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ON THE MECHANISM OF CHORISMATE MUTASES: REVISITING STRUCTURAL REQUIREMENTS FOR CATALYSIS

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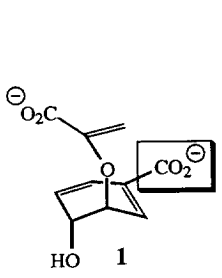
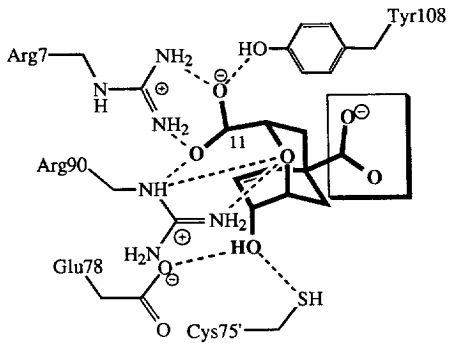
Abstract: The behavior of nor-chorismic acid **7** has now been tested against three different chorismate mutases. Notably, **7** is not a substrate for *Bacillus subtilis* mutase, but is a weak competitive inhibitor (K_I = 0.5 mM).

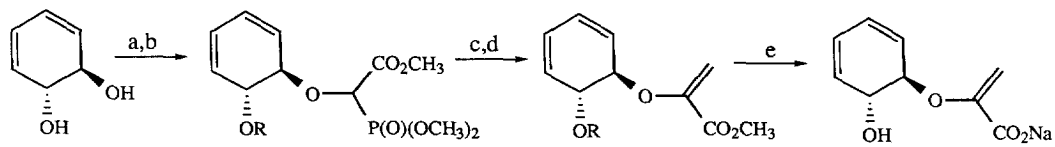
The Claisen rearrangement of chorismic acid **1**, which is a central step in the biosynthesis of phenylalanine and tyrosine in bacteria, fungi, and higher plants, has intrigued chemists and biochemists for almost forty years.[1] In 1989, Pawlak et al. reported that besides the allyl vinyl ether in **1**, the only structural features absolutely required for binding and catalysis by the *Escherichia coli* enzyme (EcCM) were the two carboxylic acid groups.[2] Recent X-ray crystallographic studies on EcCM,[3] as well as on mutases from *Bacillus subtilis* (BsCM)[4] and *Saccharomyces cerevisiae* (ScCM)[5] suggest a number of common structural, and perhaps catalytic, features.[6] One intriguing difference involves the environment of the ring carboxyl group (highlighted) of **1**. Unlike EcCM and ScCM, whose active sites circumscribe **1** with stabilizing electrostatic and H-bonding interactions, the binding pocket of BsCM is a shallow surface cleft that orients the substrate's alicyclic carboxyl towards bulk water. Because that carboxyl makes no apparent contact with active site residues on the protein, judging from the crystal structure, we wondered whether BsCM exhibited the same absolute requirement for both carboxylic acid groups as EcCM. Here we report an improved synthesis of the previously reported[2] nor-chorismic acid (±)-**7** and a kinetic examination of its role as a substrate or inhibitor of BsCM.





Active Site of BsCM
Cocrystallized with Inhibitor

Rhodium-catalyzed insertion of trimethylphosphonodiazoacetate with the known[7] diol **2** afforded **3** (75%) which, after protection as its silyl ether **4** (98%), underwent smooth olefination to give **5** (73% after chromatography). After desilylation of **5** using fluorosilicic acid, hydroxyester **6** (64%) was saponified directly to **7** (99%), whose spectroscopic data matched reported values.[2] The 5-step conversion of **2** to **7** in 46% overall yield represented a significant improvement over the earlier 4-step route to **7** in 10% overall yield.



2 **3** R = H, **4** R = TBDMS, **5** R = TBDMS, **6** R = H, **7**

(a) $CH_3O_2CC(N_2)PO(OCH_3)_2$ (1.3 equiv), cat $Rh_2(OCOC_7H_{15})_4$, C_6H_6, 80 °C, 2 h; (b) TBDMSCl (1.2 equiv), imidazole (1.5 equiv), DMF, rt, 4 h; (c) $LiN(TMS)_2$ (1.2 equiv), THF, -20 °C, 10 min, then CH_2=O (20 equiv); (d) H_2SiF_6 (3 equiv) CH_3CN, rt, 45 min; (e) NaOH (1 equiv), THF-H_2O, 0 °C, 30 min.

Pawlak et al. reported that **7** underwent a facile Claisen rearrangement/dehydration to phenylpyruvate, with a rearrangement half-life of 1.7 h (30 °C, pD 7.4). Rearrangement was not catalyzed by the *E. coli* bifunctional enzyme chorismate mutase-prephenate dehydrogenase, but **7** was found to be a modest competitive inhibitor of the mutase component (K_I = 0.4 mM; K_m = 0.16 mM for **1**).[2] We measured a rearrangement half-life of 3.1 ± 0.3 h (37 °C, pH 7.8) for **7**. Using standard assay conditions[8] [37 °C, 50 mM tris (pH 7.8), 2.5 mM EDTA, 20 mM β-mercaptoethanol, 0.1 mg/mL BSA], we determined that **7** was not a substrate for the 109-residue N-terminal chorismate mutase domain engineered from the bifunctional *E. coli* chorismate mutase-prephenate dehydratase.[3] In further accord with earlier work, **7** proved to be a modest competitive inhibitor of the monofunctional EcCM (K_I = 0.4 mM; K_m = 0.32 mM for **1**). At concentrations up to 1 mM, **7** was not a substrate for BsCM, but was a weak competitive inhibitor of the enzyme (K_I = 0.5 mM; K_m = 0.28 mM for **1**).

Besides establishing similarities between both *E. coli* mutases, our findings indicate that the ring carboxyl group of **1** is essential for mutase activity in BsCM. In this context we call attention to studies by Cload et al. who found that mutagenesis of Arg116, which is proximal to the exposed face of the BsCM active site, substantially increased K_m while only weakly affecting k_{cat}.[9] That region, which is poorly resolved crystallographically, might well be the locus of important electrostatic and H-bonding interactions.

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