Synthesis of a Potential Transition-State Analogue Inhibitor of Isochorismate Synthase

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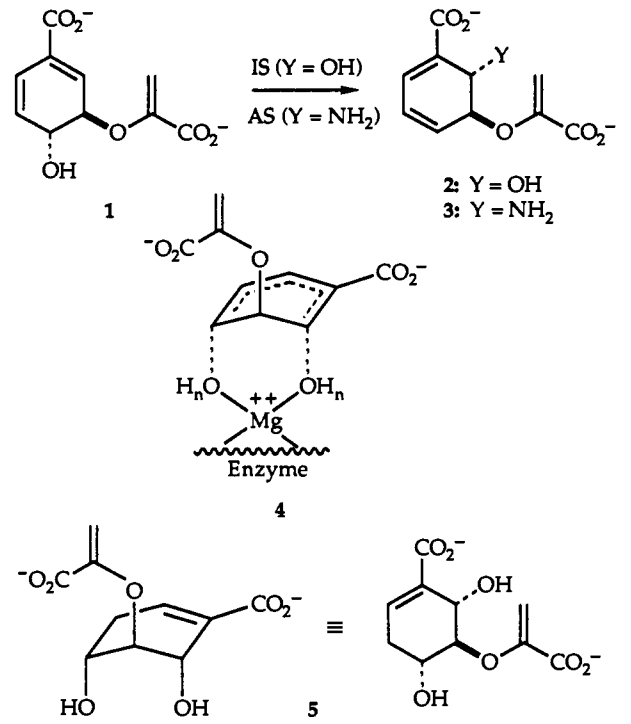
The conversion of chorismic acid (**1**) to isochorismic acid (**2**) occurs at a branch in the shikimate pathway that leads to enterobactin, menaquinone, salicyclic acid, (*m*-carboxyphenyl)alanine, and the arene oxides.[1,2] The intriguing 1,5-substitution reaction involved in this conversion is catalyzed by isochorismate synthase (IS, EC 5.4.99.6).[3] ^{18}O labeling studies have shown that the incoming hydroxyl is derived from solvent, not via direct transfer from the 4-position; moreover, the enzyme requires divalent magnesium for activity.[3] Several mechanisms consistent with these data have been proposed, including (1) concerted (S_N2'') or stepwise (S_N1'') displacement mediated by magnesium chelation (**4**, with neutral or cationic character in the pentadienyl system), (2) Michael-type addition followed by 1,4-elimination (**4**, with anionic character), and (3) S_N2' attack at C-2 by an enzymatic "X"-group or the enolpyruvyl carboxylate, followed by S_N2' displacement of this moiety by attack at C-6.[4]

Anthranilate synthase (AS) and *p*-aminobenzoate synthase (PABS) show significant sequence similarity to IS,[5,6] and their chorismate-binding subunits catalyze similar transformations. AS catalyzes the conversion of chorismate to anthranilate via *trans*-6-amino-5-[(1-carboxyethenyl)oxy]-1,3-cyclohexadiene-1-carboxylic acid (**3**); i.e., the amino version of the 1,5-substitution catalyzed by IS.[7-9] As a bisubstrate analogue that is potentially capable of mimicking the transition-state species **4**, the dihydro diol derivative **5** was proposed as an IS inhibitor. In this communication, we describe the synthesis of this material and its evaluation as an inhibitor of IS and AS.

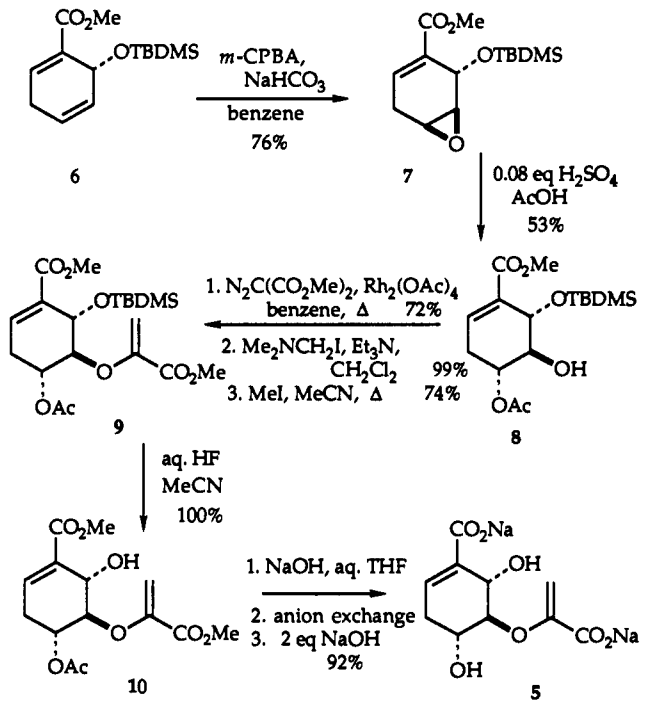
The synthesis of **5** is depicted in Scheme II. 1-[(*tert*-Butyldimethylsilyl)oxy]-1,3-butadiene is prepared from crotonaldehyde (61%)[10] and condensed with methyl propiolate to provide cyclohexadiene **6** (71%).[11] Epoxidation of this material with *m*-chloroperoxybenzoic acid affords monoepoxide **7** with high stereo- and regioselectivity. Acetolysis of the epoxide is carried out under carefully defined conditions involving catalytic H_2SO_4 in acetic acid to give the differentially protected triol **8**. Introduction of the enolpyruvyl side chain is carried out according to Ganem's procedure to give **9**.[12]

Conventional fluoride-induced desilylation (e.g., tetrabutylammonium fluoride in THF) induces elimination of the acetoxy moiety in **9**; however, this side reaction can be circumvented with aqueous hydrofluoric acid, which gives alcohol **10** in quantitative yield. Complete deprotection is accomplished with sodium hydroxide to give inhibitor **5** in 92% yield (14% overall) as the disodium salt.

Scheme I



Scheme II



The inhibition of IS was evaluated in the forward direction by using a coupled assay with isochorismatase;[3,13] inhibition of AS was determined with a fluorescent assay.[15] Diol **5**, as the

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(13) Under the conditions of the assay, diol **5** is neither an inhibitor nor a substrate for the coupling enzyme, isochorismatase.[14] Doubling the amount of IS resulted in a doubling of the rate in assays with or without inhibitor, indicating that the enzymatic reaction was properly coupled. Incubation of inhibitor **5** in the presence of IS resulted in no spectrophotometrically detectable amount of either chorismate or isochorismate, which confirms that the inhibitor is not a substrate for IS. Incubation of inhibitor **5** in the presence of IS, isochorismatase, lactase dehydrogenase, and NADH (0.2 mM) did not lead to any consumption of NADH (as measured at 340 nm), indicating that the enolpyruvyl side chain of compound **5** is not hydrolyzed by isochorismatase under standard assay conditions.
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racemate, behaves as a strong, competitive inhibitor of IS, with a K_i value of 0.36 ± 0.05 μM (compare to chorismate K_m = 7.0 ± 1.3 μM). In contrast, **5** is a weak competitive inhibitor of AS (K_i of 195 ± 15 μM; chorismate K_m = 5.4 ± 0.3 μM). The affinity of **5** for IS is not proof that the isomerization catalyzed by this enzyme involves the transition state **4** nor does it suggest whether such a species is cationic or anionic in nature; however, inhibition by **5** is not supportive of a mechanism which requires covalent attachment of the substrate to the enzyme or transient lactonization. That compound **5** is more effective as an inhibitor of IS than of AS, in spite of the homology of the two proteins, may reflect the specificity of AS for ammonia over water as cosubstrate and suggests that the 6-amino analogue of **5** may be a selective inhibitor of AS.

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Supplementary Material Available: Complete experimental details for the synthesis and enzymatic evaluation of **5** (8 pages). Ordering information is given on any current masthead page.

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Biosynthesis of Blasticidin S from Cytosylglucuronic Acid (CGA). Isolation of Cytosine/UDPglucuronosyltransferase and Incorporation of CGA by *Streptomyces griseochromogenes*

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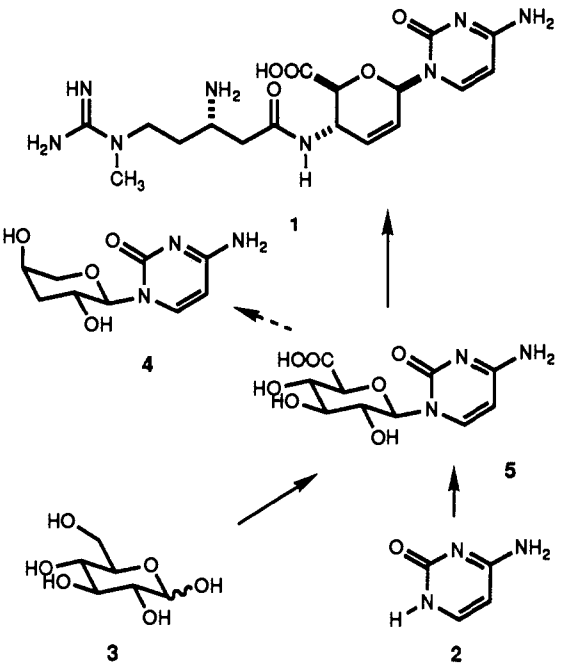
Blasticidin S, **1**, an antifungal antibiotic produced by *Streptomyces griseochromogenes* used commercially for the control of *Piricularia oryzae* (rice blast), was first isolated by Takeuchi et al. in 1958.[1] Its structure and absolute stereochemistry were elucidated by chemical means[2-5] and confirmed by X-ray diffraction.[6,7] Seto et al.[8] established that **1** is biosynthesized from cytosine, **2**, D-glucose, **3**, L-α-arginine, and L-methionine. This group has also reported the characterization of a number of structurally related metabolites from *S. griseochromogenes*.[9-13]

Scheme I



We have shown that L-arginine is converted to L-β-arginine and have established the stereochemistry of the arginine-2,3-aminomutase reaction.[14] We now report the identity of the first intermediate committed to the biosynthesis of the remainder of **1** and the isolation of the responsible enzyme.

For our further studies we adopted an approach of altering the fermentation conditions to block potential biosynthetic steps and accumulate intermediates.[15] When aminooxyacetic acid (a transaminase inhibitor)[16] was included in a fermentation with a chemically defined medium,[9] increases in the concentration of pentopyranine C, **4** (54 mg, 5.70-fold), and of another, unidentified metabolite ("UK", 247 mg, 82.4-fold) as well as a 33% decrease in the concentration of **1** (345 mg) were observed by HPLC (Waters Assoc. C_{18} RadialPak column, 97% H_2O, 3% CH_3CN, 0.1% TFA, detection at 278 nm). Alternatively, inclusion of arginine hydroxamate (an inhibitor of arginine biosynthesis)[17] (1.2 g/L) and cytosine (500 mg/L) decreased production of **1** 85% and increased production of **4** and "UK" 25-fold and 163-fold, respectively, while inclusion of L-ethionine (a methyltransferase inhibitor)[18] (400 mg/L) and cytosine (1 g/L) only reduced **1** 10%, but increased **4** 12-fold and "UK" 443-fold (to 1.33 g/L)!

The unknown metabolite was determined to be cytosylglucuronic acid (CGA) **5** (Scheme I).[19] Seto et al. had isolated **5** as a minor fermentation product and named it pentopyranic acid.[11] A cell-free extract of *S. griseochromogenes* was next prepared and incubated with cytosine and either UDPglucose, UDPgalactose, UDPgalacturonic acid, or UDPglucuronic acid.[20] In only the last incubation was substrate consumed and a product generated, which corresponded to **5** by HPLC; a preparative-scale incubation (100 mL) yielded enough **5** to obtain a ^1H NMR

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