J. Antibiot. 59(9): 607–610, 2006

NOTE

THE JOURNAL OF ANTIBIOTICS

Biosynthetic Study of FR-900848: Origin of the Aminodeoxynucleoside Part

Hiroaki Watanabe, Tetsuo Tokiwano, Hideaki Oikawa

Received: April 21, 2006 / Accepted: September 8, 2006

Abstract Biosynthetic studies of the antifungal agent, FR-900848, were undertaken by feeding experiments with D-[U-$^{13}C_6$]glucose, L-[4-^{13}C]aspartate, [5,5-$^{2}H_2$]dihydrouridine and [5,5-$^{2}H_2$]dihydrouracil. The 5″-amino-5″-deoxy-5′,6′-dihydrouridine moiety was derived from ribose and aspartate. Based on the feeding experiments, a detailed biosynthetic pathway producing the aminodeoxydihydrouridine moiety of FR-900848 was proposed.

Keywords FR-900848, antifungal agent, polycyclopropane, aminodeoxynucleoside

FR-900848 (**1**) is a polyketide-nucleoside produced by *Streptoverticillium fervens* HP-891, and shows potent activity against phytopathogenic fungi [1]. Its structure consists of a cyclopropanated fatty acid and 5″-amino-5″-deoxy-5′,6′-dihydrouridine (Fig. 1). FR-900848 (**1**), and a closely related compound U-106305 [2] contains four or five contiguous and one isolated cyclopropanes which have the same stereochemistry [3]. In order to elucidate the mechanism for enzymatic construction of **1**, we started on a biosynthetic study of **1**, and found that the fatty acid backbone of **1** was biosynthesized *via* a polyketide pathway [4]. Herein we describe our investigation for the biosynthesis of the dihydrouridine moiety in **1**.

In the previous report, we have found that the polyketide backbone of FR-900848 (**1**) was constructed by acetyl-CoA derived from glucose and glycerol, and methylenes of cyclopropanes were derived from L-methionine (Fig. 1) [4]. Further studies of feeding experiments with ^{13}C- and ^{2}H-labeled precursors have determined the origin of the dihydrouridine moiety of **1**. Before conducting NMR analysis of the labeled products, **1** in crude mycelial extracts was converted to a less-polar diacetate **2** to improve its recovery in purification. In chromatographic steps, recovery of **1** was 20∼30 μg per 100 ml of the culture broth on a small scale. Treatment of the crude extracts with Ac_2O, pyridine and 4-dimethylaminopyridine provided **2** in improved yield (1∼2 mg/100 ml) after purification. The reverse reaction, the methanolysis of **2** with K_2CO_3, regenerated **1** in 53% yield.

It is well known that pyrimidines are biosynthesized from aspartate and carbamoyl phosphate. In order to confirm this pathway in FR-900848 biosynthesis, we performed a feeding experiment with ^{13}C-labeled aspartate. In the ^{13}C NMR spectrum of **2** provided by feeding L-[4-^{13}C]aspartate, enhanced signals were observed at C4′ (Fig. 2-b). It clearly shows that aspartate is origin of dihydrouracil of **2**. Although unexpected enhancements of signals at C14 and C15 were also observed, the reason for the unusual labeling was currently unknown. In the feeding experiment with D-[U-$^{13}C_6$]glucose, we previously reported that incorporation of $^{13}C_2$- and $^{13}C_3$-units into the dihydrouracil part was observed [4] (Fig. 2-c). This experimental result has supported incorporation of the aspartate precursor derived from D-[U-$^{13}C_6$]glucose *via* phosphoenolpyruvate.

Incorporation of L-[4-^{13}C]aspartate indicates that the nucleoside moiety of **1** is biosynthesized *via* similar routes to those of normal pyrimidine nucleosides. In the feeding experiment with [5,5-$^{2}H_2$]-5,6-dihydrouridine, the ^{2}H-NMR spectrum of **2** in $CHCl_3$ showed the signal corresponding to

H. Oikawa (Corresponding author)**, H. Watanabe, T. Tokiwano:** Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan,
E-mail: hoik@sci.hokudai.ac.jp

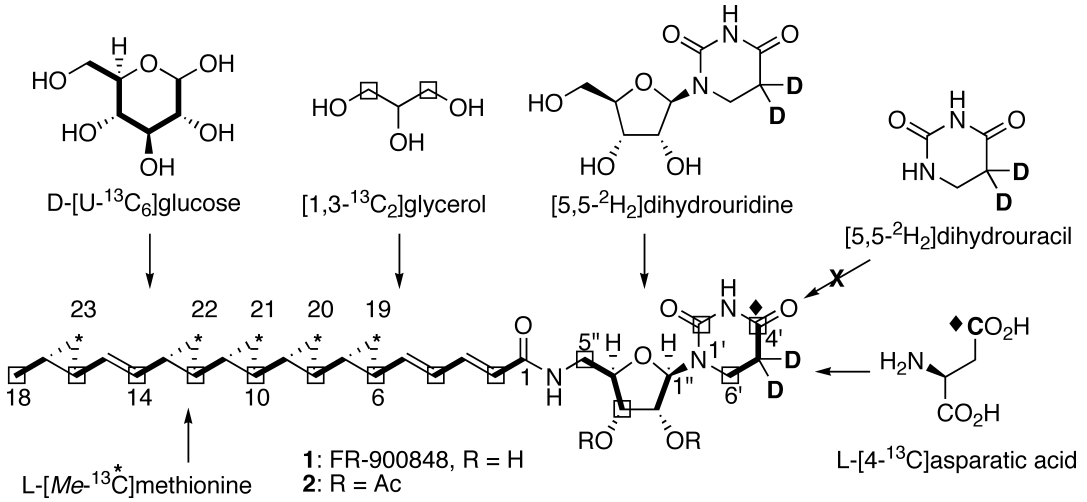


Fig. 1 Incorporation of D-[U-$^{13}C_6$]glucose, [1,3-$^{13}C_2$]glycerol, L-[*Me*-^{13}C]methionine, L-[4-^{13}C]aspartate and [5,5-$^{2}H_2$]dihydrouridine into FR-900848 (**1**).

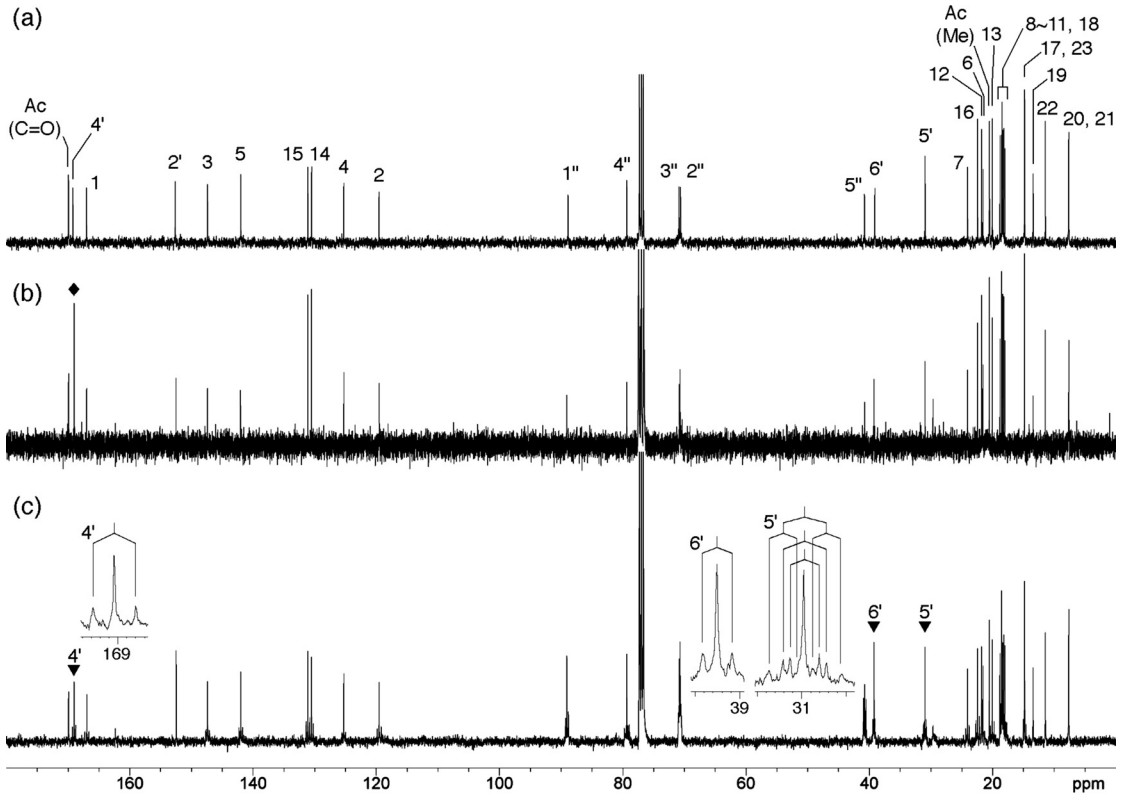


Fig. 2 The ^{13}C NMR spectra of **2**.

(a) a natural abundance sample; (b) a sample from the feeding experiment with L-[4-^{13}C]aspartate; (c) a sample from the feeding experiment with D-[U-$^{13}C_6$]glucose.

deuteriums at H5′ (Fig. 3). [5,5-$^{2}H_2$]-5,6-dihydrouridine was derived from uridine by palladium-catalyzed hydrogenation (4.5 MPa H_2 in MeOH - H_2O) followed by ^{1}H-^{2}H exchange (MeONa in $^{2}H_2O$) in 11% yield. [5,5-$^{2}H_2$]-5,6-dihydrouridine: $[\alpha]_D^{30}$ −22 (*c* 0.57, MeOH); ^{13}C NMR (75 MHz, D_2O containing 5% CD_3OD) δ 174.6, 155.3,

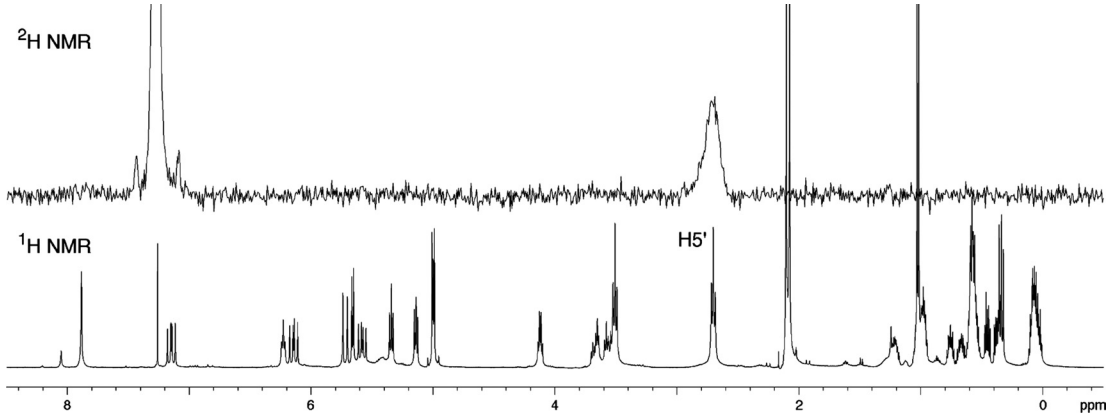
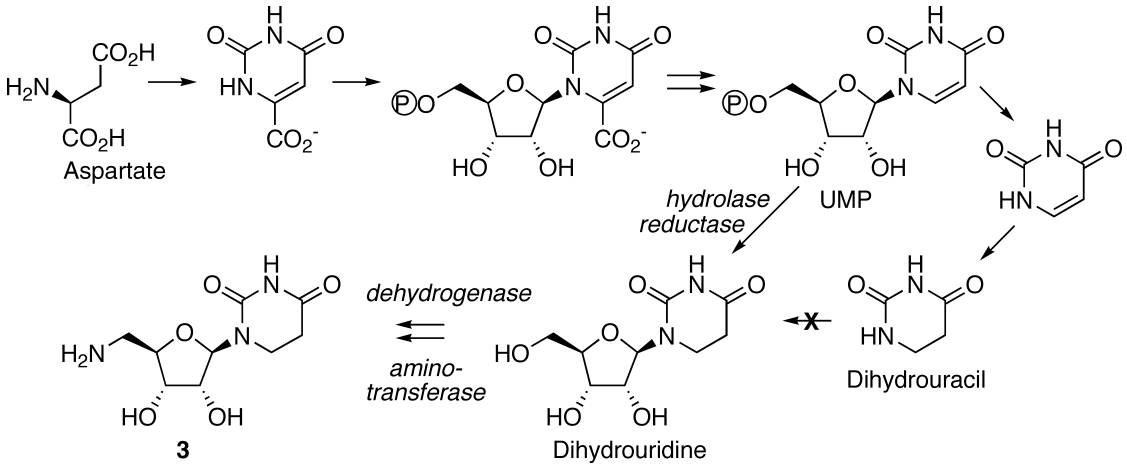


Fig. 3 The ^{1}H and ^{2}H NMR spectra of **2** from the feeding experiment with [5,5-$^2H_2$]dihydrouridine.

Incorporation of the labeled dihydrouridine into **2** was 0.27% as determined from the ^{2}H NMR spectrum with reference to the natural abundance solvent peak of $CHCl_3$.



Scheme 1 A proposed biosynthetic pathway to 5′-amino-5′-deoxydihydrouridine **3**.

88.5, 84.5, 71.6, 71.1, 62.4, 37.3, 31.2. It is known that dihydrouracil is a degradation product of uridine. The unusual dihydrouridine moiety may be constructed by condensation of dihydrouracil with the ribose part. To test the possibility, ^{2}H-labeled dihydrouracil was synthesized by ^{1}H-^{2}H exchange of dihydrouracil (MeONa in ^2H_2O) in 8.2% yield. In the ^{2}H-NMR spectrum of labeled **2** provided by feeding [5,5-$^2H_2$]dihydrouracil, a number of signals of cyclopropane and methylenes at dihydrouracil were observed. This result indicates that dihydrouracil degraded into acetyl-CoA which was reincorporated. In the biosynthesis of other nucleoside antibiotics, blasticidine S and nikkomycins, their nucleoside cores were derived from common nucleosides, cytidine 5′-monophosphate (CMP) and uridine 5′-monophosphate (UMP), respectively [5, 6]. In the case of blasticidin S, it was found that intracellular pyrimidine bases exist almost exclusively at the nucleoside level and free cytosine is not a precursor to cytidine or the cytidine phosphates [5]. Based on these observations, we proposed that the dihydrouridine moiety is derived from UMP and is not provided by *de novo* synthesis as shown in Scheme 1. Uridine or UMP is directly reduced to give dihydrouridine followed by C5″-oxidation and transamination to afford 5′-amino-5′-deoxydihydrouridine **3**. These transformations may require four enzymes, hydrolase, reductase, dehydrogenase, and aminotransferase, and the corresponding genes may be useful as markers to search the gene cluster in biosynthesis of **1**.

Feeding Experiments with Isotopically Labeled Compounds

Culture medium and growth conditions for

Streptoverticillium fervens HP-891 were as described by Yoshida *et al.* [1, 4]. On the fourth day after inoculation, the sterilized aqueous solution of a isotope-labeled compound was added to the fermentation cultures (100 ml) in a 500-ml Erlenmeyer flask. The quantities of isotope-labeled compounds supplied to 100 ml of the cultures were as follows: 50 mg of D-[U-$^{13}C_6$]glucose, 50 mg of L-[4-^{13}C]aspartic acid, 60 mg of [5,5-$^{2}H_2$]-5,6-dihydrouridine and 250 mg of [5,5-$^{2}H_2$]-5,6-dihydrouracil. After further incubation for 5 days, the diacetate **3** (1～2 mg/100 ml of the cultures) was isolated as described above.

Acknowledgements We are grateful to Dr. Hideyuki Muramatsu of Astellas Pharmaceutical Co. Ltd. for supplying *S. fervens* HP-891 and the fermentation conditions, and to High Resolution NMR Laboratory, Faculty of Science, Hokkaido University, for NMR measurement.

References

1. Yoshida M, Ezaki M, Hashimoto M, Yamashita M, Shigematsu N, Okuhara M, Kohsaka M, Horikoshi K. A novel antifungal antibiotic, FR-900848. I. Production, isolation, physico-chemical and biological properties. J Antibiot 43: 748–754 (1990)
2. Kuo MS, Zielinski RJ, Cialdella JI, Marschke CK, Dupuis MJ, Li GP, Kloosterman DA, Spilman CH, Marshall VP. Discovery, isolation, structure elucidation, and biosynthesis of U-106305, a cholesteryl ester transfer protein inhibitor from Uc-11136. J Am Chem Soc 117: 10629–10634 (1995)
3. Barrett AGM, Doubleday WW, Kasdorf K, Tustin GJ. Stereochemical elucidation of the pentacyclopropane antifungal agent FR-900848. J Org Chem 61: 3280–3288 (1996)
4. Watanabe H, Tokiwano T, Oikawa H. Biosynthetic study of FR-900848: unusual observation on polyketide biosynthesis that did not accept acetate as origin of acetyl-CoA. Tetrahedron Lett 47: 1399–1402 (2006)
5. Cone MC, Yin XH, Grochowski LL, Parker MR, Zabriskie TM. The blasticidin S biosynthesis gene cluster from Streptomyces griseochromogenes: Sequence analysis, organization, and initial characterization. Chembiochem 4: 821–828 (2003)
6. Ginj C, Ruegger H, Amrhein N, Macheroux P. 3′-Enolpyruvyl-UMP, a novel and unexpected metabolite in nikkomycin biosynthesis. Chembiochem 6: 1974–1976 (2005)