experiments are in progress to determine whether 19,19-difluoroandrost-4-ene-3,17-dione has in vivo activity. Although the inactivation by this compound occurs more slowly than that caused by other reported inactivators of aromatase (Covey et al., 1981; Metcalf et al., 1981), the difluoromethyl group is chemically stable and resistant to enzymatic degradative processes. Specific action in vivo as a result of hydroxylation at C-19 of the steroid structure by aromatase is therefore a possibility.

Acknowledgments

We thank Joseph Bauer for technical assistance, Joseph Cousins and Dr. L. S. Kan for the 300-MHz NMR spectra obtained at the Johns Hopkins Biomedical Research Center (National Institutes of Health Grant GM 27512), and Sharon Pallante, David Heller, and Dr. C. Fenselau for mass spectra obtained at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation shared instrumentation facility.

References

Berkoz, B., Denot, E., & Bowers, A. (1963) *Steroids 1*, 251–269.

Blohm, T. R., Metcalf, B. W., Laughlin, M. E., Sjoerdsma, A., & Schatzman, G. L. (1980) *Biochem. Biophys. Res. Commun. 95*, 273–280.

Brodie, A. M. H., Schwarzel, W. C., Shaikh, A. A., & Brodie, H. J. (1977) *Endocrinology (Philadelphia) 100*, 1684–1695.

Brodie, A. M. H., Marsh, D. A., & Brodie, H. J. (1979) *J. Steroid Biochem. 10*, 423–429.

Brodie, A. M. H., Garrett, W. M., Hendrickson, J. R., Morris, C.-H. T., Marcotte, P. A., & Robinson, C. H. (1981) *Steroids 38*, 693–702.

Covey, D. F., Hood, W. F., & Parikh, V. D. (1981) *J. Biol. Chem. 256*, 1076–1079.

Jen, T., & Wolff, M. E., (1963) *J. Org. Chem. 28*, 1573–1575.

Kalvoda, J., Heusler, K., Ueberwasser, H., Anner, G., & Wettstein, A. (1963) *Helv. Chim. Acta 46*, 1361–1369.

Kitz, R., & Wilson, I. B. (1962) *J. Biol. Chem. 237*, 3245–3249.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Marcotte, P. A., & Robinson, C. H. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 1867.

Metcalf, B. W., Wright, C. L., Burkhart, J. P., & Johnston, J. O. (1981) *J. Am. Chem. Soc. 103*, 3221–3222.

Middleton, W. J. (1975) *J. Org. Chem. 40*, 574–578.

Morato, T., Hayano, M., Dorfman, R. I., & Axelrod, L. R. (1961) *Biochem. Biophys. Res. Commun. 6*, 334–338.

Motohashi, T., Wu, C. H., Abdel-Rahman, H. A., Marymor, N., & Mikhail, G. (1979) *Am. J. Obstet. Gynecol. 135*, 89–95.

Reed, K. C., & Ohno, S. (1976) *J. Biol. Chem. 251*, 1625–1631.

Ryan, K. J. (1959) *J. Biol. Chem. 234*, 268–272.

Schade, K., & Schubert, K. (1979) *Endokrinologie 74*, 90–99.

Schwarzel, W. C., Kruggel, W., & Brodie, H. J. (1973) *Endocrinology (Philadelphia) 92*, 866–880.

Thompson, E. A., & Siiteri, P. K. (1974) *J. Biol. Chem. 249*, 5364–5372.

Inhibition of Chorismate Mutase Activity of Chorismate Mutase-Prephenate Dehydrogenase from *Aerobacter aerogenes*†

Herbert S.-I. Chao and Glenn A. Berchtold*

ABSTRACT: Inhibition data (I_{50} values) have been obtained for inhibitors of the chorismate mutase activity of chorismate mutase-prephenate dehydrogenase from *Aerobacter aerogenes*. Several 1-substituted adamantane derivatives were investigated; the order of decreasing inhibitory activity with the various substituents was $-PO_3^{2-} \gg -P(OCH_3)O_2^- > -CO_2^- > -CH_2CO_2^- > -SO_2^- > -SO_3^-$. 3-Chloroadamantane-1-acetic acid was slightly less effective than adamantane-1-acetic acid. 2-(1-Carboxy-1,4-dihydrobenzyl)acrylic acid (**19**), an analogue of prephenate, was an effective inhibitor. Other substances investigated, including 2,4,10-trioxaadamantane-1-acetic acid (**15**), 5-enolpyruvylshikimic acid (**20**), and 1-(carboxyethyl)-1,4-dihydrobenzoic acid (**18**), failed to inhibit chorismate mutase activity under the conditions investigated.

The classic work of Gibson that established the importance of chorismic acid in the biosynthesis of aromatic amino acids, and numerous other substances, in bacteria, fungi, and higher plants has been reviewed (Gibson & Pittard, 1968; Haslam, 1974; Lingens, 1968; Weiss & Edwards, 1980). Of the metabolic transformations available to chorismate (**1**), the rearrangement to prephenate (**2**), catalyzed by chorismate mutase, has attracted most attention since such enzyme-catalyzed reactions, formally oxy-Cope rearrangements, are rare in biochemical systems. The transformation **1** → **2** (Figure 1) is the first step from the chorismate branch point for the biosynthesis of tyrosine and phenylalanine in the plant world.

Chorismate mutase exists as a monofunctional enzyme in many species of microorganisms and higher plants. *Streptomyces aureofaciens* possesses such a mutase (Görisch & Lingens, 1973, 1974; Görisch, 1978). The occurrence of bifunctional enzymes with chorismate mutase activity is common. The enteric bacteria *Aerobacter aerogenes* and *Escherichia coli*, for example, have a chorismate mutase-prephenate dehydrogenase and a chorismate mutase-prephenate dehydratase. The rearrangement of **1** to **2** occurs in the ab-

†From the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. *Received September 22, 1981; revised manuscript received February 10, 1982.* We are grateful to the National Institutes of Health (Grant GM 19103) for financial support.

0006-2960/82/0421-2778$01.25/0

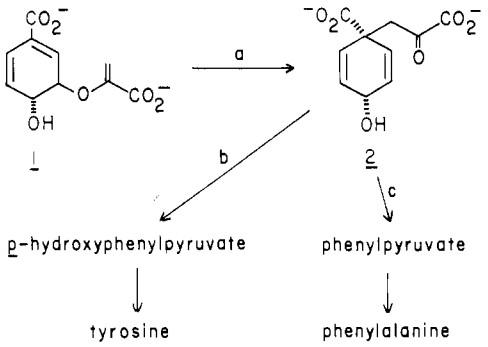

FIGURE 1: Biosynthesis of tyrosine and phenylalanine from chorismate: (a) chorismate mutase; (b) prephenate dehydrogenase, NAD^+; (c) prephenate dehydratase.

sence of enzymatic catalysis; chorismate mutase-prephenate dehydrogenase from *A. aerogenes* enhances the reaction rate by a factor of 1.9×10^6 at pH 7.5, 37 °C (Andrews et al., 1973).

Since the chorismate pathway is unique to the plant world, the discovery of effective inhibitors of chorismate mutase, prephenate dehydrogenase, or other enzymes in the chorismate scheme could provide a new approach to antibacterial agents, antifungal agents, or herbicides. It has not been possible to determine the inhibition pattern for CO_2 with chorismate mutase-prephenate dehydrogenase from *A. aerogenes* (Heyde & Morrison, 1978). One possibility for suicide inactivation of chorismate mutase, prephenate dehydrogenase, or prephenate dehydratase involves replacement of the ring carboxyl group of **1** or **2** with substituents such as $-PO_3^{2-}$, $-P(OCH_3)O_2^-$, or $-SO_3^-$. Fragmentation analogous to the decarboxylation reaction would afford an electrophilic species (PO_3^-, CH_3OPO_2, or SO_3) that might reasonably be expected to undergo covalent bond formation at the active site and effect inactivation. Since **5** is an effective inhibitor of chorismate mutase, we decided to compare initially the effect on inhibition of chorismate mutase activity by replacing the carboxylate group of adamantane-1-carboxylate with other acid anions ($-PO_3^-$, $-SO_3^-$, etc.). Described below are the results obtained from investigation of various adamantane derivatives and other substances as inhibitors of the mutase activity of chorismate mutase-prephenate dehydrogenase from *A. aerogenes* poly 3.

Experimental Procedures

Melting points were determined with a Thomas-Hoover Unimelt and are corrected. ^1H NMR spectra were obtained at 60 MHz with a Perkin-Elmer R-24B spectrometer. Chemical shift values (δ) are reported in parts per million downfield from tetramethylsilane. Infrared spectra were determined with a Perkin-Elmer 238B spectrophotometer. Ultraviolet spectra and kinetic measurements were obtained with a Perkin-Elmer 552 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

Chorismic acid (**1**) was prepared as described previously (Gibson, 1970) from *A. aerogenes* kindly provided by F. Gibson. Adamantane-1-carboxylic acid (**5**), adamantane-1-acetic acid (**7**), and glutaric acid (**21**) were purchased from Aldrich Chemical Co. Adamantane-1,3-dicarboxylic acid (**6**) was a gift from Dr. H. F. Gilbert, Biochemistry Department, Baylor College of Medicine. The following were available by literature procedures: 3-chloroadamantane-1-acetic acid (**10**) (Aigami et al., 1975), adamantane-1-phosphonic acid (**11**) (Stetter & Last, 1969), adamantane-1-sulfinic acid (**13**) (Stetter et al., 1969), adamantane-1-sulfonic acid (**14**) (Stetter et al., 1969), 2,4,10-trioxaadamantane-1-acetic acid (**15**) (Bohlman & Sucrow, 1964), and propane-1,3-diphosphonic acid (**22**) (Moedritzer & Irani, 1961). 2-(1-Carboxy-1,4-dihydrobenzyl)acrylic acid (**19**) was a gift from Dr. H. Pan, Department of Chemistry, M.I.T. (−)-5-Enolpyruvylshikimic acid (compound Z, **20**) was a gift from D. A. McGowan, Department of Chemistry, M.I.T.

Methyl Adamantane-1-phosphonate (**12**). Adamantane-1-phosphonic dichloride (7.0 g, 28 mmol) (Stetter & Last, 1969) was dissolved in 150 mL of methanol, and sodium methoxide (15.0 g) was added portionwise. The mixture was refluxed under nitrogen for 7 h. After the mixture was cooled, the solid material was removed by filtration, and the filtrate was concentrated. The residue was dissolved in 100 mL of carbon tetrachloride and washed with 100 mL of water. The aqueous layer was acidified to pH 2 by dropwise addition of 0.1 N HCl to precipitate **12** which was recrystallized from ethyl acetate to afford 4.1 g (63%) of pure **12**: mp 181–182 °C; IR (KBr) 2900, 1450, 1340 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.75–1.97 (m, 15 H), 3.67 (d, 3 H), 11.56 (s, 1 H). Anal. Calcd for $C_{11}H_{19}O_3P$: C, 57.38; H, 8.32; P, 13.45. Found: C, 57.40; H, 8.39; P, 13.44. Dimethyl adamantane-1-phosphonate [1.1 g (16%), mp 47–48 °C] could be obtained from the carbon tetrachloride solution.

1,4-Dihydrobenzenephosphonic Acid (**16**). Diethyl benzenephosphonate (15 g, 70 mmol) (Toy, 1948) was dissolved in 350 mL of ammonia at −78 °C, and lithium (1.2 g) was added in small pieces. The mixture was stirred for 0.5 h after addition was complete. The mixture was carefully quenched with ammonium chloride until the red-blue color disappeared. A few milligrams of hydroquinone was added, and the ammonia was allowed to evaporate. The residue was taken up into 200 mL of $CHCl_3$, and the solution was washed with 40 mL of water. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Distillation of the residue gave 10.1 g (67%) of diethyl 1,4-dihydrobenzenephosphonate, bp 92–94 °C (0.25 mmHg), that contained less than 5% starting material. To 1.9 g of the diethyl ester in 10 mL of CH_2Cl_2 was added 3 mL of bromotrimethylsilane. The mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was stirred with 10 mL of water until the solution became clear. The solvent was removed under vacuum, and the residue was recrystallized from ether to afford **16**: mp 142–144 °C; IR (KBr) 1300, 1200 (br), 1080 (br), 1010 (br) cm^{-1}; ^1H NMR (acetone-d_6) δ 2.2–3.1 (m, 2 H), 3.50 (br s, 1 H), 5.74 (br s, 4 H), 10.80 (s, 2 H). Anal. Calcd for $C_6H_9O_3P$: C, 45.01; H, 5.67; P, 19.35. Found: C, 45.17; H, 5.86; P, 19.42.

1-Ethyl-1,4-dihydrobenzenephosphonic Acid (**17**). The monoethyl ester of **17** was obtained by rearrangement of the anion of diethyl 1,4-dihydrobenzenephosphonate (generated with *n*-butyllithium in tetrahydrofuran at −78 °C) during an attempt to effect 1,4 addition of the anion to diethyl vinylphosphonate at room temperature. A similar rearrangement is reported in the literature (Redmore, 1969). The crude material was converted to the phosphonic acid with bromotrimethylsilane, as described above for preparation of **16**. Recrystallization from ether afforded pure **17** (35%): mp 149 °C; IR (KBr) 3030, 1020, 1000 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.80 (t, 3 H), 1.80 (m, 2 H), 2.30–2.95 (br d, 2 H), 5.15–6.20 (m, 4 H), 8.5 (br s, 2 H). Anal. Calcd for $C_8H_{13}O_3P$: C, 51.07; H, 6.96; P, 16.46. Found: C, 51.07; H, 7.16; P, 16.18.

1-(Carboxyethyl)-1,4-dihydrobenzoic Acid (**18**). The dimethyl ester of **18** (1.9 g) (Subba Rao et al., 1980) was stirred overnight with 10 mL of 10% aqueous NaOH at room temperature. The solution was diluted with 5 mL of water and

Table I: Inhibition of Mutase Activity of Chorismate Mutase-Prephenate Dehydrogenase from *A. aerogenes*

inhibitors	I_{50} (mM)[a] [1] = $1K_M^{app}$	[1] = $3K_M^{app}$
adamantanes		
5	1.2	2.2
6		[b]
7	1.6	
10	2.1	3.4
11	0.070	0.40
12	0.75	1.2
13	2.2	4.3
14	8.0	13.2
others		
15-18, 20	[c]	
19	1.3	1.5
21, 22	[b]	

[a] I_{50} is the inhibitor concentration that gives 50% inhibition with the substrate concentration indicated. [b] Acted as an activator under conditions investigated. [c] No inhibition observed; highest concentration used (mM): 15, 13.0; 16, 10.0; 17, 22.4; 18, 13.4; 20, 7.5.

acidified with 5% HCl to pH 1 to precipitate the diacid as white crystals that were filtered, washed with water, and dried to afford 0.75 g (78%) of pure **18**: mp 163–164 °C; IR (KBr) 1720, 1410, 1270 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.10 (m, 4 H), 2.66 (br, 2 H), 5.84 (m, 4 H), 8.10 (br, 2 H). Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.01; H, 5.96.

Chorismate Mutase-Prephenate Dehydrogenase from A. aerogenes Poly 3. A. aerogenes poly 3 was a gift from Professor F. Gibson, Department of Biochemistry, The John Curtin School of Medical Research, Canberra City, Australia. Cells of *A. aerogenes* poly 3 were grown as previously described (Cotton & Gibson, 1970). Cell extracts were prepared by a French press in tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.5) containing L-tyrosine, ethylenediaminetetraacetic acid (EDTA), and dithiothreitol (1 mM). The enzyme preparation was carried out as previously described (Ife et al., 1976). The enzyme stock solution was made by suspending the precipitate in the buffer solution used for extraction (2 mL/10 g of cells). The specific activity for chorismate mutase was 0.42 unit/mg. The K_M^{app} for **1**, 1.3 mM, was determined from double-reciprocal plots which were linear.

Assay of Chorismate Mutase Inhibition. Stock solutions of **1** and inhibitors were prepared by dissolving appropriate quantities in Tris-HCl buffer (0.1 M, pH 9.0) containing 1 mM EDTA. A solution of **1** (100 μL) was placed in a disposable culture tube, followed by addition of inhibitor solution, and the total volume was brought to 290 μL by addition of buffer solution. The solution temperature was maintained at 30 °C, and 10 μL of enzyme solution (30 °C) was added. After being quickly mixed (~30 s), the solution was transferred to a cuvette (1-mm path length) and placed in the thermostatted cell compartment (30 °C) of the UV spectrophotometer. Initial rate data were obtained by following the absorbance of **1** at 278 nm. Because plots of $1/v$ vs. [I] were not linear, we have chosen to describe inhibition results in terms of I_{50} values obtained from the plots where I_{50} represents [I] required to give 50% inhibition at substrate concentration equal to $1K_M^{app}$ or $3K_M^{app}$.

Results

Double-reciprocal plots of the mutase activity of chorismate mutase-prephenate dehydrogenase from *A. aerogenes* are linear (Koch et al., 1970; Heyde & Morrison, 1978). The K_M^{app} for chorismate that we observed at pH 9 was 1.3 mM,

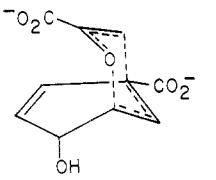

FIGURE 2: Chairlike transition state for chorismate → prephenate.

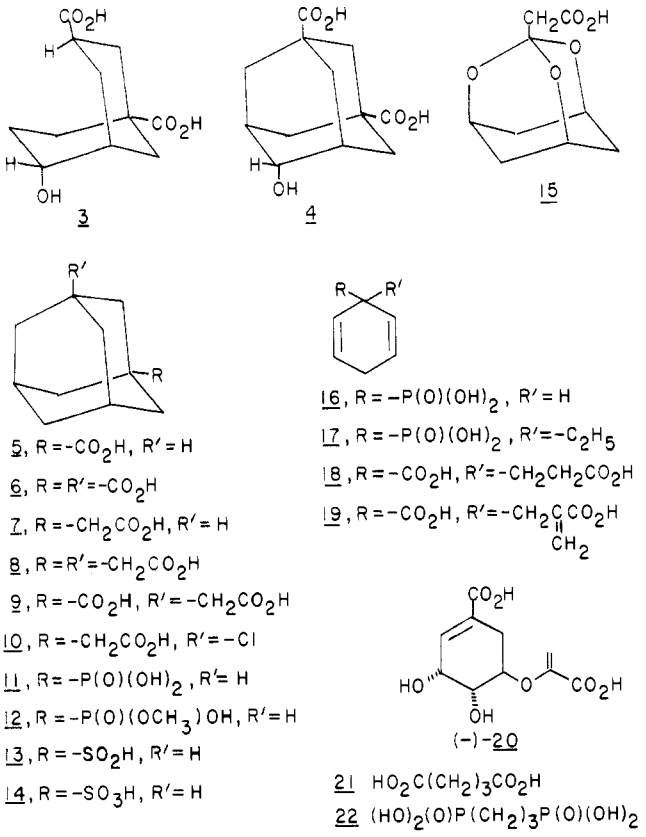

FIGURE 3: Chemical structures of substances investigated as inhibitors of mutase activity of chorismate mutase-prephenate dehydrogenase from *A. aerogenes* (work herein) and *E. coli* (Andrews et al., 1977).

in agreement with the value reported previously (Koch et al., 1970; Ife et al., 1976), but greater than the value of 0.12 mM observed at pH 7.5 after more extensive purification (Heyde & Morrison, 1978). The substances investigated as inhibitors of the mutase activity are listed in Table I. Attempts to determine the K_i^{app} for inhibitors by following the initial velocity as a function of chorismate concentration at various inhibitor concentrations gave results that were not consistent with simple competitive inhibition, so we have described inhibition in terms of I_{50} values which were obtained from plots of 1/velocity vs. inhibitor concentration at a chorismate concentration of $1K_M^{app}$ and $3K_M^{app}$ and represent the concentration of inhibition for 50% inhibition under the conditions investigated. Morrison (1981) has observed that **5** is a slow-binding inhibitor of chorismate mutase-prephenate dehydrogenase from *E. coli*. Slow-binding inhibition may be general for the inhibitors described in our work; and consequently, the relative effectiveness observed for the inhibitors is limited to the precise conditions described.

Discussion

Investigation of the isomeric, racemic 6-hydroxybicyclo-[3.3.0]nonane-1,3-dicarboxylic acids as transition-state analogues for the mutase activity of chorismate mutase-prephenate dehydrogenase from *E. coli* supports a chairlike transition state (Figure 2) for **1** → **2** (Andrews et al., 1977; Andrews, 1979).

Isomer **3** (Figure 3) and the corresponding adamantane analogue (**4**) were effective inhibitors, but other isomers of 6-hydroxybicyclo[3.3.1]nonane-1,3-dicarboxylic acid were not inhibitors of mutase activity. Adamantane derivatives **5–9** were inhibitors with K_i^{app} = 0.15, 5.5, 0.12, 0.62, and 1.3, respectively. Comparison of the K_i^{app} (IC_{50}) values of Andrews et al. (1977) with our I_{50} values listed in Table I indicates that **5** and **7** show essentially the same inhibitory effect on the mutase activity of the enzyme from both microorganisms. Introduction of a chlorine atom at C-3 of adamantane-1-acetic acid (**10**) resulted in only a slight decrease in inhibitory activity compared to **7**.

Adamantane-1-phosphonic acid (**11**) was the most potent inhibitor of mutase activity observed in this study. At a chorismate concentration equal to K_M^{app}, **11** gave 50% inhibition at a concentration $\sim^1/_{20}$ of that required to observe similar inhibition by **5**. The monomethyl ester of adamantane-1-phosphonic acid (**12**) was also a potent inhibitor. Although it was significantly less effective than **11**, it was more effective than **5**. Adamantane-1-sulfinic acid (**13**) was a weaker inhibitor than **5**, and the corresponding sulfonic acid (**14**) was a poor inhibitor. The order of decreasing effectiveness as inhibitor in the monosubstituted adamantane derivatives, $-PO_3^{2-} \gg -P(OCH_3)O_2^- > -CO_2^- > -CH_2CO_2^- > -SO_2^- > -SO_3^-$, suggests that increasing the polar and hydrophilic character of the anionic substituent enhances binding to the enzyme.

The adamantane hydrocarbon skeleton appears to play a significant role in the binding to chorismate mutase-prephenate dehydrogenase. The importance of van der Waals interactions between the adamantane ring and the enzyme from *E. coli* has been discussed (Andrews et al., 1977). Our studies confirm that such binding is equally important with the enzyme from *A. aerogenes*. Whereas **7** was a good inhibitor, the more polar skeletal derivative, trioxaadamantane-1-acetic acid (**15**), failed to inhibit mutase activity at a concentration as high as 13.0 mM. In addition, although **11** was a potent inhibitor, cyclohexadienephosphonic acid analogues **16** and **17** were ineffective as inhibitors under our experimental conditions.

Prephenate analogue **18** also failed to inhibit mutase activity, but introduction of a methylene group on the side chain provided a prephenate analogue (**19**) that was as effective an inhibitor as adamantane-1-carboxylic acid. The difference in inhibitor activity of **18** and **19** is surprising and would not be expected to be the consequence of the small difference in electron density at the side-chain carboxylate groups.

Synthetic (−)-5-enolpyruvylshikimic acid (**20**), a secondary transformation product in the biosynthesis of chorismic acid, was not an inhibitor of mutase activity. Ife et al. (1976) found that glutaric acid (**21**) was ineffective as an inhibitor of mutase activity of chorismate mutase-prephenate dehydrogenase from *A. aerogenes*. Under the conditions of our investigation, **21** and phosphonic acid analogue **22** were weak activators of chorismate mutase activity.

Andrews (1979) has presented a thorough analysis of possible transition-state analogues for inhibition of chorismate mutase activity based on his studies with carboxylic acid derivatives of adamantane and bicyclo[3.3.1]nonane. In view of the potent inhibitory activity we observed for phosphonic acid **11** compared to the corresponding carboxylic acid and acetic acid derivatives, phosphonic acid analogues should be considered in the search for more potent transition-state inhibitors of chorismate mutase activity. Phosphonic acid analogues of chorismate or prephenate may show active-site binding, and if so, it is conceivable that they could be suicide inactivators.

Acknowledgments

We thank Professor C. T. Walsh for technical assistance and Professor A. J. Sinskey for technical assistance and use of facilities for culture growth.

References

Aigami, K., Inamoto, Y., Takaishi, N., & Hattori, K. (1975) *J. Med. Chem. 18*, 713–721.

Andrews, P. R. (1979) *ACS Symp. Ser. No. 112*, 149–159.

Andrews, P. R., Smith, G. D., & Young, I. G. (1973) *Biochemistry 12*, 3492–3498.

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

Bohlman, F., & Sucrow, W. (1964) *Chem. Ber. 97*, 1839–1845.

Cotton, R. G. H., & Gibson, F. (1970) *Methods Enzymol. 17A*, 567–568.

Gibson, F. (1970) *Methods Enzymol. 17A*, 362–364.

Gibson, F., & Pittard, J. (1968) *Bacteriol. Rev. 32*, 465–492.

Görisch, H. (1978) *Biochemistry 17*, 3700–3705.

Görisch, H., & Lingens, F. (1973) *J. Bacteriol. 114*, 645–651.

Görisch, H., & Lingens, F. (1974) *Biochemistry 13*, 3790–3794.

Haslam, E. (1974) *The Shikimate Pathway*, Wiley, New York.

Heyde, E., & Morrison, J. F. (1978) *Biochemistry 17*, 1573–1580.

Ife, R. J., Ball, L. F., Lowe, P., & Haslam, E. (1976) *J. Chem. Soc., Perkin Trans. 1*, 1776–1783.

Koch, G. L. E., Shaw, D. C., & Gibson, F. (1970) *Biochim. Biophys. Acta 212*, 375–386.

Lingens, F. (1968) *Angew. Chem., Int. Ed. Engl. 7*, 350–360.

Moedritzer, K., & Irani, R. R. (1961) *J. Inorg. Nucl. Chem. 22*, 297–304.

Morrison, J. F. (1981) Biochemistry Seminar, Massachusetts Institute of Technology, Cambridge, MA.

Redmore, D. (1969) *J. Org. Chem. 34*, 1420–1425.

Stetter, H., & Last, W.-D. (1969) *Chem. Ber. 102*, 3364–3366.

Stetter, H., Michael, K., & Last, W.-D. (1969) *Chem. Ber. 102*, 3357–3363.

Subba Rao, G. S. R., Ramanathan, H., & Raj, K. (1980) *J. Chem. Soc., Chem. Commun.*, 315–316.

Toy, A. D. F. (1948) *J. Am. Chem. Soc. 70*, 186–188.

Weiss, U., & Edwards, J. M. (1980) *The Biosynthesis of Aromatic Compounds*, Wiley, New York.