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Author(s)	Watanabe, Hiroaki; Tokiwano, Tetsuo; Oikawa, Hideaki
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Biosynthetic Study of FR-900848: Origin of the Aminodeoxynucleoside Part

Hiroaki Watanabe, Tetsuo Tokiwano, Hideaki Oikawa

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Abstract Biosynthetic studies of the antifungal agent, FR-900848, were undertaken by feeding experiments with D-[U-¹³C₆]glucose, L-[4-¹³C]aspartate, [5,5-²H₂]dihydrouridine and [5,5-²H₂]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 ¹³C- and ²H-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 Ac₂O, pyridine and 4-dimethylaminopyridine provided **2** in improved yield (1~2 mg/100 ml) after purification. The reverse reaction, the methanolysis of **2** with K₂CO₃, 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 ¹³C-labeled aspartate. In the ¹³C NMR spectrum of **2** provided by feeding L-[4-¹³C]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-¹³C₆]glucose, we previously reported that incorporation of ¹³C₂- and ¹³C₃-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-¹³C₆]glucose *via* phosphoenolpyruvate.

Incorporation of L-[4-¹³C]aspartate indicates that the nucleoside moiety of **1** is biosynthesized *via* similar routes to those of normal pyrimidine nucleosides. In the feeding experiment with [5,5-²H₂]-5,6-dihydrouridine, the ²H-NMR spectrum of **2** in CHCl₃ 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

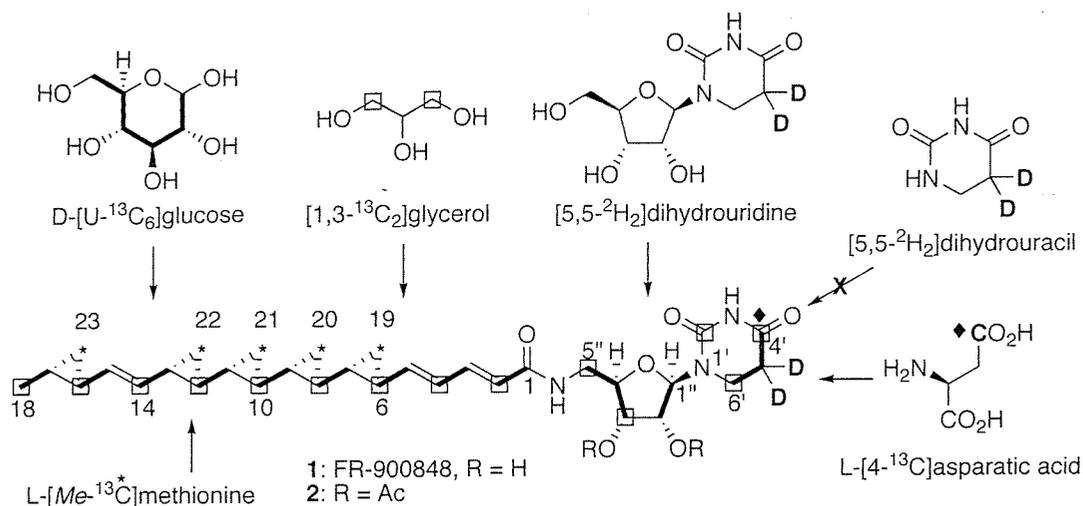


Fig. 1 Incorporation of D-[U- $^{13}\text{C}_6$]glucose, [1,3- $^{13}\text{C}_2$]glycerol, L-[Me- ^{13}C]methionine, L-[4- ^{13}C]aspartate and [5,5- $^2\text{H}_2$]dihydrouridine into FR-900848 (**1**).

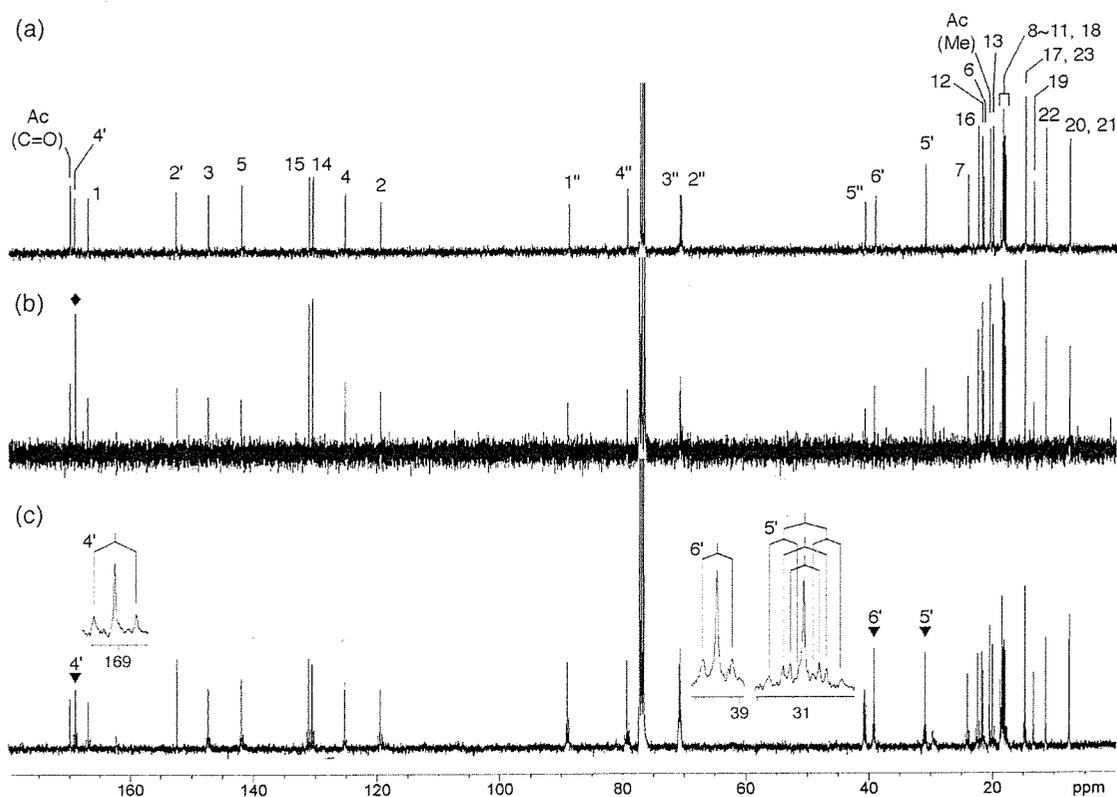


Fig. 2 The ^{13}C NMR spectra of **2**.

(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- $^{13}\text{C}_6$]glucose.

deuteriums at H5' (Fig. 3). [5,5- $^2\text{H}_2$]-5,6-dihydrouridine was derived from uridine by palladium-catalyzed hydrogenation (4.5 MPa H_2 in MeOH- H_2O) followed by

^1H - ^2H exchange (MeONa in $^2\text{H}_2\text{O}$) in 11% yield. [5,5- $^2\text{H}_2$]-5,6-dihydrouridine: $[\alpha]_{\text{D}}^{30} -22$ (c 0.57, MeOH); ^{13}C NMR (75 MHz, D_2O containing 5% CD_3OD) δ 174.6, 155.3,

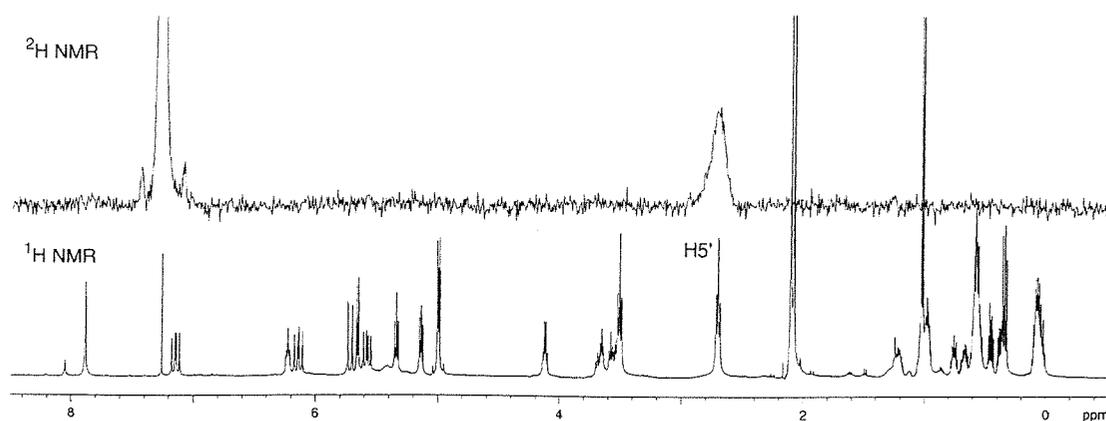
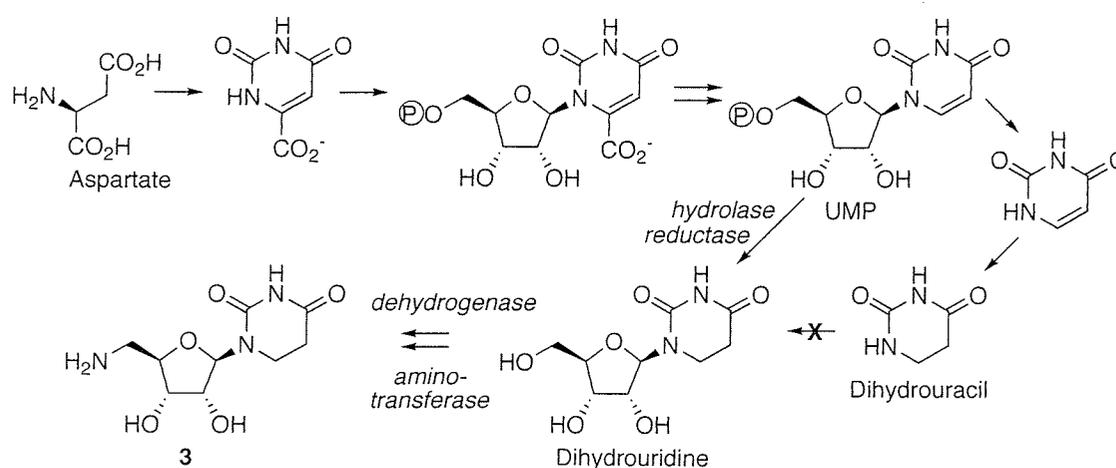


Fig. 3 The ^1H and ^2H NMR spectra of **2** from the feeding experiment with $[5,5\text{-}^2\text{H}_2]$ dihydrouridine.

Incorporation of the labeled dihydrouridine into **2** was 0.27% as determined from the ^2H 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, ^2H -labeled dihydrouracil was synthesized by ^1H - ^2H exchange of dihydrouracil (MeONa in $^2\text{H}_2\text{O}$) in 8.2% yield. In the ^2H -NMR spectrum of labeled **2** provided by feeding $[5,5\text{-}^2\text{H}_2]$ 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-¹³C₆]glucose, 50 mg of L-[4-¹³C]aspartic acid, 60 mg of [5,5-²H₂]-5,6-dihydrouridine and 250 mg of [5,5-²H₂]-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.

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