CYTOSAMINOMYCINS, NEW ANTICOCCIDIAL AGENTS PRODUCED BY *Streptomyces* sp. KO-8119

I. TAXONOMY, PRODUCTION, ISOLATION AND PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

KATSUJI HANEDA,† MAYUMI SHINOSE, AKIO SEINO, NORIKO TABATA, HIROSHI TOMODA, YUZURU IWAI and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

(Received for publication February 14, 1994)

Streptomyces amakusaensis KO-8119, a soil isolate, was found to produce a series of new anticoccidial compounds. Four active compounds, designated as cytosaminomycins A, B, C and D, were isolated from the fermentation broth of the producing strain by solvent extraction, silica gel column chromatography and preparative HPLC. Cytosaminomycins inhibited the growth of *Eimeria tenella* in an *in vitro* assay system using primary chicken embryonic cells as a host. No schizont in the cells was observed at concentrations ranging from 0.3 to 0.6 μg/ml for cytosaminomycins A, B and C, and at 2.5 μg/ml for cytosaminomycin D.

Coccidiosis in poultrys is a disease caused by a group of parasitic protozoa including *Eimeria*. Polyether ionophores such as monensin, salinomycin and lasalocid have been used as anticoccidial agents. However, polyether-resistant *Eimeria* sp. are emerging with increasing frequency[1]. Therefore, the search for new anticoccidial agents against polyether-resistant *Eimeria* sp. is being intensified. We established an *in vitro* assay system using BHK-21 cells[1~7] as a host and monensin-resistant *E. tenella* as a parasitic protozoan for a high-throughput screen of microbial anticoccidial agents. As a result, xanthoquinodins[2,3], diolmycins[4,5], hynapenes[6,7], and arohynapenes[8] were discovered.

Recently, another *in vitro* assay system was established using primary chicken embryonic cells as a host instead of BHK-21 cells, since the cell line of BHK-21 cells was derived from baby hamster kidney cells and the various characteristics including the infectivity of *E. tenella* might be different from those of chicken cells[9]. This new assay was expected to yield new leads showing effective efficacy in an *in vivo* test. As a result, an actinomycetes strain KO-8119 was found to produce a series of new anticoccidial agents. Four active compounds termed as cytosaminomycins A, B, C and D were isolated. A structurally related known antibiotic (compound E), oxyplicacetin[10], was also isolated accompanying with cytosaminomycins. In the present paper, the taxonomy of the cytosaminomycin-producing strain, the production, isolation and physico-chemical and biological properties of these compounds are described.

Materials and Methods

General Experimental Procedures

Kieselgel 60 (E. Merck) and ODS (Senshu Sci. Co., ODS-SS-1020T) were used for column chromatography. Analytical and preparative HPLC were carried out using ODS packed columns (Senshu Sci. Co., ODS-1251-N, 4.6 × 250 mm and ODS-H-5251, 20 × 250 mm, respectively).

† Present address: Asahi Chemical Industry Co., 2–1, Samejima, Fuji, Shizuoka 416, Japan.

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Melting points were measured with a Yanaco micro melting point apparatus. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. Elemental analysis was done with a Yanaco MT-5 corder and a Kyoto Electronics AT-310J potentiometric automatic titrator. FAB-MS spectra were recorded on JEOL JMS-DX300 (positive) and JMS-AX505HA (positive and negative) mass spectrometers.

Taxonomic Studies

The type of diaminopimeric acid (DAP) was determined by the established method[11]. The International Streptomyces Project (ISP) recommended by SHIRLING and GOTTLIEB[12] and those by WAKSMAN[13] were used to investigate the cultural and physiological characteristics. Cultures were routinely observed after 2-week incubation at 27°C. Color names and hue numbers were determined according to the Color Harmony Manual[14]. The utilization of carbon sources was tested by growth on PRIDHAM and GOTTLIEB's medium containing 1% carbon at 27°C[15]. The morphological properties were observed with a scanning electron microscope (Hitachi, model S-430).

In Vitro Anticoccidial Activity

Anticoccidial activity using BHK-21 cells as a host was assayed according to the established method[2]. Primary chicken embryonic cells prepared by the established method were also used as a host instead of BHK-21 cells.

Antimicrobial Activity

Antimicrobial Activity was tested using paper disks (i.d. 6 mm, Toyo Roshi Co.). Bacteria were grown on Mueller-Hinton agar medium (Difco), and fungi and yeasts were grown on potato broth-agar medium. Antimicrobial activity was observed after 24-hour incubation at 37°C for bacteria and after 48-hour incubation at 27°C for fungi and yeasts.

Results

Taxonomy of the Producing Strain KO-8119

The strain KO-8119 was isolated from a soil sample collected in Nagoya, Aichi, Japan.

The vegetative mycelium grew abundantly on sucrose-nitrate agar, inorganic salts-starch agar, oatmeal agar, yeast extract-malt extract agar, and nutrient agar, and did not show fragmentation into coccoid forms or bacillary elements. The aerial mycelium grew abundantly on inorganic salts-starch agar. The spore chains were *Spirales* type and each had more than 20 spores per chain. The spores were cylindrical in shape, $0.8 \times 0.6\,\mu m$ in size and had a smooth surface (Fig. 1). Whirls, sclerotic granules, sporangia and flagellate spores were not observed.

Fig. 1. Scanning electron micrograph of spore chains of strain KO-8119 grown on inorganic salts-starch agar for 14 days.

Bar represents $1.0\,\mu m$.

The type of DAP isomers in the whole cell of strain KO-8119 were determined to be LL.

The cultural characteristics and the physiological properties are shown in Tables 1 and 2. The vegetative mycelia showed brown color on various media. The aerial mass color showed white to bluish gray color. Melanoid pigment and other soluble pigment were not produced.

The utilization of carbon sources is shown in Table 3. Interestingly, the strain utilized glycerol and

Table 1. Cultural characteristics of strain KO-8119.

Medium	Growth	Reverse color	Aerial mass color	Soluble pigment
Yeast extract malt extract agar[a]	Good, colorless	Bright gold (2nc)	Abundant, white~aqua gray (a~19fe)	None
Oatmeal agar[a]	Good, colorless	Biscuit~light mistletoe gray (2ec~241/2fe)	Abundant, white~aqua gray (a~19fe)	None
Inorganic salts starch agar[a]	Good, bamboo (2gc)	Bamboo (2gc)	Abundant, white~light gray (a~c)	None
Glycerol asparagine agar[a]	Moderate, bamboo (2gc)	Bamboo (2gc)	Poor, white~light gray (a~c)	None
Glucose asparagine agar[b]	Moderate, bamboo (2gc)	Bamboo (2gc)	Moderate, white ~light gray (a~c)	None
Peptone-yeast extract iron agar[b]	Moderate, mustard (2le)	Mustard (2le)	None	None
Tyrosine agar[a]	Moderate, light ivory	Light ivory (2ca)	Poor, white (a) (2ca)	None
Sucrose-nitrate agar[b]	Good, colorless	Colorless	Abundant, blue tint ~no name (15ba~15fe)	None
Glucose-nitrate agar[b]	None	None	None	None
Glycerol-calcium malate agar[b]	Moderate, biscuit (2ec)	Bamboo (2gc)	Moderate, white~aqua gray (a~19fe)	None
Glucose-peptone agar[b]	None	None	None	None
Nutrient agar[b]	Good, light ivory (2ca)	Light wheat (2ea)	Moderate, white~light gray (a~c)	None

[a] Medium recommended by International Streptomyces Project.
[b] Medium recommended by S. A. WAKSMAN.

Table 2. Physiological properties of strain KO-8119.

Melanin formation	−
Tyrosinase reaction	−
H_2S production	−
Liquefaction of gelatin (21~23°C)	+
Peptonization of milk (27°C)	+
Coagulation of milk (27°C)	−
Cellulolytic activity	−
Hydrolysis of starch	+
Nitrate reduction	+
Temperature range for growth	9~32°C

+, Active; −, not active.

Table 3. Utilization of carbon sources by strain KO-8119.

D-Glucose	−	D-Xylose	−
D-Fructose	−	Sucrose	−
L-Rhamnose	−	Melibiose	−
D-Mannitol	−	Starch	+
L-Arabinose	−	Dextrin	+
i-Inositol	−	Glycerol	+
Raffinose	−		

+, Utilized; −, not utilized.

polysaccharides such as starch and dextrin but did not utilize mono- and disaccharides.

Based on the taxonomic properties described above, strain KO-8119 is considered to belong to the genus *Streptomyces*. Among the known species of this genus, strain KO-8119 showed the closest resemblance to *S. amakusaensis* NAGATSU, ANSAI, OHKUMA and SUZUKI[16].

The taxonomic characteristics of strain KO-8119 were very similar to those of *S. amakusaensis* IFO-12835^T on the direct comparison except for the coagulation of milk and nitrate reduction. In the carbon utilization test, direct comparison of the type strain of *S. amakusaensis* with strain of KO-8119 gave the same data with description of SHIRLING and GOTTLIEB[17]. Therefore, strain KO-8119 was identified as a strain of *S. amakusaensis* and designated *S. amakusaensis* KO-8119. The strain was deposited in National Institute of

Bioscience and Human Technology, Japan, under the name *Streptomyces* sp. KO-8119 and the accession No. is FERMP-13398.

Production

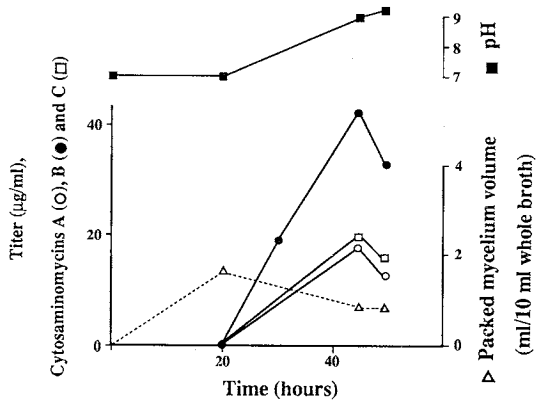
A slant culture of *S. amakusaensis* KO-8119 grown on an agar medium (soluble starch 1.0%, N-Z amine 0.3%, yeast extract 0.1%, meat extract 0.1%, $CaCO_3$ 0.3%, agar 1.0%, pH 7.0) was inoculated into 500-ml Erlenmeyer flasks containing 100 ml of seed medium (glucose 0.1%, soluble starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, pH 7.0). The flasks were shaken on a rotary shaker for 3 days at 27°C. Four hundred ml of the seed culture were transferred into 20 liters of the production medium (soluble starch 2.0%, dry yeast 1.0%, "Ebios" (Asahi Beer Co.) 2.0%, yeast extract 0.5%, peptone 0.3%, NaCl 0.3%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.4%, pH 7.0) in a 30-liter jar fermentor. The fermentation was carried out under the conditions as follows: aeration at 10 liters/minute, agitation at 250 rpm and temperature at 27°C.

A typical time course of cytosaminomycin production is shown in Fig. 2. The production of cytosaminomycins A, B, C and D was measured by analytical HPLC (22% CH_3CN in 0.02% H_3PO_4; 1.0 ml/minute; 255 nm). Cytosaminomycins A, B, C and D were eluted with retention times of 9.3, 13.0, 10.6 and 8.7 minutes, respectively. Under the same conditions, compound E (identified as oxyplicacetin, described below) structurally related to cytosaminomycins was eluted with a retention time of 5.8 minutes. The concentrations of cytosaminomycins reached a maximum at 45 hours.

Isolation

The supernatant of 48-hour cultured broth (9 liters) was extracted with 9 liters of ethyl acetate at pH 8.5. The extracts were evaporated *in vacuo* to dryness to yield a brown oil (5.03 g). After *n*-hexane precipitation, methanol soluble materials (2.27 g) were applied on a silica gel column (100 g). The column was washed with 500 ml of chloroform, and materials were eluted step-wise with chloroform-methanol solutions (98:2, 96:4, 94:6, 92:8, 90:10 and 0:100; each 500 ml). The fractions were collected into three pools; F-I, -II and -III. F-I (160 mg) recovered from 94:6 solution was enriched with cytosaminomycin D, F-II (362 mg) from 92:8 was enriched with cytosaminomycins A, B and C, and F-III (102 mg) from 90:10 was with compound E (oxyplicacetin). After ODS treatment (each fraction dissolved in 50% aq methanol was charged onto an ODS column, and materials were recovered by elution with 62.5% aq methanol), cytosaminomycins were finally purified by preparative HPLC (62.5% methanol in 1 mM phosphate buffer, pH 7.75; 8.0 ml/minute; 255 nm). Cytosaminomycin D was isolated from F-I fraction as a white powder (7.5 mg). From F-II fraction, cytosaminomycin A was obtained as a pale yellow powder (6.5 mg), cytosaminomycin B was obtained as pale yellow crystals (41.9 mg), and cytosaminomycin C was obtained as a white powder (9.5 mg). Compound E was also obtained from F-III as a pale yellow powder (8.4 mg).

Fig. 2. A typical time course of cytosaminomycin production in a 30-liter jar fermentor.



Physico-chemical Properties

The physico-chemical properties of cytosami-

Table 4. Physico-chemical properties of cytosaminomycins A, B, C and D.

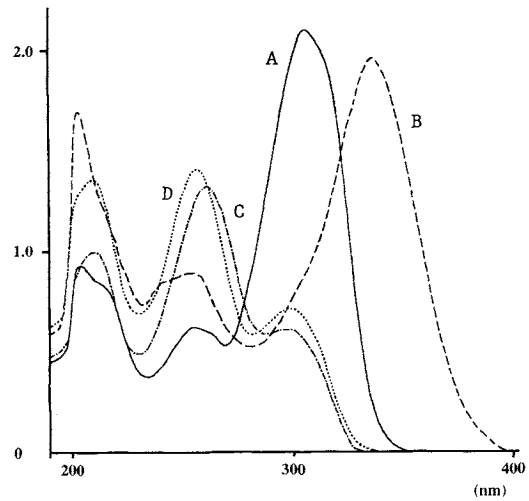
	Cytosaminomycin A	Cytosaminomycin B
Appearance	Pale yellow powder	Pale yellow crystal
Molecular formula	$C_{22}H_{34}N_4O_8S$	$C_{26}H_{37}N_5O_8$
Molecular weight		
FAB-MS	515 $(M+H)^+$, 537 $(M+Na)^+$, 513 $(M-H)^-$	548 $(M+H)^+$, 570 $(M+Na)^+$
HRFAB-MS (*m/z*)		
Found:	515.2190 $(M+H)^+$	548.2740 $(M+H)^+$
Calcd:	515.2176 (for $C_{22}H_{35}N_4O_8S$)	548.2720 (for $C_{26}H_{38}N_5O_8$)
$[\alpha]_D$ (*c* 0.1, MeOH)	+144° (24°C)	+122° (25°C)
UV λ_{max}^{MeOH} nm (ε)	204 (14,200), 215 (sh 12,100), 256 (9,300), 264 (sh 8,600), 309 (30,600), 318 (sh 29,000)	204 (23,000), 242 (sh 11,400), 255 (11,800), 339 (26,500)
IR ν_{max}^{KBr} cm^{-1}	3400, 2910, 1650, 1570, 1490, 1330, 1240, 1140, 1070, 1040	3400, 2930, 1660, 1600, 1560, 1480, 1330, 1250, 1180, 1070, 1040

	Cytosaminomycin C	Cytosaminomycin D
Appearance	White powder	White powder
Molecular formula	$C_{23}H_{36}N_4O_8$	$C_{23}H_{36}N_4O_8$
Molecular weight		
FAB-MS	497 $(M+H)^+$, 519 $(M+Na)^+$	497 $(M+H)^+$, 519 $(M+Na)^+$, 495 $(M-H)^+$
HRFAB-MS (*m/z*)		
Found:	497.2601 $(M+H)^+$	497.2611 $(M+H)^+$
Calcd:	497.2611 (for $C_{23}H_{37}N_4O_8$)	497.2611 (for $C_{23}H_{37}N_4O_8$)
$[\alpha]_D$ (*c* 0.1, MeOH)	+105° (25°C)	+144° (24°C)
UV λ_{max}^{MeOH} nm (ε)	210 (12,600), 262 (16,500), 300 (7,700)	201 (16,900), 258 (17,500), 300 (8,900)
IR ν_{max}^{KBr} cm^{-1}	3400, 2930, 1640, 1560, 1490, 1330, 1130, 1070, 1040	3400, 2910, 1640, 1560, 1480, 1380, 1330, 1250, 1070, 1040

nomycins are summarized in Table 4. The molecular formulas of cytosaminomycins A, B, C and D were determined to be $C_{22}H_{34}N_4O_8S$, $C_{26}H_{37}N_5O_8$, $C_{23}H_{36}N_4O_8$ and $C_{23}H_{36}N_4O_8$, respectively, on the basis of HRFAB-MS data. The same fragment ion peak (*m/z*) 304 was observed in the FAB-MS spectra of each compound, suggesting the presence of a common moiety in their structures. UV spectra of cytosaminomycins A, B, C and D are shown in Fig. 3.

Fig. 3. UV spectra of cytosaminomycins.

A: cytosaminomycin A, B: cytosaminomycin B, C: cytosaminomycin C, D: cytosaminomycin D.



These data indicated that cytosaminomycins are new compounds. However, compound E (molecular formula, $C_{25}H_{35}N_5O_8$; HRFAB-MS calcd 534.2564, found 534.2537 for $(M+H)^+$; UV λ_{max} nm (ε) 204 (25,100), 215 (sh 17,100), 240 (sh 11,700), 254 (12,800), 326 (27,700) in methanol) was identified as a known antibiotic, oxplicacetin[10]. The structure elucidation of cytosaminomycins will be described in the accompanying paper[18].

Table 5. Anticoccidial activity of cytosaminomycins in *in vitro* assays using chicken embryonic and BHK-21 cells.

Compound	Minimum effective concentration (μg/ml)			
	Chicken embryonic cells		BHK-21 cells	
	Anticoccidial activity[a]	Cytotoxicity[b]	Anticoccidial activity[a]	Cytotoxicity[b]
Cytosaminomycin A	0.31	10	0.16	0.31
Cytosaminomycin B	0.62	5	1.25	2.5
Cytosaminomycin C	0.62	5	1.25	5.0
Cytosaminomycin D	2.5	10	10	>10[c]
Oxyplicacetin	5.0	>10[c]	1.25	5.0
Monensin[d]	—	0.03	—	0.03

After infection of sporozoites and addition of drugs, primary chicken embryonic cells or BHK-21 cells were incubated at 41°C in an atmosphere of 2% CO_2-98% air. After three days, primary chicken embryonic cells or BHK-21 cells stained with hematoxylin solution were microscopically observed. In control experiment (no drug), infected sporozoites grew in the cells to form mature schizonts. [a] No schizont in the cells was observed at the indicated concentration of drugs. [b] No host cells was observed at the indicated concentration of drugs. [c] Cytotoxicity was not observed at 10 μg/ml of the drug. [d] Only cytotoxicity was observed.

Biological Properties

Anticoccidial Activity

The anticoccidial activity of cytosaminomycins was evaluated in the two *in vitro* assays using chicken embryonic and BHK-21 cells as host (Table 5). Comparison of the minimum effective concentrations causing inhibition of mature schizont formation in the two host cells revealed the same hierarchy of potency; cytosaminomycin A>B=C>D. Cytotoxicity of cytosaminomycins B, C and D to the two cells was analogous. However, cytosaminomycin A showed strong cytotoxicity to BHK-21 cells (0.31 μg/ml) but not so to ckicken embryonic cells (10 μg/ml). As a result, cytosaminomycin A showed the highest selectivity (minimum effective dose for cytotoxicity/minimum effective dose for anticoccidial activity) among these drugs in the assay using chicken embryonic cells as a host. The anticoccidial and cytotoxic activities of oxyplicacetin were similar to those of cytosaminomycins B, C and D in the two assays. However, monensin had no anticoccidial activity because monensin-resistant *E. tenella* was used as a protozoan in the assays.

Antimicrobial Activity

Antimicrobial activity of cytosaminomycins and oxyplicacetin was tested at a concentration of 5 μg/i.d. 6 mm paper disk. Cytosaminomycins A, B, C and D and oxyplicacetin showed antibacterial activity against *Micrococcus luteus* PCI1001 (diameter of inhibition zone: 20, 14, 21, 10 and 18 mm), *Escherichia coli* NIHJ (14, 7, 14, 0 and 12 mm), *Xanthomonas oryzae* (14, 0, 17, 0 and 16 mm), *Acholeplasma laidlawii* PG-8 (24, 14, 24, 11 and 17 mm) and *Bacteroides fragilis* ATCC 23745 (10, 0, 12, 0 and 0 mm), respectively. Thus, antibacterial activities of cytosaminomycins A and C and oxyplicacetin were similar and more potent than those of cytosaminomycins B and D. However, neither of them showed antifungal nor anti-yeast activity against *Candida albicans*, *Saccharomyces sake*, *Pyricularia oryzae*, *Aspergillus niger* and *Mucor racemosus* at the concentration tested.

Discussion

We have discovered four kinds of microbial anticoccidial agents in our *in vitro* assay system using

BHK-21 cells as a host. However, it seems that there has been a discrepancy between the *in vitro* and *in vivo* efficacies of the anticoccidial agents. Therefore, chicken embryonic cells were introduced into the assay instead of BHK-21 cells because chicken cells might be a more reasonable host to evaluate *in vitro* anticoccidial activity[9]. Consequently, we discovered cytosaminomysins as described in this paper. When the anticoccidial activities were compared in the two assays using the different host cells, analogous results were obtained (Table 2). Comparison of many compounds in the two assays also indicated that the anticoccidial activities were essentially similar (data not shown) but that cytotoxic effects of some compounds including cytosaminomycin A to chicken cells were less potent than those to BHK-21 cells. These data suggest that screening by these assays can yield similar compounds.

The *in vivo* testing of the cytosaminomycins is in progress.

Acknowledgments

We express our thanks to Mr. I. HASHIURA for excellent technical assistance and to Research Center for Biologicals, the Kitasato Institute, for routine supply of chicken embryonic cells.

References

1) CHAPMAN, H. D.: Anticoccidial drug resistance. *In* The Biology of the Coccidia, *Ed.*, LONG, P. L., pp. 429~452, University Park Press, 1983
2) TABATA, N.; Y. SUZUMURA, H. TOMODA, R. MASUMA, K. HANEDA, M. KISHI, Y. IWAI & S. ŌMURA: Xanthoquinodins, new anticoccidial agents produced by *Humicola* sp. Production, isolation and physico-chemical and biological properties. J. Antibiotics 46: 749~755, 1993
3) TABATA, N.; H. TOMODA, K. MATSUZAKI & S. ŌMURA: Structure and biosynthesis of xanthoquinodins, new anticoccidial agents. J. Am. Chem. Soc. 115: 8558~8564, 1993
4) TABATA, N.; H. TOMODA, Y. TAKAHASHI, K. HANEDA, Y. IWAI, H. B. WOODRUFF & S. ŌMURA: Diolmycins, new anticoccidial agents produced by *Streptomyces* sp. I. Production, isolation and physico-chemical and biological properties. J. Antibiotics 46: 756~761, 1993
5) TABATA, N.; T. SUNAZUKA, H. TOMODA, T. NAGAMITSU, Y. IWAI & S. ŌMURA: Diolmycins, new anticoccidial agents produced by *Streptomyces* sp. II. Structure elucidation of diolmycins A1, A2, B1 and B2, and synthesis of diolmycin A1. J. Antibiotics 46: 762~769, 1993
6) TABATA, N.; H. TOMODA, R. MASUMA, K. HANEDA, Y. IWAI & S. ŌMURA: Hynapenes A, B and C, new anticoccidial agents produced by *Penicillium* sp. I. Production, isolation and physico-chemical and biological properties. J. Antibiotics 46: 1849~1853, 1993
7) TABATA, N.; H. TOMODA, Y. IWAI & S. ŌMURA: Hynapenes A, B and C, new anticoccidial agents produced by *Penicillium* sp. II. Structure elucidation. J. Antibiotics 46: 1854~1858, 1993
8) MASUMA, R.; N. TABATA, H. TOMODA, K. HANEDA, Y. IWAI & S. ŌMURA: Arohynapenes A and B, new anticoccidial agents produced by *Penicillium* sp. Taxonomy, fermentation, and structure elucidation. J. Antibiotics 47: 46~53, 1994
9) HOFMANN, J. & W. Raether: Improved techniques for the *in vitro* cultivation of *Eimeria tenella* in primary chick kidney cell. Parsit. Res. 76: 479~486, 1990
10) YONG-LE, C.; A. ZEECK, C. CHENG-XIANG & H. ZAHNER: Studies on metabolites produced by *Streptomyces ramuloses* Tue-34. II The structural elucidation of oxyplicacetin, a new member of amicetin group. Kangshengsu 10: 285~295, 1985
11) TAKAHASHI, Y.; Y. IWAI, H. TOMODA, N. NIMURA, T. KINOSHITA & S. ŌMURA: Optical resolution of 2,6-diaminopimelic acid stereoisomer by high performance liquid chromatography for the chemotaxonomy of actinomycetes strains. J. Gen. Appl. Microbiol. 35: 27~32, 1989
12) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340. 1966
13) WAKSMAN, S. A. (*Ed.*): Classification, Identification and Description of Genera and Species. *In* The Actinomycetes. Vol. 2, Williams & Wilkins Co., 1961
14) Container Corporation of America: Color Harmony Manual, 4th. *Ed.* Chicago, 1958
15) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56: 107~114, 1988
16) NAGATSU, J.; K. ANSAI, K. OHKUMA & S. SUZUKI: Studies on a new antibiotic, tuberin. IV. Taxonomic studies on the tuberin producing organism, *Streptomyces amakusaensis*. J. Antibiotics Ser. B 16: 207~210, 1963

17) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. III. Additional species description from first and second studies. Int. J. Syst. Bacteriol. 18: 290～291, 1968

18) SHIOMI, K.; K. HANEDA, H. TOMODA, Y. IWAI & S. ŌMURA: Cytosaminomycins, new anticoccidial agents produced by *Streptomyces* sp. KO-8119. II. Structure elucidation of cytosaminomycins A, B, C and D. J. Antibiotics 47: 782～786, 1994