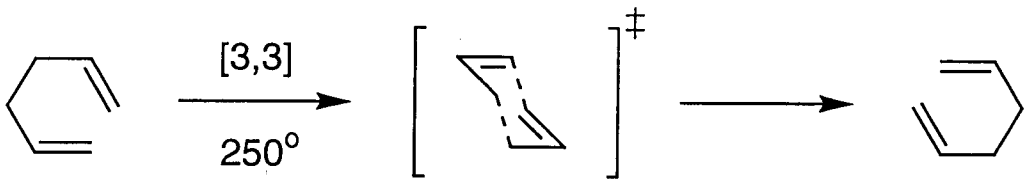
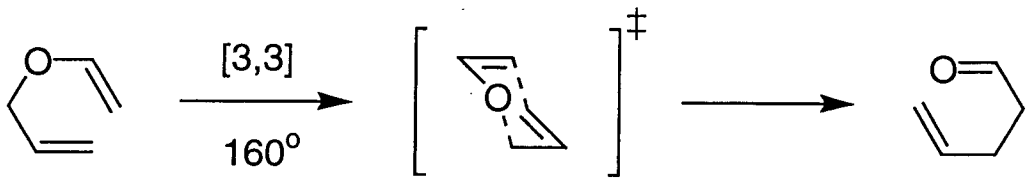
The Mechanism of Chorismate Mutase

- Physical organic chemistry has found huge number of new problems in the realm of enzymology.
- However, it is often overlooked that enzymes are simply macromolecular catalysts, not black boxes.
- Both are governed by the same physical organic principals which can fully describe the *chemical* processes mediated by the enzyme.

Cope versus Claisen Rearrangements



ΔH = 33.5 kcal / mol
ΔS = -13.8 eu

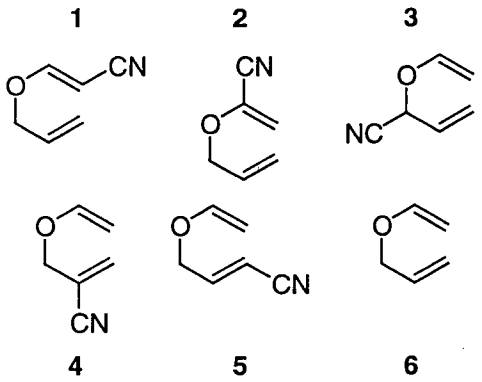


ΔH = 30.6 kcal / mol
ΔS = -7.7 eu

- [3,3] sigmatropic shifts favor chair-like transition states:
  - Cope: ΔG = 5.7 kcal / mol
  - Claisen: ΔG = 3.0 kcal / mol
- Only a small change in activation energy is observed despite an overall 17 kcal / mol exothermicity, suggesting an early transition state.
- The decreased energy difference observed in the Claisen process is most likely due to a looser transition state when compared to the Cope rearrangement.

Gajewski *J. Am. Chem. Soc.* **1979**, *101*, 6693.

Substituent Effects on the Claisen Rearrangement



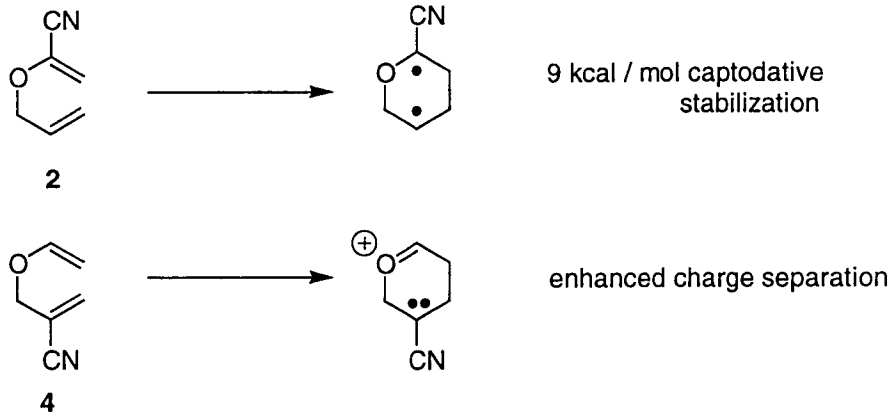


Table I. Kinetic Data for Compounds 1-6

compd	temp range, °C	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/(mol K)	k_{rel}, 100 °C
1	124–174	27.08 ± 0.09	−11.6 ± 0.2	0.90
2	66–115	22.84 ± 0.19	−13.4 ± 0.5	111
3	55–101	22.33 ± 0.20	−13.0 ± 0.6	270
4	90–140	22.58 ± 0.26	−18.0 ± 1.3	15.6
5	135–185	28.76 ± 0.54	−11.2 ± 1.2	0.11
6	113–173	25.40 ± 0.65	−15.9 ± 1.5	(1)

Table I. Predictions of Qualitative Substituent Effects on the Aliphatic Claisen Rearrangement[a]

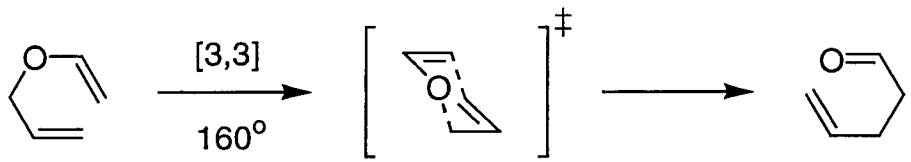
substituent location	$\Delta\Delta E_{\pi}$	
	D	A
1	−	+
2	−	−
3	−	−
4	+	(+)[b]
5	+	+

[a] D represents a π-donor substituent, and A represents a π acceptor. A negative sign means that the substituent should lower $\Delta H^{\ddagger}$ while a positive sign means that the substituent should raise $\Delta H^{\ddagger}$. [b] See text for explanation.

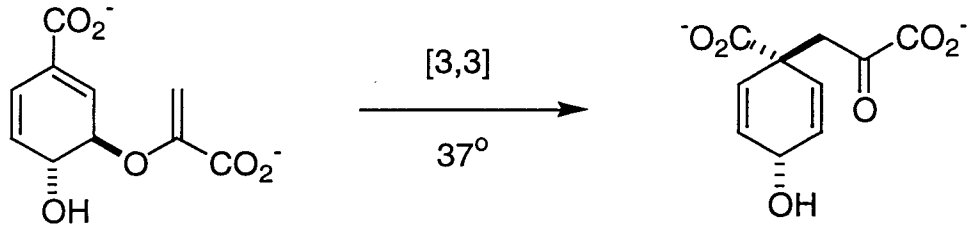
- Installation of electron withdrawing substituents accelerates the reaction by:
  - stabilizing the 2,5 diyl intermediate
  - polarizing the substrate
- This leads to a greater degree of bond formation in the transition state.

Carpenter *J. Am. Chem. Soc.* **1981**, *103*, 6983.

Claisen Rearrangements



ΔH = 30.6 kcal / mol
ΔS = -7.7 eu
k = 1.98 x 10^{-5} s^{-1}



ΔH = 20.5 kcal / mol
ΔS = -12.9 eu
k = 2.6 x 10^{-5} s^{-1}

chorismate

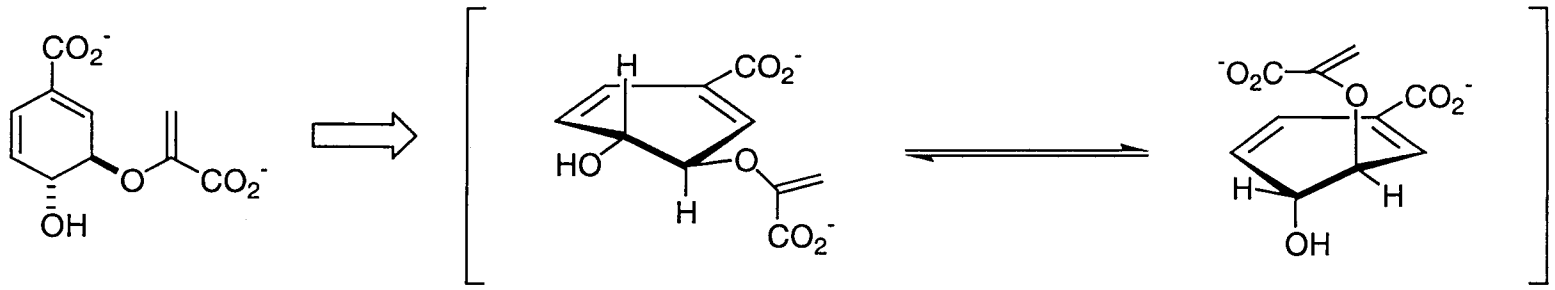
prephenate

- Relative to the parent Claisen rearrangement of allyl vinyl ether (AVE), chorismate rearranges at a greatly enhanced rate, even in the absence of the enzyme.
- What structural factors contribute to the facility of this rearrangement?
  - Conformation
  - Electronic Perturbation

Gajewski *J. Am. Chem. Soc.* **1979**, *101*, 6693.

Smith *Biochemistry* **1973**, *12*, 3492.

Solution Structure of Chorismate



psuedo-equatorial
$J_{a,b}$ = 12.6 Hz

psuedo-axial
$J_{a,b}$ = 2.8 Hz

- NMR reveals an 88 : 12 mixture of equatorial and axial conformers in H_2O at 25°C.
- In methanol, the increase in this ratio is attributed to enhanced H-bonding between the hydroxyl and carboxylate groups.
- This leads to a 100 fold attenuation in the rate of the rearrangement.
- This H-bond is responsible for the large energetic difference between the conformers in chorismate.

Table III. Conformational Preferences and Relative Rearrangement Rates of Chorismic Acid, Chorismate, and 4-*O*-Methylchorismate

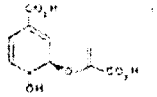
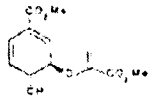
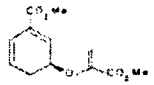
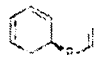
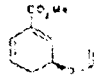
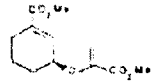
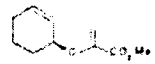
compound	solvent[a]	proportion of pseudo-diequatorial conformer[b]	k_{H_2O}/k_{MeOH}[c]
chorismate[d]	water	~0.88	100
chorismate	methanol	>0.98	
chorismic acid	water	~0.83	11
chorismic acid	methanol	~0.87	
4-*O*-methylchlorismate[d]	water	~0.60	7
4-*O*-methylchorismate	methanol	~0.65	

[a] Perdeuterio solvents were used for NMR measurements of conformational equilibria. Unlabeled solvents were used for rearrangement rate measurements. [b] 25 °C. [c] 50 °C. [d] Bis(tetra-*n*-butylammonium) salt.

Knowles *J. Am. Chem. Soc.* **1987**, *109*, 5008.

Substituent Effects in Chorismate

Table II. Relative Rate Constants for Rearrangement and Elimination of Chorismate Analogues

compound	k_{rel} (rearr)[a]	k_{rel} (elim)[a]
C1 	[1]	0.50
C6 	0.78	0.40
C7 	76	66
C8 	~10[5]	~10[6]
C9 	2.4	3.1
C10 	0.002	
C11 	0.09	

[a] All rate constants determined in 2:1 *v:v* methanol/water at 75 °C. [b] Instability of compound C8 precluded accurate determination of rate constants.

- Formation of the esters leads to a minor decrease in rate.
- Again, installation of the hydroxyl group leads to a drastic decrease in rate.
- However, these increases in the rate of rearrangement are counterbalanced by a loss of stability towards elimination.

Gajewski *J. Am. Chem. Soc.* **1987**, *109*, 1170.

Isotope Effects in the Enzyme Catalyzed Reaction

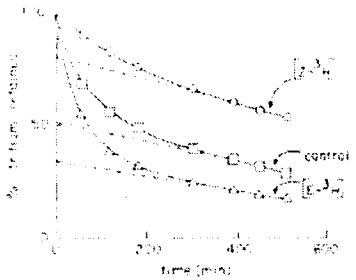


Figure 1. Rate of tritium loss from incubations of enzymically produced[5] (*Z*)-[9-^{3}H]chorismate (○), (*E*,*Z*)-[9-^{3}H]chorismate (□), and (*E*)-[9-^{3}H]chorismate (△) with chorismate mutase and phenylpyruvate tautomerase at pH <6.

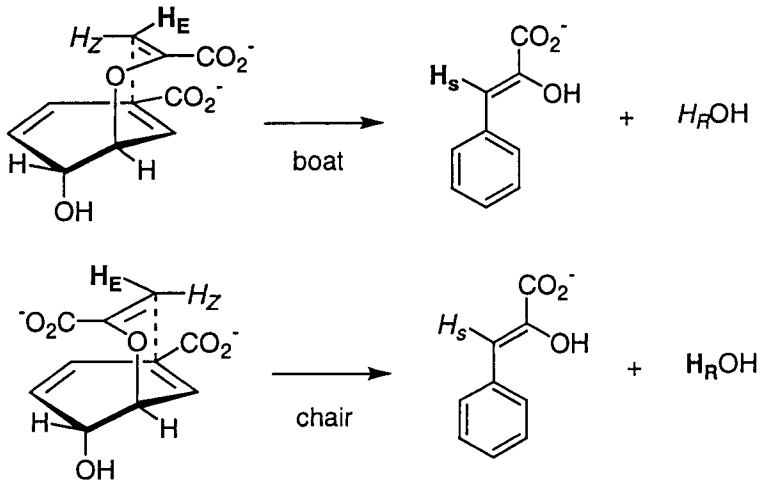
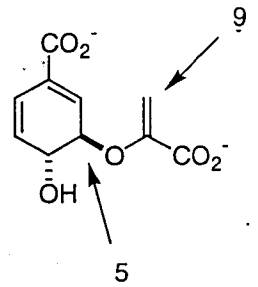


Table II. Tritium Kinetic Isotope Effects on the Nonenzymic and Enzymic Rearrangement of [5-^{3}H,7-^{14}C]- and [9-^{3}H,7-^{14}C]Chorismate[a]

rearrangement	position of ^{3}H label	r'	r	extent of reaction (%)	k_H/k_T
nonenzymic	5	1.106 ± 0.004	0.909 ± 0.002	50.1	1.149 ± 0.012
	5	1.072 ± 0.003	0.905 ± 0.003	42.8	
	9	1.003 ± 0.003	*b*	48.3	0.992 ± 0.012
	9	0.989 ± 0.004	*b*	43.7	
enzymic	5	0.987 ± 0.003	0.984 ± 0.004	39	1.003 ± 0.020
	5	1.016 ± 0.004	0.983 ± 0.005	25	
	5	0.984 ± 0.002	1.014 ± 0.003	68.9	
	9	1.005 ± 0.005	0.988 ± 0.003	46.5	1.012 ± 0.004

[a] r' and r are as defined under Results. [b] Under the conditions of the thermal reaction, the tritium label is not stably bound in the product due to enolization. In this case, therefore, the isotope effect is obtained only from the specific radioactivity of the recovered substrate.

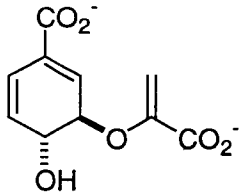


EIE = 1.21 (75°)

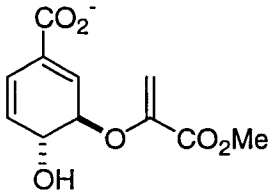
- Lack of KIE in enzymatic reaction suggests rearrangement is not the RDS.
- Small inverse KIE (k_H / k_D = 0.969) at 4 position is related to a conformational bias.

Knowles *Biochemistry* **1983**, *22*, 4494.

Structural Requirements for Catalysis

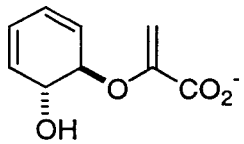


K_m = 0.14 mM
k_{cat} = 29 s^{-1}
ERA = 2.3 x 10^6



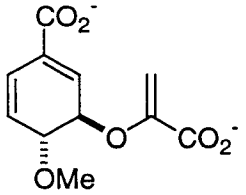
no promotion

not an inhibitor

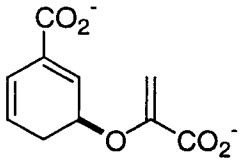


no promotion

K_i = 0.4 mM



K_m = 1.9 mM
k_{cat} = 0.56 s^{-1}
ERA = 2.0 x 10^4

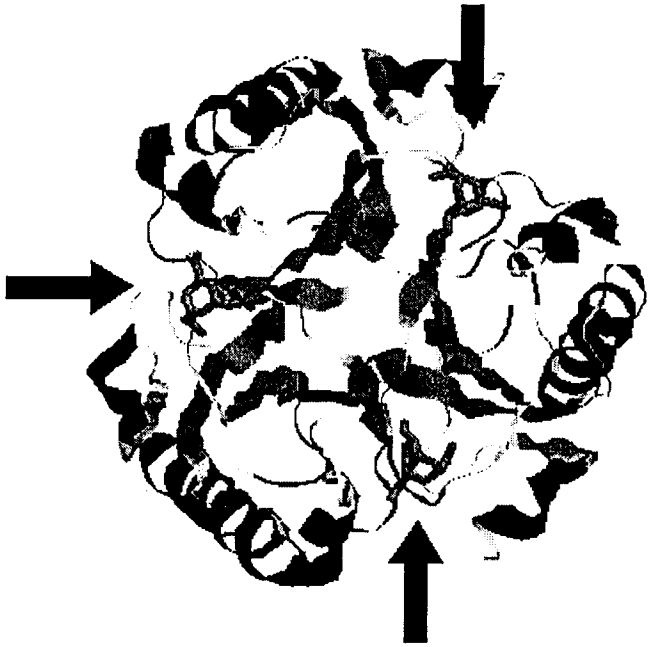


K_m = ND
k_{cat} = 0.35 s^{-1}
ERA ~ 100

- Both carboxylates are required for catalysis by the enzyme.
- The contribution of the free hydroxyl at C4 actually has a negative effect on the reaction rate (possibly a conformation bias).
- The C4 oxygen functionality is not required for catalysis, which:
  - Casts doubt on Paths C and D
- This leaves Paths A, B and E.

Berchtold *J. Am. Chem. Soc.* **1989**, *111*, 3374.

Crystal Structure of the Enzyme-Inhibitor Complex



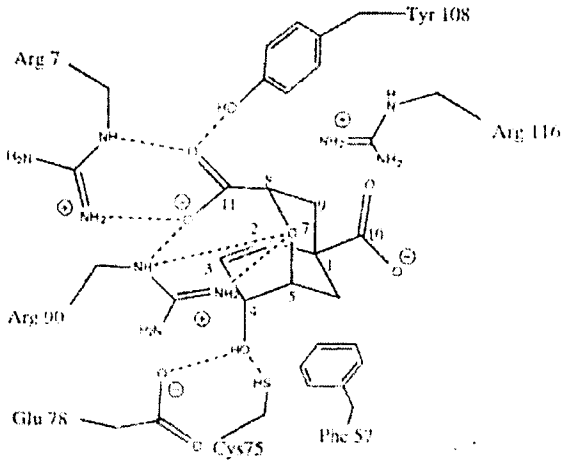
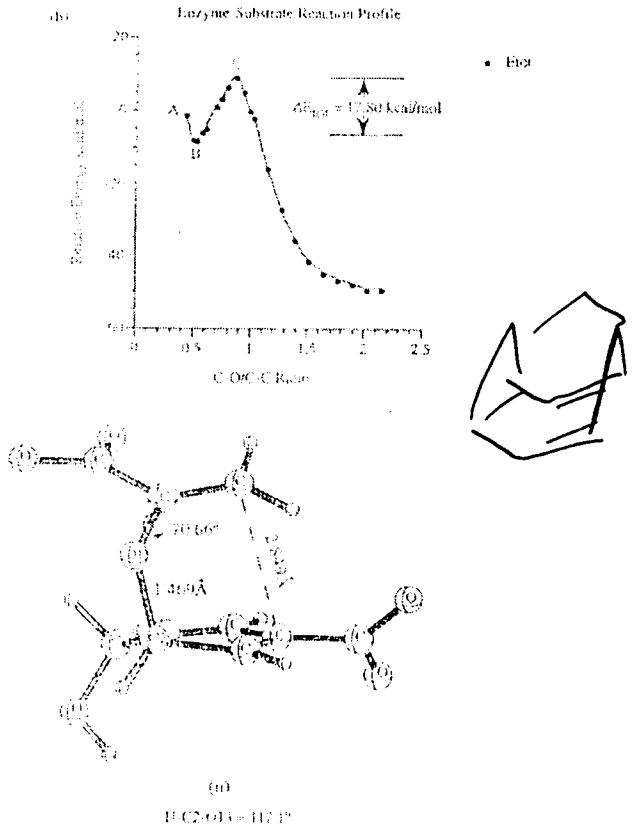


Figure 1. Schematic diagram of transition state analogue 3 bound in the BsCM active site.

- The enzyme is a monofunctional homotrimer.
- Three binding sites exist in the interfaces between the individual chains.
- The active site is identified by a positively charged wall flanked by some closely spaced aromatic residues.
- Little conformational change is observed around the active site upon binding of the inhibitor.

Lipscomb *Proc. Natl. Acad. Sci.* 1993, *90*, 8600.

Spatiotemporal Principle



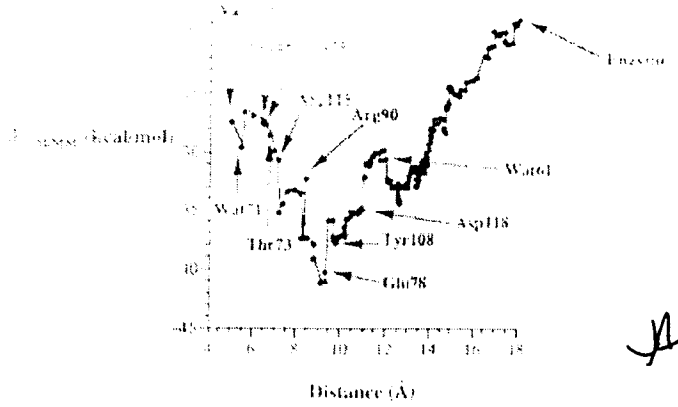


Figure 3. Estimate of the residue contributions to the energy along the reaction pathway. The abcissa represents an ordering of the amino acid residues by distance of their center of mass from the reaction center which has been defined as C4 of chorismate (refer to Figure 1 for numbering). The ordinate corresponds to the difference in E_{system}[7] of the minimum structure (point B in Figure 2) and the maximum structure (point C in Figure 3). The profile was constructed by sequentially deleting amino acid residues in decreasing order of their distance from the reaction center and recalculating the energy after each deletion. Protein corresponds to the energy difference of the entire simulation system; "vacuum" refers to the energy difference of the system after all the MM atoms have been deleted; specific points have been labeled and correspond to the energy difference after the residue indicated has been deleted.

- Two major structural changes exist between the gas phase transition state and the enzyme bound transition state:
  - ΔC-O = +0.03 Å
  - ΔC-C = -0.449 Å
- This indicates that compression is an important part of catalysis, not only conformational restriction.

Menger JACS, 1999, 11831