JOC Article

Investigation of the Enzymatic and Nonenzymatic Cope Rearrangement of Carbaprephenate to Carbachorismate

Andreas Aemissegger, Bernhard Jaun, and Donald Hilvert*

Laboratorium für Organische Chemie, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

hilvert@org.chem.ethz.ch

Received June 24, 2002

The dimethyl esters of carbaprephenate and 4-*epi*-carbaprephenate were prepared by modification of published procedures. In methanol these compounds are converted quantitatively to isomeric 6-hydroxytricyclo[3.3.1.$0^{2,7}$]non-3-en-1,3-dimethyl esters via a two-step sequence involving an initial Cope rearrangement, followed by intramolecular Diels−Alder reaction of the dimethyl carbachorismate or 4-*epi*-carbachorismate intermediates. Carbaprephenate and its epimer were obtained by alkaline hydrolysis of the corresponding dimethyl esters. These compounds, in contrast to their ester precursors, undergo spontaneous acid-catalyzed decarboxylation in aqueous solution. Only at high pH does the Cope rearrangement compete with decarboxylation. At pH 12 and 90 °C, carbaprephenate slowly rearranges to carbachorismate, which rapidly loses water to give 3-(2-carboxyallyl)benzoic acid as the major product. A small amount of the intramolecular Diels−Alder adduct derived from carbachorismate is also observed by NMR as a minor product. Carbaprephenate is not a substrate for the enzyme chorismate mutase from *Bacillus subtilis* (BsCM), nor does carbaprephenate inhibit the normal chorismate mutase activity of this enzyme, even when present in 200-fold excess over chorismate. Its low affinity for the enzyme-active site is presumably a consequence of placing a methylene group rather than an oxygen atom proximal to the essential cationic residue Arg90. Nevertheless, BsCM variants that lack this cation (R90G and R90A) do not accelerate the Cope rearrangement of carbaprephenate either, and a catalytic antibody 1F7, which exhibits modest chorismate mutase activity, is similarly inactive. Poor substrate binding and the relatively high barrier for the Cope compared to the Claisen rearrangement presumably account for the lack of detectable catalysis. Acceleration of this sigmatropic rearrangement apparently requires more than an active site that is complementary in shape to the reactive substrate conformer.

Chorismate mutases (EC 5.4.99.5) accelerate the pericyclic Claisen rearrangement of chorismate **1** to prephenate **2**, the first committed step in the biosynthesis of the aromatic amino acids phenylalanine and tyrosine,[1] by a factor of 10^6-10^7. The uncatalyzed[2] as well as the enzymatic[3] reactions proceed through chairlike transition states (Figure 1A). Kinetic isotope effects show that C−O bond cleavage precedes C−C bond formation in both cases,[4,5] indicating a concerted but asynchronous process. X-ray structures[6−8] of chorismate mutases from different sources complexed to a transition state analogue show that the active site is configured to constrain the flexible substrate molecule in a reactive conformation in which the trans C-4 hydroxyl group and C-3 enol pyruvate substituents are pseudodiaxial (e.g., Figure 1A,B). Mutagenesis studies[9−13] and computation[14−16] suggest that a positively charged active site residue (either Lys or Arg) located proximal to the ether oxygen of bound substrate[6,7] (Figure 1B) is essential for catalysis, presumably because it is able to stabilize the polarized transition state

* To whom correspondence should be addressed. Phone: +41-1-632-3176. Fax: +41-1-632-1486.

(1) Haslam, E. *Shikimic Acid Metabolism and Metabolites*; John Wiley & Sons: New York, 1993.
(2) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 5306−5308.
(3) Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2701−2703.
(4) Addadi, L.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494−4501.
(5) Gustin, D. J.; Mattei, P.; Kast, P.; Wiest, O.; Lee, L.; Cleland, W. W.; Hilvert, D. *J. Am. Chem. Soc.* **1999**, *121*, 1756−1757.
(6) Chook, Y. M.; Gray, J. V.; Ke, H.; Lipscomb, W. N. *J. Mol. Biol.* **1994**, *240*, 476−500.
(7) Lee, A. Y.; Karplus, P. A.; Ganem, B.; Clardy, J. *J. Am. Chem. Soc.* **1995**, *117*, 3627−3628.
(8) Sträter, N.; Schnappauf, G.; Braus, G.; Lipscomb, W. N. *Structure* **1997**, *5*, 1437−1452.
(9) Kast, P.; Grisostomi, C.; Chen, I. A.; Li, S.; Krengel, U.; Xue, Y.; Hilvert, D. *J. Biol. Chem.* **2000**, *275*, 36832−36838.
(10) Kast, P.; Asif-Ullah, M.; Jiang, N.; Hilvert, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5043−5048.
(11) Liu, D. R.; Cload, S. T.; Pastor, R. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 1789−1790.
(12) Cload, S. T.; Liu, D. R.; Pastor, R. M.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 1787−1788.
(13) Zhang, S.; Kongsaere, P.; Clardy, J.; Wilson, D. B.; Ganem, B. *Bioorg. Med. Chem.* **1996**, *4*, 1015−1020.
(14) Wiest, O.; Houk, K. N. *J. Org. Chem.* **1994**, *59*, 7582−7584.
(15) Lyne, P. D.; Mulholland, A. J.; Richards, W. G. *J. Am. Chem. Soc.* **1995**, *117*, 11345−11350.
(16) Davidson, M. M.; Hillier, I. H. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1415−1417.

10.1021/jo026096s CCC: $22.00
Published on Web 08/24/2002

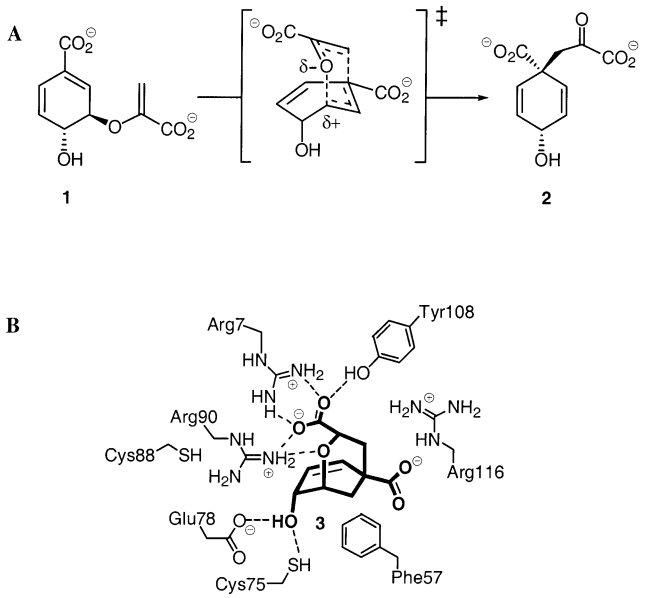
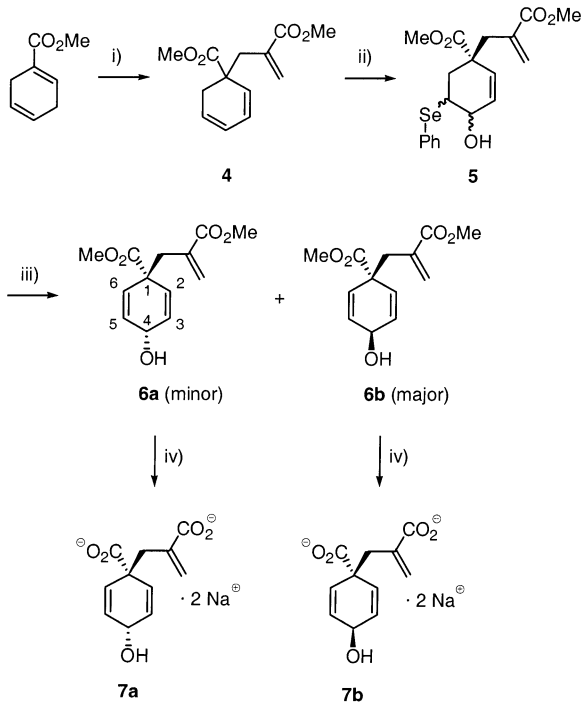


FIGURE 1. (A) Rearrangement of chorismate to prephenate. (B) Active site of BsCM with a bound transition state analogue (**3**, bold).[6] Inhibitor **3** binds chorismate mutases with K_i values in the range 0.1 to 4 μM.[21,23]

electrostatically. Interestingly, an antibody with weak chorismate mutase activity (1F7), which was raised in response to transition state analogue **3**,[17] preferentially binds the pseudodiaxial substrate conformer[18] but lacks this electrostatic feature.[19]

The Cope rearrangement of "carbachorismate", in which the ether oxygen of chorismate is replaced with a methylene group, to give "carbaprephenate", in which the carbonyl group of prephenate is replaced with an olefin, would be expected to have a substantially less polarized transition state than the Claisen rearrangement of chorismate to prephenate. This aspect makes this reaction potentially interesting as a probe of the relative importance of electrostatic[9] versus conformational[20] effects in chorismate mutase catalysis. The "carba" analogue of inhibitor **3** binds only 250 times less tightly to a bifunctional chorismate mutase from *E. coli*,[21] raising the possibility that the enzymes would also recognize carbachorismate and carbaprephenate and promote their interconversion. The catalytic antibody or variants of the natural enzyme containing an uncharged amino acid in place of the critical cationic residue might be even better catalysts since they combine an active site of appropriate shape for constraining the substrate in a reactive geometry with a more suitable electrostatic environment. To test these ideas, we have prepared carbaprephenate from the corresponding dimethyl ester, which has been reported in the literature,[22] and have examined its reactivity in the presence and absence of various proteins with chorismate mutase activity, including the natural enzyme from *Bacillus subtilis* (BsCM),[23] the R90G and R90A BsCM variants which lack the essential active site cation,[9] and the catalytic antibody 1F7.

SCHEME 1[a]



[a] Conditions: (i) LiN(^{i}Pr)(^{c}Hx), $BrCH_2C(CO_2CH_3)=CH_2$; (ii) AgO_2CCF_3, PhSeCl; (iii) (A) H_2O_2, (B) Δ; (iv) NaOH.

Results and Discussion

Synthesis. Unlike the Claisen rearrangement of chorismate to prephenate, which is highly exothermic,[24] the Cope rearrangement of the corresponding carba analogues should have an equilibrium constant closer to 1 and hence be reversible. For this reason, synthetically more accessible carbaprephenate was chosen in preference to carbachorismate for study with the enzymes.

Carbaprephenate and 4-*epi*-carbaprephenate were synthesized as shown in Scheme 1. The corresponding dimethyl esters **6a** and **6b** are known compounds[22] and were prepared in racemic form by minor modification of published protocols. In the conversion of methyl 3,6-dihydrobenzoate to **4**, the cyclic urea DMPU was employed as a cosolvent in place of HMPT because of its lower toxicity.[25] With 2 equiv of DMPU, only a modest decrease in the yield of **4** (64%) was observed compared to the published value (71%). Treatment of **4** with PhSeCl afforded **5** as a 3:1 mixture of diastereoisomers, as previously described, but comparison of its ^{1}H NMR spectrum with the published data[22] showed that the major and minor isomers were inverted. Subsequent selenoxide elimination gave the isomeric esters **6a** and

(17) Hilvert, D.; Carpenter, S. H.; Nared, K. D.; Auditor, M.-T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4953−4955.
(18) Campbell, A. P.; Tarasow, T. M.; Massefski, W.; Wright, P. E.; Hilvert, D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8663−8667.
(19) Haynes, M. R.; Stura, E. A.; Hilvert, D.; Wilson, I. A. *Science* **1994**, *263*, 646−652.
(20) Khanjin, N. A.; Snyder, J. P.; Menger, F. M. *J. Am. Chem. Soc.* **1999**, *121*, 11831−11846.
(21) Bartlett, P. A.; Nakagawa, Y.; Johnson, C. R.; Reich, S. H.; Luis, A. *J. Org. Chem.* **1988**, *53*, 3195−3210.
(22) Gajewski, J. J.; Jurayj, J.; Kimbrough, D. R.; Gande, M. E.; Ganem, B.; Carpenter, B. K. *J. Am. Chem. Soc.* **1987**, *109*, 1170−1186.
(23) Gray, J. V.; Eren, D.; Knowles, J. R. *Biochemistry* **1990**, *29*, 8872−8878.
(24) Kast, P.; Tewari, Y. B.; Wiest, O.; Hilvert, D.; Houk, K. N.; Goldberg, R. N. *J. Phys. Chem. B* **1997**, *101*, 10976−10982.
(25) *Chimia* **1985**, *39*, 147−148.

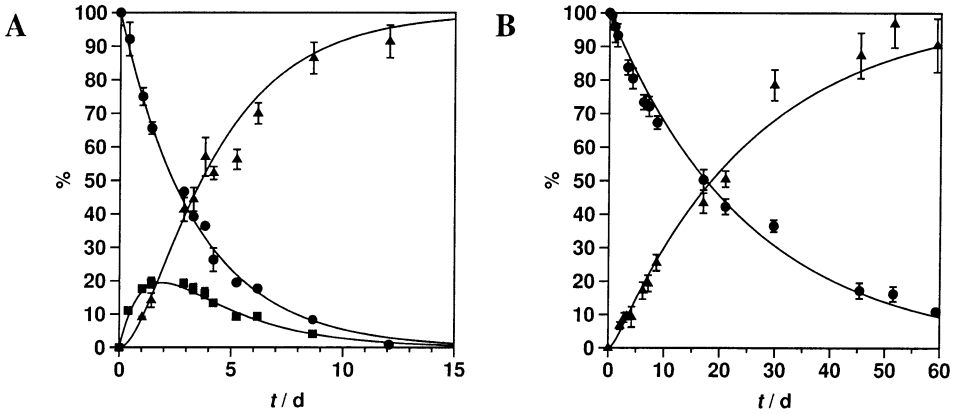


FIGURE 2. Time course for the rearrangement of dimethyl carbaprephenate (**6a**, A) and dimethyl 4-*epi*-carbaprephenate (**6b**, B) in methanol at 60 °C. Relative amounts of starting material (●), product of the Cope rearrangement (■), and product of the Diels−Alder rearrangement (▲). Percent conversion was determined by averaging NMR peak areas; error bars indicate deviation from the average value.

6b, which were separated by normal-phase preparative HPLC. The latter procedure proved more effective in our hands than flash chromatography.

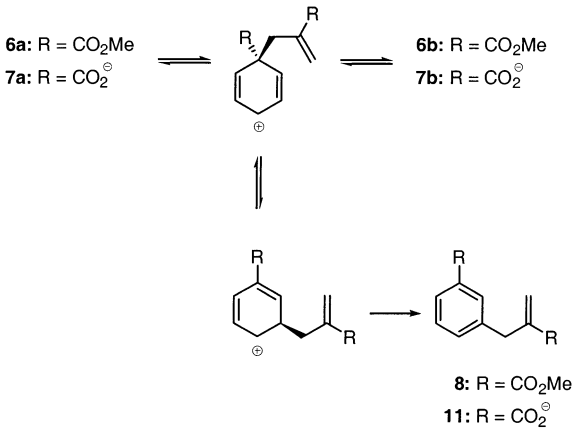
Esters **6a** and **6b** were separately hydrolyzed to carboxylates **7a** and **7b** with NaOH in a 1:3 mixture of water and THF. Excess base was used to suppress acid-catalyzed decarboxylation of the products. The reactions, which were monitored by NMR spectroscopy, proceeded quantitatively. Because **7a** and **7b** decarboxylate readily, even at neutral pH, they were used directly for kinetic measurements without further purification.

Assignment of 6a and 6b. The major and minor products of the selenoxide elimination of **5** have very similar ^{1}H NMR spectra, making unambiguous assignment of **6a** and **6b** difficult. Spectral data for dimethyl carbaprephenate, allegedly the minor product of the reaction, have been published,[22] although how this structure was assigned was not described. Because the reported coupling constants for the vinylic ring protons correspond more closely to those of our major isomer, and also to the related values for dimethyl 4-*epi*-prephenate,[26] additional support for this assignment was sought.

Observation of an NOE between the side chain methylene group at C1 and the hydrogen atom at C4 would have provided convincing evidence for structure **6a**, but magnetization transfer was not detected with either isomer, suggesting that the centers of interest are too far apart. For this reason, the Cope rearrangement of **6a** and **6b** was examined. In analogy with chorismate and 4-*epi*-chorismate,[26] carbachorismate and 4-*epi*-carbachorismate should be relatively easy to distinguish. However, in previous work[22] dimethyl carbaprephenate was found to epimerize and to rearrange to **8** in aqueous methanol. Both reactions are strongly acid-catalyzed, which was rationalized by an ionic mechanism involving carbocation formation (Scheme 2). To suppress this undesired pathway, the reactions of **6a** and **6b** were carried out in CD_3OD in base-washed NMR tubes.

Rearrangement of the two epimers was monitored by ^{1}H NMR at 60 °C (Figure 2). Under these conditions, the minor isomer is slowly converted via a transient intermediate to a single product having the same molecular mass as the starting material but only one double bond. The major isomer is quantitatively converted to a closely related compound, but it reacts about 7 times slower than its epimer and only trace amounts of the intermediate are observed during the reaction. Although isolation of the transient intermediate was not attempted in either case, the peaks observed in the NMR spectra correspond to those expected for dimethyl (4-*epi*)-carbachorismate. For example, a resonance at 6.9−7 ppm is characteristic of the hydrogen at C-2 in the conjugated cyclohexadiene ring system and compares well with the values observed for dimethyl chorismate and dimethyl 4-*epi*-chorismate.[26] The structure of the final rearrangement product of the minor isomer was established unambiguously as racemic **10a** (Scheme 3) by a complete 1D difference NOE and 2D NMR spectroscopic analysis (see Supporting Information for details). The rearrangement product of the major isomer was assigned to structure **10b** on the basis of the close similarity of its spectra and that of **10a** and the fact that **6a** and **6b** are epimers at C-4.

SCHEME 2



These observations can be rationalized by a two-step process in which dimethyl (4-*epi*)-carbaprephenate undergoes an initial Cope rearrangement to racemic dimethyl (4-*epi*)-carbachorismate, followed by an intramolecular Diels−Alder reaction to give either **10a** or **10b** (Scheme 3). Because the Cope rearrangement and Diels−Alder

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

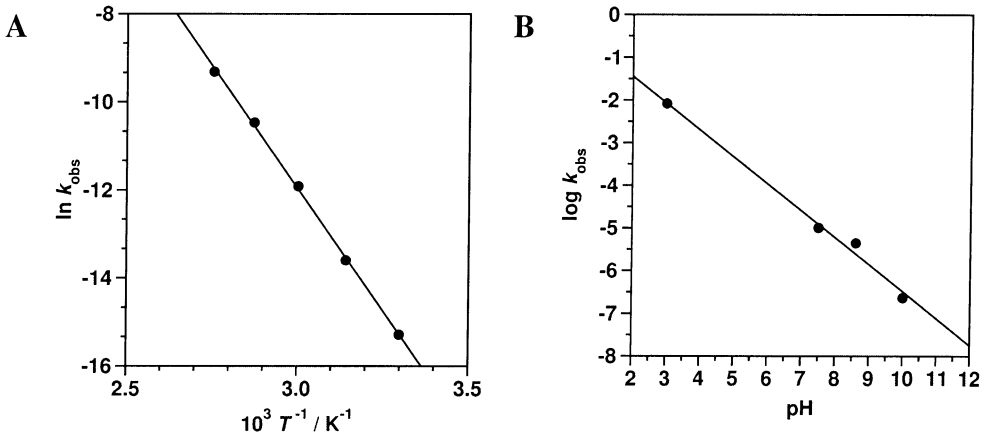
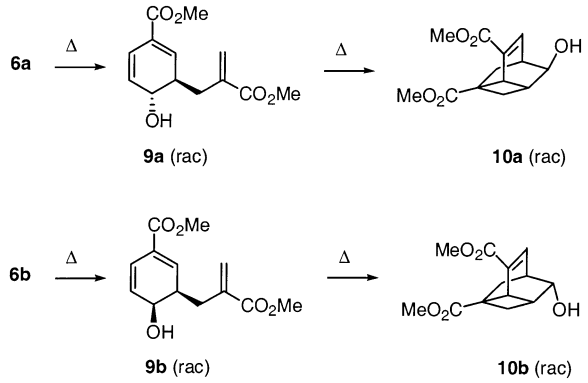


FIGURE 3. (A) Arrhenius plot for the decarboxylation of carbaprephenate. Reactions were monitored in deuterated phosphate buffer (ca. 70 mM, pD 10). $\Delta H^{\ddagger} = 22.0$ kcal mol^{-1}; $\Delta S^{\ddagger} = 18.4$ cal mol^{-1} K^{-1}. (B) pH dependence of carbaprephenate decarboxylation at 30 °C. The rate constant for the value at pH 3 was extrapolated from data obtained at 20 °C with the Arrhenius equation.

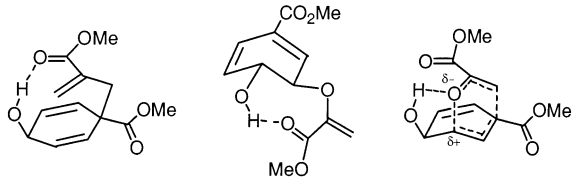
SCHEME 3



cycloaddition occur in a stereochemically defined manner, the structure of the epimeric tricyclononene derivatives allows unambiguous assignment of the minor and major products of the selenoxide elimination to structures **6a** (dimethyl carbaprephenate) and **6b** (dimethyl 4-*epi*-carbaprephenate), respectively.

Fitting the NMR data to a two-step kinetic model (A → B → C) gives rate constants of 3.4×10^{-6} and 1.0×10^{-5} s^{-1} for the Cope rearrangement of dimethyl carbaprephenate and the subsequent intramolecular Diels–Alder cycloaddition, respectively. The corresponding values for dimethyl 4-*epi*-carbaprephenate are 4.6×10^{-7} and 1.3×10^{-5} s^{-1}. Thus, while the intramolecular Diels–Alder reactions occur at comparable rates, the Cope rearrangement of dimethyl carbaprephenate is roughly an order of magnitude faster than that of dimethyl 4-*epi*-carbaprephenate.

The slower rearrangement of dimethyl 4-*epi*-carbaprephenate compared to dimethyl carbaprephenate may reflect, in part, misalignment of the reacting π-system as a consequence of intramolecular hydrogen bonding or steric interactions between the C-4 hydroxyl group and the C-1 methacrylate ester substituent (e.g., **12**). Such an interaction is not possible in dimethyl carbaprephenate, where these groups are trans to one another and oriented pseudodiaxially in the reactive conformation. Interestingly, the order of reactivity for the Claisen rearrangement of dimethyl chorismate and dimethyl 4-*epi*-chorismate is reversed: dimethyl chorismate is stable in $CDCl_3$ at 30 °C, whereas dimethyl 4-*epi*-chorismate rearranges to dimethyl 4-*epi*-prephenate with a half-life of 2.3 h under these conditions. Hydrogen bonding between the trans C-3 and C-4 substituents in dimethyl chorismate, which would stabilize the unreactive pseudodiequatorial substrate conformer (**13**), has been invoked to explain this difference in reactivity.[26] It is also conceivable that the polarized transition state for Claisen rearrangement of dimethyl 4-*epi*-chorismate would be directly stabilized by a hydrogen bond between the C-4 hydroxyl group and the ether oxygen (rather than the ester group) of the enol pyruvate substituent (**14**), leading to faster rearrangement of this isomer.[27]



Nonenzymatic Reaction of 7a and 7b. Like prephenate,[28] carbaprephenate and 4-*epi*-carbaprephenate undergo spontaneous acid-catalyzed decarboxylation in aqueous solution (Figure 3). At pH 3 and 20 °C, the half-life for prephenate is 18 min, whereas **7a** and **7b** have half-lives of 4.6 and 5.5 min, respectively. Even raising the pH does not completely suppress this undesired side reaction, although the half-life of carbaprephenate increases to >800 h at pH 10 (30 °C). NMR analysis of product mixtures obtained at pH 10 at different temperatures reveals <5% **11**, the product of Cope rearrangement and aromatization (Scheme 2).[22] However, when **7a** is heated at 90 °C for 35 h in phosphate buffer (50 mM) at pH 12 or in 1 M NaOH, compound **11** is the dominant product. A small amount of the Diels–Alder adduct (ca. 10%) is also observed in the NMR spectra of the crude product. Under these conditions, rearrangement followed

(27) Carlson, H. A.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1996**, *118*, 8475–8484.
(28) Zamir, L. O.; Tiberio, R.; Jensen, R. A. *Tetrahedron Lett.* **1983**, *24*, 2815–2818.

by dehydration or intramolecular Diels−Alder reaction thus occurs in preference to decarboxylation. Assuming that the Cope rearrangement of **7a** is pH independent, we estimate that it is roughly 10−100-times slower than decarboxylation in the pH range 8 to 9.

Enzymatic Assays. Carbaprephenate **7a** was tested as a substrate for *Bacillus subtilis* chorismate mutase (BsCM), the R90G and R90A variants of this enzyme, and the catalytic antibody 1F7. The latter proteins, which are either inactive or relatively poor chorismate mutases,[9,19] have active sites that are complementary in shape to the constrained substrate conformer needed for reaction. However, they lack the strategically placed cationic group that interacts with the ether oxygen of chorismate in wild-type BsCM.[6,9]

All reactions were monitored by HPLC with *p*-ethoxybenzoic acid as an internal standard. High concentrations of enzyme (ca. 100 μM) and antibody (22 μM) were employed to maximize the chances of detecting even modest levels of catalysis. Because the catalysts are nearly pH independent in the range 5 to 9,[23,29] the assays were performed at pH 8.6 (50 mM Tris·HCl buffer, 30 °C) to minimize the competing spontaneous decarboxylation of substrate. Under these conditions, the half-life for decarboxylation is approximately 50 h. Assuming a half-life of 500 to 5000 h for the uncatalyzed Cope rearrangement of **7a** and a K_m value much larger than the substrate concentration used in the assays, a rate acceleration as small as 10-fold would have been detectable. In the event, even after several days no significant difference in product yield or distribution was observed between the enzyme solutions and the blanks.

Not unexpectedly, BsCM has a relatively low affinity for carbaprephenate. However, even at 1 mM concentrations, this compound does not detectably inhibit the BsCM-catalyzed rearrangement of 50 μM chorismate. Replacement of the carbonyl oxygen in prephenate with a methylene group thus appears to be substantially more destabilizing than replacement of the ether oxygen in inhibitor **3** with a methylene group, which results in a 250-fold decrease in affinity.[21] If carbaprephenate were to bind to BsCM in an orientation analogous to that of prephenate[6] (which binds to the enzyme with a similar affinity as chorismate[23]), the guanidinium cation of Arg90 would be juxtaposed with an apolar methylene group rather than an oxygen atom capable of accepting a hydrogen bond.

Nevertheless, removal of this destabilizing interaction does not promote catalysis. BsCM derivatives in which Arg90 is replaced with a glycine or an alanine and the catalytic antibody 1F7, which lacks an analogous cationic group, do not accelerate the Cope rearrangement of carbaprephenate. Apparently, an active site configured simply to constrain the substrate in a conformation favorable for reaction, but lacking specific interactions capable of stabilizing the pericyclic transition state, is insufficient to overcome the energy barrier of the Cope reaction.

Experimental Section

General Methods. All reactions were carried out in dried glassware. Methanol was freshly distilled from magnesium methoxide, THF from potassium, and CH_2Cl_2 from phosphorus pentoxide. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) was distilled from calcium hydride at reduced pressure, sealed in septum-capped vials, and stored at 4 °C. All other commercially available reagents and solvents were used as received. Butyllithium was titrated against diphenyl acetic acid in THF prior to use.[30] Organic extracts were dried over magnesium sulfate, filtered, and concentrated with use of a rotary evaporator. Nonvolatile oils and solids were dried under vacuum at 0.01 Torr. Reactions were monitored by thin-layer chromatography on precoated E. Merck silica gel 60 F_{254} or on C18 reverse-phase plates (0.25 mm) and visualized either by short-wave UV or by staining with 10% ethanolic phosphomolybdic acid. Flash chromatography[31] was performed on E. Merck silica gel 60. NMR tubes used for kinetic measurements were soaked in concentrated NH_4OH solution for several hours and dried at 110 °C overnight. Chemical shifts are expressed in ppm relative to tetramethylsilane or the solvent signal. All melting points are uncorrected. Wild-type *B. subtilis* chorismate mutase, the R90G and R90A BsCM variants, and the catalytic antibody 1F7 were produced and purified as previously described.[9,17]

1-Methoxycarbonyl-1-[2-(methoxycarbonyl)allyl]cyclohexa-2,4-diene (4). *N*-Butyllithium (1.6 M in hexane, 1.87 mL, 3 mmol) was added to a −78 °C solution of *N*-cyclohexylisopropylamine (0.5 mL, 3 mmol) in THF (15 mL). After the solution was stirred for 15 min, methyl 3,6-dihydrobenzoate (0.4 mL, 3.1 mmol) in DMPU (0.8 mL) was added and stirring was continued for another 3 min. The dark red solution was transferred by cannula to a stirred solution of methyl 2-(bromomethyl)acrylate (0.48 mL, 3.9 mmol) in THF (20 mL) at −78 °C. After 1 min, the reaction was quenched with 1 mL of acetic acid. The solution was washed with water, and the aqueous layer was extracted with diethyl ether. The combined organic phases were washed twice with 3.2 M HCl and once with saturated $NaHCO_3$. The product was purified by flash chromatography (ethyl acetate/hexane, 1:9) to give **4** (470 mg, 64%) as a colorless oil. 300-MHz ^{1}H NMR ($CDCl_3$) δ 6.18 (d, J = 1.55 Hz, 1 H), 5.95−5.71 (m, 4 H), 5.46 (d, J = 1.24 Hz, 1 H), 3.68 (s, 3 H), 3.62 (s, 3 H), 2.74−2.54 (m, 3 H), 2.32 (dd, J = 5.4, 17.9 Hz, 1 H). 75-MHz ^{13}C NMR ($CDCl_3$) δ 174.96, 167.31, 136.09, 128.31, 128.20, 125.20, 124.18, 123.21, 51.78, 51.74, 45.58, 37.35, 30.23.

4-Hydroxy-1-methoxycarbonyl-1-[2-(methoxycarbonyl)allyl]-*trans*-3-phenylselenocyclohexa-5-ene (5). A mixture of silver trifluoroacetate (318 mg, 1.44 mmol) and phenylselenyl chloride (220 mg, 1.15 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 20 min and then cooled to −78 °C. The mixture was added rapidly to a solution of **4** (260 mg, 1.1 mmol) in CH_2Cl_2 (5 mL), and the solution was warmed to room temperature over 5 h. After adding a saturated solution of K_2CO_3 in 4:1 H_2O/MeOH (25 mL), followed by concentrated NH_4OH (10 mL), the bright yellow organic layer was separated, and the aqueous phase was extracted three times with dichloromethane. The crude product was purified by flash chromatography (acetone/hexane, 1:4) to give **5** (380 mg, 84%) as a 3:1 mixture of diastereoisomers. 300-MHz ^{1}H NMR ($CDCl_3$) δ 7.65−7.58 (m, 2 H), 7.35−7.25 (m, 3 H), 6.20 (d, J = 1.25 Hz, 0.25 H), 6.15 (d, J = 1.24 Hz, 0.75 H), 5.83−5.74 (m, 2 H), 5.46 (d, J = 0.93 Hz, 0.25 H), 5.34 (d, J = 0.93 Hz, 0.75 H), 4.02 (d, J = 9.33 Hz, 1 H), 3.70 (s, 3 H), 3.62 (s, 3 H), 3.27−3.18 (m, 1 H), 2.72−2.60 (m, 3 H), 2.35 (m, 0.5 H), 2.00 (m, 0.5 H).

Dimethyl Carbaprephenate and Dimethyl 4-*epi*-Carbaprephenate (6a, 6b). Compound **5** (370 mg, 0.9 mmol) in CH_2Cl_2 (40 mL) was cooled to 0 °C, and a solution of 30% H_2O_2 (1 mL) in THF (10 mL) was added. The mixture was stirred for 3.5 h and, after warming to room temperature, washed

(29) Shin, J. A.; Hilvert, D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2945−2948.

(30) Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879−1880.

(31) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

twice with a saturated solution of K_2CO_3. The aqueous layers were subsequently extracted twice with CH_2Cl_2. The combined organic fractions yielded crude 4-hydroxy-1-methoxycarbonyl-1-[2-(methoxycarbonyl)allyl]-*trans*-3-phenylseleninylcyclohexa-5-ene (388 mg) as an amorphous, pale yellow solid that was used without further purification. The solid was dissolved in CH_2Cl_2 (10 mL), and after addition of diethylamine (220 μL, 2.1 mmol), the solution was transferred to hexane (40 mL) at reflux. Removal of solvent after 2.5 h and flash chromatography (ethyl acetate/hexane, 1:4) afforded a mixture of dimethyl carbaprephenate and dimethyl 4-*epi*-carbaprephenate (205 mg). The two isomers were separated by preparative HPLC on a column (20 × 250 mm) packed with Kromasil 5 μm silica gel and eluted with ethyl acetate/hexane (1:2) at a flow rate of 15 mL/min. Dimethyl carbaprephenate **6a** (25 mg, 11%) and dimethyl 4-*epi*-carbaprephenate **6b** (66 mg, 29%) had retention times of 28.6 and 23.7 min, respectively.

Dimethyl carbaprephenate (6a): Viscous, colorless oil. 300-MHz ^{1}H NMR ($CDCl_3$) δ 6.18 (d, J = 1.37 Hz, 1 H), 6.01 (dd, J = 2.8, 10.50 Hz, 2 H), 5.94 (dd, J = 1.26, 10.50 Hz, 2 H), 5.46 (d, J = 1.28 Hz, 1 H), 4.37 (s, 1 H), 3.71 (s, 6 H), 2.79 (d, J = 0.69 Hz, 2 H). 75-MHz ^{13}C NMR ($CDCl_3$) δ 173.38, 167.86, 135.38, 129.93, 129.10, 128.97, 61.69, 52.56, 51.90, 48.91, 39.90. MS (EI) m/z ($M^+ - H_2O$) calcd 234.0892, found 234.0886.

Dimethyl 4-*epi*-carbaprephenate (6b): Amorphous, colorless solid. Mp 37−39 °C. 300-MHz ^{1}H NMR ($CDCl_3$) δ 6.15 (d, J = 1.24 Hz, 1 H), 5.99 (dd, J = 3.6, 10.45 Hz, 2 H), 5.88 (dd, J = 1.14, 10.45 Hz, 2 H), 5.42 (d, J = 1.15 Hz, 1 H), 4.37 (s, 1 H), 3.72 (s, 6 H), 2.84 (d, J = 0.73 Hz, 2 H). 75-MHz ^{13}C NMR ($CDCl_3$) δ 173.15, 168.41, 136.23, 129.36, 129.00, 128.53, 61.36, 52.61, 52.10, 49.05, 39.38. MS (EI) m/z ($M^+ - H_2O$) calcd 234.0892, found 234.0896.

Disodium Carbaprephenate (7a). Aqueous NaOH (1 M, 45 μL) was added to a solution of **6a** (5 mg, 20 μmol) in 0.5 mL of THF/H_2O (3:1) at 0 °C. After 30 min the solution was warmed to room temperature, stirred for 60 min, and then lyophilized. Hydrolysis was incomplete as judged by ^{1}H NMR, so the residue was redissolved in 0.5 mL of THF/H_2O (3:1), cooled to 0 °C and treated with NaOH (1 M, 45 μL). After an additional 45 min at 0 °C and 90 min at room temperature, solvent was removed by lyophilization to give **7a** as a white solid. 400-MHz ^{1}H NMR (D_2O) δ 5.91 (dd, J = 1.46, 10.35 Hz, 2 H), 5.83 (dd, J = 3.23, 10.37 Hz, 2 H), 5.58 (d, J = 1.87 Hz, 1 H), 5.13 (m, 1 H), 4.45 (m, 1 H), 2.68 (s, 2 H). 100-MHz ^{13}C NMR (D_2O) δ 183.99, 180.16, 145.73, 134.81, 129.88, 124.64, 64.28, 54.44, 43.99. MS (ESI) m/z $(M + Na)^-$ calcd 245.0426, found 245.0427.

Disodium 4-*epi*-Carbaprephenate (7b). Aqueous NaOH (1 M, 45 μL) was added to a solution of **6b** (5 mg, 20 μmol) in 0.5 mL of THF/H_2O (3:1) at 0 °C. After being stirred for 30 min, the solution was warmed to room temperature and stirred for another 60 min. Lyophilization afforded **7b** as a pale yellow solid. 400-MHz ^{1}H NMR (D_2O) δ 5.83 (dd, J = 1.57, 10.37 Hz, 2 H), 5.74 (dd, J = 3.13, 10.39 Hz, 2 H), 5.53 (d, J = 1.75 Hz, 1 H), 5.11 (m, 1 H), 4.37 (m, 1 H), 2.67 (s, 2 H). 100-MHz ^{13}C NMR (D_2O) δ 183.72, 180.49, 145.85, 134.62, 129.21, 124.32, 64.13, 54.36, 43.12. MS (ESI) m/z $(M + Na)^-$ calcd 245.0426, found 245.0426.

6-Hydroxytricyclo[3.3.1.0^{2,7}]non-3-en-1,3-dimethyl Ester (10a, 10b). Solutions of dimethyl carbaprephenate (5 mg, 20 μmol) and dimethyl 4-*epi*-carbaprephenate (5.2 mg, 21 μmol) in CD_3OD (0.6 mL) were sealed in NMR tubes and heated to 60 °C in a thermostated oil bath. ^{1}H NMR spectra were recorded daily for the first two weeks and, subsequently, once a week. After completion of the reaction, solvent was evaporated to yield the products in quantitative yield.

***endo*-6-Hydroxytricyclo[3.3.1.0^{2,7}]non-3-en-1,3-dimethyl ester (10a):** Viscous colorless oil. 500-MHz ^{1}H NMR (CD_3OD) δ 7.44 (dd, J = 2.11, 6.72 Hz, 1 H), 3.94 (d, J = 4.23 Hz, 1 H), 3.89 (m, 1 H), 3.75 (s, 3 H), 3.60 (s, 3 H), 3.40 (mb, 1 H), 2.53 (m, 1 H), 2.05 (m, 1 H), 1.83 (dd, J = 3.73, 12.73 Hz, 1 H), 1.35−1.32 (m, 1 H), 1.22 (d, J = 9.70 Hz, 1 H). 125-MHz ^{13}C NMR δ 176.34, 167.02, 147.45, 132.78, 73.31, 52.41, 52.14, 46.64, 41.00, 40.74, 40.10, 37.36, 32.98. MS (EI) m/z $(M + H)^+$ 223.

***exo*-6-Hydroxytricyclo[3.3.1.0^{2,7}]non-3-en-1,3-dimethyl ester (10b):** Viscous colorless oil. 500-MHz ^{1}H NMR (CD_3OD) δ 7.51 (dd, J = 2.35, 7.42 Hz, 1 H), 3.85 (m, 1 H), 3.73 (s, 3 H), 3.61 (s, 3 H), 3.53 (m, 1 H), 2.93 (mb, 1 H), 2.30 (dd, J = 3.83, 12.39 Hz, 1 H), 2.22−2.17 (m, 2 H), 2.00 (d, J = 8.51 Hz, 1 H), 1.36 (dd, J = 2.09, 12.41 Hz, 1 H). 125-MHz ^{13}C NMR δ 176.52, 167.16, 149.05, 131.84, 70.33, 52.38, 52.21, 47.32, 42.660, 41.81, 33.77, 31.62, 31.41. MS (EI) m/z $(M + H)^+$ 223.

Kinetics. NMR tubes for kinetic measurements were soaked in concentrated ammonia for several hours and dried overnight at 110 °C. After sample loading, the tubes were sealed and heated to the desired temperature in a thermostated oil bath. Rearrangement of compounds **6a** and **6b** in CD_3OD at 60 °C was monitored daily for the first week and weekly afterward. Decarboxylation of compounds **7a** and **7b** (3 mM) was monitored spectrophotometrically at 255 nm at pH 3 (100 mM citrate buffer), 7 (70 mM phosphate buffer), 8.6 (50 mM Tris-HCl), and 10 (70 mM phosphate buffer). This reaction was also monitored by NMR in deuterated phosphate buffer (ca. 70 mM, pD 10) at 30, 45, 60, 75, and 90 °C, respectively. Rearrangement to 3-(2-carboxyallyl)benzoic acid **(11)** was observed under the latter conditions but to a minor extent (<5%) that could not be quantified accurately by NMR. Carrying out the reaction in 50 mM phosphate buffer at pH 12 or in 1 M NaOH resulted predominantly in conversion to **11** within 35 h at 90 °C as judged by analytical HPLC (Waters Nova-Pak C-18, 10% to 90% acetonitrile over 30 min with 0.1% TFA in water, using *p*-ethoxybenzoic acid as an internal standard).

Enzymatic Reactions. The enzymatic reactions were carried out in Tris-HCl buffer (50 mM, pH 8.6) in a total volume of 0.5 mL. Substrate **7a** (880 μM) was incubated with high concentrations of antibody (22 μM) or enzyme (100 μM) at 30 °C and the product distribution was analyzed periodically by analytical HPLC. The reaction mixture (10 μL) was separated on a Waters Nova-Pak C18 column (30 min gradient from 10% to 90% acetonitrile in 0.1% TFA, total flow 1 mL/min). Product distribution was determined at 232 nm by integrating peak areas for **11**, the dehydrated product of the Cope rearrangement (retention time 10.1 min), and 2-benzyl-2-propenic acid (retention time 13.4 min), the product of decarboxylation. *p*-Ethoxybenzoic acid was used as an internal standard (retention time 11.9 min).

Enzyme Inhibition. The BsCM-catalyzed rearrangement of chorismate was performed in 50 mM potassium phosphate buffer at pH 7.5 by monitoring the decrease in absorption at 274 nm as previously described.[9] The concentrations of BsCM and chorismate were 50 nM and 50 μM, respectively. Inhibitor **7a** was present at concentrations of 10, 100, and 1000 μM.

Acknowledgment. This work was supported in part by the Swiss National Science Foundation and Novartis Pharma AG. We are grateful to Dr. F. Oesterhelt from F. Hoffmann-La Roche AG (Basel, Switzerland) for high-resolution ESI mass spectra and Dr. A. Flohr for helpful discussions.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **6a**, **6b**, **7a**, **7b**, **10a**, and **10b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO026096S