LANTHIOPEPTIN, A NEW PEPTIDE ANTIBIOTIC

PRODUCTION, ISOLATION AND PROPERTIES OF LANTHIOPEPTIN

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A strain of *Streptoverticillium cinnamoneum* produced a peptide antibiotic named lanthiopeptin, which contained four unusual amino acids, *erythro*-β-hydroxyaspartic acid, *meso*-lanthionine, *threo*-β-methyllanthionine and lysinoalanine. Lanthiopeptin showed antiviral activity against herpes simplex virus type 1 KOS strain infection in Vero cells by cytopathic effect reduction assay. The structure of lanthiopeptin is similar to that of ancovenin.

In a systematic search for microbial metabolites effective against herpes simplex virus type 1, an actinomycete strain No. L337-2, isolated from a soil sample collected in India, was found to produce a new peptide antibiotic, lanthiopeptin. The producing strain was later identified as *Streptoverticillium cinnamoneum* by taxonomical studies.

The antibiotic was extracted from the fermentation liquor by use of non-ionic porous polymer resin and purified by silica gel and reversed phase silica gel chromatography. Lanthiopeptin inhibited proliferation of herpes simplex virus type 1 KOS strain in Vero cells. It also showed antibacterial activity against *Bacillus subtilis* PCI 219 and anaerobic bacteria and weak activity against certain fungi.

It contained *erythro*-β-hydroxyaspartic acid, *meso*-lanthionine, *threo*-β-methyllanthionine and lysinoalanine in addition to seven common amino acids. The structure of this antibiotic was determined to be a novel peptide[1] closely related to ancovenin.[2]

This paper reports information on the producing organism, as well as the production, isolation and physico-chemical and biological properties of lanthiopeptin.

Producing Organism

The producing organism, strain L337-2, was isolated from a soil sample collected in Dohad, Gujarat State, India. Morphological observation of the culture showed that both substrate and aerial mycelia are formed on the agar slant; they are well-branched and non-fragmentary. On most agar media, the aerial mycelium bears a tuft of short straight spore chains on biverticillate sporophores.

The biverticils are arranged in regular intervals on single hypha like a barbed wire. The spore chains contain 3 to 10 spores in each chain. The spores are oblong (0.6 by 1.0 to 2.0 μm), with a smooth surface (Fig. 1). As for cultural characteristics, good or moderate growth is observed on almost all agar media, but poor growth on CZAPEK's sucrose - nitrate agar. The color of the aerial mycelium is grayish pink, grayish yellowish pink or brownish pink after sporulation, hence it belongs to the Red-series. The reverse color is colorless to light yellowish brown or olive brown. Melanoid and other distinct pigments are not produced (Table 1). Strain L337-2 was characterized physiologically according to the descriptions of WILLIAMS *et al.*[3] The results are shown in Table 2. The strain lacks the ability to utilize D-glucose and most other sugars. The whole cell hydrolysate contains LL-diaminopimelic acid, glucose, mannose and ribose, and hence is placed in cell wall Type I_{NC}. The phospholipids include

Fig. 1. Scanning electronmicrograph showing verticillate spore chains of strain L337-2 grown on CZAPEK - DOX agar.

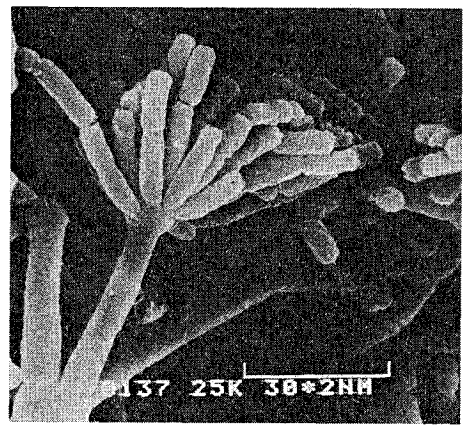

Table 1. Cultural characteristics of strain L337-2.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Sucrose - nitrate agar (CZAPEK - DOX agar)	Poor	Poor; grayish pink (8)	Colorless	None
Tryptone - yeast extract broth (ISP No. 1)	Poor; not turbid	None	Colorless	None
Yeast extract - malt extract agar (ISP No. 2)	Good	Good, V; grayish yellowish pink (32)	Dark orange yellow (72)	None
Oatmeal agar (ISP No. 3)	Moderate	Moderate, V; grayish pink (8)	Colorless	None
Inorganic salts - starch agar (ISP No. 4)	Moderate	Moderate, C; grayish pink (8)	Light olive brown (94)	None
Glycerol - asparagine agar (ISP No. 5)	Moderate	Moderate, V; grayish yellowish pink (32)	Light yellow (86)	None
Peptone - yeast extract - iron agar (ISP No. 6)	Moderate	None	Moderate olive yellow (71)	None
Tyrosine agar (ISP No. 7)	Good	Good, V; light grayish reddish brown (45)	Moderate yellowish brown (77)	None
Glucose - asparagine agar	Moderate	Moderate, V; brownish pink (33)	Dark olive brown (96)	None
Nutrient agar	Moderate	None	Light yellow (86)	None
BENNETT's agar	Good	Good, V; reddish gray (22)	Dark olive yellow (72)	None

Observation after incubation at 28°C for 3 weeks.
Color name used: ISCC-NBS Color-Name Charts.
V: Velvety, C: cottony.

Table 2. Physiological characteristics of strain L337-2.

Temperature:		Utilization of:	
Growth range	18~45°C	Coumarin	−
Optimal growth	29~40°C	L-Methionine	−
No growth	13 and 48°C	L-Proline	+
Tolerance to:		Shikimic acid	−
Lysozyme, 0.01%	+	DL-α-Aminobutyric acid	−
NaCl, 1~7%	+	Acid from:	
8% or more	−	D-Galactose	−
pH 4.5~11.5	+	Inositol	+
Production of:		D-Fructose	−
Gelatinase	+	D-Ribose	+
Amylase	+	D-Trehalose	+
Nitrate reductase	−	Degradation of:	
Tyrosinase	−	Aesculin	−
Utilization of:[a]		Citrate	+
Glycerol	+	DNA	+
D-Arabinose	−	Hypoxanthine	+
L-Arabinose	−	L-Tyrosine	+
D-Xylose	−	Tween 80	+
D-Ribose	+	Production of:	
L-Rhamnose	−	H_2S	+
D-Glucose	−	Growth with:	
D-Galactose	−	1-Phenolethanol	+(w)[b]
D-Fructose	−	Potassium tellurite	−
D-Mannose	+(w)[b]	Crystal violet	−
L-Sorbose	−	Malachite green	−
Sucrose	−	Resistance to:	
Lactose	−	Carbenicillin	+
Cellobiose	−	Cephaloridine	+
Melibiose	−	Cephalothin	+
Trehalose	+	Cefamandole	+
Raffinose	−	Colistin	+
D-Melezitose	−	Antibiosis to:	
Soluble starch	+	*Aspergillus niger*	+
Cellulose	−	*Bacillus subtilis*	+
Dulcitol	−	*Candida albicans*	+
Inositol	+		
D-Mannitol	−		
D-Sorbitol	−		
Salicin	−		
Production of:			
Melanin	−		

[a] Basal medium: PRIDHAM - GOTTLIEB's inorganic salts medium (ISP No. 9 medium).
[b] (w): Weakly positive.

phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol, hence it belongs to Type P-II. According to the descriptions of BALDACCI and LOCCI,[4] SHIRLING and GOTTLIEB,[5,6] and WILLIAMS *et al.*,[3] the taxonomic position of strain L337-2 is similar to those of *Streptoverticillium griseocarneum*, *S. cinnamoneum*, *Streptoverticillium hachijoense*, *Streptoverticillium lilacinum* and *Streptoverticillium kashmirense*. Further descriptive or direct comparisons of strain L337-2 to the above five species indicated that the strain is classified as *S. cinnamoneum*. The type strain of *S. cinnamoneum* is reported to produce cinnamycin with a non-mobile factor and fungichromin.

Fermentation

A well-grown agar slant of *S. cinnamoneum* strain No. L337-2 was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of the seed medium consisting of glucose 3%, soybean meal (Nihon Kouyu) 2%, distiller's solubles (Sungrowth Co.) 1.5%, fish meal (Hokuyo Suisan) 0.2% and $CaCO_3$ 0.6% (pH 7.0). The seed culture was incubated at 28°C for 4 days on a rotary shaker (200 rpm) and 5 ml of the culture was transferred into a 500-ml Erlenmeyer flask containing 100 ml of a fermentation medium having the same composition as the seed medium. The fermentation was carried out at 28°C for 6 days on a rotary shaker (200 rpm). Fermentation study in a jar-fermenter was also carried out. A 500-ml portion of the seed culture prepared by the flask fermentation was transferred into a 20-liter jar-fermenter containing 12 liters of the fermentation medium (pH 7.0). The fermentation was carried out at 28°C for 64 hours with agitation of 250 rpm and an aeration rate of 12 liters per minute. The antibiotic activity in the fermentation broth was determined by the cytopathic effect reduction assay against herpes simplex virus type 1 in parallel with the antibacterial activity by the paper-disc agar diffusion assay against *B. subtilis* PCI 219.

Isolation and Purification

The harvested fermentation broth (30 liters) was separated to supernatant (28 liters) and mycelial cake by use of a Sharples centrifuge (Kokusan Seiko Co., No. 4A). The mycelial cake was vigorously stirred with methanol (6 liters) and filtered. The filtrate was concentrated to 2 liters under reduced pressure and mixed with broth supernatant. After adjusting to pH 7.0 with 6 N HCl, the mixed solution was stirred with Diaion HP-20 resin (Mitsubishi Chemical Industries Limited, 3 liters) for 2 hours. The resin was collected by filtration (the filtrate still showed antibacterial activity which was shown to arise from co-produced streptomycin), and packed in a column 70 i.d. × 1,000 mm. The column was washed with water and methanol - water (5 : 5) (5 liters each), and eluted with methanol - water (8 : 2). The eluate was collected in fractions which were examined by paper-disc assay using *B. subtilis* PCI 219. The active fractions were pooled and concentrated to dryness *in vacuo* to yield a crude solid of lanthiopeptin (21.3 g). The solid was purified by column chromatography on Silica gel 60 (E. Merck, Darmstadt, No. 9385, 40 i.d. × 350 mm). The column was first eluted with propanol to remove co-produced methylpentaene antibiotic and then with propanol - water (8 : 2) using FMI medium pressure LAB pump (Fluid Metering, Inc.). The bioactive fractions eluted with the latter solvent were pooled and evaporated *in vacuo* to give a yellow solid (8.3 g). It was again subjected to medium pressure liquid chromatography with Silica gel 60 (40 i.d. × 240 mm). Elution was performed by mixtures of acetone - methanol with stepwise increasing methanol content (20~70%). The crystals deposited in the active fractions eluted with acetone - methanol (3 : 7) were collected (750 mg) and applied to a column of LiChroprep RP-18 (E. Merck, 22 i.d. × 700 mm) which had been equilibrated with acetonitrile - 0.01 M SØRENSEN phosphate buffer pH 7.0 (2 : 8). The column was washed with the same solvent mixture and then eluted with 3 : 7 mixture. The bioactive fractions were collected, evaporated *in vacuo* to remove acetonitrile and desalted using Diaion HP-20 chromatography to give lanthiopeptin as a white amorphous powder (734 mg). The side fractions of the second silica gel chromatography were combined and chromatographed again on Silica gel 60 under the same condition. The relevant fractions were pooled, evaporated *in vacuo* (3.98 g) and finally purified by chromatography on LiChroprep RP-18. The fractions that contained pure antibiotic were pooled, desalted and freeze-dried to give 3.61 g of a white powder of lanthiopeptin. It was crystallized as colorless needles from methanol.

Physico-chemical Properties

Lanthiopeptin is soluble in aqueous lower alcohols, dimethyl sulfoxide (DMSO), dimethylformamide and acidic water, slightly soluble in water and lower alcohols, but insoluble in hexane. It showed positive response to iodine, ninhydrin, Sakaguchi and Rydon-Smith reagents but negative response to anthrone, Fehling and $FeCl_3$ tests. Lanthiopeptin did not show a definite melting point and decomposed above 245°C. The molecular formula of $C_{89}H_{125}N_{25}O_{25}S_3$ was assigned to this antibiotic based on its fast atom bombardment mass spectrum (FAB-MS) and NMR spectra, amino acid analysis and elemental analysis. The IR spectrum of lanthiopeptin showed intense amide bands at 1655 and 1520 cm^{-1}. The UV spectrum in methanol - water (1 : 1) gave λ_{max} nm (ε) 207 (46,100) along with less intense bands at 252 (710), 258 (690), 264 (510) and 268 (sh), typical for a phenyl chromophore. From these data, lanthiopeptin appeared to be a phenylalanine-containing polypeptide. The peptide structure was further confirmed by the ^1H NMR spectrum in DMSO-d_6 at 60°C, which showed the presence of amide NH signals between δ 7.2 and 10.9, and a complex set of multiplets between δ 3.0 and 5.3 due to α-protons of the peptidic amino acids. The spectrum also showed multiplets assignable to three phenyl groups, and doublets due to four aliphatic methyl groups, δ 0.83 (J=6.8 Hz), 0.96 (J=6.4 Hz), 1.17 (J=7.7 Hz) and 1.20 (J=7.7 Hz). In the ^{13}C NMR spectrum (DMSO-d_6, 60°C), a total of 22 carbonyl signals were observed between δ 167.8 and 177.2. By distortionless enhancement by polarization transfer (DEPT) pulse sequence, 36 methine (including 15 aromatic carbon signals), 23 methylene and 4 methyl signals were identified. These signals, along with 4 additional quaternary carbon signals at δ 137.8, 138.0, 138.5 (aromatic) and 157.0 (guanidino group) indicated that a total 89 carbons were present in lanthiopeptin (Table 4).

Acid hydrolysis of lanthiopeptin with 6 N HCl gave several ninhydrin-positive components, which were isolated by ion exchange and cellulose chromatography. As shown in the Table 5, seven common amino acids (Asp, Glu, Pro, Gly, Val, Phe and Arg) were isolated and their absolute configurations were determined to be 'L' from their specific optical rotations. In addition to these, four unusual amino acids were isolated from the hydrolyzate. They were determined to be β-hydroxy-L-aspartic

Table 3. Physico-chemical properties of lanthiopeptin.

Nature	Colorless needles	
MP	>245°C (dec)	
$[\alpha]_D^{22.5}$	−120° (c 1.0, 50% aq MeOH)	
Microanalysis	Calcd for $C_{89}H_{125}N_{25}O_{25}S_3 \cdot 5H_2O$:	Found:
	C 50.15	C 49.90
	H 6.38	H 6.20
	N 16.43	N 16.31
	S 4.51	S 4.59
FAB-MS (m/z)	2,043 (MH^+, chemical mass value)	
HPLC[a] (retention time)	19.4 minutes	
TLC[b] (Rf)	0.20 (propanol - H_2O, 7 : 3)	
	0.14 ($CHCl_3$ - ethanol - H_2O, 4 : 7 : 2)	
	0.13 (butanol - acetic acid - H_2O, 3 : 1 : 1)	

[a] Column: Microsorb C18, 80-299 (10 i.d. × 250 mm, 5 μm), mobile phase: solvent A: H_2O containing 0.1% TFA, solvent B: 2-propanol - acetonitrile (7 : 3) containing 0.1% TFA, A/B: 100/0→0/100 linear gradient, 30 minutes, flow rate: 4 ml/minute, detection: UV absorption at 210 nm.

[b] SiO_2.

Table 4. ^{13}C NMR data for lanthiopeptin.[a]

Chemical shift[b]	Functional groups	Chemical shift[b]	Functional groups
18.1	CH_3	57.8	CH
18.2	CH_3	57.9	CH×2[c]
18.5	CH_3	60.7	CH
20.9	CH_3	61.1	CH
22.7	CH_2	72.3	CH
24.5	CH_2	125.7	CH=C
24.8	CH_2	125.8	CH=C
28.3	CH_2	126.0	CH=C
28.5	CH_2	127.6	CH=C×2[c]
29.5	CH_2	127.8	CH=C×2[c]
31.0	CH_2×2[c]	128.0	CH=C×2[c]
31.7	CH×2[c]	128.2	CH=C×2[c]
32.0	CH_2×2[c]	128.7	CH=C×2[c]
33.9	CH_2	129.1	CH=C×2[c]
34.6	CH_2	137.8	C=C
35.0	CH_2	138.0	C=C
37.2	CH_2	138.5	C=C
38.2	CH_2	157.0	C
39.0	CH_2	167.8	C=O
39.3	CH_2×2[c]	168.3	C=O
40.8	CH_2×2[c]	168.7	C=O
41.2	CH	169.1	C=O
42.0	CH_2	169.7	C=O
46.0	CH_2	169.9	C=O
47.1	CH_2	170.0	C=O
47.8	CH	170.1	C=O×2[c]
48.7	CH	170.19	C=O
50.4	CH	170.23	C=O
51.2	CH	170.4	C=O
51.9	CH	170.6	C=O
52.2	CH	170.7	C=O×2[c]
52.9	CH	172.6	C=O
53.5	CH	173.2	C=O
54.5	CH	173.5	C=O×2[c]
55.3	CH	175.2	C=O
56.2	CH	177.2	C=O×2[c]
56.5	CH		

[a] In DMSO-d_6, 100 MHz at 60°C.
[b] δ in ppm relative to DMSO-d_6 as internal reference (39.50 ppm).
[c] Overlapping signal.

acid,[7] *meso*-lanthionine, *threo*-β-methyllanthionine[8] and lysinoalanine by their melting point, ^1H and ^{13}C NMR, specific optical rotation values and comparison with authentic samples. The unique sulfide amino acids lanthionine and β-methyllantionine have been found in some peptide antibiotics such as subtilin,[9,10] cinnamycin,[11] duramycin,[12] nisin,[13] gardimycin,[14] ancovenin,[15] and epidermin.[16] By a comparison of the specific optical rotation with the literature value,[17] lysinoalanine obtained from lanthiopeptin appeared to be a racemic mixture of the alanine moiety and the lysine fragment to be 'L'. This amino acid was reported to easily racemize at the alanine portion under acid hydrolysis conditions to yield two diastereoisomers.[18] Lysinoalanine was also found in cinnamycin and duramycin. The ratios of the constituent amino acids were determined as shown in Table 5 by recovered

Table 5. The amino acid composition of lanthiopeptin.

Amino acid	Molar ratio		
	Isolated amount[a]	Amino acid analyzer	Estimated number of residues
L-HyAsp[b]	0.99	1.00	1
L-Asp	1.00	1.02	1
L-Glu	1.11	1.12	1
Gly	2.38	2.00	2
L-Val	1.24	0.82	1
L-Pro	1.13	1.10	1
L-Phe[c]	2.87	2.94	3
MeLan[d]	1.91	1.87	2
Lan[e]	0.35	1.01[f]	1
L-Arg	1.02	1.00	1
LAL[g]	0.98	1.00	1

[a] 1.1 g of lanthiopeptin was hydrolyzed for 24 hours at 110°C in a sealed tube.

[b] *erythro*-β-Hydroxy-L-aspartic acid. $[\alpha]_D^{25.5}$ +43.3° (*c* 0.60, H_2O); mp 225~245°C (dec). Literature[7] $[\alpha]_D$ +41.4° (*c* 2.43, H_2O); mp 225~245°C (dec). TLC Rf 0.41 (SiO_2, 10% CH_3COONH_4 - CH_3OH - 10% NH_4OH, 9:10:1); *threo*-DL-HyAsp, Rf 0.53.

[c] 'L'-configuration was further confirmed by HPLC analysis using a chiral recognition column. Retention time: 10.7 minutes (TSK gel Enantio L1, 4.6 i.d.×150 mm, 1 mM $CuSO_4$ aqueous solution as mobile phase, at 50°C); D-Phe 6.2 minutes.

[d] *threo*-β-Methyllanthionine. $[\alpha]_D^{25.5}$ −31.4° (*c* 0.35, 1 N HCl). Literature[8] of (2*R*)-*S*-[(2*S*,3*S*)-3-methylalanin-3-yl]cysteine, $[\alpha]_D^{23}$ −36° (*c* 0.22, 1 N HCl).

[e] *meso*-Lanthionine.

[f] The value was corrected taking into account that epimerization of *meso*-lanthionine during the acid hydrolysis of pure *meso*-lanthionine for 24 hours caused epimerization of 25%.

[g] Lysinoalanine. $[\alpha]_D^{25.5}$ +15.7° (*c* 2.09, 2 N HCl). Literature[18] of N^{ε}-DL-(2-amino-2-carboxyethyl)-L-lysine, $[\alpha]_D^{25}$ +15° (*c* 2, 2 N HCl).

weight of each component from the acid hydrolysate coupled with amino acid analysis. The total structure has been established by the Edman degradation of its modified peptides to be a novel cyclic peptide related to ancovenin (Fig. 2).[1]

Antimicrobial Activity

The MICs of lanthiopeptin against various test organisms were determined by the serial 2-

Table 6. Antibacterial activity of lanthiopeptin.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	100
S. aureus Smith	100
S. aureus D136	>100
S. aureus No. 52-34	>100
S. aureus A20239	>100
S. aureus BX1633-2 A9606	>100
S. aureus A15097	>100
S. epidermidis D153	>100
S. epidermidis A22152	>100
Streptococcus faecalis A9612	>100
S. pyogenes A20201	100
Micrococcus luteus No. 1001	100
Bacillus subtilis PCI 219	0.8
Escherichia coli NIHJ	>100
E. coli Juhl A15119	>100
E. coli JR/C600 A20665	>100
E. coli A9624	>100
E. coli TEM A20341-1	>100
Enterobacter cloacae A9659	>100
Klebsiella pneumoniae No. 22-3038 A20680	>100
Pseudomonas aeruginosa A9930	100
P. aeruginosa A9843A	>100
Proteus vulgaris A9436	>100
P. mirabilis A9554	>100
Morganella morganii A9553	>100
Serratia marcescens A20222	>100

Fig. 2. The structure of lanthiopeptin.

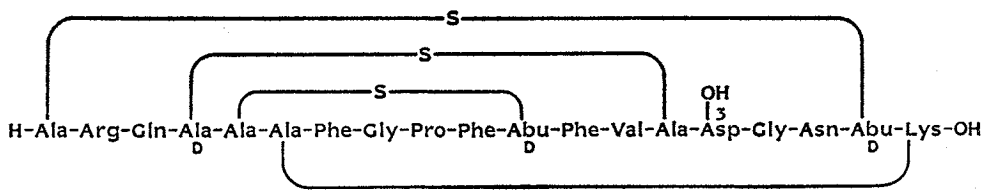

Table 7. Anti-anaerobic bacterial activity of lanthiopeptin.

Test organism	MIC (μg/ml)
Clostridium difficile A21675	>100
C. perfringens A9635	100
Peptostreptococcus anaerobius A21905	3.1
Bacteroides fragilis A22693	6.3
B. fragilis A22035	6.3
B. fragilis A22021	3.1
B. fragilis A21916	3.1
B. fragilis A22534	6.3
B. fragilis A22695	6.3
B. fragilis A22533	3.1
B. fragilis CUH-67	6.3

Table 8. Antifungal activity of lanthiopeptin.

Test organism	MIC (μg/ml)
Candida albicans IAM 4888	50
C. albicans A9540	100
Cryptococcus neoformans D49	50
C. neoformans IAM 4519	50
Aspergillus fumigatus IAM 2530	>100
A. fumigatus IAM 2034	>100
A. flavus FA 21436 NRRL 484	>100
Piricularia oryzae D91	>100
Trichophyton mentagrophytes D155	100
T. mentagrophytes No. 4329	>100
Blastomyces dermatitidis IFO 8144	>100
Sporothrix schenckii IFO 8158	100
Petriellidium boydii IFO 8078	100

fold dilution method with overnight incubation at 37°C. Nutrient agar medium was used for Gram-positive and Gram-negative bacteria, GAM agar medium for anaerobic bacteria and Sabouraud dextrose agar medium for fungi. As shown in Tables 6~8 lanthiopeptin showed specific inhibition of *B. subtilis* PCI 219, *Bacteroides fragilis* and *Peptostreptococcus anaerobius* strains. It also exhibited weak inhibitory activity against some strains of Gram-positive and Gram-negative bacteria and fungi.

Table 9. Activity against herpes simplex virus type 1.

	Activity vs. HSV-1[a] (ID_{50}, μg/ml)	Cytotoxicity vs. Vero cell (TD_{50}, μg/ml)
Lanthiopeptin	0.05	12.5
Acyclovir	0.46	>100

[a] HSV-1: KOS strain, host cell: Vero cells.

Antiviral Activity

The cytopathic effect (CPE) reduction assay was used to evaluate the *in vitro* antiviral activity of lanthiopeptin against herpes simplex virus type 1 (HSV-1) infection in Vero cells. A 1-ml aliquot of cell suspension containing 8×10^4 cells was placed in each well of the 24-well microplate. After incubation at 37°C for 48~72 hours under humidified 5% CO_2 - 95% air atmosphere, the growth medium was drained from the wells and replaced with fresh medium containing lanthiopeptin at various doses. Wells which contained only the medium were used as the control in the assay. Medium (0.1 ml) containing approximately $2\times50\%$ tissue culture infective dose ($TCID_{50}$) of the test virus was added to each well and the microplate was then incubated for 72 hours. CPE reduction activity was expressed as 50% inhibitory dose (ID_{50}) which is defined as the concentration of compound required to reduce CPE by 50% as compared to the control. Wells without viral inoculation were incubated to determine cytotoxicity. The concentration of the compound which showed 50% inhibition of Vero cell growth is defined as TD_{50}. Acyclovir, which was used as the standard for this assay, showed potent activity against the virus and no cytotoxicity. The ID_{50} value for lanthiopeptin against herpes simplex virus was 0.05 μg/ml and the TD_{50} against Vero cells was 12.5 μg/ml (Table 9). Lanthiopeptin did not show inhibitory activity against influenza virus type A/MDCK cell by the CPE reduction assay.

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