Communications to the editor

LAIDLOMYCIN, A NEW ANTIMYCOPLASMAL POLYETHER ANTIBIOTIC

Sir:

Screening of *Streptomyces* culture filtrates for antimycoplasmal activities as conducted in our laboratory has been found to be a very useful system to isolate new antibiotics prossessing characteristic biological activities.[1,2] Laidlomycin, one of the antibiotics which were isolated in our laboratory by such means, was obtained from the culture filtrate of a *Streptomyces* species indexed as S-822 in our culture collection. *Streptomyces* S-822 was isolated from a soil sample collected at Lake Saiko, Yamanashi Prefecture, Japan. The strain is very similar to *Streptomyces eurocidicus* var. *asterocidicus*, if not identical (unpublished).

Laidlomycin was purified and determined to be a new polycyclic polyether antibiotic possessing inhibitory activity against various *Mycoplasma* species, especially against *Acholeplasma laidlawii* at the concentration of 0.16 mcg/ml, but not against bacteria, fungi or yeast. Furthermore, the antibiotic was cytotoxic to several established tissue culture cells, and was effective in controlling coccidiosis in chickens. In this paper, we describe the results of the isolation and characterization of laidlomycin.

Production and Isolation

For laboratory production, 100 ml of seed culture medium consisting of 2% soy bean meal, 2% starch, 0.5% dry yeast, 0.25% NaCl, 0.2% $CaCO_3$, 0.005% $MnSO_4$, 0.005% $CuSO_4$ and 0.005% $ZnSO_4$ (pH 7.4) was inoculated with the strain S-822 grown on KRAINSKY's glucose-asparagine agar slants and cultured at 27°C for 16 hours on a reciprocating shaker at 120 strokes/minute. Three ml each of this seed culture was transferred into 500-ml SAKAGUCHI flasks containing 100 ml of the production medium consisting of 2% glucose, 0.5% peptone, 0.5% NaCl, 0.2% $CaCO_3$ and 0.1% CH_3COONa (pH 7.2), and cultured for 80 hours at 27°C under the same shaking condition. The titer, when assayed by the pulp disc diffusion method against *A. laidlawii*, reached a maximum of 60 mcg/ml. The pH of the medium was 5.6~6.0 at the time of maximum potency.

To isolate the antibiotic, 30 liters of culture filtrate was adjusted to pH 3.0 with 5 N HCl and extracted twice with 5 liters of ethyl acetate. The combined extracts were then washed with 2 liters of water, dried by adding anhydrous sodium sulfate and concentrated *in vacuo*. The darkbrown syrup (3.0 g) which was obtained contained almost all of the original antimycoplasmal activity.

Further purification of the antibiotic was carried out by gel filtration on Sephadex LH-20 and silica gel column chromatography. The syrup was dissolved in a small amount of ethanol and charged on a column of Sephadex LH-20 (200 ml, 24 × 400 mm) formed with ethanol. The active fractions eluted with ethanol were concentrated to dryness *in vacuo*, then dissolved in a small volume of benzene and applied onto a silica gel column (Merck Kieselgel, 20 g, 22×150 mm). After washing the column with 200 ml of benzene-ethylacetate (5:1), the active principle was eluted from the column with benzene-ethylacetate (5:3). Active fractions were concentrated to dryness *in vacuo*, and crystallized from ethyl acetate. After recrystallization from chloroform-ethyl acetate, 40 mg of laidlomycin free acid was obtained as a colorless prisms.

The sodium salt of laidlomycin can be isolated by the two ways; one is by extracting with organic solvent after adjusting the culture filtrate to pH 8.0 with 1 N NaOH, followed by the previously described isolation procedure. Another is by converting pure acid form to the salt form with sodium hydroxide. Both free acid and salt forms of laidlomycin are readily extractable from water into organic solvents.

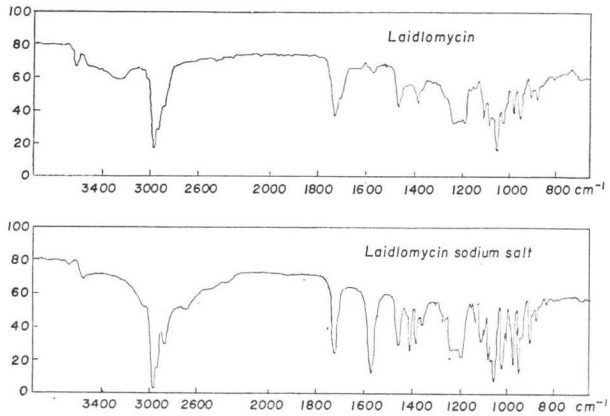
Chemical Characterization

Laidlomycin is characterized as a monocarboxylic acid which forms monomethyl ester when treated with diazomethane in diethylether. It shows no ultraviolet absorption. Mass spectra and elemental analysis favored a molecular formula of $C_{37}H_{62}O_{12}$ (M^+, 698) for

Table 1. Properties of laidlomycin and monensin A.

Property	Laidlomycin	Laidlomycin Na salt	Monensin A	Monensin A Na salt
Melting point (°C)	151~153	277~279	103~105	267~269
$[\alpha]_D^{22}$(0.2 %, $CHCl_3$)	+51.3	+78.9	+47.7	+57.3
Ultraviolet	None	None	None	None
Infrared (C=0)	1725, 1710	1725, 1560	1695	1563
Molecular weight	698 (M^+)	720 (M^+) 721 (MH^+)	670 (M^+)	692 (M^+)
Empirical formula	$C_{37}H_{62}O_{12}$	$C_{37}H_{61}O_{12}Na$	$C_{36}H_{62}O_{11}$	$C_{36}H_{61}O_{11}Na$
Nuclear magnetic resonance (-OCH_3)	None		3.37	
(-CO-O-CH-)	5.12		None	
Colour reaction Positive	Vanillin-H_2SO_4		Vanillin-H_2SO_4	
Negative	MOLISCH, $FeCl_3$, LIEBERMANN-BURCHARD		MOLISCH, $FeCl_3$, LIEBERMANN-BURCHARD	
Solubility Soluble	$CHCl_3$, acetone, alcohols		$CHCl_3$, acetone, alcohols	
Insoluble	H_2O, *n*-hexane		H_2O, *n*-hexane	

Fig. 1. IR spectra of laidlomycin and laidlomycin sodium salt in chloroform.



the acid, $C_{37}H_{61}O_{12}Na$ (M^+, 720) for the sodium salt (Chemical ionization mass spectrometry with iso-butane revealed a relatively intense protonated molecular ion at *m/e* 721.).

Anal. Calc'd for $C_{37}H_{62}O_{12}$: C,63.59; H,8.94; O,27.47
Found: C,63.55; H,8.58; O,27.87
Anal. Calc'd for $C_{37}H_{61}O_{12}Na$: C,61.64; H,8.53; O,26.64; Na,3.19
Found: C,61.62; H,8.36; O,26.74; Na,3.28

Other properties of laidlomycin and its sodium salt are shown in Table 1. The infrared absorption spectrum (in $CHCl_3$) and nuclear magnetic resonance spectrum (in $CDCl_3$) are shown in Fig. 1 and Fig. 2, respectively.

Laidlomycin seems to be a new polyether antibiotic containing a lactone group and no methoxyl group (from its NMR). Laidlomycin seems to be more closely related to monensin A, B and C[3] than to such polyether antibiotics as nigericin (polyetherin)[4], dianemycin[5], salinomycin[6], X-537A[7], X-206[8] and A-204A.[9] However, laidlomycin differs from monensins A, B and C as follows: (1) Molecular weight of the

Fig. 2. NMR spectra of laidlomycin and laidlomycin sodium salt ($CDCl_3$ solution).

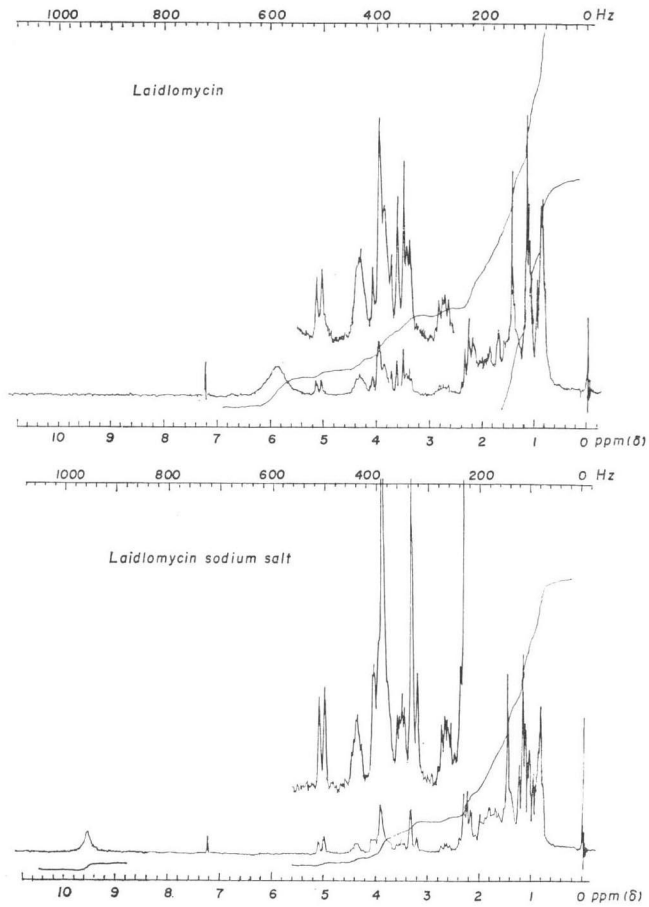


Table 2. Antimicrobial spectrum of laidlomycin (Agar dilution streak method).

Test organisms	Minimum inhibitory concentration (mcg/ml)
Staphylococcus aureus FDA 209 P	50
Bacillus subtilis PCI 219	100
Sarcina lutea PCI 1001	>1,000
Proteus mirabilis	>1,000
Escherichia coli NIHJ	>1,000
Shigella flexneri 2a	>1,000
Salmonella paratyphi B	>1,000
Pseudomonas aeruginosa	>1,000
Saccharomyces fragilis AHU 3174	>1,000
Hansenula anomala IFO	>1,000
Candida albicans	>1,000
Piricularia oryzae	>1,000
Xanthomonas oryzae	>1,000
Trichophyton rubrum	>1,000

three antibiotics is 670, 656 and 684 respectively, while, that of laidlomycin is 698. (2) These antibiotics have one methoxyl group, while laidlomycin has none. (3) Nuclear magnetic resonance spectrum of laidlomycin is different from that of monensins at lower field (5 ppm). Accordingly it can be concluded that laidlomycin is a new polyether antibiotic.

Biological Characterization

As shown in Table 2 laidlomycin inhibited growth of some Gram-positive bacteria only at high concentrations such as 50~100 mcg/ml, but was not active against Gram-negative bacteria, yeast and fungi even at concentration of 1,000 mcg/ml. In broth dilution, laidlomycin was active against several *Mycoplasmas* (Table 3) and most active against the sterol-nonrequiring mycoplasma, *Acholeplasma laidlawii*. The antibiotic was active against *Acholeplasma* at 0.16 mcg/ml in the serum-free medium, whereas some 100 times this concentration was required to inhibit the organism in the serum-containing medium. The mode of action of this antibiotic will be described in a separate paper.

Table 3. Inhibitory activity of laidlomycin on the growth of *Mycoplasmas*.

Test organisms	MIC* (mcg/ml)
Mycoplasma pneumoniae	>100
M. fermentans	4
M. hominis	20
M. orale type 2	100
M. salivarium	20
M. gallisepticum	20
M. plumonis	4
M. hyorhinis	4
Acholeplasma laidlawii A	20
A. laidlawii A (-S)**	0.16

* MIC=Minimum inhibitory concentration by broth dilution method in PPLO broth supplemented with 20% horse serum.
** serum free PPLO broth

Table 4. Cytotoxic activity of laidlomycin.

Cells	Medium	Minimum degenerative conc. (mcg/ml)
HeLa S3	YLE	0.01
Rabbit kidney	MEM	0.15
P_3HR-1	MEM	0.10
CEF	YLE	0.10

Cytotoxicity of laidlomycin was determined in various tissue culture cells including HeLa S3, RK (rabbit kidney cells), P_3HR-1 (BURKITT lymphoma cells), CEF (chick embryo fibroblasts cells) and NC-37 (human lymphoblastoid cells). As shown in Table 4, the minimum degenerative concentration of the antibiotic against these cells was in the order of 0.1~0.01 mcg/ml.

The acute toxicity of laidlomycin, expressed as LD_{50}, was 5 mg/kg (intraperitoneally), 1 mg/kg (intravenously) and 2.5 mg/kg (subcutaneously) in mice.

Antitumor activity against EHRLICH ascites carcinoma and Sarcoma 180 solid tumors in mice and antiviral activity against several viruses *in vitro* were examined but showed no significant effect.

Chemical structure and anticoccidial activity of laidlomycin will be described in a separate paper.

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FUMIO KITAME
KUNIKO UTSUSHIKAWA
TOMOAKI KOHAMA
TAKUYA SAITO
MIKIO KIKUCHI
NAKAO ISHIDA

Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan

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