

Biosynthesis of the Lipophilic Side Chain in the Cyclic Hexadepsipeptide Antibiotic IC101

Kazuo Umezawa,^{*,†} Yoko Ikeda,[‡] Hiroshi Naganawa,[‡] and Shinichi Kondo[‡]

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-0061, Japan, and Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

Received May 1, 2002

Antibiotic IC101 is a cyclic hexadepsipeptide having a C₁₅ lipophilic side chain. The side chain was shown to be synthesized in *Streptomyces* from acetate, propionate, and 3-methylbutyrate derived from leucine. Thus, the terminal isopentyl structure came from leucine and not from the mevalonate pathway.

Although many anticancer agents are known to induce apoptosis in cultured cells, human solid carcinoma cells are often resistant to anticancer drug-induced apoptosis. In the course of our screening for potent apoptosis inducers,¹ we isolated novel cyclic peptides, polyoxypeptins A and B,^{2,3} and chloptosin⁴ from a *Streptomyces* strain. Recently, we also isolated a novel cyclic hexadepsipeptide, pipalamycin, from a culture broth of *Streptomyces* sp. ML297-90F8, and determined its structure by spectral analysis.⁵ Pipalamycin was isolated as a minor component, and the same strain also produced a large amount of the known antibiotic IC101 (*N*-hydroxypipalamycin).⁶

There are two types of terminal alkyl groups in these cyclic hexadepsipeptide antibiotics, i.e., the 2-methylbutyl and 3-methylbutyl (isopentyl) chains. The former includes variapentin,⁷ L-156,602,⁸ and polyoxypeptins A and B,^{2,3} and the latter, IC101⁶ and pipalamycin.⁵ In our biosynthetic studies on polyoxypeptin A,⁹ we found that the novel amino acid (2*S*,3*R*)-3-hydroxy-3-methylproline and the C₅ lipophilic side chain moieties were both derived from isoleucine (Figure 1). Antibiotic IC101 consisted of two molecules each of *N*-hydroxyalanine and piperazine acid, and one each of glycine and 3-hydroxyisoleucine. The 3-hydroxyisoleucine moiety was *N*-acylated to the lipophilic side chain. In comparison with that of polyoxypeptin A, the C₅ terminal side chain of IC101 and pipalamycin was suggested to be derived from leucine instead of isoleucine. Therefore, we studied the biosynthesis of the antibiotic IC101 by incorporation of stable isotope-labeled leucine, isoleucine, acetate, and propionate.

The incorporation of ¹³C-labeled compounds into antibiotic IC101 by the producing organism is summarized in Table 1. The ¹³C chemical shift assignments in CDCl₃ were made by 2D NMR techniques, such as HMQC and HMBC. When L-[U-¹³C]leucine was added to the culture, not only all six carbons of the 3-hydroxyisoleucine moiety but also the terminal five carbons (considered to come from 3-methylbutyryl-CoA) of the lipophilic side chain were strongly enriched in ¹³C, and a pair of ¹³C–¹³C spin couplings was observed. L-[U-¹³C]isoleucine was incorporated into two three-carbon sets (considered to come from propionyl-CoA) having ¹³C–¹³C spin couplings, CO:C-2:2-Me and C-11:C-12:C-13, in the C₁₅ side chain. It is known that leucine is converted to 3-methylbutyryl-CoA and acetyl-CoA, and

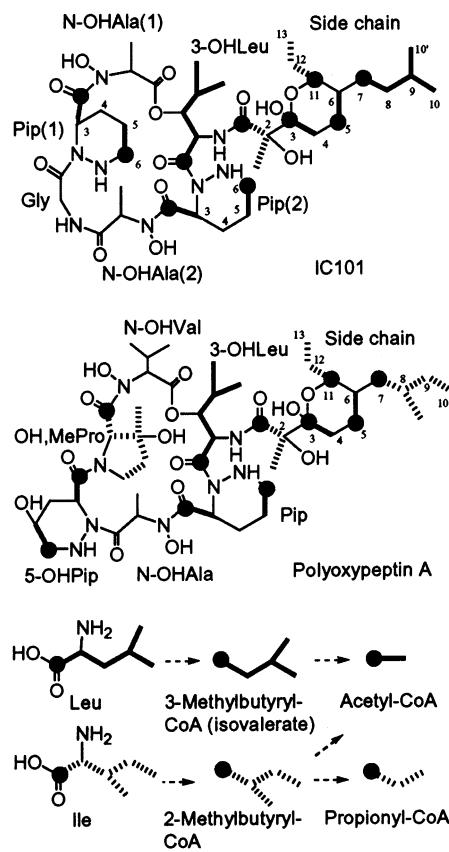


Figure 1. Incorporation of ¹³C-labeled leucine and isoleucine into hexadepsipeptide antibiotics.

isoleucine, to 2-methylbutyryl-CoA and propionyl-CoA (Figure 1).¹⁰ These incorporations were confirmed by the addition of [1-¹³C]propionate, which gave significant enrichments at the C-1 and C-11 of the side chain. Addition of [1-¹³C]acetate showed that the CO and C-6 carbons of the two piperazine acid moieties, as well as the C-3 and C-5 carbons of the side chain, were derived from acetate. Thus, as shown in Figure 1, the terminal C₅ unit is derived from Leu via 3-methylbutyryl-CoA, while that of polyoxypeptin A from Ile via 2-methylbutyryl-CoA.⁹

In conclusion, the 3-methylbutyl terminal of the C₁₅ lipophilic side chain was shown to be derived from leucine via 3-methylbutyryl-CoA, rather than from isoleucine, as in the case of polyoxypeptin A. The isopentyl group of pipalamycin should come from leucine, too. These results

* To whom correspondence should be addressed. Tel/Fax: 81-45-566-1558. E-mail: umezawa@appc.keio.ac.jp.

[†] Keio University.

[‡] Institute of Microbial Chemistry.

Table 1. Incorporation of ^{13}C -Labeled Compounds into Antibiotic IC101

carbon	δ in CDCl_3	enrichment factor ^a			
		[U- ^{13}C]Leu	[U- ^{13}C]Ile	$\text{CH}_3^{13}\text{CO}_2\text{H}$	$\text{CH}_3\text{CH}_2^{13}\text{CO}_2\text{H}$
N-OHAla(1) CO	169.0	1.0	0.8	1.1	1.2
2	53.1	1.0	1.1	1.0	1.0
3	13.0	1.0	0.9	1.0	1.1
Pip(1) CO	172.8	1.3	1.0	1.9	2.2
3	48.7	1.0	1.1	1.0	1.0
4	23.2	1.3	1.0	1.3	0.8
5	20.2	OL ^c	1.1	0.9	1.0
6	46.7	OL	1.0	3.5	1.0
Gly CO	172.6	1.0	0.9	1.3	1.3
2	42.2	1.2	1.2	1.6	1.4
N-OHAla(2) CO	170.3	1.0	0.8	1.2	1.0
2	53.5	1.0	1.0	1.0	1.0
3	11.9	1.2	1.3	1.0	1.2
Pip(2) CO	172.3	1.0	1.1	1.6	2.0
3	49.4	1.0	1.1	0.9	1.0
4	24.7	1.2	1.2	1.1	1.3
5	21.5	OL	OL	OL	OL
6	47.1	1.1	0.9	3.2	1.0
3-OHLeu CO	171.2	13.9 (88) ^b	1.0	3.9	1.2
2	46.1	10.1 (92) ^b	1.0	1.0	1.0
3	79.5	9.4 (95) ^b	1.0	1.0	1.0
4	30.2	13.8 (87) ^b	1.1	0.9	1.0
5	18.3	11.0 (87) ^b	1.0	1.0	1.0
5'	20.0	10.7 (84) ^b	1.1	0.9	0.9
side chain CO	175.5	1.1	1.8 (38) ^b	1.5	11.0
2	77.6	1.0	1.7 (37) ^b	1.0	1.0
2-Me	21.5	OL	1.9 (48) ^b	OL	OL
3	98.7	0.9	0.8	3.5	1.0
4	27.2	1.2	1.1	0.9	1.1
5	24.6	0.9	1.1	2.9	1.2
6	39.6	1.1	1.1	1.1	1.1
7	29.2	14.2 (81) ^b	1.2	1.0	1.2
8	35.5	11.1 (86) ^b	0.9	0.8	0.9
9	28.5	9.7 (86) ^b	1.0	1.0	1.0
10	23.0	11.7 (88) ^b	1.2	1.1	1.3
10'	22.3	11.1 (86) ^b	1.1	1.0	1.1
11	76.6	1.0	1.4 (37) ^b	1.2	7.0
12	25.0	0.9	1.9 (69) ^b	0.9	0.8
13	11.2	1.2	3.5 (43) ^b	1.0	1.1

^a Enrichment factor (peak area of enriched sample/natural abundance peak area) was calculated from spectral run under essentially identical conditions. ^b The enrichment factor includes ^{13}C – ^{13}C spin-coupling peak area. The coupling peak areas (%) are shown in parentheses. ^c OL: The coupling peaks overlapped. Underlining shows significantly enriched signals.

may also suggest that the terminal 2-methylpropyl (isobutyl) group in aurantimycins A, B, and C¹¹ and a related antibiotic¹² is derived from valine.

Experimental Section

Stable Isotope-Labeled Compounds and Spectral Analysis. Sodium [1- ^{13}C]acetate (99 atom %) and sodium [1- ^{13}C]propionate (99%) were purchased from Sigma and Aldrich, respectively. L-[U- ^{13}C]leucine (98%) and L-[U- ^{13}C]isoleucine (98%) were obtained from Cambridge Isotope Lab. ^1H and ^{13}C spectra were measured in CDCl_3 with a JEOL JNM-EX400 spectrometer.

Fermentation. Mycelia of *Streptomyces* strain ML297-90F8 were inoculated into a 500 mL baffled Erlenmeyer flask containing a medium (110 mL; pH 7.4) composed of 2.0% D-galactose, 2.0% dextrin, 1.0% peptone (Soytone Peptone, Difco), 0.5% corn steep liquor (Ajinomoto), 0.2% $(\text{NH}_4)_2\text{SO}_4$, and 0.2% CaCO_3 . The mycelia were cultured at 30 °C for 5 days on a rotatory shaker at 180 rpm. The seed culture (2.2 mL) was transferred to each of three Erlenmeyer flasks containing 110 mL of a medium composed of 1.0% glycerol, 0.5% peptone (Soytone Peptone, Difco), 0.15% yeast extract (Difco), 0.1% $(\text{NH}_4)_2\text{SO}_4$, and 0.1% CaCO_3 , and the mixture was then incubated at 28 °C for 4 days on a rotatory shaker at 180 rpm. Each stable isotope-labeled compound was added to 24 h cultures (amino acids, 5 mg, and alkanolic acids, 20 mg, in each flask).

Isolation of Labeled IC101. The whole culture broth combined from the three flasks (330 mL, pH 6.8–7.8) was

extracted with an equal volume of EtOAc, and the extract was evaporated to a syrup. The syrup was purified by column chromatography on silica gel 60 (Kanto Chemical, particle size 40–60 mm; 7–14 g) with $\text{CHCl}_3/\text{MeOH}$ (50:1) as the eluent. Fractions were monitored by TLC ($\text{CHCl}_3/\text{MeOH}$, 10:1) and detected by phosphomolybdic acid/ H_2SO_4 reagent consisting of 12 g of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 7.5 mL of 85% H_3PO_3 , 25 mL of concentrated H_2SO_4 , and 500 mL of H_2O . Fractions containing IC101 (R_f 0.50) were collected and concentrated to yield pure labeled IC101 (11.5–33.6 mg). The physicochemical data of labeled compound were compared with those of known IC101.⁶

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