Screening of Antianaerobic Antibiotics and Anticoccidial Antibiotics from Microorganisms

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1. INTRODUCTION

The discovery of Penicillin has contributed not only to public health as a medicine but also to the development of life science. It promoted progressive research in microbiology, screening of microbial products, fermentation, biochemistry and synthetic organic chemistry, etc. As this suggests, it is considered that discovery of new substances is the origin of creative research.

When we started screening in 1970's, it was often discussed that the discovery of new antibiotics has reached an end. However, it was actually a new approach that was needed. Factors necessary for discovery of microbial products are listed in Table 1. These factors closely interact with each other and their appropriate balance is essential to the discovery of new compounds. Among all factors, assay system is the most important.

Therefore, we established new assay systems and

Table 1. The points of screening for new bioactive compounds from microorganisms.

- (1) Sources: actinomycetes, fungi, bacteria, etc.
- (2) Strain improvement
- (3) Culture conditions: constituents of media, pH, temperature, oxygen, etc.
- (4) Assay: *in vitro* (agar plate, enzyme, receptor, cell, etc.), *in vivo*
- (5) Isolation and Structure determination
- (6) Chemical modification and Organic synthesis

Table 2. List of new bioactive compounds from microorganisms.

- (1) Penicillinase inhibitor: KA-107¹⁾
- (2) Microbial alkaloids: Dityromycin²⁾, 1,3-Diphenethylurea³⁾, Staurosporine⁴⁾, Neoxaline⁵⁾, Herquiline⁶⁾
- (3) Herbicidal compounds: Ara-A⁷⁾, Herbimycins⁸⁾, Karabemycin⁹⁾
- (4) Antiviral antibiotics: Virantomycin¹⁰⁾, Virustomycin¹¹⁾
- (5) Antimycoplasmal antibiotics: 2'-Amino-2'-deoxyadenosine¹²⁾, Cervinomycins¹³⁾, Deoxyfrenolicin¹⁴⁾, Frenolicin B¹⁴⁾, OM-173¹⁵⁾
- (6) Antianaerobic antibiotics: Clostomicins¹⁶⁾, Luminamicin¹⁷⁾, Lustromycin¹⁸⁾, Thiotetromycin¹⁹⁾
- (7) Anticoccidial antibiotics: Cytosaminomycins²⁰⁾, Diolmycins²¹⁾, Frenolicin B²²⁾, Arohynapenes²³⁾, Fudecalone²⁴⁾, Hynapenes²⁵⁾, Xanthoquinodins²⁶⁾

*This antibiotic was isolated during the course of screening for (5) antimycoplasmal antibiotics.

screened new bioactive compounds produced by actinomycetes as main source. From this approach conducted on seven items, we found many kinds of new compounds with various structures and activities as shown in Table 2.

The screening of microbial alkaloids^{27,28)}, antiviral antibiotics²⁹⁾ and antimycoplasmal antibiotics³⁰⁾ in our institute has been reported. This review focuses on screening of antianaerobic and anticoccidial antibiotics.

2. SCREENING OF NEW ANAEROBIC ANTIBIOTICS

2-1. Background

In the course of screening for growth promoting compounds in animal, we picked up substances against *Clostridium perfringens* ATCC 3624, a kind of anaerobic bacteria. This led to the discovery of aurantin³¹⁾ with polyketide structure which is produced by *Bacillus aurantinus* KM-214. At that time, anaerobic bacteria such as *Bacteroides fragilis* and *Fusobacterium varium* were clinically problematic causing opportunistic infections, and *Clostridium difficile* causing pseudomembranous colitis and antibiotic-associated diarrhea.

This is how we started screening of antianaerobic antibiotics as a measure for preventing bacterial infections.

2-2. Assay

Since a simple and quick assay system for samples of broths, crude materials, partially purified materials, etc. is essential for the antibiotic screening, we employed a paper-disc agar diffusion method.

(1) Anaerobic culture

In those days, apparatus for anaerobic culture was not in common use and we employed the following two methods. We initially used Steel-wool method as shown in Fig. 1. Though the cost was low, the procedure was complicated and it was impossible to assay many agar plates at one time. Because of these disadvantages, we switched to BBL anaerobic system when it became easily available in the market.

(2) Assay for anti-*Clostridium difficile* compounds

As the name suggests, *Clostridium difficile* is difficult to culture. This bacterium is extremely sensitive to oxygen and is culturable only under strictly anaerobic condition. We studied various assay systems by a plate method and devised a Reverse layer method as shown in Fig. 2, which

Fig. 1. Steel-wool method, an assay procedure for antibiotics active against *Clostridium perfringens*.

Reverse phase double layer:

Fig. 2. Reverse layer method, an assay procedure for antibiotics active against *Clostridium difficile*.

has a basal layer and a seed layer in reverse order from ordinary one for screening³²⁾.

2-3. Screening

Table 3 shows four novel compounds and many kinds of known antibiotics isolated from screening with 14,003 actinomycete strains as a source using *Bacteroides fragilis*

ATCC23745, *Fusobacterium varium* ATCC 8501 and *Clostridium difficile* ATCC 9689 as test organisms.

Fig. 3 shows the structures of three new antianaerobic antibiotics, clostomicins, lunimamicin and thiotetromycin. The structural study of lustromycin is in progress.

(1) Clostomicins A, B₂, C and D¹⁶⁾

Clostomicins were isolated from the culture broth of an actinomycete strain, KMR-593, a soil isolate which was identified as a new subspecies of the genus *Micromonospora* and designated *Micromonospora echinospora* subsp. *armeniace* subsp. nov.³³⁾. The antibiotics were found to have five components, A, B₁, B₂, C and D, which are structurally related compounds with two chlorine atoms. Clostomicins A, B₂, C and D are new antibiotics and B₁ is identical with lipiamycin³⁴⁾. The structural difference of each component was determined by NMR analysis. Clostomicins exhibited potent antibacterial activities against Gram-positive bacteria especially against *Clostridium* sp., but showed less activities against Gram-negative bacteria.

(2) Luminamicin¹⁷⁾

Luminamicin was isolated from the culture broth of an actinomycete strain OMR-59, a soil isolate which is provisionally classified in the genus *Streptomyces*, compared with genus *Nocardioide*s³⁵⁾. The antibiotic showed no ac-

Table 3. Screening for antianaerobic antibiotics produced by actinomycete strains.

Screening system	Number of actinomycete strains as source	New antibiotic	Known antibiotic
Anti- <i>Bacteroides fragilis</i> compound	6820	Thiotetromycin	Althiomycin, Angolamycin, Chloramphenicol, Echinomycin, Labilomycin, Lankacidin C, Mikamycin A, Mocimycin, NCIB 8697, Neothramycins A, B, Pentalenolactone, RP-13252, Streptolydigin, Tirandamycins A, B, VD-844
Anti- <i>Fusobacterium varium</i> compound	2454	none	Althiomycin, Hygromycin A, Neothramycins A, B, Sarkomycin
Anti- <i>Clostridium difficile</i> compound	4729	Clostomicins Luminamicin Lustromycin	Aurodox, Dihydromocimycin, Lipiarmycin, Mocimycin, Pentalenolactone, Tirandamycin

Fig. 3. Structures of antianaerobic antibiotics, clostomicins, luminamicin and thiotetromycin.

tivity against aerobic bacteria but selective activity against anaerobic bacteria, especially against *Clostridium* sp. Luminamicin was reported to be identical to coloradocin³⁶⁾ discovered later by the Abbott group. It is assumed that the Abbott group and our group were working on the screening of antianaerobic antibiotics almost simultaneously. We released the discovery of luminamicin first but the Abbott elucidated the structure before us. This episode illustrates the competitive tension in the world of screening.

(3) Lustromycin¹⁸⁾

Lustromycin was isolated from the culture broth of *Streptomyces* sp. SK-1071, a soil isolate. The antibiotic shows a selective activity against *Clostridium* sp. but no activity against aerobic bacteria, except for *Micrococcus luteus*. Activity and structure of lustromycin are similar to those of luminamicin. The difference in molecular formula between lustromycin (C₃₂H₃₈O₁₃) and luminamicin (C₃₂H₃₈O₁₂) is one oxygen atom. Judging from their NMR

spectra, lustromycin has two methoxy and one methyl groups in the structure while luminamicin has one methoxy and two methyl groups. Recently, its structure is under study again.

(4) Thiotetromycin^{19,37)}

This compound contains a thiotetronic acid in the molecule and is produced by *Streptomyces* sp. OM-674 which was isolated from a soil sample of Luxor, Egypt. Thiotetromycin exhibits a selective activity against *B. fragilis* but no activity against many kinds of bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Clostridium perfringens*, *Fusobacterium varium*, etc. The antibiotic has inhibitory activity against type II fatty acid synthase (FAS)³⁸⁾. Recently, it was found that thiotetromycin induces modulation of actomyosin ATPase activity mediated through a conformational change in action³⁹⁾. An analogue of this compound, thiolactomycin, is the focus of attention as it is reported to exhibit anti-malarial activity by inhibiting type

II fatty acid biosynthesis in apicoplasts⁴⁰⁾ and anti-mycoplasmal activity by specifically inhibiting fatty acid and mycolic acid biosynthesis^{41), 42)}.

3. SCREENING OF ANTICOCCIDIAL ANTIBIOTICS

3-1. Background

Coccidiosis in chickens, turkeys, geese or ducks is a widespread disease caused by a group of parasitic protozoa. Chicken coccidiosis caused by *Eimeria tenella*, etc. is especially a major problem in broiler and other poultry industry. Polyether ionophores such as monensin⁴³⁾, lasalocid⁴⁴⁾, salinomycin⁴⁵⁾, etc. have been used successfully as anticoccidial agents. However, polyether-resistant *Eimeria* spp. are emerging with increasing frequency. Therefore, the search for new anticoccidial antibiotics against polyether-resistant *Eimeria* spp. has been intensified.

3-2. Assay and Screening

Assay system of anticoccidial antibiotics *in vivo* was already established. However, it was not suitable for screening because it required comparatively large quantity of sample of more than 100 mg and, moreover, it was an expensive system that took long time for detection. Then, we established the following two *in vitro* assay systems for screening. *Eimeria tenella*, the most hazardous protozoa to the industry, was used for evaluation.

(1) Screening of anti-sporozoite compounds (*In vitro* screening I)

E. tenella is known to have life cycle shown in Fig. 4. We screened known antibiotics showing sporozoite-cidal activity. Anti-sporozoite activity was judged by the number of living sporozoites after incubating them with an antibiotic in the phosphate buffer (pH7) for eight hours. As a result, frenolicin B⁴⁶⁾ which we found previously as an antimycoplasmal antibiotic, althiomycin⁴⁷⁾, bundlin A⁴⁸⁾ and tirandamycin A⁴⁹⁾ were picked up as anti-sporozoite compounds.

(2) Screening of schizont production inhibitors (*In vitro* screening II)²⁶⁾

Schizonts are produced from sporozoite as shown in Fig. 4. We screened for inhibitors of schizont production from sporozoite. We adapted the tissue culture *in vitro* assay system by Onaga et al.⁵⁰⁾ using BHK-21 cells, baby hamster kidney cells, as a host and modified it into a system for bioactive compound screening as shown in Fig. 4. The arrows in left picture show the mature schizont. In the right picture no mature schizonts are observed. This system is useful for picking up agents that exhibit schizont production inhibitory activity from sporozoite. Anticoccidial activity was judged by microscopic observation of the production of mature schizont in BHK cells. To perform both *in vitro* and *in vivo* experiment in parallel as soon as possible, we also used primary chicken embryonic cells instead of BHK-21 cells, baby hamster kidney cells, as a host.

With this assay system using two kinds of host, we examined a total of 29,800 strains; 27,100 actinomycetes, 2600 fungi and 100 bacteria. Consequently, we succeeded

Fig. 4. *In vitro* assay procedure for anticoccidial antibiotics in cell system.

Table 4. *In vitro* activity of anticoccidial antibiotics discovered by the screening of schizont production inhibitor in cell assay system

New antibiotic	Minimum effective dose (μM)		Specificity (B/A)
	Anticoccidial* activity (A)	Cytotoxicity** (B)	
Xanthoquinodin A1	0.035	3.50	100
Xanthoquinodin A2	0.035	0.35	10
Xanthoquinodin A3	0.035	3.50	100
Xanthoquinodin B1	0.035	3.50	100
Xanthoquinodin B2	0.035	3.50	100
Diolmycin A1	0.067	0.6	10
Diolmycin A2	0.67	6.7	10
Diolmycin B1	73.0	>73.0	>1
Diolmycin B2	73.0	>73.0	>1
Hynapene A	123.0	>123	>1
Hynapene B	34.7	139	4
Hynapene C	34.7	69	2
Arohynapene A	35.0	140	4
Arohynapene B	7.0	140	20
Arohynapene D	0.5	1.0	2
Fudecalone	16.0	160	10
Cytosaminomycin A	0.3	0.6	2
Cytosaminomycin B	2.3	4.6	2
Cytosaminomycin C	2.5	10	4
Cytosaminomycin D	20.0	ND	ND

ND: Not determined

*No mature shizonts were observed in BHK-21 cells as a host at the indicated drug concentration.

**No BHK-21 cell was observed at the indicated drug concentration.

Fig. 5. Anticoccidial antibiotics discovered in cell assay system *in vitro*.

Table 5. *In vivo* anticoccidial activity of frenolicin B in combination with ionophores against monensin-resistant *Eimeria tenella*

Treatment	Level ppm	Weight gain %	Feed conversion	Mortality %	Lesion score
UUC * ¹	0	100	1.69	0	0.0
IUC* ²	0	68	2.47	30	3.1
Frenolicin B	50	107	1.79	0	1.6
	30	115	1.62	0	1.4
	10	98	1.85	10	2.6
Lasalocid	100	100	1.76	0	0.9
Lasalocid + Frenolicin B	50+50	99	1.80	0	0.3
Lasalocid + Frenolicin B	50+10	99	1.83	0	1.3
Monensin	100	59	2.73	10	2.5
Monensin + Frenolicin B	50+50	104	1.69	0	0.4
Monensin + Frenolicin B	50+10	90	2.16	0	1.8
Salinomycin	60	98	1.96	0	1.4
Salinomycin + Frenolicin B	30+30	100	1.80	0	0.4
Salinomycin + Frenolicin B	30+10	108	1.79	0	1.2

*¹, uninfected unmedicated controls; *², infected unmedicated controls; 200,000 sporulated oocysts/chicken; averages of 10 chickens treatment.

Table 6. *In vivo* anticoccidial activity of xanthoquinodin A1 against monensin-resistant *Eimeria tenella*.

	Dose (ppm)	Relative Lesion Oocyst				
		Survival (A) (%)	weight gain (B)	score (C)	counts (D)	ACI
Control						
Non-infected	0	100	100	0	0	200
Infected	0	80	65	40	40	65
Treated with						
Xanthoquinodin A1	25	100	65	0	0	165
Monensin	80	100	72	34	10	128

ACI (Anticoccidial index)=A+B-C-D

in finding two new compounds from actinomycetes, and four from fungi shown in Table 4 and Fig. 5. Cytosaminomycins was discovered by the assay system using primary chicken embryonic cells as a host and other five compounds, diolmycins, arohynapenes, fudecalone, hynapenes and xanthoquinodins, were discovered by the system using BHK cells.

(3) *In vivo* screening

In vivo anticoccidial activity of the compounds found by *in vitro* screenings I and II was evaluated in the assay of monensin-resistant *E. tenella* in chicken. Frenolisin B and xanthoquinodins were turned out to exhibit remarkable activity. Following is the description of the two compounds.

1) Frenolicin B

Frenolicin B, a benzoisochromane-quinone antibiotic, was isolated in the course of screening for antimycoplasmal antibiotics from the culture broth of *Streptomyces roseofulvus* AM-3867. Frenolicin B is an animal feed antibiotic that is particularly effective against chicken coccidiosis and an feed efficiency enhancer for swine⁵¹). This compound independently exhibited anticoccidial activity

in vivo. However, it was found that its activity was intensified when used together with polyether antibiotics, lasalocid, monensin and salinomycin as shown in Table 5.

2) Xanthoquinodins

Xanthoquinodins A₁, A₂, A₃, B₁, B₂ and B₃ were isolated from the culture broth of a fungal strain *Humicola* sp. FO-888 as anticoccidial agents in the cell assay system *in vitro*. *In vivo* test of xanthoquinodin A₁, a main component, showed that the growth of monensin-resistant *E. tenella* in ceca was completely inhibited and oocyst was not observed when the antibiotic was added to the diet at 25 ppm, but increment of body weight in host chicken was low (Table 6).

4. CLOSING REMARKS

This review deals with screening of antianaerobic antibiotics and anticoccidial antibiotics. They reflect some of my years of research on screening of microbial bioactive compounds.

It has been said that new technology leads to new findings. The screening with a new assay system often leads to discovery of a new compound. For instance, High Performance Liquid Chromatography (HPLC) enabled the detection and isolation of minor constituents comparatively more easily. When our group picked up staurosporine (Str)⁴) from alkaloid screening, we purified and isolated it by means of crystallization. Only a decade later, other researchers in Japan and in the United States discovered various Str related compounds employing new HPLC technology. Following this, we cultured the Str-producing strain and examined it with HPLC to find that it produces at least four Str related compounds in addition to Str²⁸). As described in this review, HPLC was used for screening of anticoccidial antibiotics and it brought us the discovery of various constituents, including cytosaminomycins and

xanthoquinodins etc. as shown in Table 4 and Fig. 5.

Recent research on bioactive microbial compounds has seen great changes along with the development and the spread of recombinant DNA technology. Though the classic screening method described in this paper would probably survive, new techniques such as metabolic engineering, viable but not culturable (VBNC) microorganisms, High Through Put Screening (HTS), etc. will probably be used more frequently for screening of microbial bioactive compounds. However, these advanced researches rely on the strains and information discovered in the classic way. I hope that the compounds and producing microorganisms picked up by us will be of value in the course of metabolic engineering and that they will contribute to the further progress in the screening of the novel substances.

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REFERENCES

- 1) Iwai, Y., Ohno, H., Takeshima, H., Yamaguchi, N., Ōmura, S. & Hata, T.: Screening and isolation of penicillinase inhibitor, KA-107. *Antimicrob. Agents Chemoth.* 4: 222–225, 1973.
- 2) Ōmura, S., Iwai, Y., Hirano, A., Awaya, J., Suzuki, Y. & Matsumoto, K.: A new antibiotic, AM-2504. *Agric. Biol. Chem.* 41: 1827–1828, 1977.
- 3) Iwai, Y., Hirano, A., Awaya, J., Matuo, S. & Ōmura, S.: 1,3-Diphenethylurea from *Streptomyces* sp. No. AM-2498. *J. Antibiot.* 31: 375–376, 1978.
- 4) Ōmura, S., Iwai, Y., Hirano, A., Nakayama, A., Awaya, J., Tsuchiya, H., Takahashi, Y. & Masuma, R.: A new alkaloid AM-2282 of *Streptomyces* origin. Taxonomy, fermentation, isolation and preliminary characterization. *J. Antibiot.* 30: 275–282, 1977.
- 5) Hirano, A., Iwai, Y., Masuma, R., Tei, K. & Ōmura, S.: Neoxaline, a new alkaloid produced by *Aspergillus japonicus*. Production, isolation and properties. *J. Antibiot.* 32: 781–785, 1979.
- 6) Ōmura, S., Hirano, A., Iwai, Y. and Masuma, R.: Herquiline, a new alkaloid produced by *Penicillium herquei*. Fermentation, isolation and properties. *J. Antibiot.* 32: 786–790, 1979.
- 7) Awaya, J., Matsuyama K., Iwai, Y. & Ōmura, S.: Production of 9- β -arabinofranosyladenine by new species of *Streptomyces* and its herbicidal activity. *J. Antibiot.* 32: 1050–1054, 1979.
- 8) Ōmura, S., Iwai, Y., Takahashi, Y., Sadakane, N., Nakagawa, A., Oiwa, H., Hasegawa, Y. & Ikai, T.: Herximycin, a new antibiotic produced by a strain of *Streptomyces*. *J. Antibiot.* 32: 255–261, 1979.
- 9) Ōmura, S., Nakagawa, A., Aoyama, H. & Iwai, Y.: Karabemycin, a new antimetabolite of glutamine produced by a strain of *Streptomyces*. *J. Antibiot.* 36: 1129–1135, 1983.
- 10) Nakagawa, A., Iwai, Y., Hashimoto, H., Miyazaki, N., Oiwa, R., Takahashi, Y., Hirano, A., Shibukawa, N., Kojima, Y. & Ōmura, S.: Virantomycin, a new antiviral antibiotic produced by a strain of *Streptomyces*. *J. Antibiot.* 34: 1408–1415, 1981.
- 11) Ōmura, S., Shimizu, H., Iwai, Y., Hinotozawa, K., Otaguro, K., Hashimoto, H. & Nakagawa, A.: AM-2604, a new antiviral antibiotic produced by a strain of *Streptomyces*. *J. Antibiot.* 35: 1632–1637, 1982.
- 12) Iwai, Y., Nakagawa, A., Nagai, A., Matsuyama, K., Takahashi, Y., Yamashita, M., Hirano, A. & Ōmura, S.: 2'-Amino-2'-deoxyadenosine produced by a strain of *Actinomadura*. *J. Antibiot.* 32: 1367–1369, 1979.
- 13) Ōmura, S., Iwai, Y., Hinotozawa, K., Takahashi, Y., Y. Kato, J., Nakagawa, A., Hirano, A., Shimizu, H. & Haneda, K.: Cervinomycins A₁ and A₂, new antibiotics active against anaerobes, produced by a *Streptomyces cervinus* sp. nov. *J. Antibiot.* 35: 645–652, 1982.
- 14) Iwai, Y., Kora, A., Takahashi, Y., Hayashi, T., Awaya, J., Masuma, R., Oiwa, R., & Ōmura, S.: Production of deoxyfrenolicin and a new antibiotic, frenolicin B by *Streptomyces roseofulvus* strain AM-3867. *J. Antibiot.* 31: 959–965, 1978.
- 15) Iwai, Y., Kimura, K., Takahashi, Y., Hinotozawa, K., Shimizu, H., Tanaka, H. & Ōmura, S.: OM-173, a new nanaomycin-type antibiotics produced by a strain of *Streptomyces*. Taxonomy, production, isolation and biological properties. *J. Antibiot.* 36: 1268–1274, 1983.
- 16) Ōmura, S., Imamura, N., Oiwa, R., Kuga, H., Iwata, R., Masuma, R. & Iwai, Y.: Clostomicins, new antibiotics produced by *Micromonospora echinospora* subsp. *armeniaca* subsp. nov. I. Production, isolation and physicochemical and biological properties. *J. Antibiot.* 39: 1407–1412, 1986.
- 17) Ōmura, S., Iwata, R., Iwai, Y., Taga, S., Tanaka, Y. & Tomoda, H.: Luminamicin, a new antibiotic. Production, isolation and physico-chemical and biological properties. *J. Antibiot.* 38, 1322–1326, 1985.
- 18) Tomoda, H., Iwata, R., Takahashi, Y., Iwai, Y., Oiwa, R. & Ōmura, S.: Lustromycin, a new antibiotic produced by *Streptomyces* sp. *J. Antibiot.* 39: 1205–1210, 1986.

- 19) Ōmura, S., Iwai, Y., Nakagawa, A., Iwata, R., Takahashi, Y., Shimizu, H. & Tanaka, H.: Thiotetromycin, a new antibiotic.: Taxonomy, production, isolation and physicochemical and biological properties. *J. Antibiot.* 36: 109–114, 1983.
- 20) Haneda, K., Shinose, M., Seino, A., Tabata, N., Tomoda, H., Iwai, Y. & Ōmura, S.: Cytosaminomycins, new anticoccidial agents produced by *Streptomyces* sp. KO-8119. I. Taxonomy, production, isolation and physico-chemical and biological properties. *J. Antibiot.* 47:774–781, 1994.
- 21) Tabata, N., Tomoda, H., Takahashi, Y., Haneda, K., Iwai, Y., Woodruff, H.B. & Ōmura, S.: Diolmycins, new anticoccidial agents produced by *Streptomyces* sp. I. Production, isolation and biological properties. *J. Antibiot.* 46: 756–761, 1993.
- 22) Ōmura, S., Tsuzuki, K., Iwai, Y., Kishi, M., Watanabe, S. & Shimizu, H.: Anticoccidial activity of frenolicin B and its derivatives. *J. Antibiot.* 38: 1447–1448, 1985.
- 23) Masuma, R., Tabata, N., Tomoda, H., Hanada, K., Iwai, Y. & Ōmura, S.: Arohynapenes A and B, new anticoccidial agents produced by *Penicillium* sp. *J. Antibiot.* 47: 46–53, 1994.
- 24) Tabata, N., Tomoda, H., Masuma, R., Iwai, Y. & Ōmura, S.: Fudecalone, a new anticoccidial agent produced by *Penicillium* sp. FO-2030. *J. Antibiot.* 48: 53–58, 1995.
- 25) Tabata, N., Tomoda, H., Masuma, R., Haneda, K., Iwai, Y. & Ōmura, S.: Hynapenes A, B and C, new anticoccidial agents produced by *Penicillium* sp. I. Production, isolation and physico-chemical and biological properties. *J. Antibiot.* 46: 1849–1853, 1993.
- 26) Tabata, N., Suzumura, Y., Tomoda, H., Masuma, R., Haneda, K., Kishi, M., Iwai, Y. & Ōmura, S.: Xanthoquinodins, new anticoccidial agents produced by *Humicola* sp. *J. Antibiot.* 46: 749–755, 1993.
- 27) Ōmura, S. & Iwai, Y.: Microbial alkaloid. *Hakko To Kogyo* 37: 223–234, 1979 (review article, Japanese).
- 28) Iwai, Y., Li, Z. & Ōmura, S.: Indolocarbazole alkaloids from microorganisms. *Kagaku To Seibutu* 32: 463–469, 1994 (review article, Japanese).
- 29) Iwai, Y., Shimizu, H. & Ōmura, S.: A view of the screening for antiviral antibiotics. *Kagaku To Seibutu* 23: 379–385, 1985 (review article, Japanese).
- 30) Ōmura, S.: Philosophy of new drug discovery. *Microbiological reviews* 50: 259–279, 1986.
- 31) Nishikiori, T., Masuma, R., Oiwa, R., Katagiri, M., Awaya, J., Iwai, Y. & Ōmura, S.: Aurantinin, a new antibiotic of bacterial origin. *J. Antibiot.* 31: 525–532, 1978.
- 32) Masuma, R., Okuyama, K., Tanaka, Y., Hirose, R., Tomoda, H., Iwai, Y. & Ōmura, S.: A new agar medium suitable for screening of anti-*Clostridium* agents. *J. Antibiot.* 40:1773–1775, 1987.
- 33) Takahashi, Y., Iwai, Y. & Ōmura, S.: Clostomicins, new antibiotics produced by *Micromonospora echinospora* subsp. *armeniaca* subsp. nov. II. Taxonomic study of the producing microorganism. *J. Antibiot.* 39: 1412–1418, 1986.
- 34) Coronelli, C., White, R.J., Cancini, G.C. & Parenti, F.: Lipiarmycin, a new antibiotic from Actinoplanes. II. Isolation, chemical, biological and biochemical characterization. *J. Antibiot.* 28: 253, 1975.
- 35) Taga, S., Takahashi, Y., Iwai, Y. & Ōmura, S.: Taxonomic study of luminamicin-producing actinomycetes strain OMR-59. *Kitasato Arch. Exp. Med.* 60:15–24, 1987.
- 36) Rasmussen, R., Scherr, M.H., Whittern, D.N., Buko, A.M. & Mcalpine, J.B.: Coloradocin, an antibiotic from a new Actinoplanes II. Identity with luminamicin and elucidation of structure. *J. Anticiot.* 40: 1383–1393, 1987.
- 37) Ōmura, S., Nakagawa, A., Iwata, R. & Hatano, A.: Structure of new antibacterial antibiotic, thiotetromycin. *J. Antibiot.* 36: 1781–1782, 1983.
- 38) Tomoda, H. & Ōmura, S.: Lipid metabolism inhibitors of microbial origin. *Kitasato Arch. Exp. Med.* 65: 1–12, 1993.
- 39) Nakatani, K., Murayama, T., Satoh, Y., Furukawa, K., Ōmura, S. & Ohizumi, Y.: Modification of actomyosin ATPase by thiotetromycin is mediated through conformational change of actin. *Eur. J. Pharmacol.* 383: 381–386, 1999.
- 40) Waller, R.F., Keeling, P.J., Donald, R.G.K., Striepen, B., Handman, E., Lang-Unrasch, N., Cowman, A.F., Resra, G.S., Roose, D.S. & McFadden, G.I.: Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U.S.A.* 95: 12352–12357, 1998.
- 41) Slayden, R.A., Lee, R.E., Armour, J.W., Cooper, A.M., Orme, I.M., Brennan, P.J. & Besra, G.S.: Antimycobacterial action of thiolactomycin: an inhibitor of fatty acid and mycolic acid synthesis. *Antimicrob. Ag. Chemoth.* 40: 2813–1819, 1996
- 42) Kremer, L., Douglas, J.D., Baulard, A.R., Morehouse, C., Guy, M.R., All&, D., Dover, L.G., Lakey, J.H., Jacobs, W.R.Jr., Brennan, P.J., Minnikin, D.E. & Besra, G.S.: Thiolactomycin and related analogues as a novel antimycobacterial agents targeting Kas A and Kas B condensing enzymes in *Mycobacterium tuberculosis*. *J. Biol. Chem.* 273: 16857–16864, 2000.
- 43) Shumard, R.F. & Callender, M.E.: Monensin, a new biologically active compound. VI. Anticoccidial activity. *Antimicrob. Agents Chemother-1967*: 369–377, 1968.
- 44) Mitrovic, M. & Schildknecht.: Anticoccidial activity of lasalocid (X-537) in chicks. *Poult. Sci.* 53: 1448–1455, 1974.
- 45) Yvore, Po, Paymaud, J.P., Conan, L. & Naciri, M.: Evaluation of the efficacy of salinomycin in the control of coccidiosis in chicks. *Poult. Sci.* 59: 2412–2416, 1980.
- 46) Watanabe, S., Kishi, M., Daitoh, T. & Ōmura, S.: Anticoccidial agent (frenolicin B). *Japan Kohkai*, 58–88313, 1983.
- 47) Watanabe, S., Kishi, M., Kamio, R., Daitoh, T. & Ōmura, S.: Anticoccidial agent (althiomycin) *Japan Kohkai*, 58–43922, 1983.
- 48) Watanabe, S., Kishi, M., Daitoh, T. & Ōmura, S.: Anticoccidial agent (bundlin A). *Japan Kohkai*, 58–13514, 1983.
- 49) Watanabe, S., Kishi, M., Kamio, R., Daitoh, T. & Ōmura, S.: Anticoccidial agent (tirandamycin A). *Japan Kohkai*, 58–43920, 1983.
- 50) Onaga, H., Ishii, T. & Koyama, T.: Development of *Eimeria tenella* in cultured cells from parasitized choriallantoic membrane. *Jpn. J. Vet. Sci.* 36:73–80, 1974.
- 51) Mazur, B. & Haroon, Y.: Redox mode electrochemical detection approach with chemically modified electrodes for the measurement of frenolicin B (antibiotic) and related compounds in poultry feed. *J. Agric. Food Chem.* 43: 3042–3045, 1995.