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.

Acknowledgment. We thank Prof. Christopher T. Walsh (Harvard Medical School) for sparking our interest in this project and for providing IS and isochorismatase. We thank Prof. Nicholas Amrhein (ETH Zürich) for a generous gift of anthranilate synthase. This work was supported by the National Institutes of Health (Grant No. GM-28965) and by a DOED fellowship to M.C.K.

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.

(15) Zalkin, H. *Methods Enzymol.* **1985**, *113*, 287-292.

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

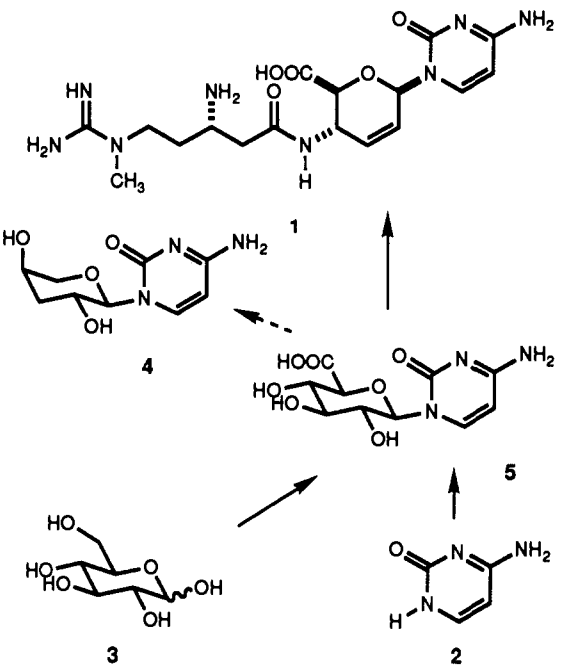
Jincan Guo and Steven J. Gould*

Department of Chemistry, Oregon State University Corvallis, Oregon 97331-4003

Received March 4, 1991

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.

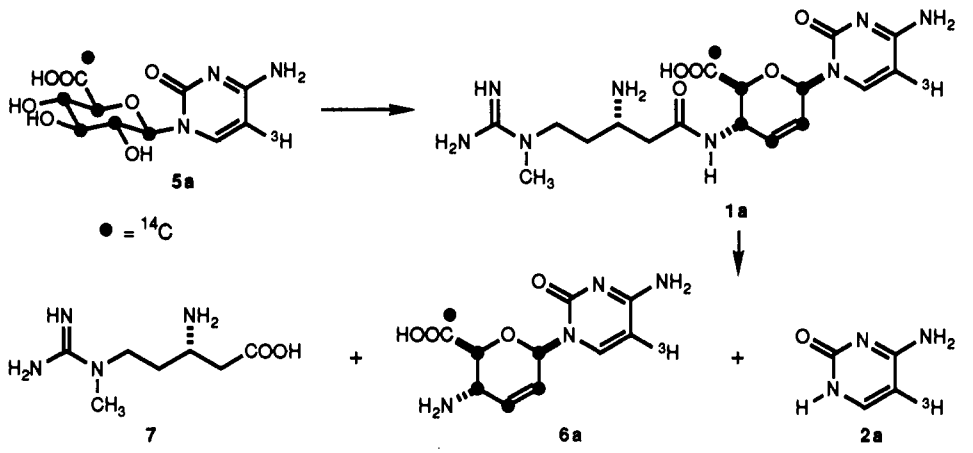
For our further studies we adopted an approach of altering the fermentation conditions to block potential biosynthetic steps and accumulate intermediates.[15] When aminooxyacetic acid (a transaminase inhibitor)[16] was included in a fermentation with a chemically defined medium,[9] increases in the concentration 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

(1) Takeuchi, S.; Hirayama, K.; Ueda, K.; Sakai, H.; Yonehara, H. *J. Antibiot.* **1958**, *11*, 1-5.
(2) Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Tetrahedron Lett.* **1965**, 1405-1410.
(3) Otake, N.; Takeuchi, S.; Endo, T.; Yonehara, H. *Tetrahedron Lett.* **1965**, 1411-1419.
(4) Fox, J. J.; Watanabe, K. A. *Tetrahedron Lett.* **1966**, 897-904.
(5) Yonehara, H.; Otake, N. *Tetrahedron Lett.* **1966**, 3785-3791.
(6) Onuma, S.; Nawata, Y.; Saito, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1091.
(7) Swaminathan, V.; Smith, J. L.; Sundaralingam, M.; Coutsogeorgopoulos, C.; Kartha, G. *Biochem. Biophys. Acta* **1981**, *655*, 335-341.
(8) Seto, H.; Yamaguchi, I.; Otake, N.; Yonehara, H. *Agric. Biol. Chem.* **1968**, *32*, 1292-1298.
(9) Seto, H.; Otake, N.; Yonehara, H. *Agric. Biol. Chem.* **1968**, *32*, 1299-1305.
(10) Seto, H. *Agric. Biol. Chem.* **1973**, *37*, 2415-2419. Seto, H.; Otake, N.; Yonehara, H. *Agric. Biol. Chem.* **1973**, *37*, 2421-2426.
(11) Seto, H.; Furihata, K.; Yonehara, H. *J. Antibiot.* **1976**, *29*, 595-596.
(12) Seto, H.; Yonehara, H. *J. Antibiot.* **1977**, *30*, 1019-1021.
(13) Seto, H.; Yonehara, H. *J. Antibiot.* **1977**, *30*, 1022-1024.
(14) Prabhakaran, P. C.; Woo, N.-T.; Yorgey, P. S.; Gould, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 5785-5796.
(15) A full description of this work will be published in detail elsewhere.
(16) (a) Wallach, D. P. (with the technical assistance of Crittenden, N. J.) *Biochem. Pharmacol.* **1961**, *5*, 323-331. (b) Kanamori, K.; Weiss, R. L.; Roberts, J. D. *J Biol. Chem.* **1988**, *263*, 2817-2823.
(17) Nakamaya, K.; Yoshida, H. *Agric. Biol. Chem.* **1972**, *36*, 1675-1684.
(18) Simmonds, S.; Keller, E. B.; Chandler, J. P.; du Vigneaud, V. *J. Biol. Chem.* **1950**, *183*, 191-195.
(19) The structure of **5** was established by ^1H NMR, ^{13}C NMR, ^1H-^1H COSY, and ^1H-^{13}C HETCOR experiments and mass spectrometric analysis.
(20) We had previously found that incorporation of [1-^{14}C]galactose into **1** was 8.14-fold higher than incorporation of [1-^{14}C]glucose. In addition, although XDPglucosyltransferases had been demonstrated in prokaryotes,[21,22] UDPglucuronosyltransferase had not.[23]

0002-7863/91/1513-5898$02.50/0

Scheme II



spectrum. As is common for nucleosides, **5** was not readily taken up by *S. griseochromogenes* mycelia (HPLC analysis), and efforts to prepare a cell-free extract or protoplasts that could metabolize **5** to **1** were unsuccessful. In order to test **5** as a biosynthetic intermediate, a sample, **5a** ($^3H/^{14}C$ = 4.25, 1.29 × 10^8 dpm/mmol ^{14}C), was prepared enzymatically from [5-^{3}H]cytosine (52.45 μCi, 19.9 mCi/μmol, Sigma Chemical Co.) and UDP[U-^{14}C]-D-glucuronic acid (10.95 μCi, 250 μCi/μmol, ICN). A portion of this (10.0 mg, 4.49 × 10^6 dpm ^{14}C) was fed to a 200-mL fermentation in the standard complex medium[14] 39 h after inoculation with a seed culture. Ninety-eight hours later workup[14] yielded 455 mg of labeled blasticidin S **1a** ($^3H/^{14}C$ = 6.75, 1.01 × 10^4 dpm/mmol ^{14}C). Most of **5a** was recovered from the broth unchanged (88.4%). On the basis of the unrecovered material, the ^{14}C incorporation was 1.8%. The change in $^3H/^{14}C$ could be explained if only 0.14% of the **5a** fed had been hydrolyzed to [5-^{3}H]cytosine, **2a**, since **2** had previously been incorporated almost quantitatively.[8] To test the specificity of the incorporation, 300 mg of **1a** were hydrolyzed[2,3] yielding labeled cytosinine **6a** (29.4 mg, $^3H/^{14}C$ = 6.26, 9.70 × 10^3 dpm/mmol ^{14}C, 96% retention of ^{14}C and 89% retention of ^3H from **1a**), labeled cytosine **2a** (21.4 mg, 5.84 × 10^4 dpm/mmol ^3H, 86% retention of tritium), and blasticidic acid, **7** (not isolated, but unlabeled vide supra). Some exchange of tritium had occurred during the hydrolysis. These results demonstrate unequivocally the specific intact incorporation of **5a** exclusively into the cytosinine portion of blasticidin S, **1**.

$$\mathbf{5a} \rightarrow \mathbf{1a} \rightarrow \mathbf{6a} + \mathbf{2a} + \mathbf{7}$$

The biosyntheses of a number of nucleoside antibiotics have been studied.[24-27] The biosynthesis of blasticidin S appears to be the first instance where the formation of a novel nucleoside has been demonstrated at the cell-free level to be the first committed step in the secondary pathway. In addition, although UDPglucuronosyl transferases are common in mammalian xenobiotic metabolism and at least two have been reported in fungi (also eukaryotes),[28,29] this is the first report of such an enzyme from a prokaryotic organism.[30]

Acknowledgment. Dr. Y. Miyazaki, Kaken Chemical Co., Ltd., Japan, is thanked for providing a culture of *S. griseochromogenes* and recipes for the standard seed and production media. This work was supported by Public Health Service Research Grant GM 32110 to S.J.G. The Bruker AC 300 NMR spectrometer used in this work was purchased in part through grants from the Public Health Service Division of Research Resources (RR04039-01) and the National Science Foundation (CHE-8712343) to Oregon State University.

(21) Grisebach, H. Biosynthesis of Sugar Components of Antibiotic Substances. In *Advances in Carbohydrate Chemistry*; Tipson, R. S., Horton, D., Eds.; Academic Press: New York, 1978; Vol. 35, pp 81-126.
(22) Zmijewski, M. J.; Briggs, B. *FEMS Microbiol. Lett.* **1989**, *59*, 129-134.
(23) The TDPglucosyltransferase described in ref 21 could utilize UDP-glucuronic acid but only poorly (only 9.5% of the rate).
(24) Parry, R. J.; Bornemann, V.; Subramanian, R. *J. Am. Chem. Soc.* **1989**, *111*, 5819-5831.
(25) For an extensive review of nucleoside antibiotics, including their biosynthesis, see: Isono, K. J. *J. Antibiot.* **1988**, *41*, 1711-1737.
(26) Hanvey, J. C.; Smal-Hawkins, E.; Baker, D. C.; Suhadolnik, R. J. *Biochemistry* **1988**, *27*, 5790-5795.
(27) Hanvey, J. C.; Smal-Hawkins, E.; Tunac, J. B.; Dechter, J. J.; Baker, D. C.; Suhadolnik, R. J. *Biochemistry* **1987**, *26*, 5636-5641.
(28) Flores-Carreon, A.; Balcazar, R.; Ruiz-Herrera, J. *Exp. Mycol.* **1985**, *9*, 294-301.
(29) Wackett, L. P.; Gibson, D. T. *Biochem. J.* **1982**, *205*, 117-122.
(30) CGA is likely to also prove to be the first committed intermediate in the biosynthesis of arginomycin,[31] the bagougeramines,[32] gougerotin,[33] and possibly Sch 36605.[34]
(31) Argoudelis, A. D.; Baczynskyj, L.; Kuo, M. T.; Laborde, A. L.; Sebek, O. K.; Truesdell, S. E.; Shilliday, F. B. *J. Antibiot.* **1987**, *40*, 750-760.
(32) Takahashi, A.; Ikeda, D.; Naganawa, H.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1041-1046.
(33) Fox, J. J.; Kuwada, Y.; Watanabe, K. A. *Tetrahedron Lett.* **1968**, 6029-6032.
(34) Cooper, R.; Conover, M.; Patel, M. *J. Antibiot.* **1988**, *41*, 123-125.

Oligoamines as Simple and Efficient Catalysts for RNA Hydrolysis

Koichi Yoshinari, Katsutoshi Yamazaki, and Makoto Komiyama*,†

Institute of Materials Science, University of Tsukuba Tsukuba, Ibaraki 305, Japan

Received February 12, 1991

Recent interest has focused on the molecular design of artificial nucleases, in which catalytic residues are attached to sequence-recognizing moieties.[1] Developments have been made in oxidative fission of specific ribose residues in DNA.[2] However, cleavage of DNA and RNA via hydrolysis of the phosphodiester linkage has been scarcely accomplished.[3-6] We report here remarkable

† Current address: Department of Industrial Chemistry, Faculty of Engineering, University of Tokyo, Hongo, Tokyo 113, Japan.

(1) (a) Dervan, P. B. *Science* (*Washington, D.C.*) **1986**, *232*, 464. (b) Barton, J. K. *Science* (*Washington, D.C.*) **1986**, *233*, 727. (c) Zuckermann, R. N.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 6592.
(2) Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2435 and references therein.
(3) Barbier, B.; Brack, A. *J. Am. Chem. Soc.* **1988**, *110*, 6880.
(4) The cooperative catalysis of two imidazoles in ribonuclease was satisfactorily mimicked: Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473, 5972 and references therein.
(5) Hydrolyses of RNAs by metal ions and metal complexes have been reported: (a) Eichhorn, G. L. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier Scientific Publishing Co.: Amsterdam, 1973; Vol. 2, Chapter 34. (b) Stern, M. K.; Bashkin, J. K.; Sall, E. D. *J. Am. Chem. Soc.* **1990**, *112*, 5357. (c) Matsumoto, Y.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1990**, 1050.

0002-7863/91/1513-5899$02.50/0