Thus, we have synthesized actinobolin stereoselectivity in 18 steps from glyoxylate **5**. We are currently in the process of preparing **5** in enantiomerically pure form and hope to use advanced intermediates **11** and/or **12** in the synthesis of bactobolin (**2**).

Acknowledgment. We thank the National Cancer Institute (CA-34303) for financial support.

An Inhibitor of Chorismate Mutase Resembling the Transition-State Conformation

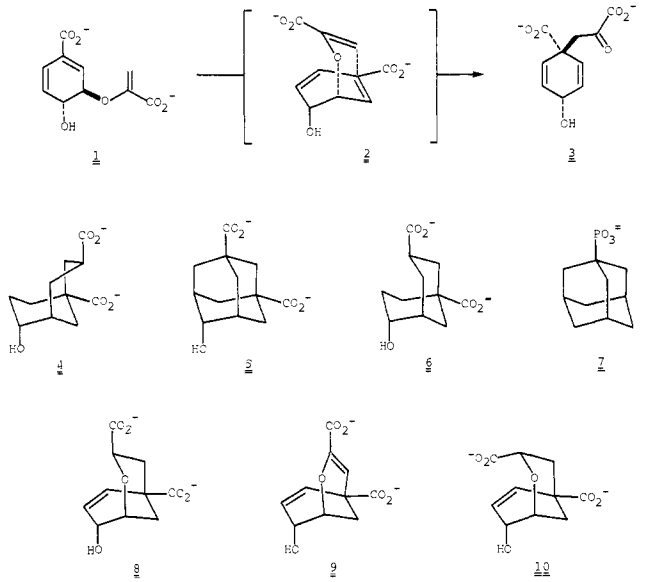
Paul A. Bartlett* and Charles R. Johnson

Department of Chemistry, University of California Berkeley, California 94720

Received July 26, 1985

The chorismate mutases are appealing targets for the design of enzyme inhibitors, since they lie on a pathway that is key for the biosynthesis of aromatic amino acids in plants and microorganisms.[1] These enzymes are unique not only for catalyzing what is formally a Claisen rearrangement[2] but also for the fact that the conversion of chorismic acid **1** to prephenic acid **3** has a *unimolecular* solution counterpart. The relationship between substrate binding forces and enzymatic rate acceleration can therefore be explored without the complications that arise from imperfect comparisons of multimolecular (solution) with unimolecular (enzymatic) transformations.[3] Indeed, it can be argued that the chorismate mutases are the ideal targets for transition-state analogue inhibitors, in that the enzymatic rate acceleration should be reflected in enhanced binding of a "perfect" transition-state analogue in comparison to substrate.[4] This factor could be as much as 2×10^6.[5]

Although a number of molecules designed or rationalized to mimic the putative transition-state conformation **2** have been



reported,[6-8] none is bound to chorismate mutase significantly more tightly than chorismic acid itself (Table I).[9] The most potent inhibitor reported to date is adamantane-1-phosphonic acid (**7**), which Chao and Berchtold have reported to have a ratio of (inhibitor I_{50})/(chorismate K_m) of 0.05 at pH 9.[7] Since the hydroxybicyclo[3.3.1]nonane- and hydroxybicyclo[3.3.1]-adamantanedicarboxylic acids **5** and **6** incorporate the polar functionality of **2** and yet are not tightly bound, Andrews et al. reasoned that the orientation of these groups is crucial.[6] In this paper, we describe syntheses of the oxabicyclic derivatives **8-10** and our discovery that the endo isomer **10** is the most potent inhibitor of chorismate mutase yet reported.

Table I. Selected Inhibitors of Mutase Activity of Chorismate Mutase/Prephenate Dehydrogenase

inhibitor	I_{50},[a] M (conditns[b])	I_{50}/K_m
4	$>2.5 \times 10^{-3}$ (A)	>20
5	1.3×10^{-3} (A)	12
6	7.8×10^{-4} (A)	7
7	4×10^{-4} (B)[c]	25
	7×10^{-5} (C)	0.05

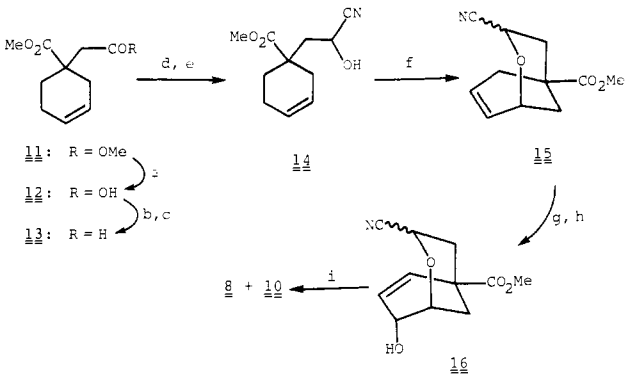
[a] I_{50} is defined as the concentration of inhibitor giving 50% inhibition when substrate concentration equals K_m; for linear competitive inhibitors, $I_{50} = 2K_i$. [b] (A) *E. coli* enzyme, pH 7.5, K_m(chorismate) = 1.1×10^{-4} M; ref 6; (B) *E. coli* enzyme, pH 6.0, K_m(chorismate) = 1.5×10^{-5} M; ref 8; (C) *A. aerogenes* enzyme, pH 9.0, K_m(chorismate) = 1.3×10^{-3} M; ref 7. [c] I_{50} value computed from $K_i = 2 \times 10^{-4}$ M.

Table II. Comparison of Oxabicyclo[3.3.1]nonenes **8-10** and Adamantane-1-phosphonic Acid **7** as Inhibitors of Chorismate Mutase/Prephenate Dehydrogenase[a]

inhibitor	I_{50}, M	I_{50}/K_m
8	5.9×10^{-5}	3.3
9	1.7×10^{-5}	0.9
7	5.5×10^{-6}	0.31
10	1.5×10^{-7}	0.008

[a] *E. coli* enzyme, pH 7.5, K_m(chorismate) = 1.8×10^{-5} M (this value determined in the presence of 0.1 mg/mL of bovine serum albumin[15]).

Scheme I[a]



[a] (a) NaOH/MeOH/H_2O, 92%; (b) ClCOCOCl/CH_2Cl_2, 95%; (c) $(Ph_3P)_2CuBH_4$/acetone, 78%; (d) Me_3SiCN/ZnI_2, 71%; (e) HCl/aqueous THF, 82%; (f) *N*-PhSe-phthalimide/*p*-TSA/CH_2Cl_2/-78 °C, then *t*-BuOOH, 83%; (g) *m*-CPBA/CH_2Cl_2/reflux, 99%; (h) $Me_3SiBr/Ph_3P/CH_2Cl_2$; DBU/MeCN/60 °C; HCl/aqueous THF, 77%; (i) KOH/H_2O/reflux, (100%).

The synthetic route leading to the racemic inhibitors **8** and **10** is outlined in Scheme I. Ester acid **12** obtained from controlled hydrolysis of the Diels-Alder adduct **11** is converted to the aldehyde **13** by the method of Fleet[10] and thence to the cyanohydrin **14** as described by Evans.[11] Selenocyclization[12] of **14** followed

(1) Ganem, B. *Tetrahedron* **1978**, *34*, 3353-3383. Haslam, E. "The Shikimate Pathway"; Halstead Press: New York, 1974.

(2) Gibson, F. *Biochim. J.* **1964**, *90*, 256. Young, I. G.; Gibson, F.; MacDonald, C. G. *Biochim. Biophys. Acta* **1969**, *192*, 62-72. Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492-3498. 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.

(3) Jencks, W. P. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 219-410. Jencks, W. P. *Mol. Biol., Biochem. Biophys.* **1980**, *32*, 3-25.

(4) Wolfenden, R. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 271-306. Schray, K.; Klinman, J. P. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 641-648.

(5) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492-3498. Goerisch, H. *Ibid.* **1978**, *17*, 3700-3705; Addadi, L.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494-4501.

(6) Andrews, P. R.; Cain, E. N.; Rizzardo, E.; Smith, G. D. *Biochemistry* **1977**, *16*, 4848-4852.

(7) Chao, H. S. I.; Berchtold, G. A. *Biochemistry* **1982**, *21*, 2778-2781.

(8) Christopherson, R. I.; Heyde, E.; Morrison, J. F. *Biochemistry* **1983**, *22*, 1650-1656.

(9) In view of the fact that chorismate mutases from different souces were employed and that inhibitors were assayed under different conditions, we present the ratio of (inhibitor I_{50})/(substrate K_m) as a normalized comparison of different inhibitors.

(10) Fleet, G. W. J.; Harding, P. J. *Tetrahedron Lett.* **1979**, 975-978.

0002-7863/85/1507-7792$01.50/0

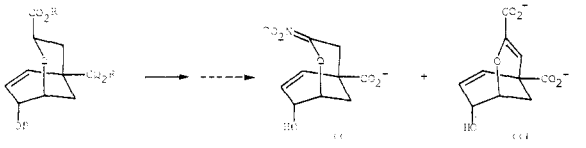
by oxidative elimination lead exclusively to the bicyclo[3.3.1] regioisomer **15** as a mixture of nitrile epimers. As precedented by work in the shikimate series,[13] this material undergoes epoxidation with a peracid from the exo face and furnishes the desired allylic alcohol **16** on rearrangement via the bromohydrin silyl ether. Alkaline hydrolysis of **16** gives a 6:1 mixture of the exo and endo isomers **8** and **10**. In addition to the expected predominance of the more stable exo isomer **8**, the stereostructure of these compounds was readily assigned on the basis of the NMR coupling pattern of the hydrogens at the 3-position: exo isomer **8**, dd, J = 3.2, 12.2 Hz; endo isomer **10**, dd, J = 3.0, 7.2 Hz. An additional compound, the unsaturated derivative **9**, was obtained on hydrolysis of a side product from a related synthesis.[14]

Compounds **8–10** as well as adamantane-1-phosphonic acid (**7**) were evaluated as inhibitors against the chorismate mutase/prephenate dehydrogenase from *E. coli*. The assays were performed at pH 7.5 using conditions similar to those reported by SampathKumar and Morrison.[15] The results detailed in Table II indicate that the exo and unsaturated derivatives **8** and **9** are not significantly better as inhibitors than their saturated carbocyclic analogue **6**. In contrast, the endo isomer **10** is bound some 100-fold more tightly, with an I_{50} value of 1.5×10^{-7} M at pH 7.5. The true K_i value could therefore be as low as 4×10^{-8} M for the active enantiomer. At this pH adamantane-1-phosphonate is a considerably weaker inhibitor than **10**. The crucial element in the efficacy of the endo isomer **10** is its chair conformation and the resulting orientation of the bridge-carboxylate moiety over the unsaturated ring. In contrast to the endo isomer of the saturated carbocycle **4**,[6] which adopts the chair–boat conformation for steric reasons, ^{1}H NMR analysis indicates that the tetrahydropyran ring of **10** is in the chair conformation as shown.[16] Although the binding enhancement observed with **10** falls short of that expected for a "perfect" transition-state analogue, these results confirm the supposition that orientational effects are critical for a chorismate mutase inhibitor and point the way for future improvements.

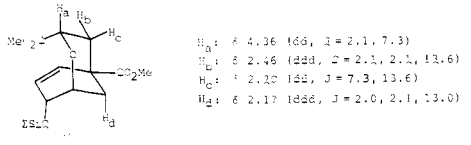
Acknowledgment. We thank Professor Jeremy Knowles (Harvard University) for generously providing us with the *E. coli* chorismate mutase/prephenate dehydrogenase. This research was funded by grants from Merck Sharp and Dohme and the National Institutes of Health (GM-28965); their support is gratefully acknowledged.

Supplementary Material Available: Experimental details of the synthesis of **8** and **10** and their enzymatic evaluation (8 pages). Ordering information is given on any current masthead page.

(11) Evans, D. A.; Truesdale, L. K. *J. Chem. Soc. Chem. Commun.* **1973**, 55–56.
(12) Nicolaou, K. C. *Tetrahedron* **1981**, *37*, 4097–4109.
(13) Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854–7860.
(14) Nitration of the enolate of i was undertaken in an attempt to produce ultimately the nitronate analogue ii. The unsaturated compound iii was



derived from a side product of this reaction.
(15) SampathKumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 212–219.
(16) The NMR datum most indicative of the chair conformation is the long-range W coupling of 2.1 Hz between H_b and H_d observed for the derivative iv



(Dr. Y. Nakagawa, unpublished results). A similar albeit less well-resolved pattern is seen for the corresponding hydrogens of the dianion **10**.

Hydration of Chloride and Bromide Anions: Determination of Relative Free Energy by Computer Simulation

Terry P. Lybrand, Indira Ghosh, and J. Andrew McCammon*

Department of Chemistry, University of Houston—University Park, Houston, Texas 77004
Received July 5, 1985

Computer-simulation techniques that can reliably predict relative free energies of reactions have great potential usefulness in chemistry, biochemistry, and pharmacology. Such techniques could be used to calculate relative solubilities, relative free energies of binding for ligand–receptor complexes, and relative free energies of activation (i.e., relative reaction rates). In particular, the ability to calculate relative free energies of solvation is of special interest. For example, relative free energies of solvation (or more precisely, relative free energies of *desolvation*) often play a major role in determining the relative binding affinity of two ligands at a common receptor site.

One simulation technique used to compute the free energy of reaction is the umbrella sampling technique.[1-4] In this approach, one uses molecular dynamics or Monte Carlo simulations to compute the free energy change as a function of reaction advancement along some predefined reaction coordinate. This method has been used to study molecular association complexes[1-3] and a chemical reaction[4] in water. In principle, this method could be used to predict relative free energies of solvation for two molecules L and M by, e.g., gradually immersing the molecules

$$\mathrm{L(g)} \rightarrow \mathrm{L(aq)} \qquad \Delta A_1 \qquad (1)$$

$$\mathrm{M(g)} \rightarrow \mathrm{M(aq)} \qquad \Delta A_2 \qquad (2)$$

and computing ΔA_1 and ΔA_2 in separate simulations. The relative free energy of solvation, $\Delta\Delta A = \Delta A_2 - \Delta A_1$, would then be computed as the difference of the two simulation results, ΔA_1 and ΔA_2. The umbrella sampling technique possesses several shortcomings, however, which limit its usefulness in relative free energy calculations.[5]

An alternative simulation approach applies perturbation theory techniques to a set of reactions forming a closed thermodynamic cycle in order to compute relative free energies of reaction.[5] The type of free energy obtained (e.g., Helmholtz free energy A, or Gibbs free energy G) depends on the type of ensemble used in the simulation (see below). In the perturbation–thermodynamic cycle approach, two hypothetical reactions would be defined:

$$\mathrm{L(g)} \rightarrow \mathrm{M(g)} \qquad \Delta A_3 \qquad (3)$$

$$\mathrm{L(aq)} \rightarrow \mathrm{M(aq)} \qquad \Delta A_4 \qquad (4)$$

Reactions 1–4 form a closed thermodynamic cycle; thus, $\Delta\Delta A = \Delta A_2 - \Delta A_1 = \Delta A_4 - \Delta A_3$, since A is a thermodynamic state function. A perturbation technique[5-7] is used to compute ΔA_4 and, if necessary, ΔA_3. Potential energy functions V_L for the L/solvent system, V_M for the M/solvent system, and V_λ for a "hybrid" system are defined, where

$$V_\lambda = \lambda V_M + (1 - \lambda) V_L \qquad (5)$$

Molecular dynamics or Monte Carlo simulations based on one or more of these potential functions are then carried out. For each simulation, the free energy for values of λ about λ_i is obtained from the perturbation result

(1) Pangali, C.; Rao, M.; Berne, B. J. *J. Chem. Phys.* **1979**, *71*, 2975.
(2) Ravishanker, G.; Mezei, M.; Beveridge, D. L. *Faraday Symp. Chem. Soc.* **1982**, *17*, 79.
(3) Berkowitz, M.; Karim, O. A.; McCammon, J. A.; Rossky, P. J. *Chem. Phys. Lett.* **1984**, *105*, 577.
(4) Chandrasekhar, J.; Smith, S. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1985**, *107*, 154.
(5) Tembe, B. L.; McCammon, J. A. *Comput. Chem.* **1984**, *8*, 281.
(6) McQuarrie, D. A. "Statistical Mechanics"; Harper and Row: New York, 1976.
(7) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; Hermans, J. In "Intermolecular Forces"; B. Pullman, Ed.; Reidel: Holland, 1981.

0002-7863/85/1507-7793$01.50/0