# LACTIMIDOMYCIN†, A NEW GLUTARIMIDE GROUP ANTIBIOTIC

## PRODUCTION, ISOLATION, STRUCTURE AND BIOLOGICAL ACTIVITY

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*Streptomyces amphibiosporus* R310-104 (ATCC 53964) produced a novel antibiotic lactimidomycin which showed inhibitory activity against fungi and prolonged the survival time of mice transplanted with experimental tumors. Structural studies clarified that lactimidomycin is a new glutarimide antibiotic having a unique unsaturated 12-membered lactone ring.

In the course of screening for new antibiotics effective against experimental tumors, we have isolated a novel antibiotic designated lactimidomycin from the culture broth of *Streptomyces amphibiosporus* R310-104 collected in Akita city[1,2]. The antibiotic exhibited strong cytotoxicity against various tumor cells and inhibitory activity against fungi but no antibacterial activity. It demonstrated prolongation of life span in mice bearing P388 leukemia and B16 melanoma. The structure of lactimidomycin was determined by spectroscopic analyses and $^{13}C$-enriched biosynthetic studies to be a novel glutarimide group antibiotic having a unique 12-membered lactone ring as a side chain.

In this paper, we describe the production, isolation, physico-chemical properties, structure determination and biological activity of lactimidomycin.

### Antibiotic Production

A loopful mature slant culture of *S. amphibiosporus* R310-104 was inoculated into 100 ml of vegetative medium consisting of soluble starch (Nichiden Kagaku) 2%, Pharmamedia (Traders Protein) 1%, $ZnSO_4 \cdot 7H_2O$ 0.003% and $CaCO_3$ 0.4% in a 500-ml Erlenmeyer flask (pH 7.0 before sterilization). The flask was incubated at 32°C for 7 days on a rotary shaker (200 rpm) and 5 ml of vegetative inoculum was added to 100 ml of sterile production medium containing Protein-S (Ajinomoto Co.) 3%, glucose 3%, Pharmamedia 0.5%, yeast extract (Oriental Yeast Co.) 0.1% and $CaCO_3$ 0.3%, pH 7.0 in a 500-ml Erlenmeyer flask. The flask was incubated at 28°C on a rotary shaker (200 rpm). Antibiotic production was monitored by the *in vitro* cytotoxicity against B16 melanoma cells and it reached maximum after 4 days incubation.

### Isolation and Purification

The fermentation broth (18 liters, pH 7.4) was stirred with 1-butanol for one hour. The solvent layer was separated from the aqueous layer and mycelial cake by use of a Sharples type centrifuge and concentrated under reduced pressure. The residue (30 g) was suspended in water (1 liter) and extracted three times with

† Lactimidomycin was originally called as BU-4146T or BMY-28886.

1 liter each of ethyl acetate. The combined organic extracts were concentrated to brown oil which was added dropwise into 600 ml of *n*-hexane to precipitate a crude antibiotic solid (2.65 g). It was applied on a column of silica gel (Wako gel C-200, 2.0 i.d. × 50 cm), which was developed with methylene chloride-methanol mixture (100 : 0 ~ 90 : 10). The eluate was monitored by antifungal activity against *Cryptococcus neoformans* IAM 4514 using paper disc assay and also by cytotoxicity against B16 melanoma cells. The active fractions were combined and evaporated *in vacuo* to yield a pale yellow solid which was chromatographed on a column of silica gel (2.0 i.d. × 35 cm) pre-equilibrated with ethyl acetate-*n*-hexane (1 : 1). The elution was performed with the same solvent and the bioactive fractions were pooled and concentrated to dryness. The solid obtained was further purified on Sephadex LH-20 chromatography (2.2 i.d. × 60 cm) developing with methanol. The appropriate fractions were combined and concentrated *in vacuo* to yield a pure solid of lactimidomycin (45 mg).

## Physico-chemical Properties

Lactimidomycin was isolated as a pale yellow solid. It is readily soluble in lower alcohols, acetonitrile,

Table 1. Physico-chemical properties of lactimidomycin.

| | |
|---|---|
| Nature | Pale yellow solid |
| MP | 121 ~ 125°C |
| $[\alpha]_D$ | −20° (*c* 0.5, DMSO) |
| Elemental analysis | Calcd for $C_{26}H_{35}NO_6 \cdot \frac{1}{4}H_2O$: C 67.58, H 7.74, N 3.03<br>Found: C 67.47, H 8.06, N 2.87 |
| FAB-MS | *m/z* 458 $(M+H)^+$, 480 $(M+Na)^+$ |
| IR $\nu_{max}^{KBr}$ $cm^{-1}$ | 3450, 3230, 3100, 2960, 2930, 1700, 1640, 1380, 1260, 1190, 1140, 1000 |
| TLC[a] | Rf 0.36 ($CH_2Cl_2$-MeOH, 95 : 5)<br>0.27 (EtOAc-*n*-hexane, 10 : 1) |
| HPLC[b] | Rt 6.0 minutes |

[a] $SiO_2$ (Merck $F_{254}$).
[b] Column: YMC-pack ODS A-301-3: $CH_3CN$-0.15% $KH_2PO_4$ (pH 3.5) (50 : 50), 1 ml/minute, UV 254 nm.

Fig. 1. IR spectrum of lactimidomycin.

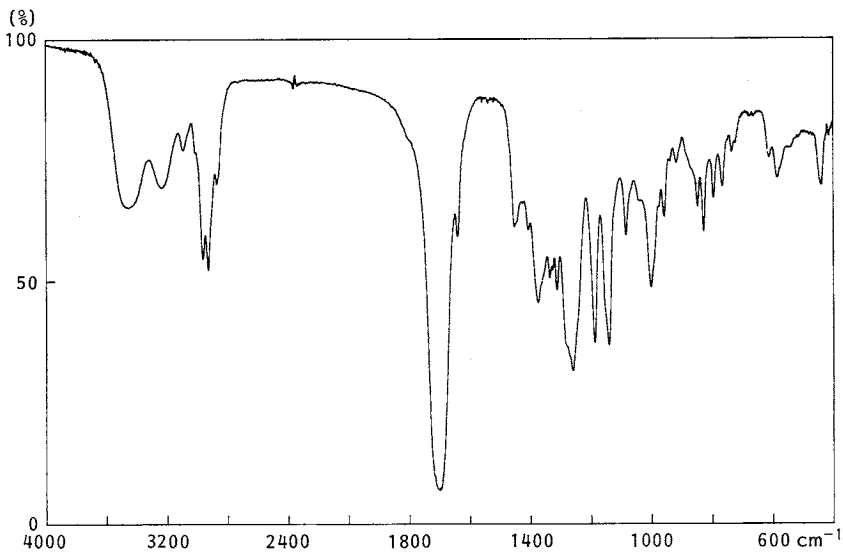
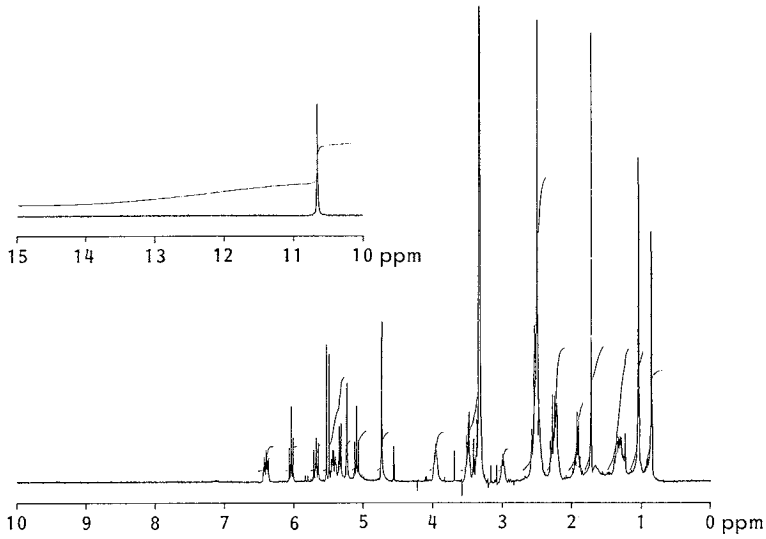


Fig. 2. $^1$H NMR spectrum of lactimidomycin.



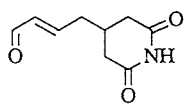

ethyl acetate, chloroform and dimethyl sulfoxide, but practically insoluble in *n*-hexane and water. It gave positive responses to iodine vapor and ammonium molybdate-sulfuric acid, but negative responses to ninhydrin and anthrone reagents.

The physico-chemical properties of lactimidomycin are summarized in Table 1. Its molecular formula was determined to be $C_{26}H_{35}NO_6$ on the basis of microanalysis and mass spectrum (*m/z* 458 $(M+H)^+$). It showed only end absorption in the UV spectrum. The IR spectrum in KBr (Fig. 1) showed strong absorption at 3230 and 1700 $cm^{-1}$ suggesting the presence of imide group. The $^1$H NMR spectrum is shown in Fig. 2.

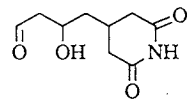
## Structure Determination

The strong absorption at 1700 $cm^{-1}$ in the IR spectrum suggested the presence of 6-membered imide ring in lactimidomycin. The FAB-MS spectrum exhibited the pseudomolecular ion peaks at *m/z* 458 $(M+H)^+$ and 480 $(M+Na)^+$ along with strong fragment ion peaks at *m/z* 180 and 198 which are commonly observed for the glutarimide group antibiotics.

*m/z*: 180



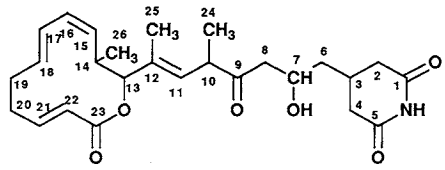

*m/z*: 198




The $^1$H NMR spectrum in DMSO-$d_6$ (Fig. 2) demonstrated three methyl groups ($\delta$ 0.86 d, 1.04 and 1.72 d), six olefinic protons ($\delta$ 5.10 t, 5.44 m, 5.51 d, 5.68 dd, 6.04 t and 6.40 ddd), one imide proton ($\delta$ 10.67 s) and one hydroxyl proton ($\delta$ 4.74 d). The $^{13}$C NMR (Fig. 3) exhibited 26 carbons including two C–$CH_3$, one =C–$CH_3$, six $CH_2$, five –$\overset{|}{C}$H, one $\succ$C=, seven –CH= and four C=O carbons ($\delta$ 165.7, 173.2, 173.3 and 209.4).

The correlation of the protons and carbons was established as shown in Table 2 by $^1$H-$^{13}$C COSY spectrum. This combined with $^1$H-$^1$H COSY spectral analyses allowed us to assign three partial structures,

Table 2. $^{1}H$ and $^{13}C$ NMR data of lactimidomycin.



| Carbon No. | $^{13}C$ NMR (100 MHz in DMSO-$d_6$) | Protons on carbon No. | $^{1}H$ NMR (400 MHz in DMSO-$d_6$) |
|---|---|---|---|
| 1 | 173.2 (s)[a] | 2 | 2.25 (m) |
| 2 | 36.9 (t)[b] | | 2.50 (m) |
| 3 | 26.6 (d) | 3 | 2.25 (m) |
| 4 | 38.0 (t)[b] | 4 | 2.25 (m) |
| 5 | 173.3 (s)[a] | | 2.50 (m) |
| 6 | 41.6 (t) | 6 | 1.25 (m) |
| 7 | 63.7 (d) | 7 | 3.96 (m) |
| 8 | 48.7 (t) | 8 | 2.50 (m) |
| 9 | 209.4 (s) | 10 | 3.42 (dq, 10.4, 6.9) |
| 10 | 45.4 (d) | 11 | 5.33 (d, 10.4) |
| 11 | 130.0 (d) | 13 | 5.24 (d, 4.8) |
| 12 | 132.3 (s) | 14 | 2.99 (ddq, 4.8, 10.9, 6.9) |
| 13 | 82.4 (d) | 15 | 5.10 (t, 10.9) |
| 14 | 35.5 (d) | 16 | 6.04 (t, 10.9) |
| 15 | 131.3 (d) | 17 | 5.68 (dd, 10.9, 15.7) |
| 16 | 128.9 (d) | 18 | 5.44 (m) |
| 17 | 134.0 (d) | 19 | 1.92 (m) |
| 18 | 128.1 (d) | | 2.50 (m) |
| 19 | 30.6 (t) | 20 | 1.92 (m) |
| 20 | 31.7 (t) | | 2.50 (m) |
| 21 | 147.0 (d) | 21 | 6.40 (ddd, 5.2, 10.9, 16.1) |
| 22 | 127.6 (d) | 22 | 5.51 (d, 16.1) |
| 23 | 165.7 (s) | 24 | 1.04 (d, 6.9) |
| 24 | 15.7 (q) | 25 | 1.72 (d, 1.2) |
| 25 | 14.6 (q) | 26 | 0.86 (d, 6.9) |
| 26 | 17.0 (q) | –NH | 10.67 (s) |
| | | –OH | 4.74 (d, 5.7) |

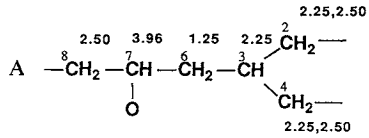
[a,b] Assignments may be interchanged.

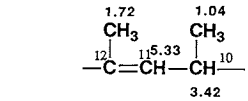
A, B and C of lactimidomycin as illustrated in Fig. 3. The geometrical configurations of C moiety were determined to be 15*Z*, 17*E* and 21*E* by coupling constants of olefinic protons ($J_{15\text{-}16}=10.9$ Hz, $J_{17\text{-}18}=15.7$ Hz, $J_{21\text{-}22}=16.1$ Hz) and that of B moiety to be 11*E* by NOE observed between 10-H ($\delta$ 3.42) and 24, 25-H ($\delta$ 1.04 and 1.72) in NOESY spectrum.

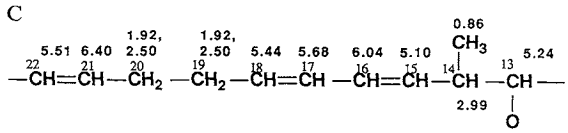
In order to clarify the sequences of the three partial structures in lactimidomycin, $^{1}H$-$^{13}C$ long range COSY spectra were measured and analyzed (Fig. 3). Clear correlation was observed from two methylene protons (2-H, 4-H) to imide carbonyl (C-1, C-5) and a methine carbon (C-3) establishing a glutarimidylhydroxyethyl group which is the common functional group of glutarimide antibiotics. Three methyl protons displayed contour with following their relevant carbons, 24H (C-9, C-10 and C-11), 25H (C-11, C-12 and C-13) and 26H (C-13, C-14 and C-15) to clarify connectivities from C1 to C22. The two methine protons (13-H, $\delta$ 5.24 and 22-H, $\delta$ 5.51) showed correlation with the ester carbonyl carbon (C-23, $\delta$ 165.7) establishing the unique 12-membered lactone ring structure. Thus, the total structure of lactimidomycin was determined as in Fig. 4.

Fig. 3. $^{1}H$-$^{1}H$ COSY and $^{1}H$-$^{13}C$-long range COSY analyses.

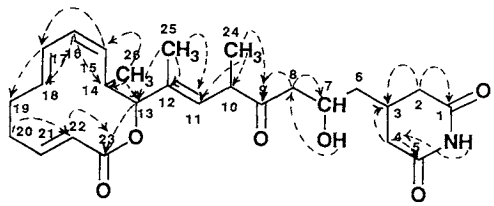
1) Partial structures analyzed by $^{1}H$-$^{1}H$ COSY spectrum




2) $^{1}H$-$^{13}C$ long range coupling observed in lactimidomycin



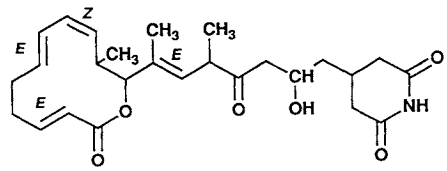
The structure was confirmed by EI-MS of hexahydrolactimidomycin which was prepared by the hydrogenation of lactimidomycin over palladium charcoal. The MS spectrum exhibited the molecular ion peak at $m/z$ 463 ($M^+$) and abundant fragment ion peaks at $m/z$ 180 ($C_9H_{10}NO_3$), 265 ($M^+ - $glutarimidylhydroxyethyl) and 308 ($265 - CH_2CO$) supporting the assigned structure.

## Biosynthetic Study

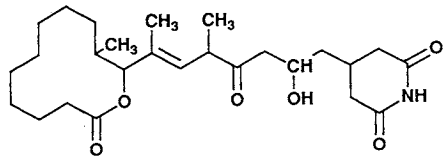
The incorporation of $^{13}C$-labeled precursors into lactimidomycin was studied to confirm the proposed structure and to clarify the biosynthetic pathway of this antibiotic. *S. amphibiosporus* R310-104 was fermented in the presence of sodium [1-$^{13}C$]acetate, sodium [2-$^{13}C$]acetate or [$CH_3$-$^{13}C$]methionine. The $^{13}C$-enriched antibiotics were isolated by the procedure as used for the natural antibiotic and analyzed by the $^{13}C$ NMR.

Table 3 shows the $^{13}C$-enrichment results. In the glutarimide ring, [1-$^{13}C$]acetate labeled at C-1,

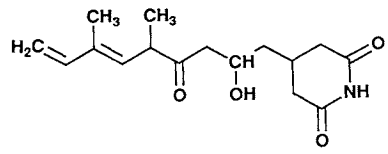
Fig. 4. Structures of lactimidomycin, hexahydrolactimidomycin and streptimidone.



Lactimidomycin

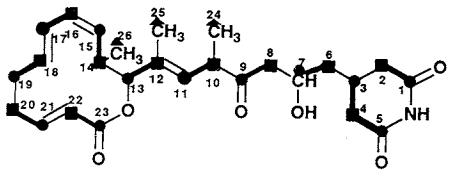


Hexahydrolactimidomycin



Streptimidone

Table 3. Labeling patterns and $^{13}C$ enrichments in lactimidomycin derived from [$^{13}C$]acetates and [$CH_3$-$^{13}C$]-L-methionine.



$CH_3{}^{13}COOH$

$^{13}CH_3COOH$

$^{13}CH_3SCH_2CH_2CH(NH_2)COOH$

| Carbon No. | $\delta$ ppm (in DMSO-$d_6$) | $^{13}C$-Enrichments | | |
|---|---|---|---|---|
| | | [1-$^{13}C$]-Acetate | [2-$^{13}C$]-Acetate | [$CH_3$-$^{13}C$]-L-Methionine |
| 1 | 173.2 | 27.33 | 1.70 | 1.78 |
| 2 | 36.9 | 0.77 | 11.81 | 1.29 |
| 3 | 26.6 | 28.33 | 1.77 | 1.73 |
| 4 | 38.0 | 1.03 | 12.11 | 1.71 |
| 5 | 173.3 | 7.92 | 1.62 | 1.82 |
| 6 | 41.6 | 0.55 | 11.26 | 1.89 |
| 7 | 63.7 | 19.57 | 1.28 | 1.55 |
| 8 | 48.7 | 0.75 | 10.25 | 1.36 |
| 9 | 209.4 | 14.55 | 0.71 | 0.71 |
| 10 | 45.4 | 0.62 | 8.72 | 1.33 |
| 11 | 130.0 | 17.36 | 1.37 | 0.95 |
| 12 | 132.3 | 0.56 | 8.11 | 1.24 |
| 13 | 82.4 | 16.04 | 1.08 | 1.24 |
| 14 | 35.5 | 0.72 | 11.54 | 1.11 |
| 15 | 131.3 | 17.36 | 1.23 | 1.33 |
| 16 | 128.9 | 0.71 | 9.61 | 1.20 |
| 17 | 134.0 | 25.62 | 1.42 | 1.75 |
| 18 | 128.1 | 0.82 | 11.91 | 1.44 |
| 19 | 30.6 | 16.80 | 1.20 | 1.20 |
| 20 | 31.7 | 0.61 | 8.55 | 0.89 |
| 21 | 147.0 | 17.67 | 1.30 | 1.22 |
| 22 | 127.6 | 0.61 | 11.67 | 1.40 |
| 23 | 165.7 | 17.86 | 0.50 | 1.00 |
| 24 | 15.7 | 0.99 | 1.31 | 35.78 |
| 25 | 14.6 | 0.92 | 0.83 | 27.75 |
| 26 | 17.0 | 1.00 | 1.00 | 30.24 |

Intensity of each peak was normalized based on the abundance at C-26 in [1-$^{13}C$] and [2-$^{13}C$]acetate additions and at C-23 in [$CH_3$-$^{13}C$]methionine addition as a standard.

C-3 and C-5, while [2-$^{13}C$]acetate at C-2 and C-4 positions. This indicates that C-3, C-4 and C-5 of the glutarimide is derived from a malonate as have been seen in the biosynthesis of cycloheximide[3]. The carbons C-6 to C-23 were enriched by [2-$^{13}C$] and [1-$^{13}C$]acetates alternately, and remaining three methyl groups (C-24, C-25, C-26) were labeled by [$CH_3$-$^{13}C$]methionine feeding.

The labeling pattern obtained was thus analyzed as in the figure (Table 3) supporting the assigned structure of lactimidomycin.

## Antimicrobial Activity

The minimum inhibitory concentrations (MIC's) were determined against various bacteria and fungi by the serial agar dilution method. Nutrient agar (Eiken) was used for bacteria and Sabouraud dextrose agar (Difco) for fungi. The inoculum size was adjusted to $10^2 \sim 10^4$ cfu/ml for bacteria and $10^3$ cfu/ml for

Table 4. Antifungal activity of lactimidomycin.

| Test organisms | | MIC (μg/ml) | |
|---|---|---|---|
| | | Lactimidomycin | Streptimidone |
| *Candida albicans* | IAM 4888 | >100 | >100 |
| *C. albicans* | A9540 | >100 | >100 |
| *Cryptococcus neoformans* | D49 | 3.1 | 50 |
| *C. neoformans* | IAM 4514 | 3.1 | 50 |
| *Aspergillus fumigatus* | IAM 2530 | 0.8 | >100 |
| *A. fumigatus* | IAM 2034 | 1.6 | >100 |
| *A. flavus* | FA 21436 | 3.1 | >100 |
| *Fusarium moniliforme* | A2284 | 0.8 | 50 |
| *Piricularia oryzae* | D91 | 3.1 | >100 |
| *Trichophyton mentagrophytes* | D155 | 100 | >100 |
| *T. mentagrophytes* | No. 4329 | 100 | >100 |
| *Blastomyces dermatidis* | IFO 8144 | 12.5 | >100 |
| *Sporothrix schenckii* | IFO 8158 | 100 | >100 |
| *Petriellidium boydii* | IFO 8078 | 3.1 | >100 |
| *Mucor spinosus* | IFO 5317 | 1.6 | >100 |

fungi. Streptimidone, a related glutarimide group antibiotic was used as the reference compound.

Lactimidomycin did not show inhibitory activity against Gram-positive and Gram-negative bacteria at 100 μg/ml. As summarized in Table 4, it exhibited potent activity against *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Fusarium moniliforme* and *Mucor spinosus*, and practically no activity against *Candida albicans* and *Trichophyton mentagrophytes*. As a whole, the antifungal activity of lactimidomycin was 30~100 times more potent than that of streptimidone.

## Antitumor Activity

Lactimidomycin and its hexahydro derivative were tested for their *in vitro* cytotoxicity and inhibition of macromolecule synthesis by the method described in the previous paper[4]. Mitomycin C and streptimidone were used as reference compounds and the results are summarized in Table 5.

Table 5. *In vitro* cytotoxicity and inhibition of macromolecule biosynthesis.

Cytotoxic activity

| Compound | $IC_{50}$ (μg/ml) | | |
|---|---|---|---|
| | B16-F10 | Moser | HCT-116 |
| Lactimidomycin | 0.03 | 0.047 | 0.0014 |
| Hexahydrolactimidomycin | 3.10 | ND | 3.10 |
| Streptimidone | 1.60 | 7.00 | 7.05 |
| Mitomycin C | 0.50 | 1.20 | 0.80 |

B16-F10 (murine melanoma), Moser (human colorectal carcinoma), HCT-116 (human colon carcinoma), ND (not determined).

Inhibition of macromolecule biosynthesis

| Compound | $IC_{50}$ (μg/ml) against B16-F10 melanoma | | |
|---|---|---|---|
| | DNA | RNA | Protein |
| Lactimidomycin | 0.023 | 4.2 | 0.024 |
| Mitomycin C | 1.6 | 11.0 | 60.0 |

Lactimidomycin showed potent cytotoxicity with the potency approximately 50~4,000 times greater than that of streptimidone and 20~600 times superior to mitomycin C in terms of $IC_{50}$ value. Hexahydrolactimidomycin was significantly less active than lactimidomycin.

Lactimidomycin inhibited both DNA and protein syntheses at the same extent and the $IC_{50}$ values were 200 times smaller than that of RNA synthesis.

The *in vivo* antitumor activity was determined in the experimental mouse tumor systems. Female $CDF_1$ mice and male $BDF_1$ mice were intraperitoneally inoculated with 0.4 ml of diluted ascitic fluid

Table 6. Antitumor activity of lactimidomycin.

| Tumor (site) | Treatment | | % T/C of MST[a] | |
|---|---|---|---|---|
| | Schedule | Dose, ip (mg/kg/day) | Lactimidomycin | Mitomycin C |
| P388 (ip) | Q4D × 3 | 4 | 109 | 182 |
| | | 2 | 136 | 155 |
| | | 1 | 136 | 141 |
| | | 0.5 | 109 | 132 |
| | Q1D × 9 | 1 | 145 | 170 |
| | | 0.5 | 135 | 155 |
| | | 0.25 | 140 | 130 |
| | | 0.13 | 120 | 120 |
| | | 0.063 | 110 | 110 |
| B16 (ip) | Q4D × 3 | 4 | 86 | ND |
| | | 2 | 145 | 190 |
| | | 1 | 121 | 138 |
| | | 0.5 | 117 | 103 |
| | | 0.25 | 117 | 100 |

[a] % T/C: The median survival time (MST) of treatment mice/MST of control mice, × 100. Significant activity was considered to be a T/C of ≥125% in each tumor model evaluated.
ND: Not determined.

containing $10^6$ lymphocytic leukemia P388 cells and 0.5 ml of 10% melanotic melanoma B16 brei, respectively. Test compounds were administered to the mice intraperitoneally once a day on days 1, 5 and 9 (Q4D × 3) and once daily on days 1 to 9 (Q1D × 9). Lactimidomycin demonstrated fairly good anti-P388 leukemic activity by Q1D × 9 treatment with the potency being comparable to that of mitomycin C, while it gave moderate antitumor activity against B16 melanoma (Table 6). Streptimidone showed no prolongation of life span of P388 leukemia-bearing mice. Although *in vivo* toxicity have not been tested, lactimidomycin was lethal at 32 mg/kg (Q1D × 3, ip) in tumor-bearing mice.

## Discussion

Lactimidomycin, a novel glutarimide antibiotic having a unique 12-membered lactone ring, was isolated from the fermentation broth of *S. amphibiosporus*. It showed potent antifungal activity, cytotoxicity and *in vivo* antitumor activity in mice. Among the glutarimide class antibiotics, streptimidone[5,6], 9-methylstreptimidone[7], protomycin[8] and S-632 $B_1$, $B_2$[9,10] have an acyclic unsaturated ketone side chain. However, none of this class antibiotic contains an unsaturated lactone side chain. Moreover, lactimidomycin is differentiated from the other known glutarimide antibiotics by its potent antitumor activity. When the lactone ring was saturated by hydrogenation, the resulting hexahydro derivative exhibited only weak antifungal activity and cytotoxicity. This indicated that the unsaturated 12-membered lactone in lactimidomycin plays an important role for its distinct biological activity.

## Experimental

### General

TLC was performed on precoated silica gel plates (Kieselgel $60F_{254}$). IR spectra were recorded on a Jasco IR-810 and UV spectra on a UVIDEC-610C spectrometer. $^1H$ and $^{13}C$ NMR spectra were measured on a Jeol JNM-GX 400 spectrometer operated in Fourier transform mode. EI-MS were obtained on a Hitachi M80B and FAB-MS on a Jeol JMS-AX 505H using *m*-nitrobenzyl alcohol as the matrix. Optical rotations were determined with a Jasco model DIP 140.

Hydrogenation of Lactimidomycin

Lactimidomycin (16 mg) dissolved in methanol (5 ml) was hydrogenated over 20% palladium charcoal (16 mg) for 16 hours. The catalyst was removed by filtration, and the solution was evaporated *in vacuo*. The residue was chromatographed on a column of Sephadex LH-20 developing with methanol to yield hexahydro derivative (10 mg). Hexahydrolactimidomycin: White powder; EI-MS *m/z* 463 ($M^+$), 445 $(M-H_2O)^+$, 308 $(M-C_7H_{10}NO_3)^+$, 265 $(M-C_9H_{12}NO_4)^+$ and 180 $(C_9H_{10}NO_3)^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.78 (3H, d, 7.2), 1.00 (3H, d, 6.8), 1.10 (1H, m), 1.2 (m), 1.58 (1H, m), 1.64 (1H, m), 1.67 (3H, d, 1.3), 1.78 (1H, m), 1.87 (1H, m), 2.1~2.3 (5H, m), 2.4~2.6 (m), 3.4 (1H, m), 3.93 (1H, m), 4.71 (1H, d, 6.0), 5.08 (1H, d, 3.0), 5.14 (1H, d, 9.8), 10.66 (1H, s).

Feeding Experiment with $^{13}$C-enriched Precursors

Strain R310-104 was grown at 32°C for 5 days in a 100 ml of vegetative medium composed of soluble starch 2%, Pharmamedia 1%, $ZnSO_4$ 0.003% and $CaCO_3$ 0.4%, pH 7.0 in a 500-ml Erlenmeyer flask and aliquot (5 ml) was transferred to a 100 ml of production medium containing glucose 3%, Protein S 3%, Pharmamedia 0.5%, $CaCO_3$ 0.3% and yeast extract 0.1%, pH 7.0 in a 500-ml Erlenmeyer flask. Fermentation was carried out at 28°C on a rotary shaker (200 rpm). Sodium [1-$^{13}$C]acetate (90 atom% $^{13}$C, Isotec Inc.), sodium [2-$^{13}$C]acetate (90 atom% $^{13}$C, Isotec Inc.) and [$CH_3$-$^{13}$C]-L-methionine (99 atom% $^{13}$C, Aldrich Chemical Co.) were disolved in distilled water at the concentration of 160 mg/ml, 100 mg/ml and 30 mg/ml, respectively, and sterilized by filtration. In all cases, 1 ml aliquot each of the labeled precursor solution was fed to 100 ml of the growing cultures at 40, 52 and 64 hours after inoculation. The fermentation was continued 24 hours after final addition of the labeled precursor.

The $^{13}$C-labeled lactimidomycin was extracted from the fermentation broth (1.0 liter) with 1-butanol and purified using solvent partition follow by successive chromatographies on silica gel and Sephadex LH-20 to yield a sample (3~6 mg) for $^{13}$C NMR spectroscopic analysis.

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## References

1) KONISHI, M.; K. SUGAWARA, M. OHBAYASHI & T. MIYAKI (Bristol-Myers Squibb): BU-4146T antibiotic. U.S. 5,002,959, Mar. 26, 1991
2) KONISHI, M.; K. SUGAWARA, M. OHBAYASHI & T. MIYAKI (Bristol-Myers Squibb): BU-4146T antibiotic. U.S. 5,037,836, Aug. 15, 1991
3) SHIMADA, H.; H. NOGUCHI, Y. IITAKA & U. SANKAWA: Biosynthesis of cycloheximide. Stereospecific incorporation of [1,2,3-$^{13}C_3$]malonate. Heterocycles 15: 1141~1147, 1981
4) SUGAWARA, K.; M. HATORI, Y. NISHIYAMA, K. TOMITA, H. KAMEI, M. KONISHI & T. OKI: Eponemycin, a new antibiotic active against B16 melanoma. I. Production, isolation, structure and biological activity. J. Antibiotics 43: 8~18, 1990
5) KOHBERGER, D. L.; M. W. FISHER, M. M. GALBRAITH, A. B. HILLEGAS, P. E. THOMPSON & J. EHRLICH: Biological studies of streptimidone, a new antibiotic. Antibiot. Chemother. 10: 9~16, 1960
6) FROHARDT, R. P.; H. W. DION, Z. L. JAKUBOWSKI, A. RYDER, J. C. FRENCH & Q. R. BARTZ: Chemistry of streptimidone, a new antibiotic. J. Am. Chem. Soc. 81: 5500~5506, 1959
7) SAITO, N.; F. KITAME, M. KIKUCHI & N. ISHIDA: Studies on a new antiviral antibiotic, 9-methylstreptimidone. I. Physicochemical and biological properties. J. Antibiotics 27: 206~214, 1974
8) SUGAWARA, R.: Protomycin, a new antibiotic of cycloheximide group. II. Production and properties. J. Antibiotics, Ser. A 16: 115~120, 1963
9) OTANI, T.; T. SASAKI, Y. MINAMI, T. MARUNAKA & Q.-W. YU: New glutarimide antibiotics, S-632-$B_1$ and $B_2$. I. Taxonomy of producing strain, fermentation and biological properties. J. Antibiotics 42: 647~653, 1989
10) OTANI, T.; Y. MINAMI, H. MATSUMOTO, T. MARUNAKA, Z.-X. LOU & Q.-W. YU: New glutarimide antibiotics, S-632-$B_1$ and $B_2$. II. Isolation, physico-chemical properties and chemical structure. J. Antibiotics 42: 654~661, 1989