Communications to the Editor

3-Amino-4-hydroxybenzoic Acid: the Precursor of the C_7N Unit in Asukamycin and Manumycin

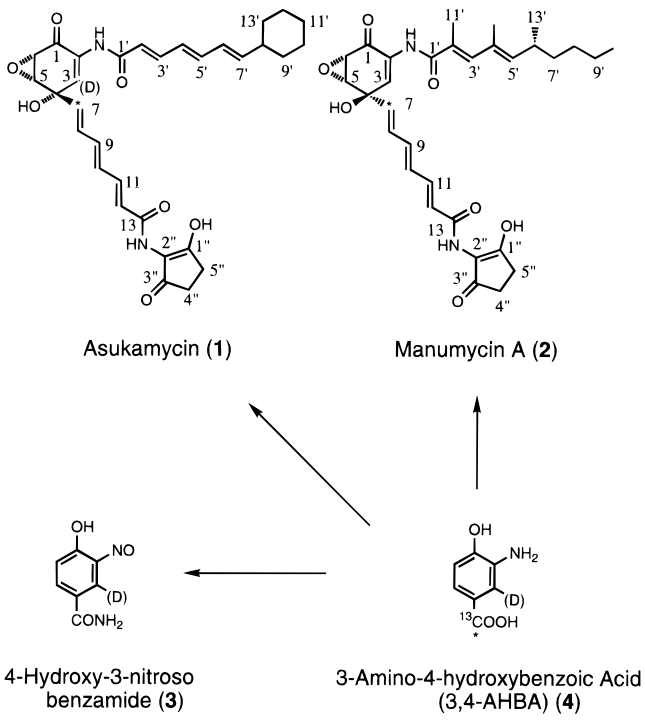
Yiding Hu,† Chris R. Melville,‡ Steven J. Gould,‡ and Heinz G. Floss*,†

Department of Chemistry, University of Washington Seattle, Washington 98195
Department of Chemistry, Oregon State University Corvallis, Oregon 97331

Received January 15, 1997

Antibiotics of the manumycin family, e.g., asukamycin (**1**)[1,2] from *Streptomyces nodosus* ssp. *asukaensis* and manumycin A (**2**)[3−7] from *Streptomyces parvulus* Tü 64, recently recognized as inhibitors of Ras protein farnesyltransferase[8] and of human interleukin-1β converting enzyme,[9,10] contain two short polyketide chains linked through a mC_7N unit which serves as the starter unit for one of the polyketide chains. Unlike the mC_7N units in ansamycin and mitomycin antibiotics, which arise from a parallel branch of the shikimate pathway,[11] the formation of the mC_7N unit in the manumycin antibiotics is not related to the shikimate pathway. Feeding experiments established that the mC_7N unit in both **1** and **2** arises from a 4-carbon dicarboxylic acid derived from succinic acid, giving rise to carbons 4−7, and a triose phosphate derived from glycerol, labeling carbons 1−3.[12] The triose phosphate is incorporated in such a way that the phosphorylated carbon gives rise to C-3, but with complete loss of deuterium attached to it.[2] The same labeling pattern was recently established[13] for 4-hydroxy-3-nitrosobenzamide (**3**), an iron-chelating metabolite isolated from *Streptomyces murayamaensis*.[14] Importantly, 3-amino-4-hydroxybenzoic acid (3,4-AHBA, **4**) had been established as a specific precursor of **3**,[14] leading to the suggestion[13] that **4** might also be a proximate precursor for the mC_7N unit of the manumycin antibiotics. In this paper, we report experimental evidence in support of this hypothesis.

Scheme 1



In an initial experiment, 15 mg of [2-^{2}H]-**4** ammonium salt (80 atom % ^{2}H) was fed to four 100 mL shake cultures of *S. nodosus* ssp. *asukaensis* ATTC 29757[15] at 24 h after inoculation. The cultures were harvested at 84 h and worked up as described,[12] except that HPLC (gradient of CH_3OH/H_2O) was substituted for the Sephadex LH-20 chromatography. ^{2}H NMR analysis of the resulting **1** (5 mg) showed a single signal at δ = 7.36 ppm corresponding to deuterium at C-3 of the mC_7N unit of **1** [16.5 atom % ^{2}H enrichment, as determined by electrospray mass spectrometry select-ion monitoring (ES-MS SIM) method]. To confirm this result, [7-^{13}C]-**4** was synthesized from 4-hydroxy-[7-^{13}C]benzoic acid[16] by nitration followed by reduction of the nitro group with N_2H_4/Raney nickel. This material (10 mg, 99% ^{13}C) was fed to ten 100 mL cultures of *S. nodosus* ssp. *asukaensis* as described above. ^{13}C NMR analysis of the **1** produced (10 mg) in $CDCl_3$ gave a single enriched signal at 136.2 ppm, corresponding to C-7 of **1** (16.5% ^{13}C enrichment determined by ES-MS SIM).

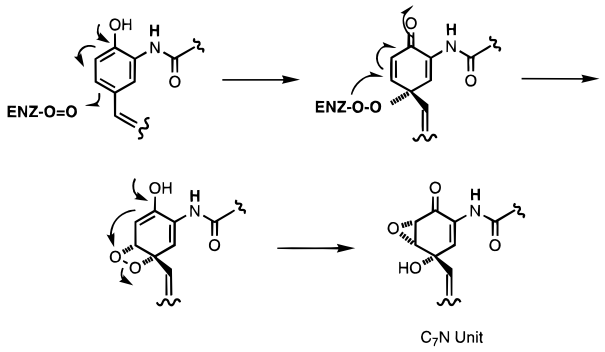
The above results demonstrate unequivocally that 3,4-AHBA is a specific precursor of the mC_7N moiety of **1**, providing the starter unit for the "southern" polyketide chain (Scheme 1). Its formation from a C_4 and a C_3 building block[13] must involve the complete loss of all deuterium from the carbon of the C_3 precursor which gives rise to C-2 of **4**. This follows from the fact that 1(*R*),2(*S*)- and 1(*S*),2(*S*)-[1-^{2}H$_1$]glycerol were incorporated into **1** with complete loss[2] of their deuterium, whereas [2-^{2}H]-**4** retained its deuterium in the process. The further

* Author to whom correspondence should be addressed at Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195-1700.
† University of Washington.
‡ Oregon State University.

(1) Kakinuma, K.; Ikekawa, N.; Nakagawa, A.; Ōmura, S. *J. Am. Chem. Soc.* **1979**, *101*, 3402.
(2) Cho, H.; Sattler, I.; Beale, J. M.; Zeeck, A.; Floss, H. G. *J. Org. Chem.* **1993**, *58*, 7925.
(3) Schröder, K.; Zeeck, A. *Tetrahedron Lett.* **1973**, *14*, 4995.
(4) Zeeck, A.; Schröder, K.; Frobel, K.; Grote, R.; Thiericke, R. *J. Antibiot.* **1987**, *40*, 1530.
(5) Zeeck, A.; Frobel, K.; Heusel, C.; Schröder, K.; Thiericke, R. *J. Antibiot.* **1987**, *40*, 1541.
(6) Thiericke, R.; Stellwaag, M.; Zeeck, A.; Snatzke, G. *J. Antibiot.* **1987**, *40*, 1549.
(7) Sattler, I.; Gröne, C.; Zeeck, A. *J. Org. Chem.* **1993**, *58*, 6583.
(8) Hara, M.; Akasaka, K.; Akimaya, S.; Okabe, M.; Nakano, H.; Gomez, R.; Wood, D.; Uh, M.; Tamanoi, F. *Proc. Nat. Acad. Sci. U.S.A.* **1993**, *90*, 2281.
(9) Uosaki, Y.; Agatsuma, T.; Tanaka, T.; Saitoh, Y. *J. Antibiot.* **1996**, *49*, 1079.
(10) Tanaka, T.; Tsukuda, E.; Uosaki, Y.; Matsuda, Y. *J. Antibiot.* **1996**, *49*, 1085.
(11) Kim, C.-G.; Kirschning, A.; Bergon, P.; Zhou, P.; Su, E.; Sauerbrei, B.; Ning, S.; Ahn, Y.; Breuer, M.; Leistner, E.; Floss, H. G. *J. Am. Chem. Soc.* **1996**, *118*, 7486 and references therein.
(12) Thiericke, R.; Zeeck, A.; Nakagawa, A.; Ōmura, S.; Herrold, R.; Wu, S. T. S.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1990**, *112*, 3979.
(13) Gould, S. J.; Melville, C.; Cone, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 9228.
(14) Cone, M. C.; Melville, C. R.; Carney, J. R.; Gore, M. P.; Gould, S. J. *Tetrahedron Lett.* **1995**, *51*, 3095.
(15) Seed cultures were grown in 500 mL Erlenmeyer flasks containing 100 mL of medium [20 g of glucose, 5 g of Bacto Peptone, 0.25 g of K_2HPO_4, 0.25 g of $MgSO_4$, 50 mg of $FeSO_4 \cdot 7H_2O$, 10 mg of $MnCl_2 \cdot 4H_2O$, 5 mg each of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, and $ZnSO_4 \cdot 7H_2O$, deionized H_2O (1 L)] for 3 days at 28 °C with rotary shaking at 300 rpm. One-tenth of a seed culture was used to inoculate each production flask containing 100 mL of the same medium; these were incubated in the same way.
(16) Cho, H.; Beale, J. M.; Graff, C.; Mocek, U.; Nakagawa, A.; Ōmura, S.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 12296.

S0002-7863(97)00101-7 CCC: $14.00

Scheme 2



conversion of the aromatic ring of **4** into the epoxyquinol structure of **1**, which probably occurs after polyketide synthesis, may plausibly involve the same dioxygenase mechanism[17] demonstrated for the oxidation of dihydrovitamin K[18−20] and the oxidation of dihydroxyacetanilide in the biosynthesis of antibiotics LL-C10037 and MPP3051[21] (Scheme 2). A necessary consequence of this mechanism is that the epoxide and the quinol oxygen in the product must be *syn* oriented. Compound **1** meets this condition,[2] as do the majority of the manumycins[7,22] and related compounds.[9,23,24] However, both manumycin A and B, the major metabolites of *S. parvulus* Tü 64, carry the epoxide and quinol oxygens in an *anti* arrangement.[6,7] It was therefore of interest to check if **4** is also a precursor to these compounds.

[7-^{13}C]3,4-AHBA (15 mg, 99% ^{13}C) was fed to ten 100 mL cultures of *S. parvulus* Tü 64, grown as described previously,[12] at 48 h after inoculation, and the cultures were harvested at 72 h. Standard workup[12] produced **2** (3 mg) which gave a single enriched ^{13}C NMR signal at 136.1 ppm corresponding to C-7. ES-MS analysis revealed 5.4% ^{13}C enrichment. A small amount of manumycin B was also isolated and showed 5.7% ^{13}C by ES-MS. Thus, **4** is a specific precursor of the mC_7N unit of manumycin type antibiotics irrespective of the relative stereochemistry of the epoxyquinol moiety.

Three possible explanations can be invoked for this surprising observation: (i) the epoxyquinol moiety of manumycins A and B undergoes a stereochemical change after its formation, (ii) the stereochemical assignment of manumycins A and B is in error, or (iii) the dioxygenase mechanism of epoxyquinol formation does not apply to the manumycin antibiotics. We are presently attempting to distinguish between these possibilities.

Acknowledgment. This work was supported by NIH grants AI 20264 (H.G.F.) and GM 31715 (S.J.G.). We thank Professor Axel Zeeck, University of Göttingen, for the culture of *S. parvulus* Tü 64 and for generous exchange of information.

JA9701016

(17) Ham, S. W.; Dowd, P. *J. Am. Chem. Soc.* **1990**, *112*, 1660.
(18) Naganathan, S.; Hershline, R.; Ham, S. W.; Dowd, P. *J. Am. Chem. Soc.* **1994**, *116*, 9831.
(19) Dowd, P.; Hershline, R.; Ham, S. W.; Naganathan, S. *Science* **1995**, *269*, 1684 and references therein.
(20) Kuliopulos, A.; Hubbard, B. R.; Lam, Z.; Koski, I. J.; Furie, B.; Furie, B. C.; Walsh, C. T. *Biochemistry* **1992**, *31*, 7722.
(21) Gould, S. J.; Kirchmeier, M. J.; LaFever, R. E. *J. Am. Chem. Soc.* **1996**, *118*, 7663.
(22) Shu, Y.; Huang, S.; Wang, R. R.; Lam, K. S.; Klohr, S. E.; Volk, K. J.; Pirnik, D. M.; Wells, J. S. *J. Antibiot.* **1994**, *47*, 324.
(23) Grote, R.; Zeeck, A.; Beale, J. M. *J. Antibiot.* **1988**, *41*, 1186.
(24) Franco, C. M. M.; Maurya, R.; Vijayakumar, E. K. S.; Chaterjee, S.; Blumbach, J.; Ganguli, B. N. *J. Antibiot.* **1991**, *44*, 1289.