BIOSYNTHETIC STUDIES OF LEPTOMYCINS

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Leptomycins are antifungal antibiotics found in the course of a screening program for activities inducing morphological abnormalities[1]. Elucidation of the chemical structures of these compounds showed them to be unique unsaturated, branched-chain fatty acids[2,3] (Fig. 1). Because of their relatively similar chemical structures to macrolide antibiotics, especially ebelactones[4], we assumed that leptomycins might be synthesized through a similar pathway from acetic, propionic and butyric acids[5]. Thus, we determined to use [1-^{13}C]sodium acetate (91.9 atom %, Prochem), [1-^{13}C]sodium propionate (91.7 atom %, Prochem) and [1-^{13}C]sodium butyrate (91.0 atom %, Prochem) as precursors to study the biosynthesis of leptomycins.

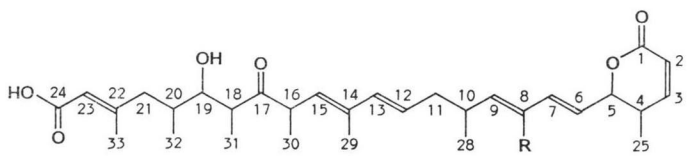
For the incorporation experiments, leptomycin-producing strain, *Streptomyces* No. 1287 was grown at 26.5°C (5% vegitative inoculum) in a medium consisting of 5% soybean flour, 3% soybean oil, 0.3% dried yeast, 0.3% KCl, 0.02% K_2HPO_4 and 0.3% $CaCO_3$ (pH 7.2) on a rotary shaker with 10 cm displacement at 100 rpm. In this medium, antibiotic production began at about 72 hours and continued until 144 hours. Neither growth nor antibiotic production were affected by addition of at least 1 mg/ml of acetic, propionic and butyric acid to the medium. Therefore, labeled precursor was added to the producing culture at 72 and 96 hours (50 mg/100 ml medium/500 ml flask at one time) and harvested at 120 hours. Mycelium was collected from five flasks by centrifugation and extracted with 200 ml of acetone three times. The extracts were concentrated to dryness and reextracted with 500 ml of EtOAc. Leptomycins A and B were purified from the concentrated EtOAc extract by silica gel column chromatography (100 ml) and Nucleosil 5C-18 silica gel HPLC (M. Nagel, 8 mm ϕ $\times$300 mm) using the solvent systems as described before[2].

The ^{13}C NMR spectra were recorded at 25.0 MHz on a Jeol FX-100 spectrometer using TMS as internal standard. The samples were dissolved in $CDCl_3$ and measurements were made at 24°C.

The isotopic enrichment was determined as the ratio of ^{13}C-enriched peaks to those of normal leptomycins. As shown in Table 1, the peaks of C-1, -5, -11 and -23 of leptomycin A were enriched in the [1-^{13}C]acetate experiment and the peaks of C-3, -7, -9, -13, -15, -17, -19 and -21 were highly enriched in the [1-^{13}C]propionate experiment, while no [1-^{13}C]butyrate was incorporated with enrichment into leptomycin A as expected. These results indicate that four molecules of acetic acid and eight molecules of propionic acid were used for biosynthesis of leptomycin A. In the case of leptomycin B (Table 2), [1-^{13}C]acetate was incorporated with enrichment into C-1, -5, -11 and -23, and the peaks of C-3, -9, -13, -15, -17, -19 and -21 were highly enriched in the [1-^{13}C]propionate experiment. On the other hand, the peak of C-7 of [1-^{13}C]butyrate-enriched leptomycin B was enriched, indicating that four molecules of acetic acid, seven molecules of propionic acid and one molecule of butyric acid were used in the biosynthesis of leptomycin B.

These results also confirmed the chemical structures of leptomycins (Fig. 2). Leptomycins include three carbonyl groups whose positions in

Fig. 1. Chemical structure of leptomycins A and B.



Leptomycin A R = CH_3

Leptomycin B R = CH_2CH_3 (26, 27)

Table 1. ^{13}C NMR spectral data for leptomycin A including enrichment from labeled precursors.

Position	Chemical shift (δ_C ppm)	Enrichment ratio[a]			Position	Chemical shift (δ_C ppm)	Enrichment ratio[a]		
		[1-^{13}C]-Acetate	[1-^{13}C]-Propionate	[1-^{13}C]-Butyrate			[1-^{13}C]-Acetate	[1-^{13}C]-Propionate	[1-^{13}C]-Butyrate
1	164.4	5.40	—[b]	0.99	18	47.0	1.80	1.8	0.72
2	120.0	1.17	1.2	0.83	19	73.8	2.10	7.5	1.65
3	151.6	2.97	27.5	2.36	20	33.5	1.08	—	0.62
4	33.5	1.08	—	0.62	21	45.7	1.23	6.5	1.81
5	81.3	4.50	—	1.51	22	160.9	—	—	—
6	123.3	1.29	—	1.05	23	117.1	6.99	—	—
7	129.6	2.22	27.7	1.25	24	171.3	—	—	—
8	135.5	1.26	—	1.28	25	12.3	0.95	—	0.86
9	138.7	2.55	26.6	0.71	26	20.5	1.08	—	0.82
10	32.3	1.62	1.3	0.93	28	13.0[c]	0.93	1.0	0.70
11	40.8	4.50	—	1.85	29	18.5	0.98	—	—
12	128.3	1.05	1.3	1.77	30	13.0[c]	0.93	1.0	0.70
13	135.3	2.10	19.1	1.28	31	18.5	1.32	1.0	1.17
14	136.5	1.52	—	0.76	32	13.6	1.00	1.0	1.00
15	128.0	1.50	14.7	1.09	33	16.0	1.65	—	0.95
16	45.6	0.99	—	1.48					
17	215.3	1.14	8.4	0.95					

a) Intensity of each peak as the ratio of enriched/normal ^{13}C abundance, to give a ratio of 1.00 for the peak of C-32.

b) Signals of these carbons were lost in the noise.

c) These peaks could not be distinguished from each other.

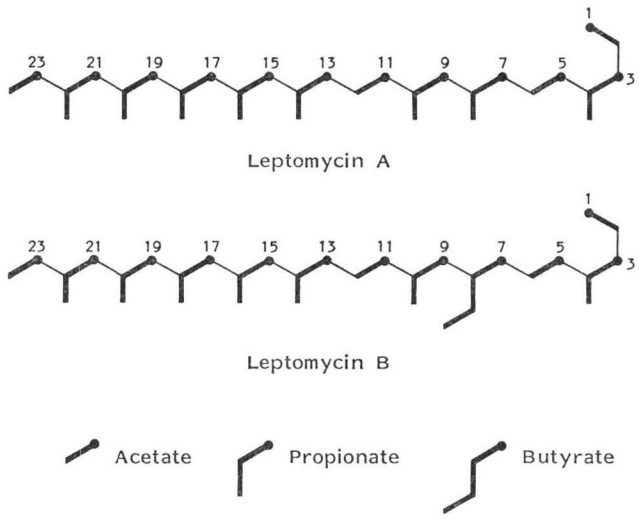
Table 2. ^{13}C NMR spectral data for leptomycin B including enrichments from labeled precursors.

Position	Chemical shift (δ_C ppm)	Enrichment ratio[a]			Position	Chemical shift (δ_C ppm)	Enrichment ratio[a]		
		[1-^{13}C]-Acetate	[1-^{13}C]-Propionate	[1-^{13}C]-Butyrate			[1-^{13}C]-Acetate	[1-^{13}C]-Propionate	[1-^{13}C]-Butyrate
1	164.4	7.80	—[b]	0.61	18	47.0	0.81	—	0.51
2	120.0	1.71	2.1	0.89	19	74.2	1.50	35.7	1.51
3	151.6	2.72	30.7	4.34	20	33.6	0.69	2.2	0.54
4	33.6	0.69	2.2	0.54	21	45.7	0.75	36.8	0.86
5	81.5	4.62	3.0	1.77	22	160.9	—	—	—
6	122.8	1.05	—	1.05	23	117.1	9.27	—	—
7	130.2	1.44	—	10.93	24	171.3	—	—	—
8	135.6	0.78	—	1.49	25	12.3	0.57	—	1.16
9	136.9	1.29	29.9	4.97	26	26.6	1.32	1.6	1.18
10	32.2	0.84	1.7	0.96	27	13.5	0.87	1.0	1.02
11	40.9	4.65	—	2.01	28	13.0[c]	1.50	1.0	1.12
12	128.2	0.66	3.0	3.26	29	18.5	1.01	—	0.54
13	135.3	0.78	19.5	3.27	30	13.0[c]	1.50	1.0	1.12
14	136.5	0.81	—	0.87	31	20.9	1.21	—	1.25
15	128.0	0.93	19.5	1.48	32	13.6	1.00	1.0	1.00
16	45.7	0.60	2.7	0.70	33	16.0	0.69	—	0.89
17	214.9	1.14	20.7	1.41					

a),b),c) See Table 1.

the chemical structure could not be determined by ^1H NMR proton spin decoupling experiment because of their lack of protons attached to the carbonyl carbons, C-1, C-17 and C-24. The structures including these carbonyl carbon atoms were elucidated with the results of ^{13}C NMR long range selective proton spin decoupling (LSPD) experiments[2]. The revealed incorporation pat-

Fig. 2. Biosynthesis of leptomycins A and B.



tern here observed indicates that the C-17 carbon atom is derived from the propionate unit. Thus, C-17 ketone carbonyl carbon atom should be placed next to C-16 and C-18 but not to C-1, C-2, C-23 and C-24, considering the spectrometric data of leptomycin that indicated existence of no carbonyl carbon atoms bonding directly to each other. On the other hand, the C-1 and C-23 carbon atoms are both enriched with [1-^{13}C]-acetate, indicating that C-1 carbonyl carbon atom should be placed next to C-2 but not to C-23 that is adjacent to C-24. These results finally confirmed the incorporation pattern of the building units into leptomycins A and B and also reconfirm the chemical structures of leptomycins.

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