme-catalyzed reaction. As discussed in the introduction, the rearrangement requires a pseudo-diaxial orientation of ring substituents, and if the equilibrium proportion of diaxial conformer had been vanishingly small, we should have had to conclude that the enzyme would bind the available diequatorial conformer and then isomerize it to the diaxial form. Thus the observed value[28] of k_{cat}/K_m is 1.2×10^6 M^{-1} s^{-1} (based upon the total chorismate concentration), and if the equilibrium proportion of the pseudo-diaxial conformer had been less than about 0.1%, the k_{cat}/K_m for this conformer as substrate would have exceeded the acceptable ceiling for an encounter-controlled enzymic process of around 10^9 M^{-1} s^{-1}. This would then have required that the enzyme first recognize and bind the more abundant pseudo-diequatorial form. However, with the knowledge that the diaxial conformer exists at reasonable levels (10-20%) in solution and that conformer interconversion is fast, there is no need to postulate an enzyme-catalyzed conformational change: the enzyme can select the diaxial conformer directly. While these arguments are tinged with teleology, mechanistic economy strongly suggests that the rate-limiting transition state of the chorismate mutase reaction does *not* involve a conformer interconversion. These arguments are developed in the following paper.[12]

(28) We have found k_{cat} = 51 s^{-1} and K_m = 41 μM, with highly purified enzyme, in 50 mM *N*-ethylmorpholine-2-(*N*-ethylmorpholino)ethanesulfonic acid buffer, pH 7.6, containing EDTA (1 mM), dithioerythritol (1 mM), and bovine serum albumin (0.1 mg/mL), at 30 °C. These values can be compared with k_{cat} = 25 s^{-1} and K_m = 60 μm reported by Sampathkumar, P. Ph.D. Thesis, Australian National University, 1978.

Acknowledgment. We are grateful to Professor M. Karplus and Dr. A. Minsky for helpful discussions, to Professor Glenn Berchtold and Dr. R. Padykula for a generous gift of 4-*O*-methylchorismate, to perceptive referees, and to the National Institutes of Health and Merck Sharp and Dohme for support.

On the Mechanism of the Chorismate Mutase Reaction

William J. Guilford,† Shelley D. Copley, and Jeremy R. Knowles*

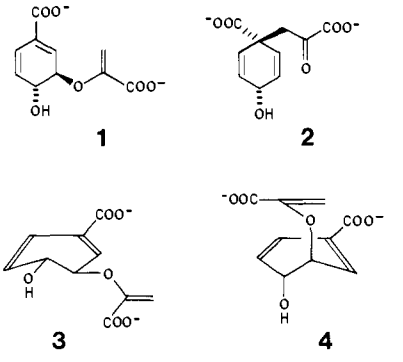
Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received November 28, 1986*

Abstract: To probe the mechanistic pathway of the reaction catalyzed by chorismate mutase, the secondary tritium isotope effect at C-4 of chorismate, k_H/k_T, has been determined. The small *inverse* effect of 0.96 rules out pathways that involve general acid catalysis at the C-4 hydroxyl group. This result, the existence of a significant (2.2-fold) solvent deuterium isotope effect (in the enzymic but *not* in the non-enzymic reaction), and all other results that bear upon this reaction are consistent with a pathway in which the rate-limiting heterolytic cleavage of the chorismate ether bond is assisted by attack of an enzymic nucleophile at C-5, to give an intermediate that collapses in an S_N2' process to yield prephenate.

The intramolecular rearrangement of chorismate (**1**) to prephenate (**2**) is catalyzed by the enzyme chorismate mutase and is a key step in the formation of tyrosine and phenylalanine via the shikimate pathway in bacteria, fungi, and higher plants.[1] This Claisen rearrangement appears to be the only example of a formal pericyclic reaction in primary metabolism. In contrast to most enzyme-catalyzed reactions, the non-enzymic rearrangement proceeds smoothly and can, therefore, be directly compared with the catalyzed reaction. Stereochemical studies have shown that the enzymic[2] and the non-enzymic[3] reaction each proceeds through a transition state of chair-like geometry. The reaction mechanism has been probed further through the use of secondary isotope effects. The non-enzymic rearrangement shows a secondary tritium isotope effect at C-5, the site of bond breaking, but none at C-9, the site of bond formation,[4] which suggests either a stepwise reaction or a very unsymmetrical transition state. However, no isotope effect at either site was observed for the enzyme-catalyzed rearrangement.[4] This result does not preclude similar pathways for the catalyzed and uncatalyzed processes, since the intrinsic isotope effects on the enzymic rearrangement could be masked by a rate-limiting transition state that precedes an isotopically sensitive rearrangement step.[4]

The activation parameters for the enzymic reaction have been determined and have been compared with those for the spontaneous thermal rearrangement. It was suggested that the enzymic rate acceleration of more than 10^6 could be the result of a decrease in the entropy of activation to near zero and a reduction in the enthalpy of activation by about 5 kcal/mol.[5] While deductions

Chart I



based on the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for reactions in structured solvents such as water are risky, it does appear that chorismate mutase is acting as more than just an entropy trap.[6]

There are (at least!) four ways in which chorismate mutase could conceivably effect the rate enhancement that is observed

† Present address: Sogetal, Inc., Hayward, CA 94545.

(1) Haslam, E. *The Shikimate Pathway*; Wiley: New York, 1974.
(2) Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2701.
(3) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 5306.
(4) Addadi, L.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494.
(5) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.
(6) Westheimer, F. H. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1962**, *24*, 441.

0002-7863/87/1509-5013$01.50/0

Chart II

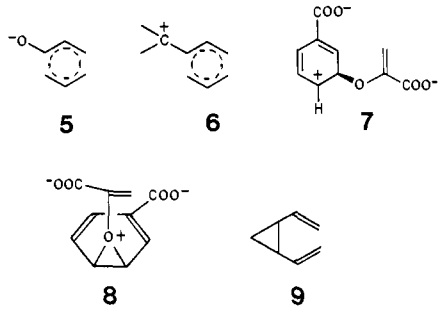
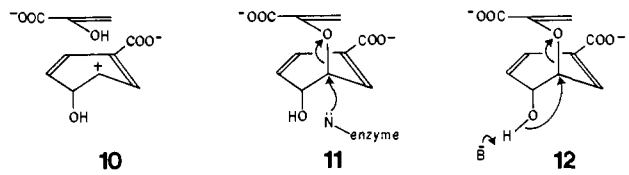


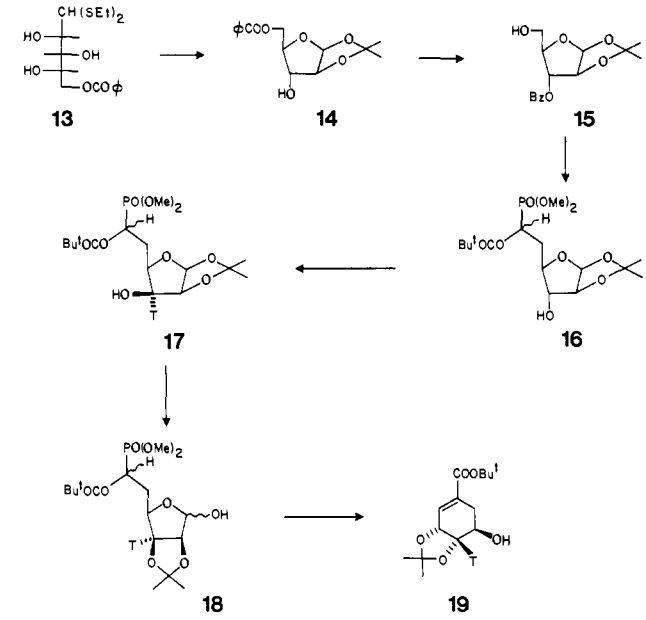
Chart III



experimentally. First, since the rearrangement can be presumed to occur from the pseudo-diaxial conformer of chorismate (**4**), the enzyme could procure the appropriate form of the substrate either by the selective binding of **4** or by first binding the predominant conformer **3** and then performing a conformational isomerization to **4**. Whether the removal of rotational degrees of freedom by this mechanism could effect an enhancement large enough to account for the observed enzymic acceleration depends upon the proportion of **3** and **4** at equilibrium in the absence of enzyme. The position of this equilibrium has been probed in the previous paper,[7] and the inadequacy of this pathway is discussed later. The second possibility hangs on the suggestions of Carpenter[8,9] concerning the effects of appropriate electron-donating and electron-withdrawing substituents on the rates of pericyclic reactions. This work extends the findings of Evans,[10] who showed that an alkoxide substituent can accelerate the rate of [3,3] sigmatropic rearrangements by factors of up to 10^{17}-fold. The Evans effect (as crudely illustrated by **5**) could have a Carpenter counterpart (as **6**), which would explain several observations in the literature such as the rapid rate of the "solvolytic" Cope rearrangement[11] and the accelerating effect of acids on the rearrangement of 2-acyl 1,5-dienes.[12] A structure analogous to **6** could be created at the active site of chorismate mutase if the hydroxyl group at C-4 were to become protonated and the water molecule were temporarily to dissociate to give **7**. The carbonium ion **7** is a Claisen analogue of the Cope cation **6** and might be expected to undergo rapid rearrangement. The third mechanistic possibility also allows the enzyme to exploit the existence of the hydroxyl group at C-4 of chorismate, by forming the oxirinium ion **8**. By analogy with the very large difference in the rate of the Cope rearrangement of 1,5-hexadiene and divinylcyclopropane **9**,[13] one could expect an acceleration of the Claisen rearrangement in **8**, even if the formation of **8** were an uphill process. Fourth, if the rearrangement of chorismate were to proceed via the heterolytic cleavage of the bond between the ether oxygen and C-5, to form the enolate of pyruvate and the cyclohexadienyl cation, one could expect acceleration of the reaction by the provision of an acidic center near the ether oxygen, as in **10**. Non-enzymic analogy for such acid catalysis exists for both Lewis[14] and Brønsted[15] acids. Cleavage of the appropriate C–O bond could also be assisted by attack of an enzyme nucleophile N (see **11**), or of the neighboring C-4 hydroxyl group as shown by **12**. In these cases, the rearrangement would be completed by the S_N2' attack of the enol pyruvate moiety at C-1 of the carbocycle.

Faced with this *embarras du choix*, we have determined the secondary tritium isotope effect at C-4 of chorismate in the enzyme-catalyzed reaction, as well as the solvent isotope effects for the enzymic and non-enzymic processes, in an effort to delimit the range of mechanistic possibility for the chorismate mutase reaction.

Scheme I



Experimental Section

Materials. Sodium [^{14}C]bicarbonate (52 mCi/mmol) and sodium boro[^{3}H]hydride were purchased from Amersham (Chicago, IL). D_2O (99.8%) was from Merck & Co. Silica gel preparative and analytical plates for thin-layer chromatography were from E. Merck (Darmstadt, Germany), cellulose plates (0.16 mm, 20 cm × 20 cm) were from Whatman (Clifton, NJ), and ribulose 1,5-bisphosphate, shikimic acid, phenyllactic acid, arabinose, and 3-phosphoglyceric acid were from Sigma (St. Louis, MO). Unlabeled chorismic acid was isolated from the fermentation of *Klebsiella pneumoniae* 62-1 by the method of Gibson[16] as modified by Addadi et al.[4] For the conversion of shikimic acid to chorismic acid, a cell-free extract from *K. pneumoniae* 62-1 was prepared as described by Addadi et al.[4]

Phosphoglycerate kinase (rabbit muscle), glyceraldehyde-phosphate dehydrogenase (rabbit muscle), ribulose-1,5-bisphosphate carboxylase (spinach), and glycerophosphate dehydrogenase (rabbit muscle) were purchased from Sigma. Enolase was obtained from Boehringer Mannheim Gmbh. Phosphoglycerate mutase was a gift from Dr. A. Minsky, and triosephosphate isomerase from Dr. J. G. Belasco. Chorismate mutase–prephenate dehydrogenase was isolated from *Escherichia coli* JFM 30 according to the procedure outlined in Addadi et al.[4]

3-Phospho[1-^{14}C]glyceric acid was synthesized as described by Addadi et al.[4] with the exception that commercial ribulose bisphosphate carboxylase (0.03 unit/mg) was used. Starting with sodium [^{14}C]bicarbonate (1.0 mCi, 52 mCi/mmol) and enzyme (1.5 units), 3-phospho[1-^{14}C]-glycerate (38 μmol, 0.96 mCi, 25.3 mCi/mmol) was isolated.

[4-^{3}H]Shikimic Acid. The dithio acetal of D-arabinose was prepared according to the method of Zinner.[17] Yield 53%; mp 125–126.5 °C, $[\alpha]_D$ (*c* 0.09, CH_3OH) −10.81°. Zinner[17] quotes, for the L-enantiomer, mp 125–125.5 °C, $[\alpha]_D$ (*c* 3.5, CH_3OH) +9.9°. The monobenzoate of this acetal (**13**) was made according to Zinner et al.[18] in 74% yield: mp

(7) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.*, preceding paper in this issue.
(8) Burrows, C. J.; Carpenter, B. K. *J. Am. Chem. Soc.* **1981**, *103*, 6983.
(9) Burrows, C. J.; Carpenter, B. K. *J. Am. Chem. Soc.* **1981**, *103*, 6984.
(10) Evans, D. A.; Golob, A. M. *J. Am. Chem. Soc.* **1975**, *97*, 4765.
(11) Breslow, R.; Hoffman, J. M. *J. Am. Chem. Soc.* **1972**, *94*, 2111.
(12) Dauben, W. G.; Chollet, A. *Tetrahedron Lett.* **1981**, *22*, 1583.
(13) Schneider, M. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 707.
(14) Borgulya, J.; Madeja, R.; Fahrni, P.; Hansen, H.-J.; Schmid, H.; Barner, R. *Helv. Chim. Acta* **1973**, *56*, 14.
(15) Widmer, U.; Hansen, H.-J.; Schmid, H. *Helv. Chim. Acta* **1973**, *56*, 2644.
(16) Gibson, F. *Biochem. Prep.* **1968**, *12*, 94.
(17) Zinner, H. *Chem. Ber.* **1951**, *84*, 780.
(18) Zinner, H.; Wessely, K.; Bock, W.; Rieckhoff, K.; Strandt, F.; Nimmich, W. *Chem. Ber.* **1957**, *90*, 500.

118.5–119.5 °C; $[\alpha]_D$ (*c* 0.073, $CHCl_3$) −48° (Lieser[19] gives, for the L-enantiomer, mp 119 °C, $[a]_D$ (*c* 0.079, $CHCl_3$) + 49.5°); ^1H NMR ($CDCl_3$) δ 8.03 (d, 2 H), 7.5 (t, 1 H), 7.4 (t, 2 H), 4.55 (dq, 2 H), 4.07 (m, 2 H), 3.87 (dd, 1 H), 3.53 (d, 1 H), 3.18 (d, 1 H), 2.90 (m, 1 H), 2.66 (m, 5 H), and 1.25 (dt, 6 H). The monobenzoate dithioethyl acetal (**13**) was converted to the acetonide benzoate, **14**, by demercaptalization by mercury(**II**) chloride in acetone (compare Fleet and Shing[20]), to yield **14** in 71% yield: mp 147.5–148.5 °C; $[\alpha]_D$ (*c* 0.04, $CHCl_3$) +25.25°; ^1H NMR ($CDCl_3$, D_2O) δ 8.03 (d, 2 H), 7.5 (t, 1 H), 7.38 (t, 2 H), 5.91 (d, 1 H, *J* = 3 Hz), 4.54 (d, 1 H, *J* = 3 Hz), 4.47 (d, 2 H, *J* = 6 Hz), 4.29 (br s, 1 H), 4.25 (m, 1 H), 1.50 (s, 3 H), and 1.29 (s, 3 H).

The benzoate **14** was converted into the benzyl acetonide **15** as follows. Sodium hydride (0.64 g, 13 mmol) was washed with petroleum ether and then dried in a stream of N_2. The flask was placed in an ice bath, and dimethylformamide (100 mL) was added. The suspension was stirred under N_2 as the solid alcohol (**14**) (3.0 g, 10 mmol) was added over 30 min. Benzyl chloride (2.57 g, 20 mmol) was then added, and the mixture was stirred at room temperature overnight. Methanol (5 mL) was then added, and the mixture was stirred for 4 h. After the addition of water, the product was extracted into ethyl acetate. The combined organic extract was washed successively with aqueous sodium bicarbonate, water, and brine. After removal of the solvent a clear oil was obtained, from which chromatography on silica gel in ethyl acetate–hexane (2:1, v/v) gave **15** (2.5 g, 83%): ^1H NMR ($CDCl_3$, D_2O) δ 7.32 (m, 5 H), 5.89 (d, 1 H, *J* = 4 Hz), 4.66 (d, 1 H, *J* = 4 Hz), 4.58 (q, 2 H), 4.18 (m, 1 H), 3.96 (d, 1 H, *J* = 3 Hz), 3.72 (m, 2 H), 1.51 (s, 3 H), and 1.32 (s, 3 H).

The alcohol **15** (2 g, 7 mmol) in methylene chloride (200 mL) was stirred with 3 Å molecular sieves (4 g) for 15 min before the portionwise addition of pyridinium chlorochromate (7.6 g, 35 mmol). The mixture was stirred for 13 h at room temperature and then washed through silica gel with ethyl acetate. The filtrate was concentrated to an oil that was chromatographed on silica gel, eluting with ethyl acetate–hexane (1:3, v/v), to yield the C-5 aldehyde (1 g): ^1H NMR ($CDCl_3$) δ 9.78 (s, 1 H), 7.36 (m, 5 H), 6.06 (d, 1 H, *J* = 3.5 Hz), 4.61 (m, 3 H), 4.52 (s, 1 H), 4.31 (s, 1 H), 1.43 (s, 3 H), and 1.29 (s, 3 H). A solution of this aldehyde (1 g, 3.6 mmol) in tetrahydrofuran (10 mL) containing *tert*-butyl dimethylphosphonacetate (0.9 g, 4 mmol) was added to a suspension of titanium tetrachloride (0.8 mL, 7.2 mmol) that had been slowly added at 0 °C with stirring to tetrahydrofuran (10 mL). This mixture was stirred at room temperature for 15 min, and *N*-methylmorpholine (1.45 g, 14.4 mmol) in tetrahydrofuran (10 mL) was then added. The reaction mixture was stirred for 90 min, aqueous sodium bicarbonate (25 mL) was then added, and the product was extracted into ether–ethyl acetate (1:1, v/v). The aqueous layer was then further extracted with ethyl acetate, and the combined organic extracts were washed successively with water and brine. After the solution was dried over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was dissolved in ethanol (40 mL) and hydrogenated over Pd/C (10% w/w, 0.27 g) after the addition of water (2 mL). After 48 h, the mixture was filtered through Celite, and the solution was concentrated under reduced pressure. Chromatography on silica gel, eluting successively with ethyl acetate–hexane (1:1, v/v), ethyl acetate–hexane (3:1, v/v), and ethyl acetate, gave a mixture of diastereoisomers of the adduct **16** (0.93 g, 65%). One diastereoisomer (A) crystallized from chloroform–hexane as white prisms (0.4 g), and the other (B) was purified by subjecting the mother liquors to preparative thin-layer chromatography on silica with acetone–hexane 0.45:0.65, v/v) as the eluant. The A isomer had mp 132–133 °C: ^1H NMR ($CDCl_3$) δ 5.87 (d, 1 H, *J* = 4 Hz), 4.49 (d, 1 H, *J* = 4 Hz), 4.11 (s, 1 H), 3.96 (dd, 1 H), 3.75 (d, 3 H), 3.72 (d, 3 H), 2.54 (ddd, 1 H), 2.20 (m, 2 H), 1.51 (s, 3 H), 1.44 (s, 9 H), and 1.26 (s, 3 H). The B isomer had the following ^1H NMR ($CDCl_3$): δ 5.86 (d, 1 H, *J* = 4 Hz), 4.51 (d, 1 H, *J* = 4 Hz), 4.10 (s, 1 H), 4.05 (m, 1 H), 3.79 (d, 3 H), 3.76 (d, 3 H), 3.14 (dt, 1 H), 2.28 (m, 2 H), 1.49 (s, 3 H), 1.45 (s, 9 H) and 1.30 (s, 3 H).

A mixture containing one or other of the diastereoisomers of **16** (64 mg, 0.16 mmol), Celite (0.2 g, oven-dried), 3 Å molecular sieves (0.2 g, oven-dried), and sodium acetate (44 mg, 0.54 mmol) in methylene chloride (15 mL) was stirred for 15 min before pyridinium chlorochromate (114 mg, 0.54 mmol) was added. The reaction mixture was stirred for 2 h and then filtered through silica gel. The silica gel was washed with methanol–acetone (1:50, v/v) and the product ketone was purified by preparative thin-layer chromatography on silica plates, eluting with methanol–chloroform (1:50, v/v). This ketone was dissolved (without further purification) in ethanol (5 mL) and sodium borohydride (40 mg, 1 mmol) was added with stirring. [When *deuteriated* material was synthesized, sodium boro[^2H]hydride was substituted in this step. The proton NMR of the labeled shikimate derivative **19** was consistent with specific deuteriation at C-4. Mass spectral analysis of this material showed it to be 18% d_0 and 82% d_1. When *tritiated* material was synthesized, sodium boro[^3H]hydride was substituted in this step. In this case, intermediates were isolated and purified by charcoal treatment rather than by silica gel chromatography. Control experiments showed that this procedure yields product **19** in a purity of >95%.] After 2 h, water was added and the product was extracted into ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and purified by preparative thin-layer chromatography on silica. The diastereoisomer **16A** gave **17A** having the following ^1H NMR ($CDCl_3$): δ 5.74 (d, 1 H, *J* = 4 Hz), 4.61 (dd, 1 H), 4.24 (m, 1 H), 4.03 (m, 1 H), 3.80 (d, 3 H), 3.77 (d, 3 H), 3.33 (dt, 1 H), 2.24 (m, 2 H), 1.62 (s, 3 H), 1.48 (s, 9 H), and 1.37 (s, 3 H). The diastereoisomer **16B** gave **17B** having the following ^1H NMR ($CDCl_3$): δ 5.70 (d, 1 H, *J* = 4 Hz), 4.63 (dd, 1 H, *J* = 4 Hz), 4.21 (q, 1 H), 4.06 (m, 1 H), 3.81 (d, 3 H), 3.78 (d, 3 H), 3.19 (dt, 1 H), 2.35 (m, 2 H), 1.60 (s, 3 H), 1.48 (s, 9 H), and 1.38 (s, 3 H).

To the alcohol **17A** or **17B** (16 mg) in acetone (10 mL) was added copper sulfate (0.2 g) and concentrated H_2SO_4 (0.2 ml), and the mixture was stirred overnight at room temperature. After the addition of sodium carbonate to neutrality, the mixture was stirred for a further 4 h and then filtered. The filtrate was concentrated under reduced pressure, and the product was purified by preparative thin-layer chromatography on silica, eluting with methanol–chloroform (1:50, v/v) to yield **18** (10 mg) as a colorless oil. ^1H NMR ($CDCl_3$): δ 5.33 (s, 1 H), 4.66 (m, 1 H), 4.60 (m, 1 H), 4.14 (m, 1 H), 3.81 (d, 3 H), 3.77 (d, 3 H), 3.23 (ddd, 0.5 H), 3.06 (m, 0.5 H), 2.27 (m, 2 H), 1.48 (s, 9 H) 1.46 (s, 3 H), and 1.31 (s, 3 H).

Sodium hydride (18 mg, 0.38 mmol) was added slowly to a stirred solution of the hemiacetal **18** (10 mg, 0.03 mmol) in dry tetrahydrofuran (3 mL). After 90 min, aqueous potassium dihydrogen phosphate (1 M, 10 mL) was added at 0 °C. The product was extracted into chloroform, and the combined organic extracts were then dried over $MgSO_4$ and concentrated under reduced pressure. After preparative thin-layer chromatography on silica in ether–hexane (2:1, v/v), the protected shikimate derivative **19** (5.7 mg) was isolated. ^1H NMR ($CDCl_3$) δ 6.80 (m, 1 H), 4.71 (m, 1 H), 4.03 (dd, 1 H), 3.80 (dddd, 1 H), 2.94 (d, 1 H), 2.74 (dd, 1 H), 2.13 (ddt, 1 H), 1.45 (s, 9 H), 1.43 (s, 3 H), and 1.37 (s, 3 H); $[\alpha]_D$ (*c* 0.0016, $CHCl_3$) −94.5° [G. W. J. Fleet and T. K. M. Shing (private communication) find $[\alpha]_D$ (*c* 0.7, $CHCl_3$) −88.3°].

Shikimic acid was obtained from **19** by stirring in aqueous trifluoroacetic acid (60%, v/v) at room temperature overnight. The solvent was then removed under reduced pressure, and ethanol was repeatedly added and removed by evaporation.

[4-^{3}H,7-^{14}C]Chorismate was prepared as follows. A solution of 200 mM Tris–HCl buffer, pH 8.0 (5.0 mL), containing EDTA (0.4 mM), dithioerythritol (10 mM), $MgSO_4$ (20 mM), KCl (50 mM), NADH (2 mM), FAD^+ (1 mM), ATP (5 mM), enolase (2 units), phosphoglycerate mutase (10 units), and spheroplast lysate (1 mL) from *K. pneumoniae* 62-1 was divided into two equal parts under argon. To one portion was added 3-phosphoglycerate (10 μmol) and [4-^{3}H]shikimate (3.2 μmol) prepared as above. To the other portion was added 3-phospho[1-^{14}C]-glycerate (9.8 μmol, 1.06×10^7 dpm) and unlabeled shikimate (5 μmol). Each portion was warmed to 37 °C for 90 min, this time having been established as optimum for the maximum production of chorismate with the particular preparation of the *Klebsiella* spheroplast lysate. At the end of the incubation the mixtures were cooled to 0 °C and then acidified to pH 1 with HCl (2 N). The protein precipitate was removed by centrifugation, and the *combined* aqueous supernatants were extracted into ether. The pooled ethereal extracts were dried and then reduced to approximately 5 mL under reduced pressure. Unlabeled chorismate (~150 mg) was then added, and the solution was treated with charcoal before being filtered through Celite into a Craig tube. The volume of solvent was reduced to about 1 mL, and water (1 vol) and methylene chloride (2 vol) were added. The solution was then cooled to 0 °C until crystallization began. Hexane (1 vol) was then added, and crystallization was allowed to proceed at 0 °C. The [4-^{3}H,7-^{14}C]chorismate was recrystallized until the isotope ratio was constant over three recrystallizations. The final yield of ^3H in the doubly-labeled chorismate was 40% based upon the protected shikimate ester, **19**.

To confirm that the ^3H label in the sample of [4-^{3}H,7-^{14}C]chorismate was indeed in the 4-position, a sample was treated with chorismate mutase–prephenate dehydrogenase[4] in the presence of NAD^+.

Methods. Ultraviolet measurements were made on a Perkin-Elmer 554 spectrophotometer. Scintillation counting was performed on a Beckman LS 1081 instrument. Aquasol (New England Nuclear, Boston, MA) was used as the scintillation cocktail. Counting efficiencies were determined by computer as a function of the "external standard ratio" by using the H-number method of Beckman (see manufacturer's in-

(19) Lieser, T.; Schweizer, R. *Liebigs Ann.* **1935**, *519*, 271.
(20) Fleet, G. W. J.; Shing, T. K. M. *Tetrahedron Lett.* **1983**, 3657.

structions). Calibration curves were prepared before the start of the experiment by using standard [^{3}H]- and [^{14}C]hexadecane samples quenched with various amounts of acetone. Typical counting efficiencies were 0.45 (channel 1) and 0.0031 (channel 2) for ^{3}H and 0.19 (channel 1) and 0.67 (channel 2) for ^{14}C. Counting efficiencies were constant from sample to sample. Isotope ratios in doubly-labeled samples were determined as follows. Samples containing approximately 2×10^4 dpm of ^{3}H were dissolved in scintillation cocktail (20 mL) and counted for 3×20 min; dpm values were calculated for ^{3}H and for ^{14}C from the average of the counts for the three counting cycles. Two separate samples were taken from each crystallization, and the mean ^{3}H:^{14}C ratio was calculated. A weighted mean of the ^{3}H:^{14}C ratios was then calculated for the different recrystallizations, provided that the average value for a given crystallization fell within the standard error of the mean value for all recrystallizations.

Kinetic Isotope Effect Measurements. For each set of experiments, samples of doubly-labeled [4-^{3}H,7-^{14}C]chorismic acid were prepared by mixing singly-labeled chorismic acid samples that were freshly prepared from shikimate and phosphoglycerate as described above. The ratio of ^{3}H:^{14}C was routinely between 4 and 6. The doubly-labeled chorismic acid was diluted with unlabeled material (approximately 140 mg) and recrystallized as described earlier. After each crystallization, two samples of approximately 0.4 mg of crystals (containing about 10^4 dpm of ^{3}H) were removed for scintillation counting after filtration and vacuum drying. The bulk of the material was immediately recrystallized. Five recrystallizations (each in 70% recovered yield) were normally performed, leaving around 25 mg for the rearrangement reaction.

For the chorismate mutase catalyzed reaction, doubly-labeled chorismic acid was dissolved in 50 mM *N*-ethylmorpholine-2-(*N*-morpholino)ethanesulfonate buffer, pH 7.5, containing EDTA (1 mM), dithioerythritol (1 mM), sodium citrate (10 mM), glycerol (10%, v/v), and bovine serum albumin (0.1 mg/mL) at 30 °C so as to make the solution about 5 mM in chorismate. An appropriate amount of enzyme (approximately 3 units) was then added so as to effect 50% conversion to prephenate in about 15 min. A portion of the incubation mixture was monitored spectrophotometrically at 275 nm and frequently exchanged with the bulk reaction mixture. The measured $\Delta\epsilon$ is 2340 M^{-1} cm^{-1}. At the appropriate extent of reaction, the mixture was cooled rapidly to 3 °C, and solid sodium borohydride (approximately 5 mg) was added with stirring. After 10 min, an additional portion of borohydride (approximately 5 mg) was added, and the mixture was then stirred in an ice bath for an additional 10 min. The solution was acidified to pH 1 with HCl (2 N) and then warmed at 30 °C for 1 h to convert the reduced prephenate to phenyllactic acid. The remaining chorismic acid and the phenyllactic acid were then extracted into ether, and a portion (20 mg) of each of these unlabeled compounds was added as carrier. The two acids were separated and purified on preparative thin-layer plates of silica gel, developing with toluene–acetic acid (12:7, v/v; chorismic acid, R_f 0.30, phenyllactic acid, R_f 0.50). Small amounts of *p*-hydroxybenzoic acid (R_f 0.61) could also be detected. The silica samples containing chorismic acid and phenyllactic acid were packed separately into small columns and eluted with ether–acetone (1:1, v/v). Each sample was diluted again with 40 mg of the appropriate unlabeled carrier. Chorismic acid was recrystallized as described above, and phenyllactic acid was recrystallized from ether–hexane at −20 °C. Each material was recrystallized at least three times, the ^{3}H:^{14}C ratio being measured after each recrystallization. The extent of reaction was obtained from the value of A_{275nm} at the time of quench and was confirmed by enzymic assay of the remaining chorismate after borohydride reduction of the prephenate product. In addition, the extent of reaction was determined by analysis of the reaction mixture before ether extraction by HPLC (Pharmacia FPLC column, Polyanion SI HR 5/5, using a gradient of 0–113 mM sodium phosphate, pH 7.0), using both ultraviolet and radioactivity detection. The results of all three methods were in agreement within experimental error (±2%).

To determine if chorismic acid, prephenic acid, phenyllactic acid, or any other intermediate is lost in an isotopically sensitive step, the following controls were carried out. Chorismic acid (^{3}H:^{14}C, 4.775 ± 0.016) was incubated for 30 min as noted above but without the addition of enzyme. The reaction was worked up as usual and the isotope ratio in the recrystallized chorismic acid was found to be unchanged (^{3}H:^{14}C, 4.743 ± 0.019). In a parallel reaction with chorismic acid from the same preparation, enzyme was added and the reaction was allowed to proceed for 3 h until all of the chorismate had been consumed. The reaction mixture was worked up as usual and the recrystallized phenyllactic acid was found to have the same ^{3}H:^{14}C ratio (^{3}H:^{14}C, 4.800 ± 0.020) as the starting chorismic acid. Therefore the only isotopically sensitive step is the conversion of chorismate to prephenate.

Solvent Isotope Effect Measurements. For determination of the solvent isotope effect on the enzymic rearrangement of chorismate to prephenate, chorismate mutase was assayed at 30 °C in reaction mixtures containing 50 mM *N*-ethylmorpholine-2-(*N*-morpholino)ethanesulfonic acid buffer containing EDTA (1 mM), dithioerythritol (1 mM), and bovine serum albumin (0.1 mg/mL) in a total volume of 1.2 mL. Reactions in H_2O were carried out at pH 7.6. For reactions in D_2O, the buffer was adjusted to pD = 7.6 by using the relationship pD = "pH" + 0.4,[21] where "pH" is the meter reading. The consumption of chorismate was determined by following the decrease in A_{274nm}. Values for K_m and V_{max} were determined from the data by using the HYPERO program of Cleland.[22]

The non-enzymic rearrangement of chorismate at 30 °C was carried out in the same buffers used for the enzymic rearrangement. At intervals, the value of A_{274nm} was measured. The rate constant, k, for disappearance of chorismate was determined by fitting the data to the equation $A_t = A_0e^{-kt} + C$ where A_t and A_0 are the absorbance values at time t and zero, respectively, and C is a constant.

Results

Samples of doubly-labeled [4-^{3}H,7-^{14}C]chorismic acid were synthesized from [4-^{3}H]shikimic acid and phosphoenol [1-^{14}C]-pyruvate by using the spheroplast lysate derived from *K. pneumoniae* 62-1, which contains all of the enzymes of the shikimate pathway up to (but not including) chorismate mutase. Labeled phosphoenolpyruvate was prepared in situ from 3-phospho[1-^{14}C]glyceric acid with the enzymes enolase and phosphoglycerate mutase. The synthesis of labeled shikimic acid was based on the enantiospecific synthesis of shikimic acid by Fleet and his group.[20] The early stages of the synthesis were modified in order to introduce the tritium label. Protected arabinose was cyclized to the furanose form with Lewis acid catalysis. Exchange of protecting groups and oxidation of the primary alcohol gave the aldehyde at C-5. Condensation of this aldehyde with *tert*-butyl dimethylphosphonoacetate in the presence of titanium tetrachloride gave **16** after reduction and deprotection with palladium on charcoal. The tritium label was introduced at C-4 by an oxidation–reduction sequence with use of sodium boro[^{3}H]hydride to give **17**. The stereospecific nature of the reduction was predicted on the basis of literature precedent and steric considerations and was proved by the ^{1}H NMR of the product derived from borodeuteride reduction. The stereochemistry was assigned from comparison of the coupling constants of the epimeric alcohols. [In the case of the ^{3}H-labeled material, the location of the isotope was confirmed by tracing the fate of ^{3}H in the reaction of [4-^{3}H]chorismate with chorismate mutase–prephenate dehydrogenase in the presence of NAD^+. More than 95% of the radioactivity was found, as expected, in the NADH.] Migration of the isopropylidene protecting group and treatment with sodium hydride gave the protected shikimate **19**. The labeled shikimate derivative was deprotected with trifluoroacetic acid just before use.

Samples of the doubly-labeled chorismate were synthesized on the day of the experiment and were crystallized to a constant ^{3}H:^{14}C ratio according to the procedure described earlier.[4] Purified, doubly-labeled chorismate was then partially converted to prephenate at 30 °C in the presence of chorismate mutase. Prephenate was converted to phenyllactate in situ by reduction with sodium borohydride followed by decarboxylative dehydration in acid. Although the hydroxyl group at C-4 of prephenate is lost in this process, no isotope discrimination is observed if the reaction is carried to completion. The remaining chorismate and phenyllactate were isolated by chromatography, and, after dilution with unlabeled material, each was recrystallized to constant ^{3}H:^{14}C ratio. The secondary kinetic isotope effects were evaluated as described earlier,[4] and the results for one of the experiments are shown in Table I. The results from both experiments are given in Table II, and they show the presence of a small *inverse* isotope effect of 0.96 in the formation of prephenate from [4-^{3}H]chorismate.

The D_2O solvent isotope effect for the enzymic reaction gives $^{D_2O}(K_m)$, 1.06 ± 0.31; $^{D_2O}(V_{max})$, 2.23 ± 0.19; and $^{D_2O}(V_{max}/K_m)$, 2.11 ± 0.26. The solvent isotope effect for the non-enzymic process, k_{H_2O}/k_{D_2O}, is 0.95 ± 0.04.

(21) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(22) Cleland, W. W. *Methods Enzymol.* **1979**, *63*, 103.

Table I. Values of the ^3H:^{14}C Ratio in the Rearrangement of [4-^3H,7-^{14}C]Chorismate[a]

compound	crystallization	^3H:^{14}C	weighted mean
chorismic acid	1	4.146 ± 0.005	4.145 ± 0.005
before reaction	2	4.149 ± 0.026	
	3	4.137 ± 0.013	
chorismic acid	1	4.022 ± 0.011	4.038 ± 0.008
remaining after	2	4.049 ± 0.016	
reaction	3	4.057 ± 0.015	
phenyllactic	1	4.252 ± 0.020	4.264 ± 0.009
acid product	2	4.252 ± 0.019	
	3	4.273 ± 0.012	

[a] These data relate to experiment 2 in Table II.

Table II. Secondary Tritium Kinetic Isotope Effects on the Enzymic Rearrangement of [4-^3H,7-^{14}C]Chorismate[a]

	r'	r	extent of reaction (%)	k_H/k_T[b]
1	0.982 ± 0.001	1.026 ± 0.002	32 ± 2	0.962 ± 0.007
2	0.974 ± 0.002	1.028 ± 0.001	38 ± 2	0.956 ± 0.008
			mean	0.96

[a] For the definition of r' and r and the method of calculation of the isotope effects, see ref 4. [b] Each experiment provides one value for k_H/k_T from r' and one from r. The value in this column is the average of those from r' and from r.

Discussion

From the fact that in the mutase-catalyzed rearrangement of chorismate, no secondary isotope effects were observed for tritium attached either to the bond-making carbon (C-9) or to the bond-breaking carbon (C-5), we were forced to conclude that the rate-limiting transition state in k_{cat}/K_m is isotopically insensitive.[4] To understand the mechanism of the chorismate mutase reaction, therefore, we must discover what is involved in this isotopically insensitive, rate-limiting transition state.

The first possibility we considered is that the enzyme binds the more abundant pseudo-diequatorial conformer and that this enzyme–substrate complex undergoes a rate-limiting isomerization to convert the substrate to its pseudo-diaxial form, which then rearranges rapidly to enzyme-bound prephenate (see Figure 1A).[4] This postulate begs two questions: (i) is there really so little pseudo-diaxial conformer present in solution that this route is necessary, and (ii) is the mere orientation of chorismate into the appropriate conformation enough to account for the rapid rate of the rearrangement on the enzyme. We have addressed the first question in the previous paper[7] and find that a respectable proportion (10–20%) of chorismate is present in solution as the pseudo-diaxial form. The conformational equilibrium is rapid on the NMR time scale. These facts seriously weaken any argument based upon the *need* for the enzyme to catalyze a conformational change, for the enzyme could merely select the appropriate diaxial form from the dynamic equilibrium mixture. In the simplest case (Figure 1B) the enzymic reaction itself would be encounter controlled, and k_{cat}/K_m would be insensitive to isotopic substitution at C-5 and C-9, as observed. This simple case is unattractive, however, for the following reasons. The value for k_{cat}/K_m that we have measured[7] for highly purified enzyme (and based on the *total* chorismate concentration) is 1.2×10^6 M^{-1} s^{-1}, and this value would only rise to around 10^7 M^{-1} s^{-1} if just the 10% or so of the substrate in the pseudo-diaxial form were handled by the enzyme. The value of 10^7 M^{-1} s^{-1} is still below what is usually considered the diffusive "on" rate for enzymic reactions,[23] though a low "on" rate constant can always derive from the docking of a rare form of the substrate or of a rare form of the enzyme, or both. However, the simple path illustrated in Figure 1B is effectively ruled out by the observed D_2O solvent isotope effect of more than 2 in both k_{cat} and in k_{cat}/K_m. While it is true that the higher viscosity of D_2O (of 1.23, compared to H_2O) would be expected to slow down a diffusive process, the observed effect is much too large to be ascribed to this cause. The identity of the solvent isotope effects on k_{cat} and k_{cat}/K_m is not well accommodated by the pathway shown in Figure 1B. We require, therefore, a second slower step that involves a transition state (ts_2 in Figure 1C) that is unaffected by isotopic substitution at C-5 or C-9, but which produces an intermediate X from which product formation is fast. Each of the remaining mechanisms that we consider therefore defines a different intermediate X (Figure 1C) and, in consequence, a different ts_2 that leads to this intermediate.

(23) Which can be estimated to be around 5×10^8 M^{-1} s^{-1}; see, e.g.,: Knowles, J. R.; Albery, W. J. *Acc. Chem. Res.* **1977**, *10*, 105.

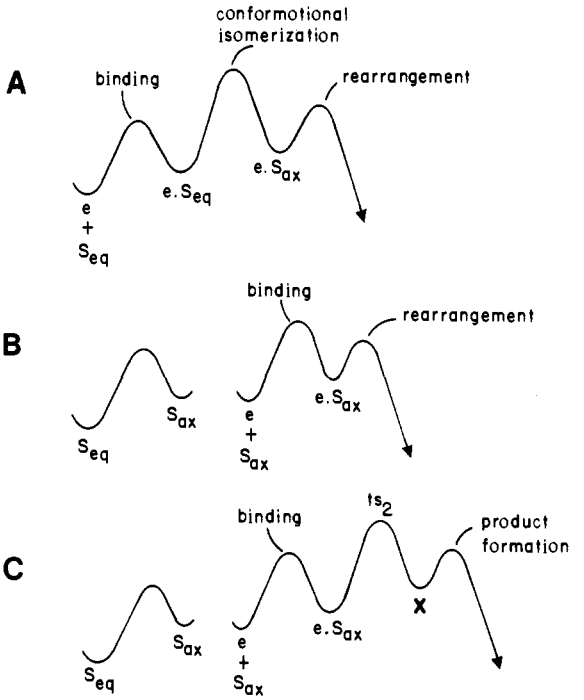


Figure 1. Free energy profiles illustrating possible modes of chorismate mutase action. (A) Preferential binding of the more abundant pseudo-diequatorial conformer of chorismate (S_{eq}) to the enzyme (e), followed by a rate-limiting conformational isomerization to the complex of enzyme with the pseudo-diaxial conformer (S_{ax}), from which rearrangment occurs rapidly. (B) Preferential binding of the less abundant S_{ax}, followed by rapid rearrangement. (C) Preferential binding of the less abundant S_{ax}, followed by a rate-determining step to the intermediate X, from which product forms rapidly.

In the second conceivable mechanism outlined in the introduction, it was suggested that the rate-limiting transition state (ts_2) might involve the protonation of the hydroxyl group at C-4 and loss of water to produce the carbonium ion 7.[8,9] In this mechanism, the intermediate X would be 7, and the rearrangement itself would follow rapidly. The secondary tritium isotope effect study reported here was designed to test this possibility by probing for any change in the hybridization at C-4 in the rate-limiting transition state for k_{cat}/K_m, ts_2. Since a heavy hydrogen isotope prefers to be a partner in a bond where the potential well is steep, tritium will prefer an sp^3 center rather than an sp^2 center, relative to hydrogen. We should therefore expect, for this mechanism, a normal secondary tritium effect (i.e., $k_H/k_T > 1$) of between 10 and 25%. This is not observed. Indeed, we see a small (4%) *inverse* isotope effect, which effectively rules out this pathway. Consistent with this conclusion are two further facts. First, when the chorismate mutase–prephenate dehydrogenase reaction is run in $H_2{}^{18}O$ and the product prephenate is trapped as *p*-hydroxyphenylpyruvate by the addition of NAD^+, *no* ^{18}O is found in the product. [In practice, the *p*-hydroxyphenylpyruvate was reduced to the more stable material *p*-hydroxyphenyllactate with sodium borohydride, and the hydroxyphenyllactate was subjected to mass spectrometric analysis after purification by ion-exchange chromatography.] This finding does not, of course, *rule out* the intermediacy of the carbonium ion 7 in the mutase-catalyzed reaction, since the liberated water molecule (from the hydroxyl group at C-4) could be sequestered at the active site and not exchange with the solvent $H_2{}^{18}O$. The negative result is, never-

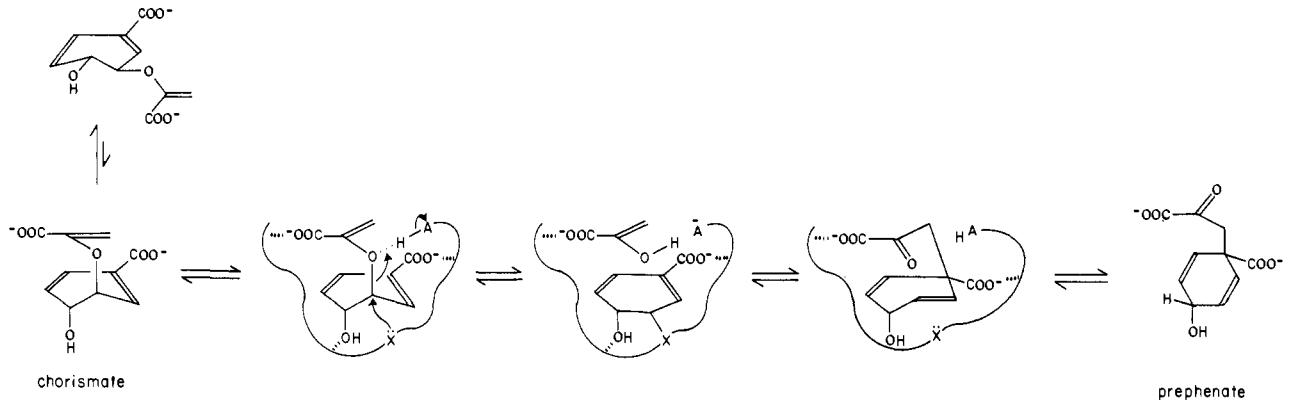


Figure 2. Possible mechanistic pathway for chorismate mutase.

theless, consistent with there being no transient loss of the C-4 hydroxyl group. The second fact that supports our rejection of the mechanism via **7** is the recent finding of J. Pawlak and G. Berchtold (private communication) that *deshydroxy*chorismate is a substrate ($k_{cat} \geq 1$ s^{-1}: compare k_{cat} for chorismate itself,[7] of 51 s^{-1}) for the enzyme. This finding shows that the C-4 hydroxyl group is not essential for catalysis by chorismate mutase. The second mechanism, via **7**, is evidently not followed by the enzyme.

The third mechanistic suggestion put forward in the introduction involves the transient formation of the oxirinium ion, **8**. In this pathway, ts_2 would involve the displacement of the C-4 hydroxyl group by the ether oxygen,[24] to give the intermediate **8**, which would be X in Figure 1C. While there are some attractive features of this proposal, it can be ruled out on the following grounds. First, the dramatic difference in the rates of the Cope rearrangement of hexa-1,5-diene and of divinylcyclopropane (**9**) relates to the *cis*-cyclopropane derivative,[13] which, in order to follow the required disrotatory pathway, must proceed via a *boat* transition state to yield cyclohepta-1,4-diene. [Disrotatory reaction via a chair transition state would necessarily yield a *trans*-cycloheptene species.] If analogous stereochemical constraints apply to species **8** (the geometrical constraints in which are, indeed, greater than in *cis*-divinylcyclopropane) then this pathway is ruled out, since rearrangement via a boat transition state is known *not* to be the pathway for the rearrangement of chorismate, either enzymically[2] or non-enzymically.[3] We have earlier shown that both of these reactions proceed via transition states of *chair*-like geometry.[2,3] The elimination of the third mechanism from consideration is consistent with the evident non-essentiality of the C-4 hydroxyl group for the enzymic reaction (J. Pawlak and G. Berchtold, private communication).

The fourth mechanism that we considered was one involving heterolytic cleavage of chorismate between C-5 and the ether oxygen. A heterolytic pathway for the enzymic reaction would parallel the recent suggestions of Gajewski et al.[25] and of Coates et al.[26] that the transition states of a variety of non-enzymic Claisen rearrangements have considerable dipolar character. This pathway has the teleological attraction that it gives the enzyme something more to do than merely to bind the appropriate conformation of the substrate, for we can readily imagine (a) that incipient protonation of the ether oxygen of chorismate would catalyze the formation of the enol of pyruvate and the cyclohexadienyl cation (**10**), (b) that an enzymic nucleophile N would attack at C-5 of the substrate, as in **11**, or (c) that an enzymic general base B would assist attack at C-5 by the *anti* hydroxyl group at C-4, as in **12**. In these formulations, the rate-limiting ts_2 (Figure 1C) would involve the heterolytic cleavage of the bond between C-5 and the ether oxygen. Of these three alternatives (**10**, **11**, and **12**), we must immediately reject **10**, since we have earlier shown[4] that there is no detectable secondary tritium isotope effect at C-5. Furthermore, we do not favor **12**, since the pathway requires the participation of the substrate's C-4 hydroxyl group, which Pawlak and Berchtold have shown is inessential. Additionally, R. Padykula and G. Berchtold (private communication) have shown that 4-*O*-methylchorismate is a substrate ($k_{cat} \sim 1$ s^{-1}) for chorismate mutase, which further rules out any major contribution from **12**. We are left, then, with **11** as a viable mechanistic candidate.

While we must recognize the dangers of arriving at a favored reaction mechanism merely by elimination of other notional pathways, we are driven in the present case to favor the path illustrated by **11**. To examine this conclusion, we determined the solvent isotope effect on the chorismate mutase reaction, to see if we could obtain evidence for *protonic motion* in the rate-limiting transition state. That is, is the mutase reaction sensitive to the solvent hydrogen isotope because of general acid and/or general base catalysis in **11**? Gratifyingly, we find that chorismate mutase does show a D_2O solvent isotope effect in k_{cat} of 2.23. There is no effect on the value of K_m. In contrast, the non-enzymic rearrangement is *unaffected* by the solvent isotope. While the interpretation of solvent isotope effects on enzyme-catalyzed reactions must always be cautious since subtle effects on enzyme structure cannot be ruled out, a solvent isotope effect of more than 2 on k_{cat} is certainly consistent with a general acid–base pathway, as shown by **11**.

Finally, we must ask how **11** accommodates the small inverse tritium isotope effect at C-4 reported in this paper and the absence of a tritium effect at C-5 reported earlier.[4] The isotope effect of 0.96 that we have observed at C-4 can be explained by the effect of tritium on the conformational equilibrium of the substrate. Thus Anet and his collaborators[27] have recently demonstrated that the effect of one deuterium on the conformational equilibrium of cyclohexane favors the conformer with deuterium *equatorial* by a factor of about 0.989. We expect, therefore, that a *tritium* at C-4 of chorismate would analogously prefer to be equatorial and that tritium substitution would slightly tip the conformational equilibium in favor of the pseudo-diaxial conformer **4**. Since, as illustrated in Figure 1C, the enzyme seems directly to select the pseudo-diaxial conformer of chorismate from solution, this would result in a slight acceleration of the rearrangement of the C-4 tritiated species, which is what we have observed.

The absence of an observable tritium effect at C-5 that we earlier reported[4] is also consistent with **11**. The kinetic effects of isotopic substitution at a carbon suffering an S_N2 displacement are, indeed, known to be very small and can be inverse or normal depending on the identity of the nucleophile and on the tightness of the transition state.[28] The overall effect of tritium substitution at C-5 may therefore derive from a small inverse conformational effect (analogous to that observed at C-4), counterbalanced by

(24) For an example of such oxygen participation in a situation of high electron demand, see: Lambert, J. B.; Larson, E. G. *J. Am. Chem. Soc.* **1985**, *107*, 7546.

(25) Gajewski, J. J.; Jurayj, J.; Kimbrough, D. R.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987**, *109*, 1170.

(26) Coates, R. M.; Rogers, B. D.; Hobbs, S. J.; Peck, D. R.; Curran, D. P. *J. Am.* Chem. *Soc.* **1987**, *109*, 1160.

(27) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 1355.

(28) Gray, C. H.; Coward, J. K.; Schowen, K. B.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4351.

a small normal effect arising from the displacement reaction that is occurring at C-5.

In summary, we have four pieces of isotopic information on the enzyme-catalyzed rearrangement of chorismate to prephenate: the solvent deuterium effect, and tritium effects at C-4, C-5, and C-9. These data, along with results from the non-enzymic reaction and from the use of chorismate analogues, limit the mechanistic possibilities for the chorismate mutase reaction. Thus we can eliminate those pathways (a) that involve a conformational isomerization of bound pseudo-diequatorial substrate, (b) that exploit the C-4 hydroxyl group to yield a carbonium ion at that position (**7**), (c) that proceed via an oxirinium ion intermediate (**8**), and probably (d) that involve anionic participation of the C-4 oxygen (**12**). The results are best accommodated by a pathway (Figure 2) in which the rate-limiting heterolytic cleavage of the ether bond of chorismate is assisted by attack of an enzymic nucleophile, to give an intermediate that collapses in an S_N2' process to yield the product prephenate.

Acknowledgment. We are especially grateful for the advice of Dr. George Fleet, for helpful discussions with Ted Widlanski, John Pawlak, R. Padykula, and Professor Glen Berchtold, for assistance with the mass spectrometer from Dr. J. M. Friedman, and for information on their work prior to publication from Professor Glenn Berchtold, Professor Bruce Ganem, and Professor Robert Coates. We also thank two of the referees, who substantially refined our mechanistic thinking. This work was supported by the National Institutes of Health and Merck Sharp and Dohme.

Communications to the Editor

Stereochemistry and Mode of Intermolecular Si–H Unsaturated Carbene Insertions†

Peter J. Stang* and Alan E. Learned

Department of Chemistry, The University of Utah
Salt Lake City, Utah 84112
Received February 11, 1987

Unsaturated carbenes, like their saturated congeners, undergo four basic types of reactions: (a) additions; (b) insertions; (c) ylide formations; (d) rearrangements.[1] Recent, concomitant theoretical and experimental studies[2,3] have clearly established the exact manner of alkylidenecarbene–olefin interactions and concurrent transition-state geometries, whereas the mode of carbene insertion reactions in general, and unsaturated carbenes in particular, is much less understood.

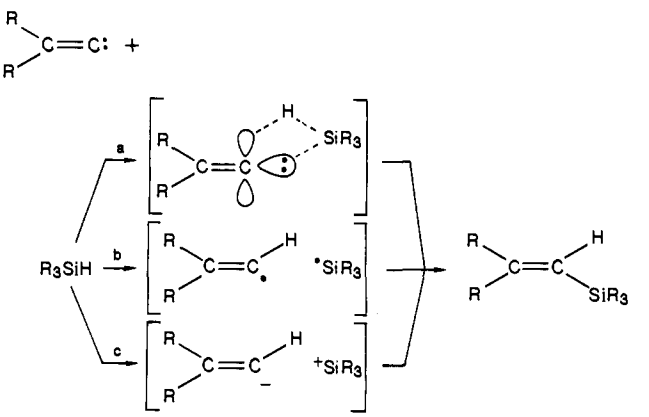
In a recent, elegant investigation, Gilbert and co-workers[4] established that the 1,5-intramolecular C–H insertion of alkylidenecarbenes occurs with retention of configuration, most likely via a concerted process involving a nonlinear transition state. Few examples of intermolecular C–H unsaturated carbene insertions are known.[1] However, these species readily insert into Si–H bonds.

A priori, intermolecular Si–H unsaturated carbene insertions may be envisioned to proceed by (a) a concerted, three-centered process or (b) a homolytic or (c) heterolytic hydrogen abstraction–recombination pathway (Scheme I).

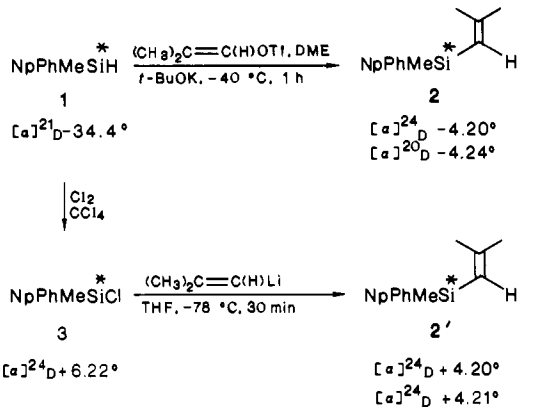
A concerted, three-centered process would require retention of chirality during the course of reaction with a chiral silane, whereas either of the abstraction–recombination pathways would predict loss of chirality. During the course of our investigations, Gilbert and Giamalva[5] predicted, by means of the temperature *independence* of the kinetic deuterium isotope effect [$(CH_3)_2C{=}C$: + $Et_3SiH(D)$], a concerted process, via a nonlinear transition state, as the most likely pathway for such intermolecular Si–H insertions. This transition state would require not only retention of chirality, but also *complete stereospecificity with retention of absolute configuration.*

In this paper, we wish to report the results of our investigation of the interaction of both $(CH_3)_2C{=}C$: and $(CH_3)_2C{=}C{=}C{=}C$: with chiral, optically pure (*S*)-α-NpPhMeSi*H (**1**).[6]

Scheme I



Scheme II

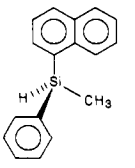


Optically pure (*S*)-α-NpPhMeSi*H (**1**) was prepared according to the procedure of Corriu and Moreau.[7] Interaction of the triflate-derived[1] $(CH_3)_2C{=}C$: with **1** in glyme (duplicate runs) gave the insertion product **2** in 78% yield with $[\alpha]_D$ −4.20° and

† Dedicated to Professors W. von E. Doering and A. Streitwieser, Jr., on the occasion of their 70th and 60th birthdays, respectively.

(1) Stang, P. J. *Acc. Chem. Res.* **1982**, *15*, 348; *Chem. Rev.* **1978**, *78*, 383.
(2) Fox, D. P.; Stang, P. J.; Apeloig, Y.; Karni, M. *J. Am. Chem. Soc.* **1986**, *108*, 750.
(3) Apeloig, Y.; Karni, M.; Stang, P. J.; Fox, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 4781.
(4) Gilbert, J. C.; Giamalva, D. H.; Baze, M. E. *J. Org. Chem.* **1985**, *50*, 2557.
(5) Gilbert, J. C.; Giamalva, D. H. *J. Org. Chem.* **1985**, *50*, 2586.

(6) (*S*)-α-NpPhMeSi*H =



(7) Corriu, R. J. P.; Moreau, J. J. E. *Bull. Soc. Chim. Fr.* **1975**, *3*, 901.

0002-7863/87/1509-5019$01.50/0