LEPTOMYCINS A AND B, NEW ANTIFUNGAL ANTIBIOTICS

II. STRUCTURE ELUCIDATION

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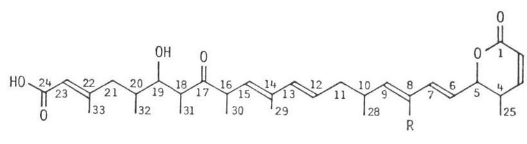
The structures of new antifungal antibiotics, leptomycins A and B produced by *Streptomyces* sp. ATS1287 were determined as described below (Fig. 1) on the basis of their spectral and chemical character. Leptomycins have unique structures which belong to the unsaturated, branched-chain fatty acids with δ-lactone rings at the end.

In the course of a screening program for new antifungal antibiotics, *Streptomyces* sp. ATS1287 isolated from a soil sample was found to produce two unique substances which caused cell elongation of Schizosaccharomyces and hyphal swelling or curling of *Mucor racemosus* and *M. rouxianus*[1]. Taxonomy of the producing strain, purification and characterization of these compounds have been reported in the preceding paper[2]. This paper deals with the structure elucidation of leptomycins A (LMA) and B (LMB).

Fig. 1. Structures of leptomycins.



Leptomycin A (LMA)	$R=CH_3$
Leptomycin B (LMB)	$R=CH_2CH_3$

Chemical Structure of Leptomycin B (LMB)

In the preceding paper, the molecular formula of LMB ($C_{33}H_{48}O_6$, MW 540) was established by the ^1H NMR, ^{13}C NMR and mass spectrometry, and it was assumed to have a hydroxyl group and three carbonyl groups by spectral analysis. The presence of these structural elements were supported by the IR (Fig. 2) and ^{13}C NMR spectra (Table 1) of methylated and acetylated LMB. In the IR spectrum of LMB methyl ester, the characteristic absorption band for a carboxylate ion (3500~3200 cm^{-1}) disappeared and the absorption band for a carbonyl group shifted by methylation (1720 cm^{-1}). The IR spectrum of *O*-acetyl LMB methyl ester lacked an OH-absorption band. In the ^{13}C NMR spectrum of LMB methyl ester, a new signal at δ_C 50 assigned as $-OCH_3$ methyl carbon atom appeared and a carbon atom at δ_C 171 showed the methylation shift to δ_C 167. The ^{13}C NMR spectrum of *O*-acetyl LMB methyl ester showed new signals at δ_C 23 and 174 consistent with the CH_3CO- group. On the other hand, the chemical shifts of carbon atoms at δ_C 164 and 214 remained almost unchanged in the spectra of the derivatives. Thus, the chemical structure of LMB were determined to have a carboxyl group (δ_C 171), an ester (164), a ketone (214) and an alcohol.

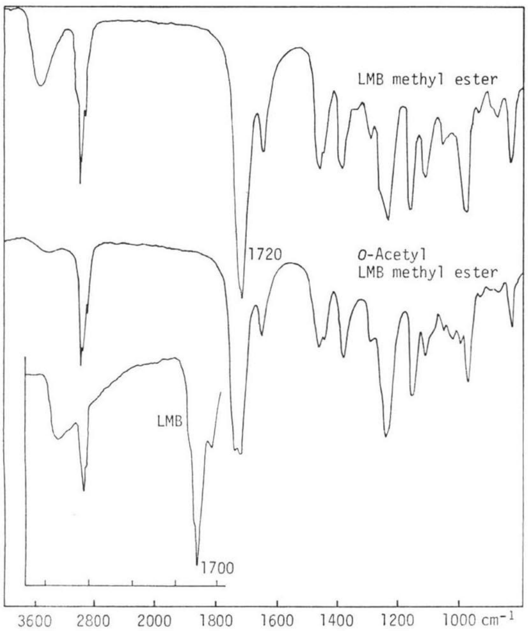
Partial structures of LMB were obtained by a number of experiments using ^1H NMR (400 MHz, in $CDCl_3$) homo-spin decoupling. The partial structures and coupling constants proved by homo-spin decoupling studies are shown in Fig. 3.

Table 1. ^{13}C NMR spectral data of LMB methyl ester and *O*-acetyl LMB methyl ester ($CDCl_3$, 25.05 MHz).

No.	LMB	LMB-Me#	LMB-MeAc##	No.	LMB	LMB-Me	LMB-MeAc
1	*214.9	215.2	**215.3	19	45.7	45.7	48.7
2	171.3	167.0	170.2	20	45.7	45.6	48.4
3	164.4	164.2	168.3	21	40.9	40.8	43.7
4	160.9	158.4	160.8	22	33.6	33.6	36.4
5	151.6	151.7	156.1	23	33.6	33.6	36.2
6	136.9	137.0	139.9	24	32.2	32.2	35.2
7	136.5	136.4	139.9	25	26.6	26.6	29.5
8	135.6	135.5	139.1	26	20.9	20.8	22.5
9	135.3	135.3	138.2	27	18.5	18.4	20.3
10	130.2	130.2	132.6	28	16.0	16.1	18.0
11	128.2	128.2	131.2	29	13.6	13.7	16.1
12	128.0	128.0	131.2	30	13.5	13.6	16.1
13	122.8	122.8	126.3	31	13.0	13.1	15.6
14	120.0	120.0	121.9	32	13.0	12.4	15.2
15	117.1	117.1	119.9	33	12.3	12.4	14.4
16	81.5	81.5	84.6	Me		50.8	53.1
17	74.2	74.2	78.6	Ac			174.2
18	47.0	47.0	49.5				23.2

#; LMB methyl ester, ##; *O*-acetyl LMB methyl ester.
*; δ_C Relative to TMS, **; in CD_3OD.

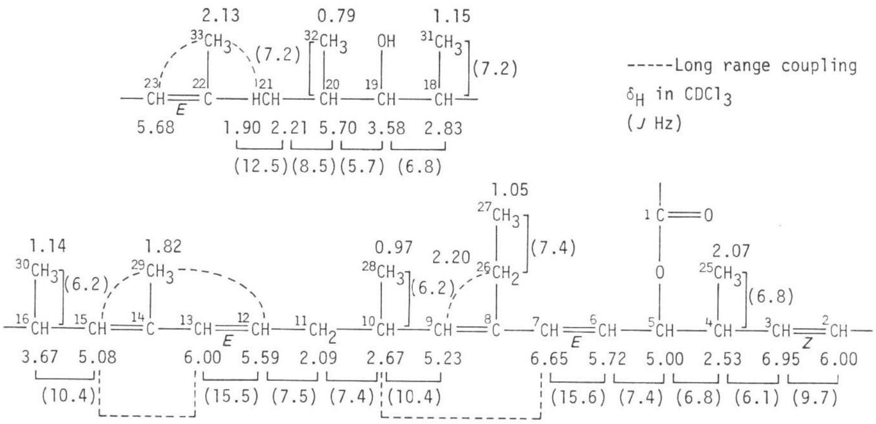
Fig. 2. IR spectra of LMB methyl ester and *O*-acetyl LMB methyl ester (film).



The configurations of three double bonds at C-2, C-6 and C-12 were revealed to be *Z*, *E* and *E* by the coupling constants of $J_{H2-H3} = 9.7$ Hz, $J_{H6-H7} = 15.6$ Hz and $J_{H12-H13} = 15.5$ Hz, respectively. The upfield chemical shifts of the methyl carbons C-29 (δ_C 18.5) and C-33 (δ_C 16.0) suggested the *E* configurations of the double bonds at C-14 and C-22. The configuration of the double bond at C-8 is still ambiguous and under investigation.

The carbonyl carbon atoms were assigned by ^{13}C-{^1H} long range selective proton decoupling (LSPD) spectra[3,4] (Fig. 4). The signal at δ_C 164 (ester carbonyl carbon atom) collapsed to a doublet-like signal when the H-2 proton atom (δ_H 6.00) was irradiated and to a singlet-like signal when the H-3 proton atom (δ_H 6.95) was irradiated. The signal at δ_C 171 (carboxylate carbon atom) became a sharp signal when the H-23 proton atom (δ_H 5.68) was irradiated. The signal at δ_C 214 (ketone carbonyl carbon atom) did not collapse by the irradiation of any proton atom so it was assigned to the C-17 carbon atom. The assignments of all the carbon atoms by ^{13}C NMR selec-

Fig. 3. Partial structures of LMB.



tive proton decoupled spectra as well as those of proton atoms of LMB are shown in Table 2.

Thus, the chemical structure of LMB was determined as described in Fig. 1.

Chemical Structure of Leptomycin A (LMA)

The chemical structure of LMA was determined from a comparison of the ^1H NMR spectrum with that of LMB. In the ^1H NMR spectrum of LMA (Fig. 5), a new signal at δ_H 1.82 (s, 3H) assigned as $=C-CH_3$ methyl protons was observed, and signals assigned to H-26 and H-27 protons were not observed. Thus, the chemical structure of LMA was determined to have a methyl group (C-26) at C-10

Table 2. ^1H NMR and ^{13}C NMR chemical shift of leptomycin B.

No.	[1)] δ_H			[2)] δ_C		No.	δ_H			δ_C	
1	—			164.4[3)]	s	18	2.83	1H	m	47.0	d
2	6.00	1H	d	120.0	d	19	3.58	1H	t	74.2	d
3	6.95	1H	d	151.6	d	20	1.75	1H	m	33.6	d
4	2.53	1H	m	33.6	d	21	1.90	1H	dd	45.7	d
5	5.00	1H	dd	81.5	d		2.21	1H	dd		
6	5.72	1H	dd	122.8	d	22	—			160.9	s
7	6.65	1H	d	130.2	d	23	5.68	1H	s	117.1	d
8	—			*135.6	s	24	—			171.3	s
9	5.23	1H	d	136.9	d	25	1.07	3H	d	♯12.3	q
10	2.67	1H	d	32.2	d	26	2.20	2H	q	26.6	t
11	2.09	2H	t	40.9	t	27	1.05	3H	t	♯13.5	q
12	5.59	1H	m	**128.2	d	28	0.97	3H	d	♯13.0	q
13	6.00	1H	d	135.3	d	29	1.82	3H	s	18.5	q
14	—			*136.5	s	30	1.14	3H	d	♯13.0	q
15	5.08	1H	d	**128.0	d	31	1.15	3H	d	20.9	q
16	3.67	1H	m	45.7	d	32	0.79	3H	d	♯13.6	q
17	—			214.9	s	33	2.13	3H	s	16.0	q

[1)] δ_H Relative to TMS ($CDCl_3$, 400 MHz), [2)] δ_C relative to TMS ($CDCl_3$, 25.05 MHz), [3)] multiplicity in off-resonance spectrum.

*, **, ♯; Assignments may be interchanged.

Fig. 4. ^{13}C NMR (LSPD) signals of LMB (in $CDCl_3$, 100 MHz).

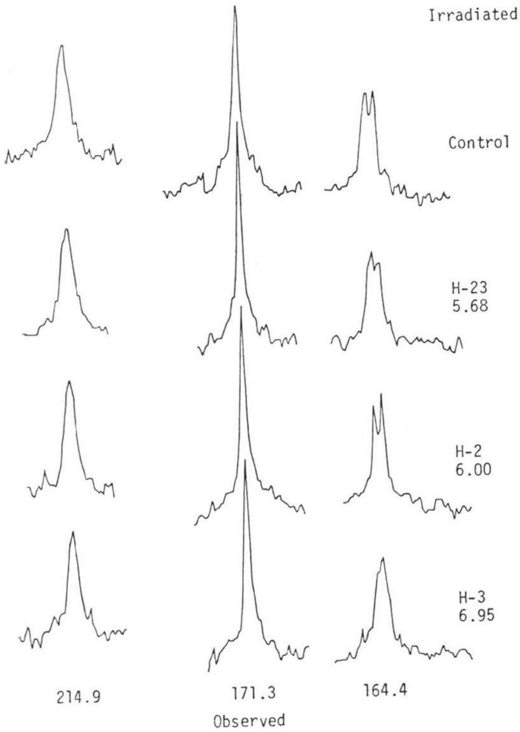


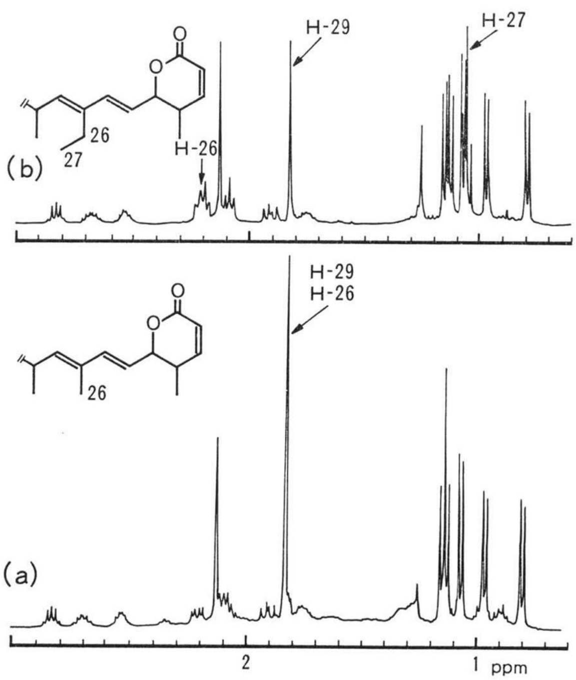
Table 3. ^1H NMR chemical shift of leptomycin A ($CDCl_3$, 400 MHz).

No.	$^*\delta_H$			No.	δ_H		
H-2	6.00	1H	d	H-19	3.58	1H	t
3	6.95	1H	d	20	1.75	1H	m
4	2.53	1H	m	21	1.90	1H	dd
5	5.01	1H	dd		2.20	1H	dd
6	5.70	1H	dd	23	5.70	1H	s
7	6.75	1H	d	25	1.07	3H	d
9	5.26	1H	d	26	1.82	3H	s
10	2.69	1H	d	28	0.98	3H	d
11	2.09	2H	t	29	1.82	3H	s
12	5.59	1H	m	30	1.14	3H	d
13	6.00	1H	d	31	1.16	3H	d
15	5.09	1H	d	32	0.79	3H	d
16	3.66	1H	m	33	2.12	3H	s
18	2.83	1H	m				

*; δ_H Relative to TMS.

whereas LMB has an ethyl group (C-26 and C-27), as described in Fig. 1. The assignments of all the proton atoms of LMA are shown in Table 3.

Fig. 5. ^1H NMR spectra of LMA (a) and LMB (b) in $CDCl_3$.



Experimental

General

The IR absorption spectra were measured on a JASCO A-202 spectrometer. The ^{1}H NMR spectra were measured on a JEOL FX-400 FT-NMR spectrometer (400 MHz) and ^{13}C NMR spectra on a JEOL FX-400 FT-NMR spectrometer (100 MHz) or a JEOL FX-100 FT-NMR spectrometer (25.05 MHz) using TMS as an internal standard.

LMB Methyl Ester

To a solution of 20 mg LMB in 1 ml of chloroform was added 0.5 ml of diazomethane ether solution, and was the mixture kept at 20°C for 15 minutes. After evaporation of the solvent, the residue was purified by preparative TLC on silica gel (chloroform - ethyl acetate, 10: 1) to give 16 mg LMB methyl ester.

O-Acetyl LMB Methyl Ester

Here, 16 mg LMB methyl ester in 2 ml of acetic anhydride - pyridine was stirred at 20°C for 16 hours. The product was extracted with ethyl acetate and purified by preparative TLC on silica gel (chloroform - ethyl acetate, 1: 2) to give 10 mg of *O*-acetyl LMB methyl ester.

References

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