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Deacetylravidomycin M, a New Inhibitor of IL-4 Signal Transduction, Produced by *Streptomyces* sp. WK-6326

I. Taxonomy, Fermentation, Isolation and Biological Activities

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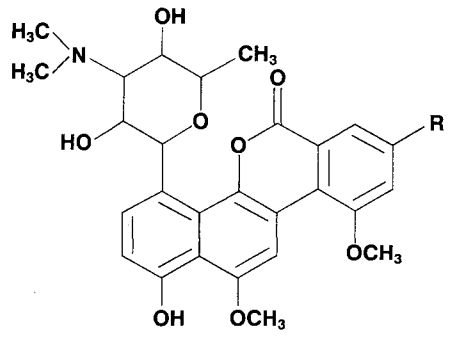
Streptomyces sp. WK-6326, a soil isolate, was found to produce an inhibitor of interleukin (IL)-4 signal transduction. Two structurally related compounds, a novel one designated deacetylravidomycin M and the known deacetylravidomycin, were isolated from the culture broth by solvent extraction, silica gel column chromatography and HPLC. Deacetylravidomycin M inhibited IL-4-induced CD23 expression in U937 cells without any cytotoxic effect, whereas deacetylravidomycin showed no inhibitory activity.

Interleukin (IL)-4 is known to be a cytokine which plays a central role in the regulation of immune response[1]. Binding of IL-4 to its receptor on B cells leads to the activation of the janus kinase (JAK)-signal transducer and activator transcription 6 (STAT6) pathway[2]. In this pathway, the receptor associated JAK1 and 3 phosphorylates tyrosine residue of STAT6. Then STAT6 forms dimers, translocates to the nucleus, binds the specific elements in the promoters of target genes, and transcriptionally activates these genes. As a results, B cells raise expression of CD23 and class switching to IgE. Production of IgE causes the release of various chemical mediators such as histamine, leukotriene, prostaglandin and so on[3]. Therefore, inhibitors of IL-4 signal transduction are expected to prevent allergic diseases.

During the course of our screening program for inhibitors of IL-4 signal transduction, we discovered and isolated a new ravidomycin-related compound, termed deacetylravidomycin M[4], along with a known compound deacetylravidomycin[5] in the culture broth of *Streptomyces* sp. WK-6326 (Fig. 1). In this paper, the taxonomy of the producing strain, fermentation, isolation and biological properties of the deacetylravidomycins are described.

Fig. 1. Structure of deacetylravidomycins.



	R
Deacetylravidomycin M	-CH_3
Deacetylravidomycin	-$CH{=}CH_2$

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Materials and Methods

General Experimental Procedures

Strain WK-6326 was isolated from a soil sample collected at Utah, USA, by using established method[6] and was used for production of deacetylravidomycins. Silica gel 60 (230~400 mesh, Merck) was used for column chromatography. HPLC was carried out using a GULLIVER system (JASCO). For determination of the amounts of deacetylravidomycins in culture broths, the samples (ethyl acetate extracts) dissolved in methanol were analyzed by HPLC as follows: column, CAPCELL PAK C18 4.6×250 mm (Shiseido); solvent, 25% acetonitrile containing 0.05% phosphoric acid; flow rate, 1.0 ml/minute; UV at 246 nm. Deacetylravidomycin M and deacetylravidomycin were eluted as a peak with a retention time of 17 and 28 minutes, respectively.

Taxonomic Studies

The International *Streptomyces* Project (ISP) media recommended by SHIRLING and GOTTLIEB[7] and media recommended by WAKSMAN[8] were used to investigate the cultural and physiological characteristics of the strain WK-6326. Cultures were routinely observed after the incubation for two weeks at 27°C. Color names and hue numbers were determined according to the Color Harmony Manual[9]. The utilization of carbon sources was tested by growth on PRIDHAM and GOTTLIEB's medium containing 1% carbon at 27°C[10]. The morphological properties were observed with a scanning electron microscope (model JSM-5600, JEOL). The type of diaminopimelic acid (DAP) isomers was determined by the method of BECKER *et al.*[11]. Menaquinones were extracted and purified by the method of COLLINS *et al.*[12], then analyzed by HPLC equipped with a CAPCELL PAK C18 column (4.6×250 mm, Shiseido)[13].

Assay for IL-4-induced CD23 Expression in U937 Cells

Assay for IL-4-induced CD23 expression in U937 cells was carried out according to the method described previously[14]. In brief, U937 cells (2×10^5 cells) were treated with 10 ng/ml of IL-4 in the presence or absence of deacetylravidomycins for 72 hours at 37°C. Then, the cells were washed with PBS and mixed with FITC conjugated anti CD23 antibody (PharMingen) at the recommended dilution as instructed in the manual on ice for 30 minutes. The CD23 of cell surface was determined by immunofluorescence analysis using flow cytometry (COULTER). Effect of drugs on CD23 expression was evaluated by calculation as follows: CD23 expression (%)=100×cell number from C to D/cell number from B to D (Fig. 6).

Assay for Cell Viability

Cytotoxicity of deacetylravidomycins to U937 cells under the same conditions of CD23 expression was measured using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) by the established method[15]. A drug concentration causing 50% inhibition of the growth of U937 cells (IC_{50}) was defined as an index of cytotoxicity.

Other Biological Assays

Antimicrobial activity was tested by the microtiter broth dilution assay[16]. *Mycobacterium smegmatis* (ATCC607) was grown on Waksman medium (glucose 1.0%, peptone 0.5%, meat extract 0.5%, NaCl 0.3%, pH 7.0), and other bacteria were grown on Nutrient medium (pepton 0.5%, meat extract 0.5%, pH 7.0), for 24 hours at 37°C. Fungi and yeasts were grown on GY medium (glucose 1.0%, yeast extract 0.5%, pH 6.0), for 24 hours at 27°C. The growth of microorganisms was measured as OD values at 660 nm with ELx 808 (BIO-TEK Instruments) The minimum inhibitory concentration (MIC) value was defined as the lowest inhibitor concentration, which prevented increasing absorbance at 660 nm.

Results

Taxonomy of the Producing Strain WK-6326

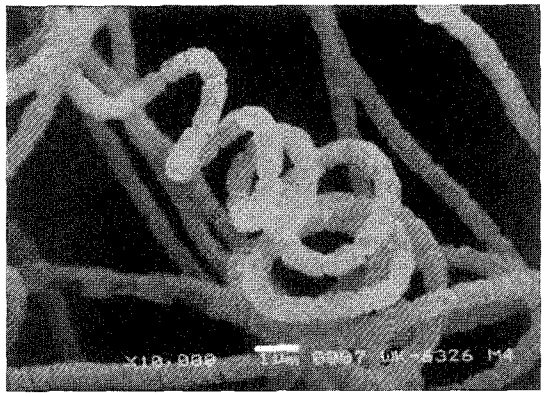
The vegetative mycelia grew abundantly on yeast extract-malt extract agar, inorganic salts-starch agar and other agar media, and did not show fragmentation into coccoid forms or bacillary elements. The aerial mycelia grew abundantly on yeast extract-malt extract agar, oatmeal agar, inorganic salts-starch agar, glycerol-asparagine agar and tyrosine agar. The spore chains were straight or flexible and each had more than 20 spores per chain. The spores were cylindrical in shape, 1.0×0.6 μm in size and had a smooth surface (Fig. 2). Whirls, sclerotic granules, sporangia and flagellate spores were not observed.

The isomer of DAP in whole-cell hydrolysates of strain WK-6326 was determined to be LL-form. Major menaquinones were MK-9(H_6) and MK-9(H_8).

The cultural characteristics, the physiological properties and the utilization of carbon sources are shown in Tables 1, 2 and 3. The color of vegetative mycelia showed yellow to brown. The aerial mass color showed gray series on various media. Melanoid pigment was produced.

Based on the taxonomic properties described above, strain WK-6326 is considered to belong to the genus *Streptomyces*[17].

Fig. 2. Scanning electron micrograph of spore chains of *Streptomyces* sp. WK-6326 grown on inorganic salt - starch agar for 14 days at 27 °C.



Bar represents 1 μm.

The strain was deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. WK-6326 and the accession No. is FERM P-17592.

Fermentation

A slant culture of the strain WK-6326 grown on Waksman agar was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of the seed medium (starch 2.4%, glucose 0.1%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.5%, pH 7.0). The flask was shaken on a rotary shaker at 27°C for 3 days. One-ml portion of the seed culture was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of the production medium (soluble starch 4.0%, solvent extracted toasted soy bean meal 2.0%, 0.1 M $Na_2S_2O_3$ 32 μl/liter, $FeSO_4 \cdot 7H_2O$ 0.05%, K_2HPO_4 0.05%, KCl 0.03%, pH 6.5). The flasks were shaken under the same condition of seed culture for 6 days. A typical

Table 1. Cultural characteristics of strain WK-6326.

Medium	Cultural characteristics	Medium	Cultural characteristics
Yeast extract-malt extract agar [a]	G: Good, bamboo (2gc) R: Light amber (3ic) AM: Abundant, beige (3ge) SP: Trace, topaz (3ne)	Tyrosine agar [a]	G: Good, honey gold (2ic) R: Light mustard tan (2ie) AM: Abundant, rose taupe (5ig) SP: None
Oatmeal agar [a]	G: Good, camel (3ie) R: Bisque (3ec) to light beige (3pl) AM: Abundant, rosewood (5ge) SP: None	Sucrose-nitrate agar [b]	G: Moderate, pearl pink(3ca) R: Pearl pink(3ca) AM: Poor, ivory (2db) SP: None
Inorganic salts-starch agar [a]	G: Good, light wheat (2ea) R: Light ivory (2ca) to adobe brown (3lg) AM: Abundant, rosewood (5ge) SP: None	Glucose-nitrate agar [b]	G: None R: None AM: None SP: None
Glycerol-asparagine agar [a]	G: Good, light wheat (2ea) R: Bamboo (2gc) AM: Abundant, ashes of rose (6ge) SP: None	Glycerol-calcium malate agar [b]	G: Good, ivory (2db) R: Ivory (2db) AM: Poor, white (a) SP: None
Glucose-asparagine agar	G: Poor, ivory (2db) R: Ivory (2db) AM: None SP: None	Glucose-peptone agar [b]	G: Poor, bamboo (2gc) R: Bamboo (2gc) AM: None SP: None
Peptone-yeast extract-iron agar [a]	G: Moderate, light mustard tan (2ie) R: Light mustard tan (2ie) AM: None SP: Covert brown (2nl)	Nutrient agar [b]	G: Moderate, bamboo (2gc) R: Bamboo (2gc) AM: Poor, white (a) SP: None

[a] Medium recommended by ISP.
[b] Medium recommended by S. A. Waksman.
Abbreviations: G, growth of vegetative mycelium; R, reverse side color; AM,aerial mycelium; SP, soluble pigment.

Table 2. Physiological properties of *Streptomyces* sp. WK-6326.

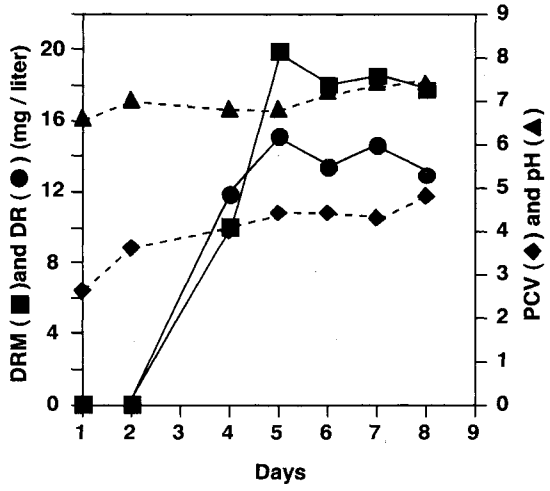
Melanin formation	
Tyrosine agar	—
Peptone-yeast extract-iron agar	+
Tryptone-yeast extract broth	+
Reduction of nitrate	—
Liquefaction of gelatin (21~23°C)	—
Hydrolysis of starch	+
Coagulation of milk (27°C)	—
Peptonization of milk (27°C)	+
Decomposition of cellulose	—
Temperature range for growth	5~32°C

+: Positive, —: Negative

Table 3. Utilization of carbon sources by strain WK-6326.

Utilized:	D-Glucose
Weakly utilized:	D-Fructose
Not utilized:	L-Arabinose, *i*-Inositol, D-Mannitol, Melibiose, Raffinose, L-Rhamnose, Sucrose, D-Xylose

Fig. 3. A typical time course of deacetylravidomycins production by *Streptomyces* sp. WK-6326.



DRM, deacetylravidomycin M; DR, deacetylravidomycin; PCV, packed cell volume (ml) from 10 ml of the whole culture broth.

time course of the fermentation is shown in Fig. 3. Deacetylravidomycin M and deacetylravidomycin were detected in the culture broth at day 3 after inoculation, and their concentrations reached levels of 20 and 15 mg/liter, respectively, at day 5.

Isolation

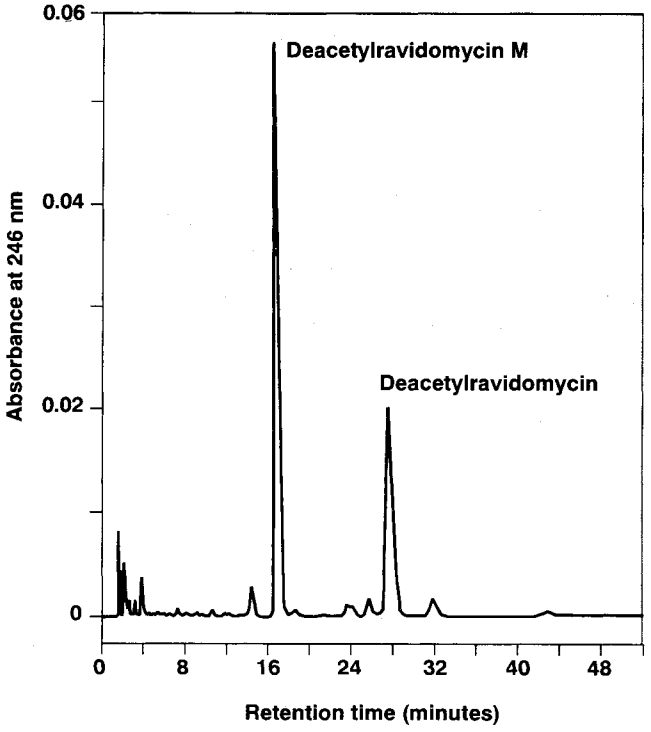
The 6-day old culture broth (5 liters) was centrifuged to separate mycelium and supernatant. The mycelium was extracted with 5 liters of acetone. After the acetone extracts were filtered and concentrated, the resulting aqueous solution was extracted with 10 liters of ethyl acetate. The supernatant was extracted with 10 liters of ethyl acetate. Both extracts of ethyl acetate were combined, dried over Na_2SO_4 and concentrated *in vacuo* to dryness to yield 560 mg of oily material. The material was dissolved in a small volume of $CHCl_3$ and subjected to an open silica gel column (30 g). The principles were eluted stepwise with $CHCl_3$-MeOH solvent system (100:0, 100:10, 100:30, 100:50, 100:70 and 0:100, 10 ml×20 fractions for each solvent mixture), and the inhibitory activity was observed in the fractions from solvent ratio 100:30-fraction 10 to solvent ratio 100:50-fraction 18 (112 mg). Furthermore, the active principles from open silica gel column were purified by HPLC (Shiseido CAPCELL PAK C18, 4.6×250 mm; 25% acetonitrile containing 0.05% phosphoric acid; flow rate 1.0 ml/minute; UV at 246 nm). Under the conditions, deacetylravidomycin M and deacetylravidomycin were eluted with a retention time of 17 and 28 minutes, respectively (Fig. 4). Each peak was collected and concentrated to yield deacetylravidomycin M (18 mg) and deacetylravidomycin (26 mg) as pale yellow powder.

Biological Properties

Cytotoxicity of Deacetylravidomycins to U937 Cell

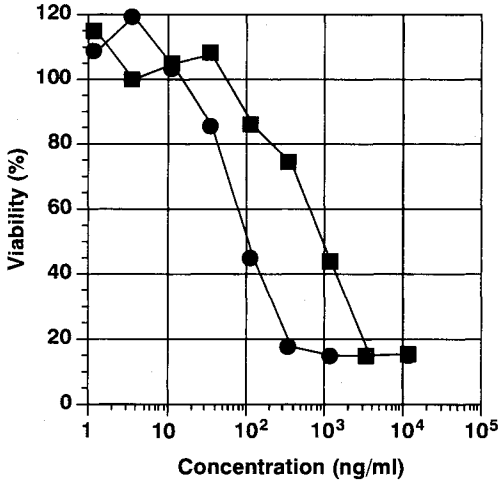
Under the condition of IL-4-induced CD23 expression, cytotoxic effect of deacetylravidomycins on U937 cells was evaluated by the MTT assay. As shown in Fig. 5, deacetylravidomycin M and deacetylravidomycin showed no cytotoxic effect up to 100 ng/ml and 10 ng/ml, and gave IC_{50} values of 1.0 μg/ml and 100 ng/ml, respectively. Thus, deacetylravidomycin M was found to give ten times lower cytotoxic effect on U937 cells than deacetylravidomycin.

Fig. 4. A chromatographic profile of deacetylravidomycins by HPLC.



Sample, 5.0 μg of active materials (obtained through Silica gel column chromatography) dissolved in 50 μl acetonitrile. Deacetylravidomycin M and deacetylravidomycin were eluted with a retention time of 17 and 28 minutes, respectively.

Fig. 5. Cytotoxicity of deacetylravidomycins to U937 cells.



U937 cells (4×10^4 cells) were incubated under the condition of CD23 expression in the presence of indicated amounts of deacetylravidomycin M (■) or deacetylravidomycin (●). After a 72-hour incubation, cell viability was measured using method of MTT as described in the "Materials and Methods".

Effect of Deacetylravidomycins on IL-4-induced CD23 Expression in U937 Cells

When U937 cells were treated with IL-4 for 72 hours, about 45% of the cells were shifted to higher fluorescent region (Fig. 6A-a *vs.* -b), indicating that CD23 was induced on the cell surface. In the presence of deacetylravidomycin M (0.05 ng/ml) (Fig. 6A-c), the distribution of the cells was essentially the same as that of Fig. 6A-a, showing that the drug completely inhibits IL-4-induced expression of CD23. However, deacetylravidomycin showed no effect on the expression at concentrations ranging from 0.005 ng/ml to 5.0 ng/ml, and a stimulative effect was observed at 5.0 ng/ml (Fig. 6A-d and B).

Antimicrobial Activity of Deacetylravidomycins

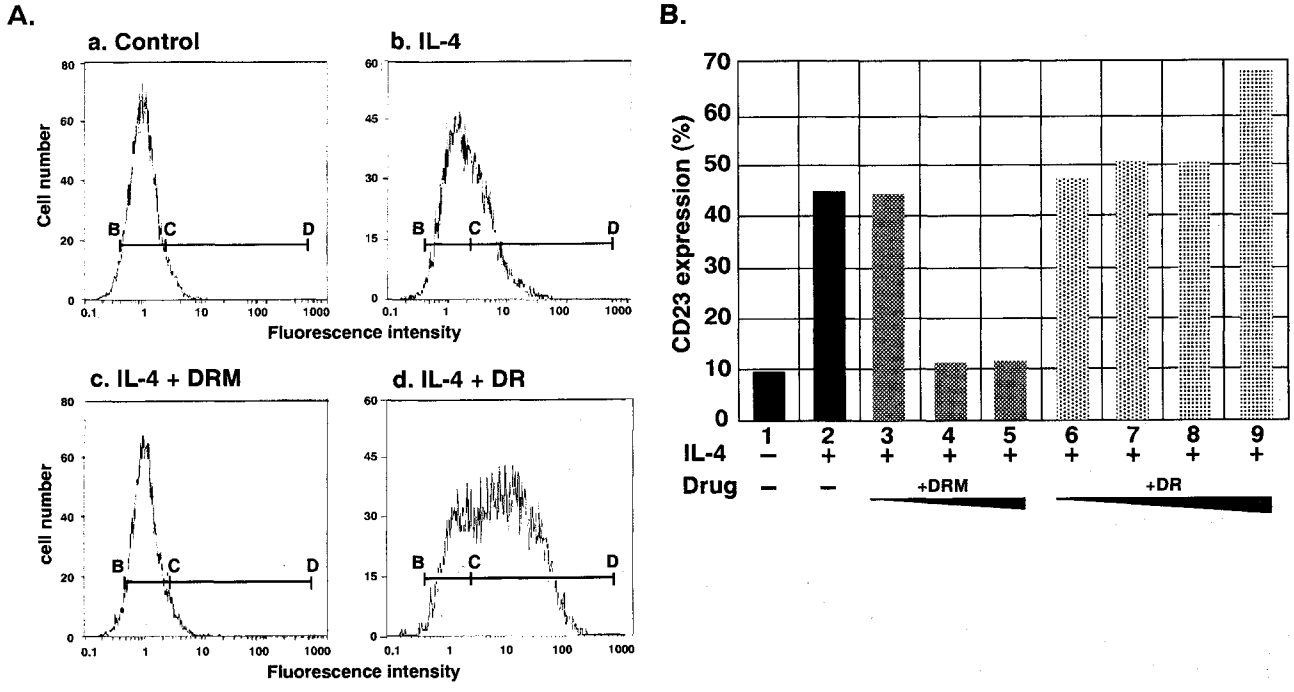
The antimicrobial activity of deacetylravidomycins is summarized in Table 4. Deacetylravidomycin M inhibited the growth of *B. subtillis* and *M. luteus* moderately, but showed almost no antimicrobial activity against the other organisms. On the other hand, deacetylravidomycin showed antimicrobial activity against Gram-positive bacteria (*B. subtillis*, *S. aureus*, *M. luteus* and *M. smegmatis*), which are comparable to those reported previously[5,18].

Discussion

Deacetylravidomycin and deacetylravidomycin M were isolated from the culture broth of *Streptomyces* sp. WK-6326. Deacetylravidomycin, reported as an antitumor antibiotic[5], with a vinyl residue at the R position (Fig. 1), showed about ten times more potent cytotoxic effect (Fig. 5) and antimicrobial activity (Table 4) than deacetylravidomycin M with a methyl residue at the position (Fig. 1). On the contrary, deacetylravidomycin M inhibited IL-4-induced signal transduction, while deacetylravidomycin showed no effect (Fig. 6).

Many ravidomycin analogs, which incorporate the same aromatic aglycon attached to different glycoside substituents, were produced by *Streptomyces* strains as antitumor or/and antimicrobial antibiotics[5,18~21]. Among

Fig. 6. Effect of deacetylravidomycins on IL-4-induced CD23 expression in U937 cells.



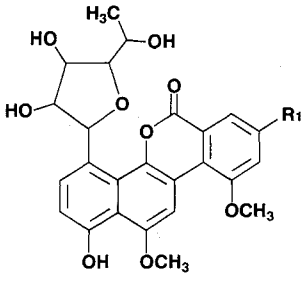
A. Distribution of U937 cells in the absence (a), in the presence of IL-4 alone (b), plus deacetylravidomycin M (DRM, 0.05 ng/ml) (c) or plus deacetylravidomycin (DR, 5.0 ng/ml) (d) analysed by flow cytometry.

B. CD23 expression (%) was calculated as described in the "Materials and Methods". Column 1, cells incubated in the absence of drugs and IL-4. Column 2, in the presence of IL-4 without drugs; columns 3, 4 and 5 with DRM (0.005, 0.05 and 0.5 ng/ml, respectively); columns 6, 7, 8 and 9 with DR (0.005, 0.05, 0.5 and 5.0 ng/ml, respectively).

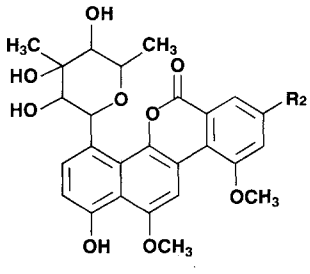
Table 4. Antimicrobial activities of deacetylravidomycins.

Test organism	MIC(μg/ml)	
	Deacetylravidomycin M	Deacetylravidomycin
Bacillus subtilis KB27 (ATCC6633)	25	3.0
Staphylococcus aureus KB210 (ATCC6538p)	125	5.0
Micrococcus luteus KB40 (ATCC9431)	25	3.0
Mycobacterium smegmatis KB42 (ATCC607)	125	3.0
Escherichia coli KB176 (IFO12734)	>125	>125
Pseudomonas aeruginosa KB105 (IFO3080)	>125	>125
Xanthomonas campestris KB88	125	25
Acholeplasma laidlawii KB174	>125	>125
Pyricularia oryzae KB180	>125	25
Aspergillus niger KF103 (ATCC6275)	>125	>125
Mucor racemosus KF223 (IFO4581)	>125	>125
Candida albicans KF1	>125	>125
Saccharomyces cerevisiaes KF26 (ATCC9763)	>125	>125

Fig. 7. Structures of ravidomycin analogs, glivocarcins M and V, and chrysomycins M and V.



	R_1
Gilvocarcin M	$-CH_3$
Gilvocarcin V	$-CH=CH_2$



	R_2
Chrysomycin M	$-CH_3$
Chrysomycin V	$-CH=CH_2$

them, gilvocarcin V and chrysomycin V with a vinyl residue at the corresponding R_1 and R_2 positions (Fig. 7) were reported to show potent activities, while glivocarcin M and chrysomycin M with a methyl residue (Fig. 7) showed moderate activities[22,23]. RAKHIT *et al.*[24] reported that reduction of the vinyl group of deacetylravidomycin resulted in an appreciable loss of antitumor and antimicrobial activities. The mechanism of action has been studied extensively[25~29]. A covalent modification occurs on DNA under the light *via* [2+2]cycloaddition between the vinyl residue of gilvocarcin V and thymine residues in DNA[30] to give strong cytotoxic effect. Interestingly, the UV spectrum of the photoadduct derived from gilvocarcin V showed the presence of a chromophore similar to that of gilvocarcin M. However, compounds having the methyl residue at the position like gilvocarcin M and chrysomycin M cannot cause such a reaction under the light, resulting in low cytotoxicity. Therefore, the presence of the vinyl residue at the R position in ravidomycin analogs is important for eliciting potent antimicrobial and antitumor activities. Accordingly, deacetylravidomycin M also having a methyl residue at the position does not seem to react with DNA. In this sense, the mechanism of inhibition of the IL-4-induced signal transduction by deacetylravidomycin M is different from that antimicrobial and antitumor activities by ravidomycin analogs.

Further study of its effect on IgE secretion from B cells and the target molecule in the JAK-STAT6 pathway remains to be investigated.

References

1) PAUL, W. E.: Interleukin-4: a prototypic immunoregulatory lymphokine. Blood 77: 1859~1870, 1991
2) HOU, J.; U. SCHINDLER, W. J. HENZEL, T. C. HO, M. BRASSEUR & S. L. MCKNIGHT: An interleukin-4-induced transcription factor: IL-4 Stat. Science 265: 1701~1706, 1994
3) MARONE, G.; V. CASOLARO, V. PATELLA, G. FLORIO & M. TRIGGIANI: Molecular and cellular biology of mast cells and basophils. Int. Arch. Allergy Immunol. 114: 207~217, 1997
4) ARAI, M.; H. TOMODA, N. TABATA, N. ISHIGURO, S. KOBAYASHI & S. ŌMURA: Deacetylravidomycin M, a new inhibitor of IL-4 signal transduction, produced by *Streptomyces* sp. WK-6326. II. Structure elucidation. J. Antibiotics 54: 562~566, 2001
5) NARITA, T.; M. MATSUMOTO, K. MOGI, K. KUKITA, R. KAWAHARA & T. NAKASHIMA: Deacetylravidomycin *N*-oxide, a new antibiotic. Taxonomy and fermentation of the producing organism and isolation, structure and biological properties of the antibiotic. J. Antibiotics 42: 347~356, 1989
6) OMURA, S. (*Ed.*): Selection of Microbial Sources of Bioactive Compounds. *In* The Search for Bioactive Compounds from Microorganisms. Springer-Verlag. New York, 1989
7) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
8) WAKSMAN, S. A. (*Ed.*): Classification, identification and description of genera and species. *In* The actinomycetes. Vol. 2. Williams and Wilkins Co. Baltimore, 1961
9) Container Corporation of America: Color Harmony Manual 4th. Ed. Chicago, 1958
10) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56: 107~114, 1948
11) BECKER, B.; M. P. LECHEVALIER & H. A. LECHEVALIER: Chemical composition of cell-wall preparation from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13: 236~243, 1965
12) COLLINS, M. D.; M. GOODFELLOW & D. E. MINNIKIN: Distribution of menaquinones in actinomycetes and corynebacteria. J. Gen. Microbiol. 100: 221~230, 1977
13) TAMAOKA, J.; Y. KATAYAMA-FUJIMURA & H. KURAISHI: Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J. Appl. Bacteriol. 54: 31~36, 1983
14) MARSHALL, L. A.; M. J. HANSBURY, B. J. BOLOGNESE, R. J. GUM, P. R. YOUNG & R. J. MAYER: Inhibitors of the p38 mitogen-activated kinase modulate IL-4 induction of low affinity IgE receptor (CD23) in human monocytes. J. Immunol. 161: 6005~6013, 1998
15) MOSMANN, T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immun. Methods 65: 55~63, 1983
16) MANDALA, S. M.; R. A. THORNTON, M. ROSENBACH, J. MILLIGAN, M. GARCIA-CALVO, H. G. BULL & M. B. KURTZ: Khafrefungin, a novel inhibitor of sphingolipid synthesis. J. Biol. Chem. 272: 32709~32714, 1997

17) WILLIAMS, S. T.; M. GOODFELLOW & G. ALDERSON: Genus *Streptomyces* Waksman and Henrici 1943. *In* BERGEY's Manual of Systematic Bacteriology. Volume 4. *Ed.*, WILIAMS, S. T. *et al.*, pp. 2452~2492, Williams & Wilkins Co., 1989
18) SEHGAL, S. N.; H. CZERKAWSKI, A. KUDELSKI, K. PANDEY, R. SAUCIER & C. VEZINA: Ravidomycin (AY-25,545), a new antitumor antibiotic. J. Antibiotics 36: 355~362, 1983
19) SINGH, K.: Studies on the mechanism of action of ravidomycin (AY-25,545). J. Antibiotics 37: 71~73, 1984
20) YAMASHITA, N.; K. SHIN-YA, K. FURIHATA, Y. HAYAKAWA & H. SETO: New ravidomycin analogues, FE35A and FE35B, apoptosis inducers produced by *Streptomyces rochei*. J. Antibiotics 51: 1105~1108, 1998
21) LORICO, A. & B. H. LONG: Biochemical characterisation of elsamicin and other coumarin-related antitumor agents as potent inhibitors of human topoisomerase II. Eur. J. Cancer 29A: 1985~1991, 1993
22) BALITZ, D. M.; F. A. O'HERRON, J. BUSH, D. M. VYAS, D. E. NETTILETON, R. E. GRULICH, W. T. BRADNER, T. W. DOYLE, E. AMOLD & J. CLARDY: Antitumor agents from *Streptomyces anandii*: gilvocarcins V, M and E. J. Antibiotics 34: 1544~1555, 1981
23) MATSON, J. A.; W. C. ROSE, J. A. BUSH, R. MYLLYMAKI, W. T. BRADNER & T. W. DOYLE: Antitumor activity of chrysomycins M and V. J. Antibiotics 42: 1446~1448, 1989
24) RAKHIT, S.; C. ENG, H. BAKER & K. SINGH: Chemical modification of ravidomycin and evaluation of biological activities of its derivatives. J. Antibiotics 36: 1490~1494, 1983
25) GREENSTEIN, M.; T. MONJI, R. YEUNG, W. M. MAIESE & R. J. WHITE: Light-dependent activity of the antitumor antibiotics ravidomycin and desacetylravidomycin. Antimicrob. Agents Chemother. 29: 861~866, 1986
26) ELESPURU, R. K. & S. K. GONDA: Activation of antitumor agent gilvocarcins by visible light. Science 223: 69~71, 1984
27) ELESPURU, R. K. & V. M. HITCHINS: Wavelength dependence for the induction of bacteriophage lambda by antitumor agent gilvocarcin V. Photochem. Photobiol. 44: 607~612, 1986
28) TSE-DINH, Y. C. & L. R. MCGEE: Light-induced modifications of DNA by gilvocarcin V and its aglycone. Biochem. Biophys. Res. Commun. 143: 808~812, 1987
29) GASPARRO, F. P.; R. M. KNOBLER & R. L. EDELSON: The effects of gilvocarcin V and ultraviolet radiation on pBR322 DNA and lymphocytes. Chem. Biol. Interact. 67: 255~265, 1988
30) MCGEE, L. R. & R. MISRA: Gilvocarcin photobiology. Isolation and characterization of the DNA photoadduct. J. Am. Chem. Soc. 112: 2386~2389, 1990