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Biosynthetic study of FR-900848: unusual observation on polyketide biosynthesis that did not accept acetate as origin of acetyl-CoA

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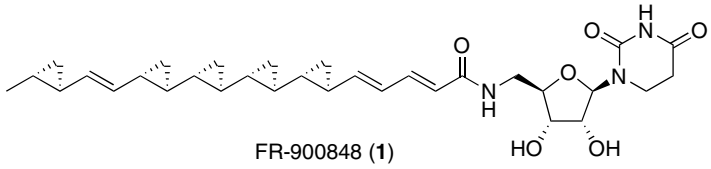
Abstract—The biosynthetic pathway of a potent antifungal agent, FR-900848, has been examined by administration of several ^{13}C-labeled precursors to *Streptoverticillium fervens* HP-891. Although none of the ^{13}C-labeled acetate was incorporated into FR-900848, the labeling pattern of FR-900848 derived from D-[U-$^{13}C_6$]glucose revealed that the fatty acid backbone of FR-900848 has been biosynthesized via a polyketide pathway. These unusual results strongly show that the major pathway to provide acetyl-CoA in this microorganism is glycolysis. Feeding experiments with D-[U-$^{13}C_6$]glucose, [1,3-$^{13}C_2$]glycerol, and L-[Me-^{13}C]methionine provided information on the biosynthetic origin of structurally unusual parts (polycyclopropane and aminonucleoside) in this antibiotic.

FR-900848 (**1**) is an antifungal agent isolated from *Streptoverticillium fervens* HP-891 by Yoshida et al. in 1990.[1] FR-900848 was found to have potent activity against phytopathogenic fungi suppressing their growth. The structure of **1** including the absolute stereochemistry was established by degradation and synthetic studies to be as shown in Figure 1.[2] FR-900848 consists of a 2,4,14-octadecatrienoic acid having four contiguous and one isolated cyclopropanes, and a 5″-amino-5″-deoxy-5′,6′-dihydrouridine. Closely related polycyclopropane compound U-106305 (**2**), a cholesteryl ester transfer protein (CETP) inhibitor, was isolated from *Streptomyces* sp. UC 11136 by Kuo et al.,[3] and was found to have the same stereochemistry for cyclopropane rings. In order to elucidate the mechanism constructing the polycyclopropane, we have started on a biosynthetic study of **1**, and herein describe the biosynthetic origins of structurally unique polycyclopropane



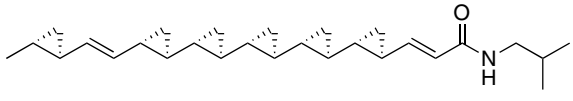


Figure 1.

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and aminonucleoside units, and an unusual observation on the polyketide biosynthesis.

Before conducting the biosynthetic study, we faced a problem in the purification of **1**. Conversion of **1** to diacetate **3** at crude mycelial extracts significantly improved its recovery in a series of chromatography. Although the ^{13}C NMR data of **1** was reported, the assignment of signals has not been done. Thus, the signals of **3** in both ^{1}H and ^{13}C NMR spectra were unambiguously assigned based on extensive 2D NMR analysis including COSY, HSQC, and HMBC.

To elucidate the biosynthetic origin of the FR-900848 carbon skeleton, incorporation studies of ^{13}C-labeled precursors were conducted.[4] Feeding experiment with L-[Me-^{13}C]methionine to *S. fervens* HP-891 gave diacetate **3**. In the ^{13}C NMR spectra of labeled **3**, strongly enhanced signals were observed at cyclopropane methylenes C19, C20, C21, C22, and C23 (Table 1). In the case of administration with [1-^{13}C]acetate, however, no incorporation was observed. Use of [1,2-$^{13}C_2$]acetate, which is expected to be more effective in detection of ^{13}C-enriched signals also failed to detect any incorporation. These results were unexpected since Kuo et al. reported that structurally related long-chain cyclopropanoid **2** is biosynthesized via a polyketide pathway.[3a]

Glucose is a universal precursor of major biosynthetic pathways of secondary metabolites such as polyketide, mevalonate, shikimate, alkaloid pathways. Thus, D-[U-$^{13}C_6$]glucose was frequently used to define the biosynthetic pathway with analysis of ^{13}C-^{13}C coupling patterns in natural labeled products.[4] When D-[U-$^{13}C_6$]glucose was administered to the cultures, the ^{13}C NMR spectra of **3** obtained showed pairs of doublet

Table 1. ^{13}C NMR data of diacetate **3** derived from ^{13}C-labeled precursors[a]

	δ (ppm)	D-[U-$^{13}C_6$-]glucose		[1,3-$^{13}C_2$]glycerol enrichment[b]	[*Me*-^{13}C]L-methionine enrichment[b]
		Multiplicity	$J_{C\text{-}C}$ (Hz)		
1	167.0	d	66.4	1.27	
2	119.6	d	65.5	2.21	
3	147.4	d	55.6	1.16	
4	125.3	d	56.4	2.53	
5	142.0	d	56.4	1.03	
6	21.6	d	56.4	2.02	
7	24.1	d	54.8	1.35	
8	18.0	d	54.7	1.64	
9	18.8		—[c]	0.91	
10	18.4		—[c]	1.63	
11	18.2	d	54.7	0.99	
12	21.8	d	56.4	1.49	
13	20.0	d	56.4	1.14	
14	130.5	d	56.4	1.60	
15	131.1	d	56.4	1.20	
16	22.4	d	55.6	1.59	
17	14.80	d	43.9	1.29	
18	18.5	d	43.1	1.57	
19-CH_2	13.4			1.31	11.0
20-CH_2	7.7			1.42	21.5
21-CH_2	7.6			1.56	21.5
22-CH_2	11.5			1.03	13.8
23-CH_2	14.82			1.21	19.3
2′	152.6			1.61	
4′	169.2	d	47.9	1.64	
5′	31.0	d	33.0	1.38	
		d	48.7		
		dd	33.2, 48.1		
6′	39.1	d	33.8	2.42	
1″	88.9	d	45.4	1.31	
2″	70.6		—[c]	0.99	
3″	70.9		—[c]	1.71	
4″	79.4	d	39.8	1.20	
		t	40.7		
5″	40.8	d	41.3	2.87	
2″-CH_3CO_2	169.9				
2″-CH_3CO_2	20.52				
3″-CH_3CO_2	170.0				
3″-CH_3CO_2	20.53				

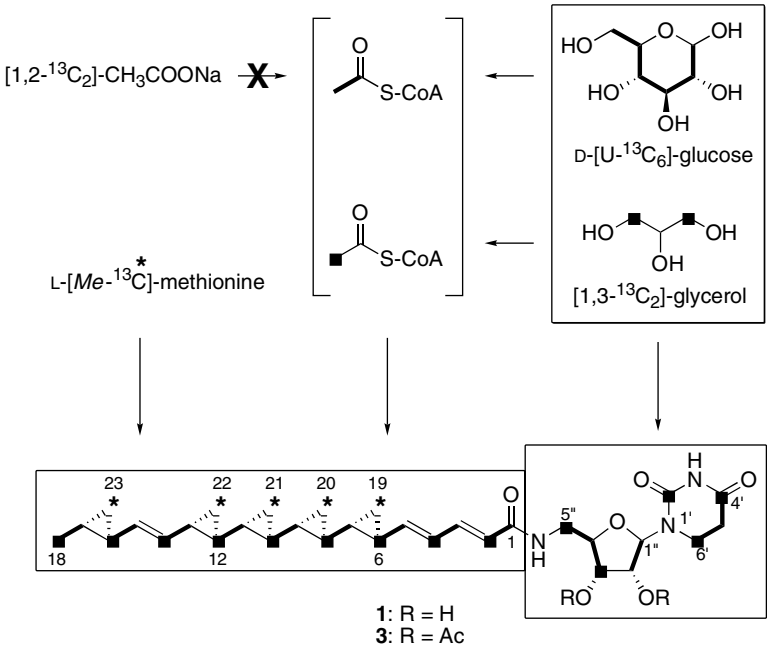
[a] The ^{13}C NMR spectra were measured in $CDCl_3$.

[b] The values of enrichments were determined by comparison of the relative peak intensities of the corresponding carbons in labeled and non-labeled spectra.

[c] The values could not be obtained since the corresponding signals were overlapped.

signals on the long-chain fatty acid at C1 through C18 (Table 1). This result can be explained by D-[U-$^{13}C_6$]glucose converted to acetyl-CoA via pyruvate and the resultant [1,2-$^{13}C_2$]acetyl-CoA was introduced to the fatty acid backbone (Scheme 1). Feeding [1,3-$^{13}C_2$]glycerol enhanced signals preferentially at C2, 4, 6, 8, 10, 12, 14, 16, and C18 (Table 1). This also supports that [1,3-$^{13}C_2$]glycerol was metabolized into [2-^{13}C]acetyl-CoA (Scheme 1), which was incorporated into the fatty acid moiety. It is generally accepted that most organisms utilize acetate as a carbon source of polyketides. In this case, acetate is converted by acetyl-CoA synthetase via adenylation and the subsequent attack of CoA-thiolate. The reason for the inability to incorporate acetate could be attributed to inactivation of acetyl-CoA synthetase. These unusual results in *S. fervens* strongly show that the major pathway to provide acetyl-CoA in this microorganism is glycolysis. In the feeding experiment with [^{13}C]acetates, lack of the ^{13}C-label in the dihydrouracil moiety of **3** agrees with this conclusion.

Analysis of the ^{13}C NMR spectrum of **3** from the feeding experiment with D-[U-$^{13}C_6$]glucose provided information on the biosynthetic pathway of the aminonucleoside moiety (Scheme 1). Although overlapped signals at C2″ and C3″ did not give useful data on the



Scheme 1. Incorporation of D-[U-$^{13}C_6$]glucose, [1,3-$^{13}C_2$]glycerol, and L-[*Me*-^{13}C]methionine into **1**.

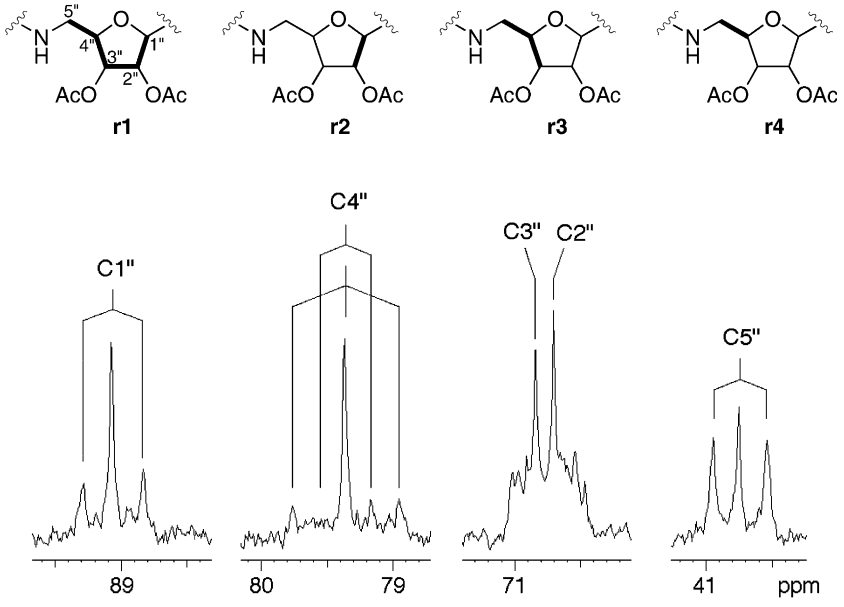


Figure 2. The ^{13}C-labeling patterns of the deoxyaminoribose unit and part of the ^{13}C NMR spectrum of **3** from feeding with D-[U-^{13}C]glucose.

coupling patterns, observation of doublets at C1″, C4″, C5″, and a triplet at C4″ (ribose unit) clearly showed the presence of ^{13}C-labels at adjacent positions as shown in Scheme 1. The observed ^{13}C-labeling pattern (Fig. 2, **r1**–**r4**) suggested that the ribose unit of **1** is derived via the pentose phosphate pathway from the D-[U-$^{13}C_6$]glucose or from partially ^{13}C-labeled glucose constructed by the $^{13}C_3$-units (glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate).[5d] On the other hand, the labeling pattern (doublets at C4′, C5′, C6′, and a double doublet at C5′) of the dihydrouracil moiety (C4′–C5′–C6′) contained a mixture of $^{13}C_3$- and two alternative $^{13}C_2$-units. This nucleoside part should originate from aspartate, which can be derived from acetyl-CoA via oxalacetate. Efficient reconstruction of oxaloacetate from two molecules of $^{13}C_2$-acetyl-CoA may explain the formation of $^{13}C_3$ units.

Enhanced signals at C4′/C6′ and C3″/C5″ in the spectrum of **1** from [1,3-$^{13}C_2$]glycerol supported intact incorporation of the C3-units in the ribose and dihydrouracil units, respectively, though the presence of ^{13}C-labels at multiple positions indicates significant scrambling occurred. Similar scrambling of ^{13}C-labels via the pentose phosphate pathway[5] and the TCA cycle has been reported.[6]

In summary, the biosynthetic origin of structurally unique polycyclopropanoid FR-900848 has been elucidated by a series of feeding experiments. During this study, we found an unusual observation that the polyketide backbone of FR-900848 was constructed by acetyl-CoA derived from glucose but not from acetate. To clarify the detailed mechanism of the polycyclopropanation, we are currently identifying the actual biosynthetic intermediate and the biosynthetic gene cluster.

Acknowledgments

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4. Feeding experiments with isotopically labeled compounds: culture medium and growth conditions for *Streptoverticillium fervens* HP-891 were as described by Yoshida et al.[1] Culture medium (100 ml) in a 500 ml Erlenmeyer flask was inoculated with a well-grown slant culture of the strain. The flask was shaken on a rotary shaker (180 rpm) at 30 °C for 3 days. The resultant cultures (10 ml per one flask) were poured into two to four Erlenmeyer flasks containing 100 ml of a fermentation medium. On the fourth day after inoculation, the sterilized aqueous solution of a labeled compound (50 mg per one flask) was added to the cultures. After further incubation for 5 day, the mycelia were extracted with acetone. The crude extracts were treated with acetic anhydride, pyridine, and dimethylaminopyridine. Purification of the products by silica gel column chromatography and reverse-phase HPLC afforded FR-900848 diacetate **3** (1.6–5.4 mg).
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