PC-766B, A NEW MACROLIDE ANTIBIOTIC PRODUCED BY *Nocardia brasiliensis*

I. TAXONOMY, FERMENTATION AND BIOLOGICAL ACTIVITY

KAZUO KUMAGAI*,††, KIYOSHI TAYA, AKIO FUKUI, MASATOMO FUKASAWA†, MASARU FUKUI† and SHIGEYASU NABESHIMA

Biotechnology Laboratory, Takarazuka Research Center,
Sumitomo Chemical Co., Ltd.,
Takatsukasa, Takarazuka, Hyogo 665, Japan
†Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd.,
Kasugadenaka, Konohana-ku, Osaka 554, Japan

(Received for publication November 20, 1992)

An actinomycete strain SC-4710, a new soil isolate, was found to produce a new macrolide antibiotic, PC-766B. Chemotaxonomic analysis of the producing organism revealed that the cells of SC-4710 had type IV cell wall, type A whole cell sugar pattern, type PII phospholipids, menaquinone MK-8(H_4), cellular fatty acids comprising straight-chain saturated, unsaturated and tuberculostearic acids, and mycolic acids. The strain was identified as *Nocardia brasiliensis* (Lindenberg) Pinoy. The antibiotic, PC-766B, was active against Gram-positive bacteria, and some fungi and yeasts, but inactive against Gram-negative bacteria. It also showed antitumor activity against murine tumor cells *in vitro* and *in vivo*, and a weak inhibitory activity against Na^+, K^+-ATPase *in vitro*.

In the course of our screening program for new antibiotics, we isolated a new 16-membered macrolide antibiotic from the culture broth of an actinomycete strain named SC-4710[1]. In this paper, we describe the taxonomy of the producing organism, fermentation and biological activity of the antibiotic, PC-766B. The isolation, physico-chemical properties and structure elucidation of the antibiotic will be reported in a separate paper[2].

Materials and Methods

Producing Organism

The strain SC-4710 was isolated from a soil sample collected in Konan-cho, Shiga Prefecture, Japan. The strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan under the assession number FERM P-8233.

Morphological, Cultural and Physiological Characteristics

The morphological observations of strain SC-4710 were made using phase-contrast and scanning electron microscopes after grown on various agar media at 27°C for 14 days. Cultural characteristics were determined according to the methods of SHIRLING and GOTTLIEB[3], and WAKSMAN[4]. Color assignments were made by using the Color Tone Manual[5]. Physiological characteristics were determined by the method of GOODFELLOW[6].

Chemotaxonomic Analyses

The cells of strain SC-4710 grown in shake culture at 27°C for 3 days in GY medium (glucose 1%, yeast extract 1%, pH 7.2) were harvested by centrifugation and washed with distilled water. Diaminopimelic

†† Present address: Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd., Takatsukasa, Takarazuka, Hyogo 665, Japan.

acid type and whole-cell sugar patterns were determined by the methods of BECKER *et al.*[7], and LECHEVALIER and LECHEVALIER[8], respectively. Whole-cell phospholipid type was determined by the method of LECHEVALIER *et al.*[9]. Cellular fatty acid methyl esters were prepared from lyophilized cells and anlyzed by the method of SUZUKI and KOMAGATA[10]. Mycolic acid methyl esters were prepared and analyzed by the method of KROPPENSTEDT[11]. Isoprenoid quinones were analyzed by the method of TAMAOKA *et al.*[12].

HPLC Assay of PC-766B

The culture broth (10 ml) of strain SC-4710 was centrifuged at 4,000 rpm for 10 minutes. The supernatant was extracted with 10 ml of ethyl acetate and the cell cake was extracted with 5 ml of acetone. Both extracts were evaporated to dryness under reduced pressure, and the residues were dissolved in 1 ml of isopropanol. Samples (10 μl) were analyzed by reversed-phase HPLC using a Waters μBondapak C_{18} column (3.9 × 250 mm) with methanol-water (80:20) as a mobile phase at a flow rate of 1 ml/minute, and detection by UV absorption at 254 nm. The retention time of PC-766B was 16.0 minutes under these conditions.

Determination of Phosphorus Content

Phosphorus contents in medium ingredients were determined by the molybdenum blue colorimetry using a commercial kit (Wako Pure Chemical Industries).

Antimicrobial Activity

The antimicrobial activity of PC-766B was determined by the agar dilution method. Mueller-Hinton agar (Nissui Pharmaceuticals) was used as the assay medium for bacteria, and SABOURAUD's agar for fungi and yeasts. Bacteria were cultivated at 37°C, while fungi and yeasts at 30°C.

Cells and Cell Culture

Murine cell lines P388 leukemia and B16 melanoma were used in this study. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 10 μM 2-mercaptoethanol (P388 leukemia) or with 10% FBS only (B16 melanoma) in a 5% CO_2 incubator at 37°C. For cytotoxicity assay, cells (1×10^5 cells/ml) were cultured with various concentrations of test compounds for 3 days and viability was determined by the trypan blue dye exclusion method.

Animal Experiments

Five male CDF_1 mice of 6 weeks old were used for each group as test animals and P388 leukemia was inoculated intraperitoneally at 1×10^6 cells per mouse. Test compounds were dissolved in sterilized saline containing 0.2% Tween 80 and administered intraperitoneally on days 1 and 5 after tumor implantation. 5-fluorouracil (5-FU) was used as a control compound.

Na^+,K^+-ATPase Inhibitory Activity

Assay of Na^+,K^+-ATPase inhibitory activity was performed by the method of HUANG *et al.*[13]. Na^+,K^+-ATPase [EC 3.6.1.3] of porcine cerebral cortex origin, oligomycin (a mixture of oligomycins A (59%), B (21%) and C (21%)) and ouabain were purchased from Sigma. Test compounds were dissolved in dimethyl sulfoxide, and then they were diluted into the assay mixture.

Results

Taxonomy

The strain SC-4710 produced well-developed and branched aerial mycelia on some agar media. The spore chains formed on the aerial mycelia contained 3 to 10 spores per chain. The mature spores were cylindrical and measured 0.3 ~ 0.4 × 0.8 ~ 1.2 μm, with a smooth surface. No endospores, sporangia, sclerotia or synnemata were formed. No fragmentation of substrate mycelia was observed in agar media, but marked fragmentation into non-motile coccoid elements was observed in liquid cultures.

Table 1. Cultural characteristics of strain SC-4710.

Medium	Growth	Aerial mycelium	Reverse side color	Soluble pigment
Yeast extract-malt extract agar (ISP-2)	Good	Abundant, white	Yellowish brown	None
Oatmeal agar (ISP-3)	Poor	Poor, grayish white	Pale reddish yellow	None
Inorganic salts-starch agar (ISP-4)	Poor	Poor, white	Dull reddish yellow	None
Glycerol-asparagine agar (ISP-5)	Moderate	Moderate, brownish white	Dull reddish yellow	None
Peptone-yeast extract-iron agar (ISP-6)	Poor	None	Dull reddish yellow	None
Tyrosine agar (ISP-7)	Moderate	Moderate, yellowish white	Dull reddish yellow	None
Nutrient agar	Poor	Poor, pale beige	Dull yellow	None
Sucrose-nitrate agar	Poor	Poor, grayish white	Grayish yellow	None
Glucose-asparagine agar	Good	Moderate, pale beige	Dull reddish yellow	None
Bennett agar	Moderate	Abundant, white	Dull reddish yellow	None

Table 2. Chemotaxonomic characteristics of strain SC-4710.

Cell wall type	IV (*meso*-diaminopimelic acid, arabinose, galactose)
Phospholipid type[a]	PII (DPG, PE, PI, PIDM)
Cellular fatty acids (%)[b]	Saturated acids: 12:0 (1.8), 14:0 (tr), 16:0 (29.7), 17:0 (1.3), 18:0 (5.0)
	Unsaturated acids: 16:1 (17.8), 18:1 (12.0)
	10-methyl acids: 10-Me-16:0 (2.2), 10-Me-18:0 (9.2)
	Cyclopropane acids: cyclo-17:0 (4.3), cyclo-19:0 (tr)
	Hydroxy acids: 2-OH-14:0 (6.3), 3-OH-14:0 (4.8)
Mycolic acids	Present
Menaquinone	MK-8(H_4)

[a] Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannoside.
[b] % of total fatty acids. tr, trace amount (less than 1%).

The cultural characteristics of strain SC-4710 on various agar media are shown in Table 1. The color of the aerial mycelia was white to pinkish or grayish white, while that of the reverse side of colony was reddish to brownish yellow. No water-soluble pigment was produced in any medium.

The chemotaxonomic characteristics of strain SC-4710 are shown in Table 2. The whole-cell hydrolysate contained *meso*-diaminopimelic acid, arabinose and galactose but not glucose, rhamnose, madurose or xylose. These results suggest that the cell wall chemotype of strain SC-4710 is type IV of LECHEVALIER and LECHEVALIER[8]. Analysis of whole-cell phospholipids revealed the presence of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol dimannoside but not phosphatidylcholine (phospholipid type PII). The major components of the cellular fatty acids were *n*-$C_{16:0}$, *n*-$C_{16:1}$ and *n*-$C_{18:1}$. 10-methyl-$C_{18:0}$ (tuberculostearic acid) was also detected as a major component. *Iso*- and *anteiso*-branched acids were not detected. Mycolic acid methyl esters were detected on TLC developed with *n*-hexane-diethyl ether (85:15), having the same Rf as the authentic sample prepared from *Nocardia asteroides* JCM 3384^T. The predominant isoprenoid quinone was tetrahydrogenated menaquinone with eight isoprene units (MK-8(H_4)). These results indicate that strain SC-4710 belongs to the genus *Nocardia*.

Next we examined 27 physiological characteristics including 19 GOODFELLOW tests[14] to identify the species of strain SC-4710. The strain was obligately aerobic and able to grow at 17 to 37°C with optimal growth at 27 to 32°C. It decomposed casein, elastin, hypoxanthine, testosterone, tyrosine and urea but

Table 3. Comparison of physiological characteristics of strain SC-4710 with those of *Nocardia brasiliensis*.

Characteristics	SC-4710	*N. brasiliensis*	Characteristics	SC-4710	*N. brasiliensis*
Decomposition of:			Growth on sole carbon source (1% w/v)		
Casein	+	+	Adonitol	−	−
Elastin	+	+	L-Arabinose	−	−
Hypoxanthine	+	+	D-Galactose	+	+
Testosterone	+	+	Inositol	+	+
Tyrosine	+	+	D-Mannitol	+	+
Xanthine	−	−	D-Mannose	+	+
Production of:			Melezitose	−	−
Nitrate reductase	+	+	L-Rhamnose	−	−
Urease	+	+	Adipic acid (0.1% w/v)	−	−
			Pimelic acid (0.1% w/v)	−	−
			Sebacic acid (0.1% w/v)	+	−

not xanthine. Hydrogen sulfide production was detected by stab culturing in triple sugar iron agar. Nitrate was reduced to nitrite. Milk was coagulated and peptonized. Starch was not hydrolyzed. Gelatin was not liquefied. The following compounds were utilized as sole carbon sources: D-fructose, D-galactose, D-glucose, inositol, D-mannitol, D-mannose and sebacic acid. The following compounds were not utilized as sole carbon sources: adonitol, L-arabinose, melezitose, raffinose, L-rhamnose, sucrose, D-xylose, adipic acid and pimelic acid. These characteristics are very similar to those of *Nocardia brasiliensis* (Table 3). Thus the strain SC-4710 was identified as *N. brasiliensis* (Lindenberg) Pinoy.

Fermentation

A stock culture of strain SC-4710 on Bennett agar slant was inoculated into a test tube (24 × 200 mm) containing 10 ml of seed medium consisted of glucose 2.5%, soy bean flour 1.5%, yeast extract 0.2% and $CaCO_3$ 0.4%, pH 7.2, and cultured at 27°C with reciprocal shaking at 300 rpm for 6 days. This culture was transferred into a 2-liter Sakaguchi flask containing 400 ml of the same medium, and cultured at 27°C with reciprocal shaking at 130 rpm for 6 days. This seed culture was then inoculated into a 30-liter jar fermentor containing 15 liters of production medium consisted of glycerol 5%, soy bean flour 3%, $CaCO_3$ 0.4% and Silicone KS-66 (Shin-Etsu Chemical) 0.05%, pH 7.2. The fermentation was carried out at 27°C with an air flow of 7.5 liters per minute and 150 rpm agitation. The typical time course of the fermentation is shown in Fig. 1. A maximum titer of 440 μg/ml of PC-766B was achieved at day 10 after inoculation. Approximately 95% of the antibiotic was found in the cell fraction.

Effects of Phosphate and Ammonium on PC-766B Production

Phosphate and ammonium are known to regulate secondary metabolism of some actinomycetes. The effects of two inorganic salts, KH_2PO_4 and $(NH_4)_2SO_4$, on PC-766B production were studied using 30-liter jar fermentors. Addition of 1 mM KH_2PO_4 to the production medium decreased PC-766B productivity to 44% of control (Table 4). On the contrary $(NH_4)_2SO_4$ did not inhibit PC-766B production up to 10 mM. The inhibition of PC-766B production at higher concentrations of $(NH_4)_2SO_4$ appears to be due to the inhibition of cell growth by the ammonium salt. These results suggest that PC-766B production by strain SC-4710 is regulated by the free phosphate level in the production medium.

Antimicrobial Activity

The antimicrobial spectrum of PC-766B is shown in Table 5. The antibiotic was active against Gram-positive bacteria, and some fungi and yeasts, but inactive against Gram-negative bacteria.

Fig. 1. Time course of PC-766B production.

PC-766B in cells (■), in culture fluid (●), wet cell mass (□) and pH (○).

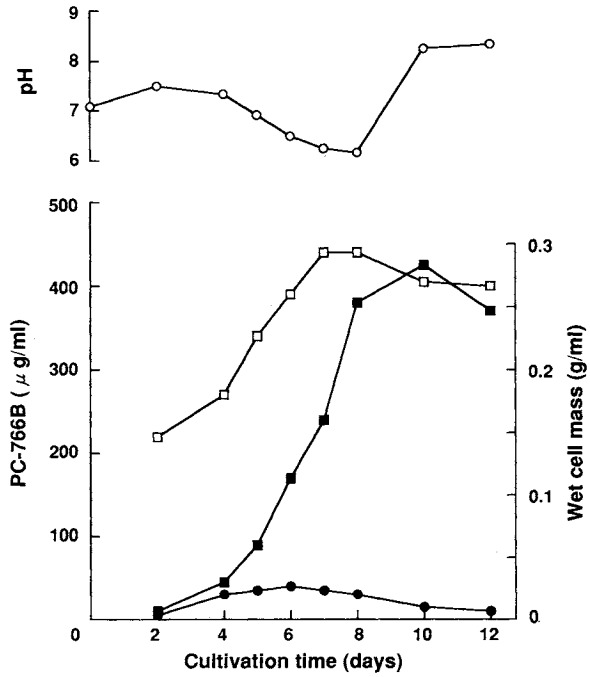

Table 4. Effect of KH_2PO_4 and $(NH_4)_2SO_4$ on PC-766B production[a].

Inorganic salt added (mM)		PC-766B (%)	Wet cell mass (%)
None		100[b]	100[c]
KH_2PO_4	0.1	69	103
	1	44	94
	10	33	86
	50	14	68
$(NH_4)_2SO_4$	0.1	98	110
	1	92	106
	10	97	117
	50	61	63

[a] Fermentation was carried out at 27°C for 10 days. Basal medium: glycerol 5%, soy bean flour 3%, $CaCO_3$ 0.4%, Silicone KS-66 0.05%, pH 7.2.
[b] 441 μg/ml.
[c] 270 mg/ml.

Table 5. Antimicrobial activity of PC-766B.

Organism	MIC (μg/ml)
Staphylococcus aureus 209 P	12.5
S. epidermidis IAM 1296	6.25
Micrococcus luteus ATCC 9341	1.56
Streptococcus pyogenes Cook	3.13
Bacillus subtilis ATCC 6633	6.25
Escherichia coli NIHJ JC-2	>100
Klebsiella pneumoniae ATCC 10031	>100
Proteus mirabilis GN 2425	>100
Pseudomonas aeruginosa T	>100
Serratia marcescens X100	>100
Candida albicans MTU 12001	>100
Cryptococcus neoformans MTU 13001	12.5
Trichophyton mentagrophytes MTU 19001	>100
Aspergillus niger MTU 16001	>100
Fulvia fulva IFO 9759	25.0

Antitumor Activity

PC-766B exhibited very potent cytotoxicity against murine tumor cell lines *in vitro*. The IC_{50} values against P388 leukemia and B16 melanoma were 0.1 and 0.5 ng/ml, respectively. The antibiotic also showed *in vivo* antitumor activity against P388 leukemia (Table 6). The T/C value was 125% when the antibiotic was administered intraperitoneally at days 1 and 5 at a dose of 0.25 mg/kg/day.

Table 6. Antitumor activity of PC-766B and 5-fluorouracil (5-FU) against murine P388 leukemia *in vivo*.

Compound	Dose[a] (mg/kg/day)	MST[b] (days)	T/C (%)
PC-766B	0.25	14.3	125
	1.0	2.1	19
			(toxic)
5-FU	30	15.4	135
	60	17.8	156

[a] Days at 1 and 5, ip.
[b] Median survival time.

Table 7. Na^+,K^+-ATPase inhibitory activity of PC-766B and known inhibitors of the enzyme.

Compound	IC_{50} (μM)
PC-766B	64
L-681,110 A_1	13 ± 1.4[a]
Oligomycin	3.1
Ouabain	0.92

[a] Data from reference 13.

Na^+,K^+-ATPase Inhibitory Activity

It has been reported that L-681,110 A_1 (=bafilomycin C_1[15]), which is structurally related to PC-766B, is an inhibitor of Na^+,K^+-ATPase[13]. Table 7 shows the comparison of the Na^+,K^+-ATPase inhibitory activity of PC-766B with that of known inhibitors of the Na^+,K^+-ATPase. PC-766B was one fifth as potent as L-681,110 A_1, and one twentieth and one seventieth as potent as oligomycin and ouabain, respectively, in inhibiting the enzyme.

Discussion

The chemotaxonomic analysis of strain SC-4710 revealed that the producing organism had type IV cell wall, type PII phospholipids, straight chain fatty acids as the predominant cellular fatty acids, menaquinone MK-8(H_4) and mycolic acids (Table 2). These results correspond to the characteristics of typical strains of "true" *Nocardia*[14]. In physiological tests, strain SC-4710 was found to be closely related to *N. brasiliensis*. The only difference was the ability to utilize sebacic acid as a sole carbon source (Table 3). Thus strain SC-4710 was considered a strain of *N. brasiliensis*. PC-766B is a new class of 16-membered macrolide antibiotics and belongs to the hygrolidin family[2]. All of the hygrolidin family antibiotics reported so far (hygrolidins[16], L-681,110[13], bafilomycins[15], leucanicidin[17], L-155,175[18], PD 118,576[19], HS-6[20]), to our knowledge, have been isolated from *Streptomyces* spp. except for setamycin (=bafilomycin B_1)[21] and HS-6, which have been isolated from *Kitasatosporia setae* and *Nocardia otitidiscaviarum*, respectively. The isolation of PC-766B from a *Nocardia* strain SC-4710 was first reported by the authors in 1987[1], which precedes the report of the isolation of HS-6[20]. Therefore PC-766B is the first example of this family isolated from a strain of *Nocardia*.

Antibiotics belonging to this family are known to have a broad spectrum of activity such as antimicrobial activity against Gram-positive bacteria and fungi, antiparasitic activity, insecticidal activity and antitumor activity. Among antibiotics of this family, L-681,110 was first reported to be an inhibitor of Na^+,K^+-ATPase[13]. PC-766B also had an inhibitory activity against the Na^+,K^+-ATPase (Table 7), but its IC_{50} value was one to three orders of magnitude higher than the MIC values against susceptible microorganisms (Table 5) and *in vitro* IC_{50} values against murine tumor cells. Recently, bafilomycin A_1 was found to be a specific inhibitor of vacuolar-type H^+-ATPase[22]. Therefore, further study on the activity of PC-766B against several types of ATPase will be required to understand the real mechanism(s) of action of PC-766B and the structure-activity relationship of these 16-membered macrolides.

PC-766B production by strain SC-4710 was inhibited by the exogenously supplied inorganic phosphate (Table 4). This result is consistent with an observation that the antibiotic production by the strain was repressed by yeast extract added to the production medium at 0.1~0.2% (data not shown). The yeast extract contained 1.6% (w/w) phosphorus, while the soy bean flour we used contained only 0.08% (w/w) phosphorus. Thus soy bean flour seems to be a suitable nitrogen source for PC-766B production.

References

1) KUMAGAI, K.; K. TAYA, S. TANAKA, K. MORIGUCHI, M. FUKASAWA & A. FUKUI (Sumitomo Chemical Co., Ltd.; Sumitomo Pharmaceuticals Co., Ltd.): Novel antibiotic PC-766B manufacture with *Nocardia*. Jpn. Kokai 5990 ('87), Jan. 12, 1987
2) KUMAGAI, K.; A. FUKUI, S. TANAKA, M. IKEMOTO, K. MORIGUCHI & S. NABESHIMA: PC-766B, a new macrolide antibiotic produced by *Nocardia brasiliensis*. II. Isolation, physico-chemical properties and structure elucidation. J. Antibiotics 46 (7): 1993, in press
3) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
4) WAKSMAN, S. A. (*Ed.*): The Actinomycetes. Vol. II. Classification, Identification and Description of Genera and Species. Williams & Wilkins Co., Baltimore, 1961
5) Nippon Shikisai Kenkyusho (*Ed.*): Color Tone Manual. Nippon Shikiken Jigyo Co., Tokyo, 1973
6) GOODFELLOW, M.: Numerical taxonomy of some nocardioform bacteria. J. Gen. Microbiol. 69: 33~80, 1971
7) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421~423, 1964
8) LECHEVALIER, M. P. & H. A. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
9) LECHEVALIER, M. P.; C. DEBIEVRE & H. A. LECHEVALIER: Chemotaxonomy of aerobic actinomycetes. Phospholipid composition. Biochem. Syst. Ecol. 5: 249~260, 1977
10) SUZUKI, K. & K. KOMAGATA: Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. Int. J. Syst. Bacteriol. 33: 188~200, 1983
11) KROPPENSTEDT, R. M.: Fatty acid and menaquinone analysis of actinomycetes and related organisms. *In* Chemical Methods in Bacterial Systematics. *Ed.*, M. GOODFELLOW & D. E. MINNIKIN, pp. 173~199, Academic Press, London, 1985
12) TAMAOKA, J.; Y. KATAYAMA-FUJIMURA & H. KURAISHI: Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J. Appl. Bacteriol. 54: 31~36, 1983
13) HUANG, L.; G. ALBERS-SCHONBERG, R. L. MONAGHAN, K. JAKUBAS, S. S. PONG, O. D. HENSENS, R. W. BURG, D. A. OSTLIND, J. CONROY & E. O. STAPLEY: Discovery, production and purification of the Na^+, K^+ activated ATPase inhibitor, L-681,110 from the fermentation broth of *Streptomyces* sp. MA-5038. J. Antibiotics 37: 970~975, 1984
14) GOODFELLOW, M. & M. P. LECHEVALIER: Genus *Nocardia* Trevisan 1989, 9^{AL}. *In* BERGEY's Manual of Systematic Bacteriology. Vol. 4. *Ed.*, S. T. WILLIAMS *et al.*, pp. 2350~2361, Williams & Wilkins Co., Baltimore, 1989
15) WERNER, G.; H. HAGENMAIER, H. DRAUTZ, A. BAUMGARTNER & H. ZÄHNER: Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. J. Antibiotics 37: 110~117, 1984
16) SETO, H.; H. AKAO, K. FURIHATA & N. ŌTAKE: The structure of a new antibiotic, hygrolidin. Tetrahedron Lett. 23: 2667~2670, 1982
17) ISOGAI, A.; S. SAKUDA, S. MATSUMOTO, M. OGURA, K. FURIHATA, H. SETO & A. SUZUKI: The structure of leucanicidin, a novel insecticidal macrolide produced by *Streptomyces halstedii*. Agric. Biol. Chem. 48: 1379~1381, 1984
18) GOETZ, M. A.; P. A. MCCORMICK, R. L. MONAGHAN, D. A. OSTLIND, O. D. HENSENS, J. M. LIESCH & G. ALBERS-SCHONBERG: L-155,175: A new antiparasitic macrolide. Fermentation, isolation and structure. J. Antibiotics 38: 161~168, 1985
19) WILTON, J. H.; G. C. HOKANSON & J. C. FRENCH: PD 118,576: A new antitumor macrolide antibiotic. J. Antibiotics 38: 1449~1452, 1985
20) MIKAMI, Y.; S. F. YU, K. YAZAWA, K. FUKUSHIMA, A. MAEDA, J. UNO, K. TERAO, N. SAITO, A. KUBO & K. SUZUKI: A toxic substance produced by *Nocardia otitidiscaviarum* isolated from cutaneous nocardiosis. Mycopathologia 112: 113~118, 1990
21) OTOGURO, K.; A. NAKAGAWA & S. ŌMURA: Setamycin, a 16-membered macrolide antibiotic. Identification and nematocidal activity. J. Antibiotics 41: 250~252, 1988
22) BOWMAN, E. J.; A. SIEBERS & K. ALTENDORF: Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. U.S.A. 85: 7972~7976, 1988