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Philosophy of New Drug Discovery

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INTRODUCTION

It is a great honor for me to have been chosen as the recipient of the Hoechst-Roussel Award. I fully realize that this award was presented to me on behalf of the antibiotic research group at the Kitasato Institute and of outside researchers who have worked so diligently with me in the discovery of new antibiotics and their practical applications. It gives me great pleasure to have this opportunity to publish my point of view about our research work and the discovery of new antibiotics and bioactive compounds.

I have been working diligently on finding new antibiotics and on chemical and biological studies of these new compounds. Table 1 shows the antibiotics and other biologically active substances which we have discovered by various screening systems.

Referring to some of these compounds, I would like to present my viewpoint and ideas on research work. These achievements have been produced by (i) believing in the great capabilities of microorganisms, (ii) establishing a well-designed screening system for desired substances, (iii) recognizing that screening is not just routine work, (iv) emphasizing basic studies, and (v) treasuring good human relationships.

CAPABILITIES OF MICROORGANISMS

Besides antibiotics, microorganisms produce a number of biologically active substances such as plant growth factors, vitamins, alkaloids, enzymes, and enzyme inhibitors. One of the keys to the discovery of new drugs from microorganisms is to have a belief that the microorganisms can produce any desired substance. I have reached this belief based on my experiences.

Organic chemists clarify the structures of substances produced by living things. They also conduct chemical synthesis of natural products. They often say that they have synthesized non-natural compounds. However, during the course of screening for bioactive compounds, we have often experienced that the synthetic substances are found in the culture broths of microorganisms. These include 2′-amino-2′-deoxyribofuranosyladenine (antiviral and antimycoplasmal) (26, 123), L-1*H*-1,2,4-triazole-3-alanine (histidine antagonistic) (20, 27), quinoline-2-methanol (hypoglycemic) (63, 118), pyrrole-2-carboxylic acid (antiviral) (42, 109), and β-carboline carboxylic acid ethyl ester (benzodiazepine receptor binding activity) (4). Long before these compounds were synthesized chemically, they must have been produced by microorganisms.

While we were studying the biosynthesis of macrolide antibiotics, we learned that microorganisms can perform simple but very interesting reactions. One example is the isomerization of isobutyrate to *n*-butyrate (Fig. 1). This explains clearly the mechanism by which valine is incorporated into the 16-membered lactone ring of leucomycin and tylosin (96). We had never known previously such an interesting isomerization reaction in organic chemistry. On the metabolic map, we can see many other reactions which are difficult to perform chemically.

These examples indicate that the microorganism is far more capable than we think of producing organic compounds with all possible structures. It is very likely that only those substances with a particular biological activity can be discovered by screening procedures.

It is also known that a natural compound can show various types of biological activity. I herewith provide some examples. The antimicrobial activity of erythromycin is well known. Besides this activity, erythromycin shows strong gastrointestinal motor stimulating activity, which is known to be the major side effect of the antibiotic (21). This activity was discovered by Professor Ito of Gunma University,

TABLE 1. Screening methods and the new compounds found by Ōmura's group at Kitasato (Dec. 1985)

Screening system	Compounds (reference)
Antibacterial	A-73A, aurantinins (44), factumycin (14), kinamycins (12), KM-8 (47), LA-1 (55), leucomycins (94), takaokamycin (69)
Bacterial cell wall synthesis inhibitor	AM-5289, asukamycin (43), azureomycin (110), nanaomycins (116), setomimycin (29), vineomycins (19)[a]
Antifolate	AM-8402 (73), diazaquinomycin (39)
Antimycoplasmal	2′-Amino-2′-deoxyadenosine (26), cervinomycins (58), frenolicin B (93), OM-173 (23)
Antianaerobic	Clostomicin, luminamycin (64), lustromycin, thiotetromycin (76)
Antiviral	Virantmycin (40), virustomycin (56)
Antifungal	AF-8 (alboleutin) (61), cerulenin (119), irumamycin (92), O-2867 (104), prumycin (49)
Antiparasitic	Avermectins (3), hitachimycin (stubomycin) (46), setamycin (79)
Anticancer	OS-3256B (105), sporamycin (120)
Herbicidal	Herbimycins (70), karabemycin (75), oxetin (72), phosalacine (53)
Penicillinase inhibitor	KA-107 (45)
Adenosine deaminase inhibitor	Adechlorin (57)
Elastase inhibitor	Elasnin (41)
Antiplatelet	OM-3209
Alkaloid (chemical screening)	AM-2504 (prolimycin) (60), 1,3-diphenethylurea (22), herquline (54), NA-337 A (97), neoxaline (33), pyrindicin (25), quinoline-2-methanol (63), staurosporine (11), TM-64 (narcoactine) (34)
Hybrid biosynthesis	Chimeramycins (80), mederrhodins (18)
Chemical modification	Rokitamycin (3″-*O*-propionylleucomycin A_5) (102), 3,3″,4″-tri-*O*-propionylspiramycin I (81)
Others	Irumanolides (101), protylonolide (90)

[a] Asukamycin, nanaomycins, setomimycin, and vineomycins do not inhibit bacterial cell wall synthesis, but were isolated as by-products from this screening system.

Japan, who is now working with us on the structure-side effect correlation (95).

The movement of the gastrointestine was recorded by setting transducers into a dog. Figure 2 shows a typical contractile wave on a polygraph when 0.1 mg of erythromycin per kg is administrated intravenously. Contraction of the gastrointestine was induced by as little as 30 μg of erythromycin per kg. This is amazingly small, i.e., 1/100 of the 3-mg/kg dose normally given to a patient with infectious disease.

Another example of microbial metabolites having different kinds of biological activities is vineomycin (Fig. 3), which was discovered in our laboratory. The antibiotic produced by *Streptomyces matensis* subsp. *vineus* (Fig. 4) is an isotetracenone antibiotic active against gram-positive bacteria. Kishi and his co-workers at Takeda Chemical Industry Ltd. found that this antibiotic inhibits prolyl hydroxylase, an important enzyme of collagen biosynthesis in animals (48). 4-Hydroxy proline, a constituent of collagen, is formed after a proline-containing polypeptide is biosynthesized. The hydroxylation step precedes the biosynthesis of collagen. As small an amount as 2 μg of vineomycin per ml inhibits the activity of prolyl hydroxylase by 50%.

We have often seen that a microorganism coproduces several substances with different activities and structures. For example, three substances, the antibiotic complex leucomycin (Fig. 5) (67), the toxic substance teleocidin (Fig. 6) (114), and LA-1 (Fig. 7) with antibacterial activity (55), were isolated from *Streptoverticillium* sp. KA-6 (Fig. 8).

In 1972, we reported for the first time a screening method for β-lactamase inhibitors of microbial origin. It was to cope with the appearance of bacterial resistance to penicillins and cephalosporins (16). Although the compounds we obtained at that time from four strains of *Streptomyces* spp. were macromolecular ones, efforts in this work led to the later discovery by other research groups of a number of β-lactamase inhibitors of low molecular weight such as thienamycin (111), olivanic acid (6), and clavulanic acid (5).

From all of these facts, we know that we shall find more biologically active substances from microorganisms, fully realizing the great capability of the microorganism.

Here I would like to recall "the laws of applied microbiology" established by the late David Perlman in 1979 (98):

1. The microorganism is { always right. / your friend. / a sensitive partner.
2. There are no stupid microorganisms.
3. Microorganisms { can / will } do anything.
4. Microorganisms are { smarter, / wiser, / more energetic } than { chemists. / engineers. / others.
5. If you take care of your microbial friends, they will take care of your future (and you will live happily ever after).

EFFECTIVE SCREENING SYSTEMS

Requirements for Biological Screening

The key to success in obtaining active substances from microorganisms depends on effective screening systems. Sometimes we have to spend as much as a year or two to devise a satisfactory one.

Biological screening requires the following: (i) the use of appropriate biological material such as bacteria, animal cells, enzymes, and others for the detection of desired activity; (ii) the ability to assay a small quantity of substance easily and quickly; and (iii) the ability to devise various conditions for the successful isolation and cultivation of microorganisms.

If any of the above is inappropriate, it is difficult to obtain a desired substance. I shall discuss some of these requirements in the screening procedures which we have established and carried out.

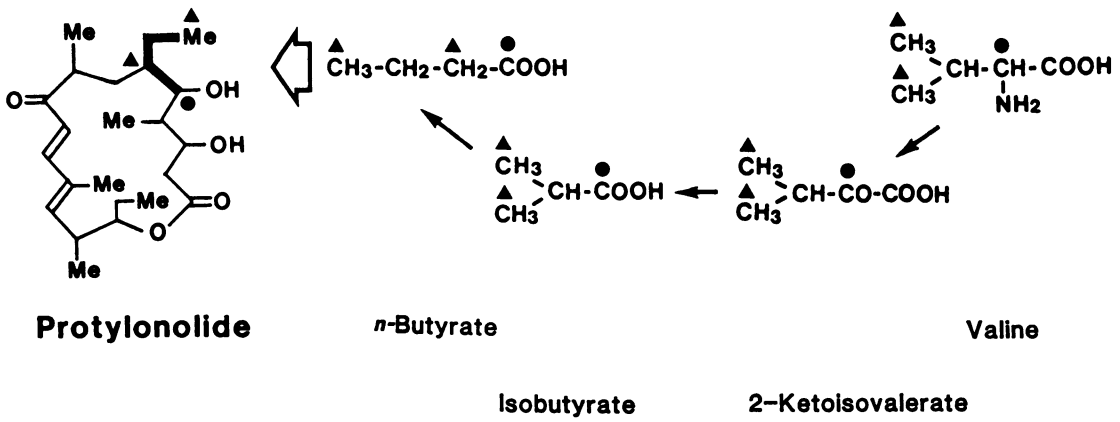


FIG. 1. Metabolism of valine to protylonolide via butyrate in *S. fradiae*.

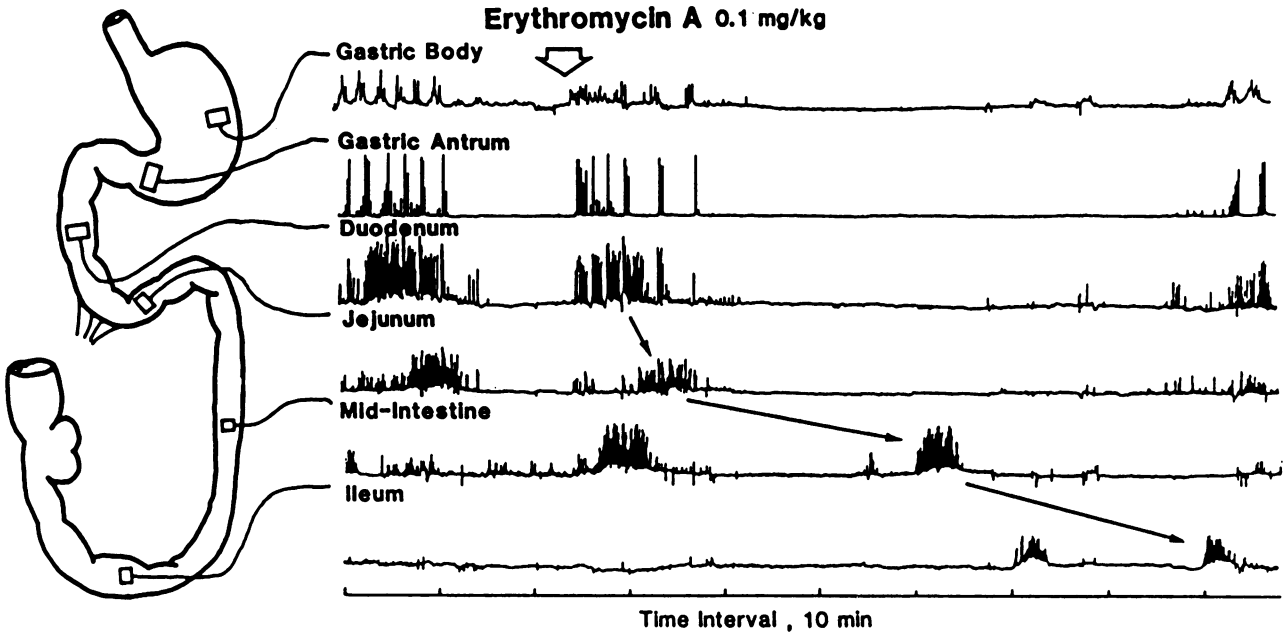


FIG. 2. Gastrointestinal contraction induced by erythromycin A.

Screening of Cell Wall Synthesis Inhibitors and Antimycoplasmal Substances

The procedure described here is an effective screening system for both bacterial cell wall biosynthesis inhibitors and antimycoplasmal substances (Fig. 9) (52).

At the first stage of this screening, we divided culture broths into three groups according to their antimicrobial activity, namely, those without activity against mycoplasma, those without activity against *Bacillus subtilis*, and those with activity against both of them. The last group was discarded from this screening process. We targeted our

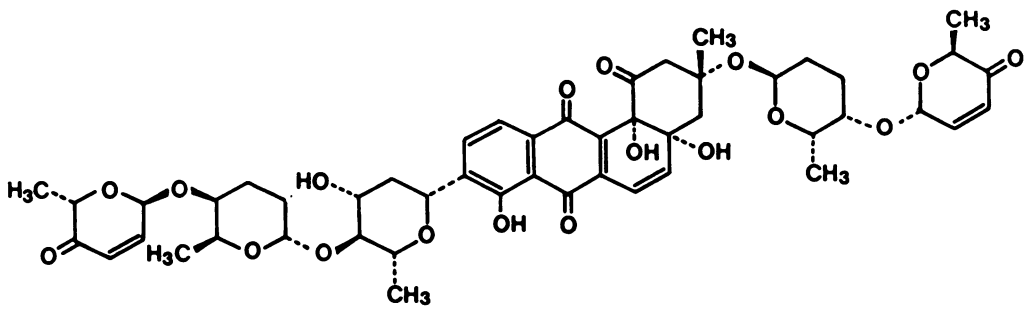


FIG. 3. Structure of vineomycin A_1.

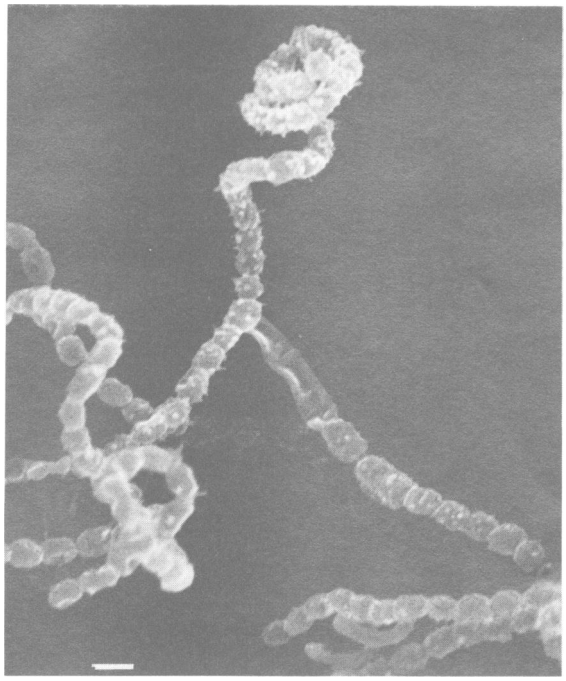


FIG. 4. *S. matensis* subsp. *vineus*. Bar, 1 μm.

screening effort on those cultures with activity against *B. subtilis* but with no activity against mycoplasma. This type of response is an indication that the culture broth contains a cell wall synthesis inhibitor, because mycoplasma do not have cell walls.

The next step was to determine if a culture broth selected above inhibits the incorporation of [^{3}H]diaminopimelic acid (Dpm) and [^{14}C]leucine into a macromolecular fraction of the bacterium. [^{3}H]Dpm is a precursor of cell wall peptidoglycan, while [^{14}C]leucine is a precursor of protein. It has been verified that a specific inhibitor of cell wall synthesis inhibits only the incorporation of [^{3}H]Dpm. By this method we found the new antibiotics azureomycins A and B and AM-5289, in addition to known cell wall synthesis inhibitors (86).

From the culture broths showing activity against mycoplasma, we found three new antibiotics: nanaomycins composed of five components which are effective against dermatomycosis of livestock (30, 32, 115); frenolicin B, an excellent anticoccidium substance (24); and cervinomycins, which are active against anaerobic bacteria (58).

Another three new antibiotics were obtained as by-products of this screening process: asukamycin (28, 68), setomimycin (29, 84), and vineomycin (19, 85).

Azureomycin is a glycopeptide antibiotic of yet unknown

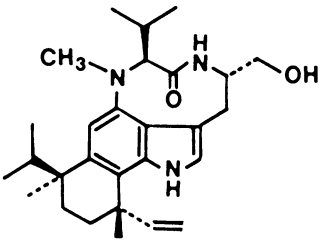


FIG. 6. Structure of teleocidin.

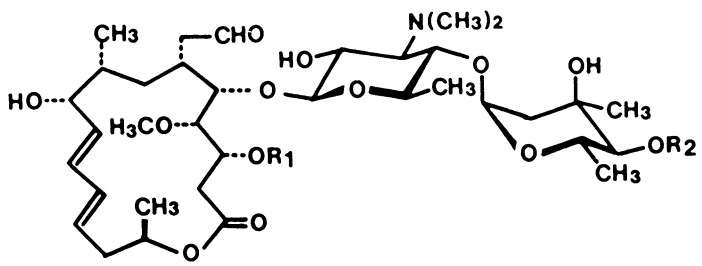
structure. This antibiotic was isolated from a culture broth of a new actinomycete, *Pseudonocardia azurea* (87, 110). It induces lysis of growing *B. cereus* at a concentration of 10 μg/ml but does not affect resting cells. A study on the mode of action showed that the primary target of the antibiotic action is the transfer of the disaccharide peptide unit, GlcNAc-MurNac-pentapeptide, from lipid-bound precursor to the acceptor, nascent peptidoglycan, as shown in Fig. 10.

Nanaomycin (Fig. 11) produced from *S. rosa* subsp. *notoensis* (Fig. 12) was named after the place where the soil sample was collected (30, 115). Nanaomycin shows moderate antibacterial activity and strong activity against mycoplasma, as well as activity against *Trichophyton* spp. in vitro (Table 2). Though we were unsuccessful in developing nanaomycin as an antimycoplasmal agent, Hamana and his co-workers (15) found that the complex has a remarkable in vivo effect against dermatomycosis of cattle. With a single application of 100 ppm of nanaomycin A, the dermatomycosis caused by *Trichophyton verrucosum* was healed in 1 month. After safety tests were conducted at Kyowa Hakko Kogyo Co. Ltd., nanaomycin A was approved as an antifungal antibiotic for veterinary use.

Biosynthesis of each component of the nanaomycin complex was studied by use of a cell-free system from *S. rosa* subsp. *notoensis* (116). Three kinds of enzymes involved in nanaomycin biosynthesis were characterized (Fig. 13). Among them, nanaomycin D reductase, a flavoenzyme, was purified to homogeneity. Nanaomycin B synthase is the first enzyme known to convert an epoxide group to a monohydroxyl.

We proposed the mechanism of action of this antibiotic in a marine bacterium, *Vibrio alginolyticus* (Fig. 14). Nanaomycin A receives electrons from respiratory flavoprotein to give a reduced form. When reduced nanaomycin A is oxidized to nanaomycin A by molecular oxygen, superoxide radical (O_2^-) is produced (17). Superoxide dismutase present in the bacterium acts as a scavenger of O_2^-, but the bacterium is killed due to insufficient digestion and toxicity of O_2^- in the presence of a significant amount of nanaomycin A.

Cervinomycin (Fig. 15), obtained from *S. cervinus* (Fig.



	R_1	R_2
A_1	H	$COCH_2CH(CH_3)_2$
A_3	$COCH_3$	$COCH_2CH(CH_3)_2$
A_4	$COCH_3$	$COCH_2CH_2CH_3$
A_5	H	$COCH_2CH_2CH_3$
A_6	$COCH_3$	$COCH_2CH_3$
A_7	H	$COCH_2CH_3$
A_8	$COCH_3$	$COCH_3$

FIG. 5. Structure of leucomycin.

TABLE 2. Antimicrobial activities of nanaomycins A and B

Test organism	Medium[a]	Minimum inhibitory concn (μg/ml)	
		A	B
Candida albicans KF-1	P	31.2	31.2
Saccharomyces sake KF-26	P	31.2	62.5
Aspergillus niger ATCC 6275	P	62.5	62.5
Piricularia oryzae KF-180	P	7.8	15.6
Trichophyton asteroides KF-50	P	1.6	12.5
T. mentagrophytes KF-213	P	0.8	25
T. purpureum KF-61	P	3.1	25
T. rubrum KF-53	P	0.1	3.1
Mycoplasma gallisepticum KP-13	H	<0.013	<0.013
M. gallisepticum 333P	H	<0.013	<0.013
M. gallinarum	H	1.56	3.12
M. pneumoniae KB-173	E	0.013	3.05
Acholeplasma laidlawii (B) Bm1	H	25	25

[a] P, Potato agar (pH 6.4, 4 days, 27°C); H, Hokken PPLO agar (pH 7.8, 8 days, 37°C); E, Eiken PPLO agar (pH 7.8, 8 days, 37°C).

16), is an antimycoplasmal substance with a unique structure. It is barely soluble in most solvents. Its structure was determined mainly by nuclear magnetic resonance spectroscopy of its methyl and triacetyl derivatives. The triacetate is highly soluble and as effective as clindamycin against various anaerobic and gram-positive bacteria (Table 3).

Screening of Inhibitors of Folate-Related Metabolism

The next screening system is that used for antibiotics affecting folate-related metabolism. A microorganism used conventionally for folate bioassay was applied as the test organism (Fig. 17).

Most microorganisms stop growing when folate biosynthesis is inhibited by sulfonamides, because they cannot take up folate from the outside. *Enterococcus faecium*, a strain which is used for folate bioassay, lacks a part of the enzyme assembly for folate biosynthesis, but it can take up folates to use for the biosynthesis of primary metabolites such as thymine and serine. Therefore, it is possible to examine whether a substance is an inhibitor of folate metabolism or not by observing the difference in antibacterial activities in the presence and absence of a folate-related substance added to a vitamin assay medium (Table 4). For example, in the case of the inhibitor of thymidylate synthase, the inhibition would be reversed by thymidine, but not by pteroic or folic acid. A cultured broth of *Streptomyces* sp. strain OM-704 (Fig. 18) was found to show such an inhibitory pattern (59). The inhibitor was named diazaquinomycin (Fig. 19) after its structure determination (74). It is barely soluble in water, and the synthesis of its soluble derivative is being attempted in our laboratory. The antibiotic inhibits thymidylate synthase from *E. faecium* and from animal cells acting competitively with the normal substrate 5,10-methylenetetrahydrofolate (Fig. 20) (39).

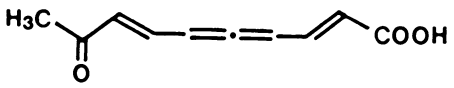


FIG. 7. Structure of LA-1.

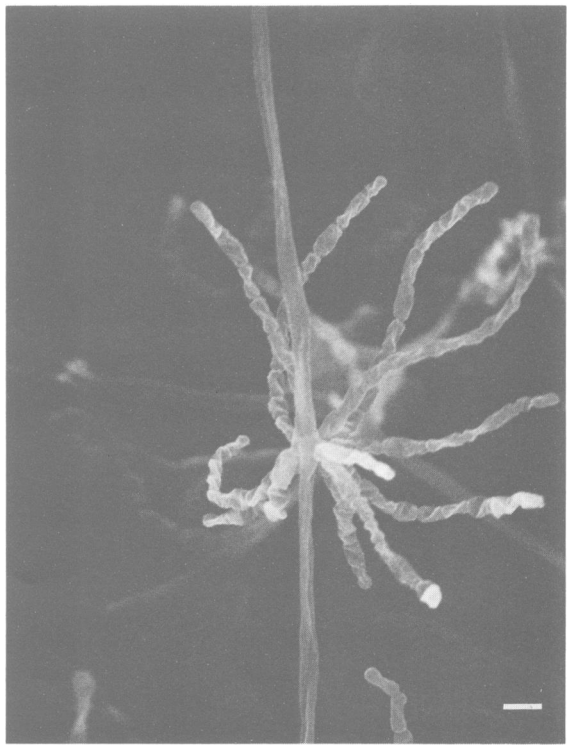


FIG. 8. *Streptoverticillium* sp. strain KA-6. Bar, 1 μm.

Screening of Herbicidal Compounds by Using a Bacterium

This screening system shows how we obtained a herbicidal substance by using *B. subtilis* as a tool to detect biological activity.

Some synthetic herbicides inhibit glutamine biosynthesis in plant tissue, accumulating NH_4^+ which kills the plant. Based on this information, a screening system was designed to find a substance which inhibits growth of *B. subtilis* in minimal medium but not in the presence of glutamine. A very interesting substance, phosalacine, was discovered from a new organism, *Kitasatosporia phosalacinea* (Fig. 21) (112). Phosalacine was determined to be L-phosphinothricyl-L-alanyl-L-leucine (Fig. 22) (71). It has a broad herbicidal activity against mono- and dicotyledons.

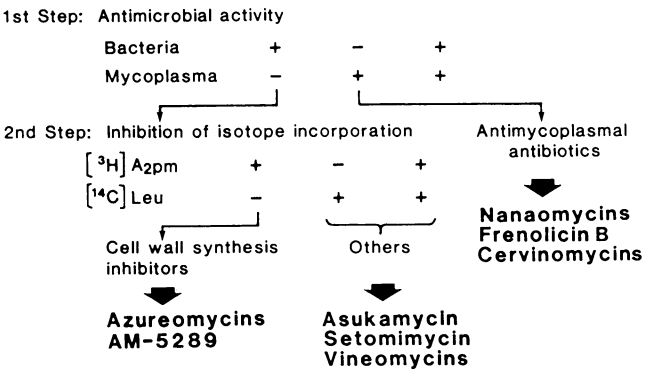


FIG. 9. Screening for inhibitors of bacterial cell wall synthesis and antimycoplasmal antibiotics. A_2pm, Diaminopimelic acid.

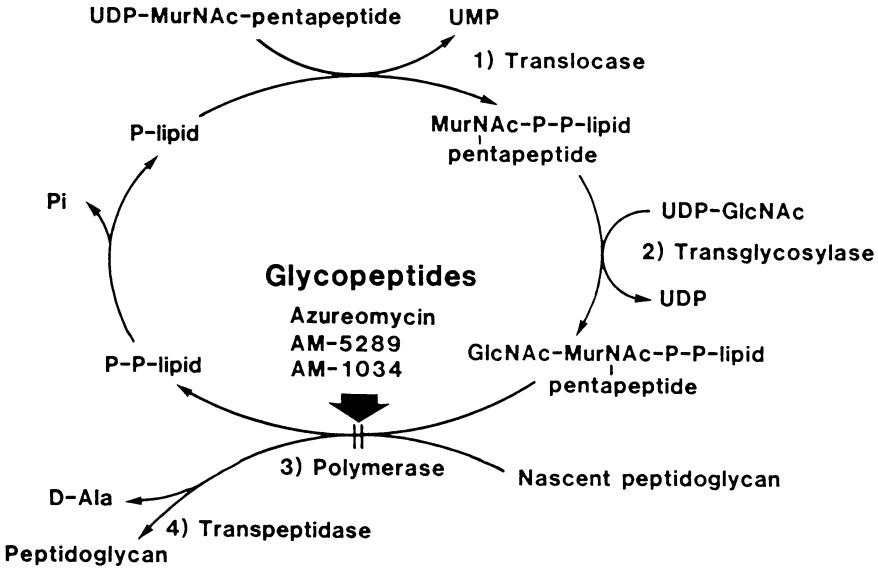


FIG. 10. Site of action of glycopeptide antibiotics in the peptidoglycan biosynthetic pathway of *B. megaterium* KM. UDP, Uridine diphosphate; UMP, uridine monophosphate.

Compounds Found by Other Screening Systems

Many other screening systems for new bioactive compounds have been established in my laboratory. Some of the new antibiotics obtained by these systems are described.

Hitachimycin (Fig. 23) was isolated at almost the same time by two different screening systems at the Kitasato Institute. As an anticancer compound, the antibiotic was isolated from *Streptomyces* sp. strain KG-2245 (Fig. 24) (121). It was also isolated from actinomycete strain KM-4927 as an antiprotozoal compound (Fig. 25) (46). Its structure, containing a large lactam ring, is unique. Biosynthetic studies with ^{13}C-labeled precursors showed that hitachimycin is built up from eight acetates, one propionate, one phenylalanine, and one methionine methyl via the polyketide pathway (Fig. 26) (78). It is of particular interest that the phenyl group with three carbon units, the starter unit of the polyketide formation, originates from phenylalanine and the nitrogen of the lactam moiety originates from the α-amino group of phenylalanine after migration to the β-position.

Irumamycin (Fig. 27) was isolated from *S. subflavus* subsp. *irumaensis* (Fig. 28) as an antifungal antibiotic (77, 91). This antibiotic shows strong activity against plant pathogenic fungi such as *Scleotinia cinerea* and *Alternaria kikuchiana*. In the screening for substances affecting the life cycle of a cellular slime mold, *Dictyostelium discoideum*, this antibiotic was found to inhibit adhesion of the cellular slime mold.

Virustomycin (Fig. 29), which was produced by *Streptomyces* sp. strain AM-2604 (Fig. 30), was found by screening for antiviral compounds (56, 82). Besides its antiviral activity, this 18-membered macrolide antibiotic showed antitrichomonal activity.

Prumycin (Fig. 31), which is produced by *S. kagawaensis* (Fig. 32), was found in the screening for antibiotics active against plant pathogenic fungi (65, 66). Later, this antibiotic was found to be active against mouse mammary adenocar-

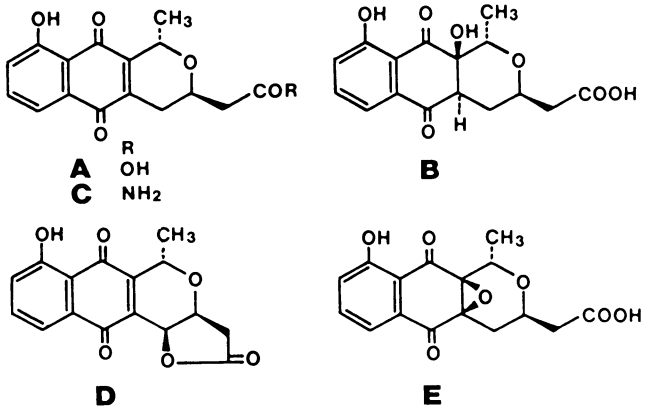


FIG. 11. Structure of nanaomycin.

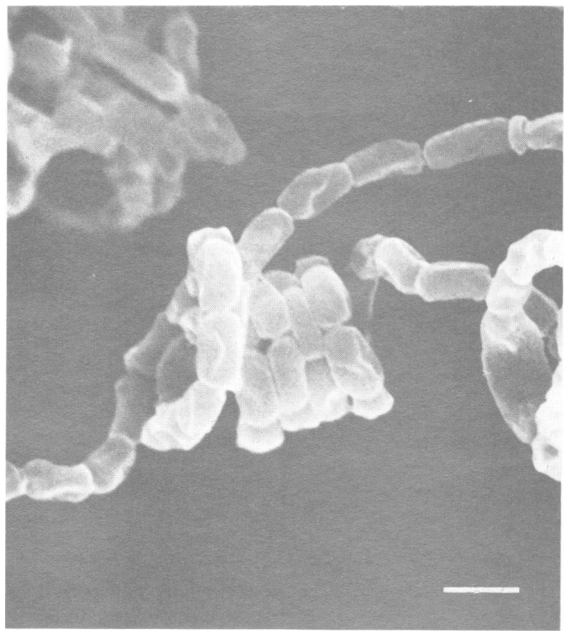


FIG. 12. *S. rosa* subsp. *notoensis*. Bar, 1 μm.

TABLE 3. Antimicrobial activities of cervinomycin A_1 triacetate and clindamycin

Test organism	Minimum inhibitory concn (μg/ml)	
	Cervinomycin A_1 triacetate	Clindamycin
Staphylococcus aureus ATCC 6538P	0.16	0.16
Bacillus subtilis ATCC 6633	0.02	2.5
B. cereus IFO 3001	0.31	0.63
Micrococcus luteus ATCC 9341	0.08	0.04
Escherichia coli NIHJ JC-2	>10	>10
Salmonella typhimurium KB20	>10	>10
Klebsiella pneumoniae ATCC 10031	>10	>10
Proteus vulgaris IFO 3167	>10	>10
Mycoplasma gallisepticum KB171	0.16	6.3
M. pneumoniae KB173	0.16	6.3
Acholeplasma laidlawii KB174	0.31	6.3
Clostridium difficile ATCC 9689	0.05	6.3
C. perfringens ATCC 13124	0.02	0.1
C. perfringens ATCC 19574	0.02	0.02
Bifidobacterium adolescentis ATCC 15703	0.02	0.01
B. bifidum ATCC 11146	0.2	0.02
Peptococcus prevotii ATCC 9321	0.1	0.05
Streptococcus mutans RK-1	0.02	0.05
Bacteroides fragilis ATCC 23745	0.1	0.02

cinoma (50). Among the many antibiotics inhibiting protein biosynthesis in eucaryotes, prumycin is unique in its mode of action (106).

ISOLATION OF MICROORGANISMS AND FERMENTATION CONDITIONS

To find new compounds from microorganisms, it is valuable to isolate strains of new species or new genera. Rare actinomycete strains especially attract us, since they have many possibilities of producing undiscovered bioactive substances.

TABLE 4. Inhibition patterns in primary screening for antifolates

Inhibition site[a]	Inhibitor[b]	Inhibition against *E. faecium*[c] in the presence of:		
		Pteroic acid	Folic acid	Thymidine
DHP synthase	Sulfonamides	−	−	−
DHF reductase	AM, MT, TP	+	+	−
TMP synthase	5FU	+	+	−
Other biochemical functions	General antibiotics	+	+	+

[a] DHP, dihydropteroate; DHF, dihydrofolate; TMP, thymidine monophosphate.
[b] AM, Aminopterin; MT, methotrexate; TP, trimethoprim; 5FU, 5-fluorouracil.
[c] The folic acid assay medium "Nissui" was used.

Taxonomic Studies in the Screening of New Compounds

We make it a rule to conduct taxonomic studies on the producing strains when we find new substances, because we can apply the results of basic research, including cultivation and fermentation physiology, in our screening. One of the taxonomic studies led us to the discovery of a new genus of the family *Actinomycetales*, which we named *Kitasatosporia*.

In the classification of actinomycetes, the type of cell wall constituent, together with morphological and physiological properties, are key characteristics (37). It is very important whether Dpm contained in the cell wall is of the *meso* or LL type. Normally, one of the two types is contained in the cell wall of an actinomycete (Table 5). An interesting finding was that *Kitasatosporia* contains both types of Dpm. We have isolated three species of this genus, *K. setae*, *K. griseola*, and *K. phosalacinea*, which are producers of setamycin and phosalacine (83, 112).

Figure 33 shows various growth levels of *K. setae* in a submerged culture and the change of the mycelium into submerged spores. It was found that the submerged spores

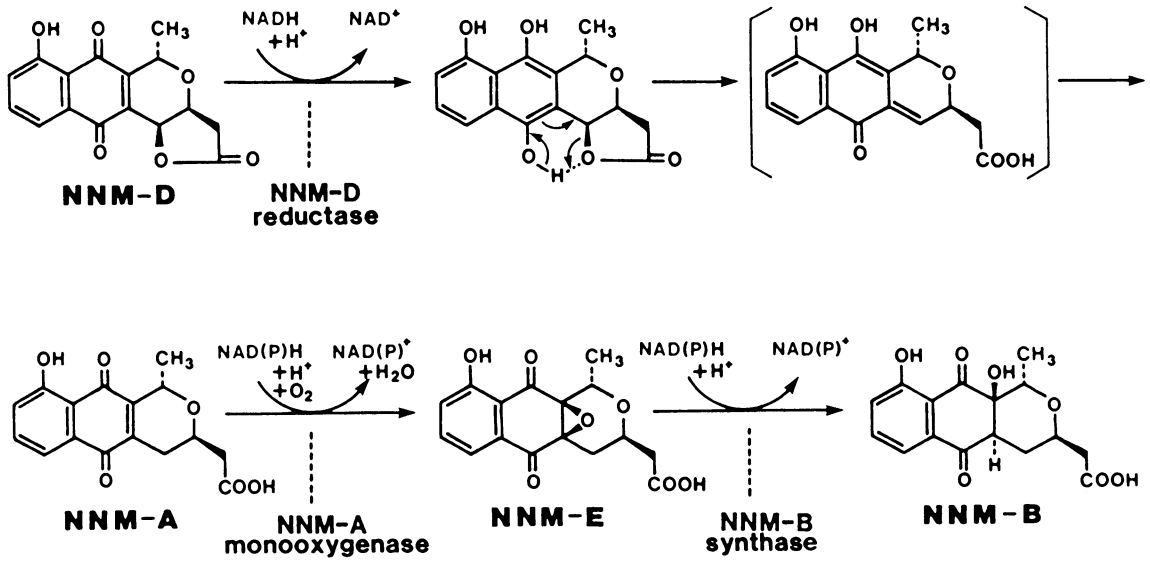


FIG. 13. Proposed biosynthetic pathway of nanaomycins (NNMs) by *S. rosa* subsp. *notoensis*. NAD, Nicotinamide adenine dinucleotide; NADH, NAD, reduced form; NADP, NAD phosphate.

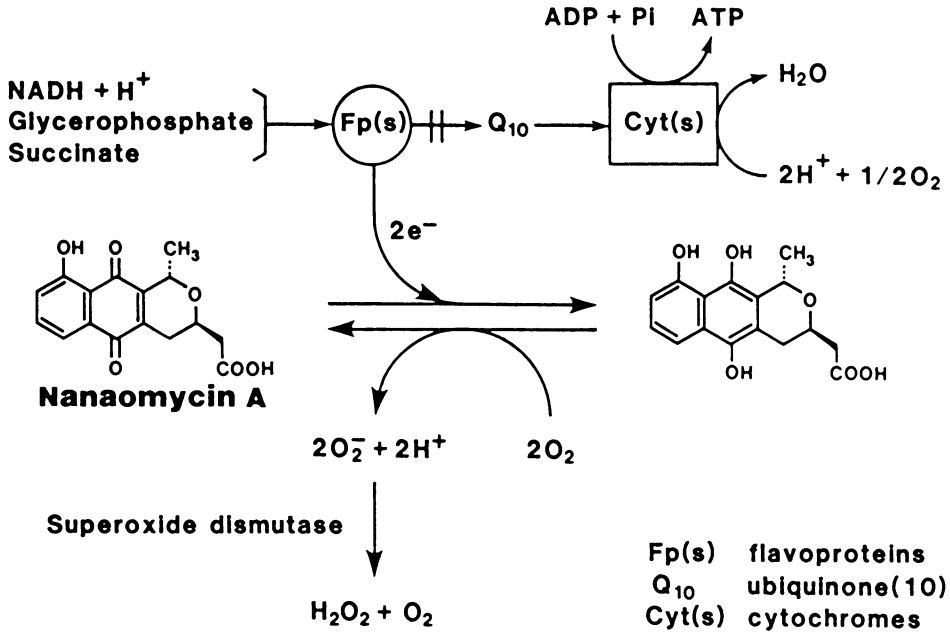


FIG. 14. Proposed mechanism of action of nanaomycin A in *Vibrio alginolyticus*. ADP, Adenosine diphosphate; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide, reduced form.

contain LL-Dpm and the mycelia contain *meso*-Dpm. This is a key characteristic of the genus *Kitasatosporia* (113).

Fermentation Conditions

During the course of our studies on macrolide biosynthesis, it was found that ammonium ion produced by the metabolism of nitrogen-containing compounds in the medium inhibits antibiotic production (89). We found a method to produce a large quantity of antibiotics by trapping NH_4 with magnesium phosphate or zeolite to release the inhibition (Table 6). We called this technique "ammonium ion-depressed fermentation" (38). By use of magnesium phosphate in a screening system, two new antibiotics, thiotetromycin (62) and new nanaomycin-related antibiotics (23), were obtained. Phosphate (PO_4^{3-}) has also been pointed out by other researchers as an inhibitory substance for antibiotic production (88). In this case, we used Kanumatsuchi, one of the allophane-containing minerals, to

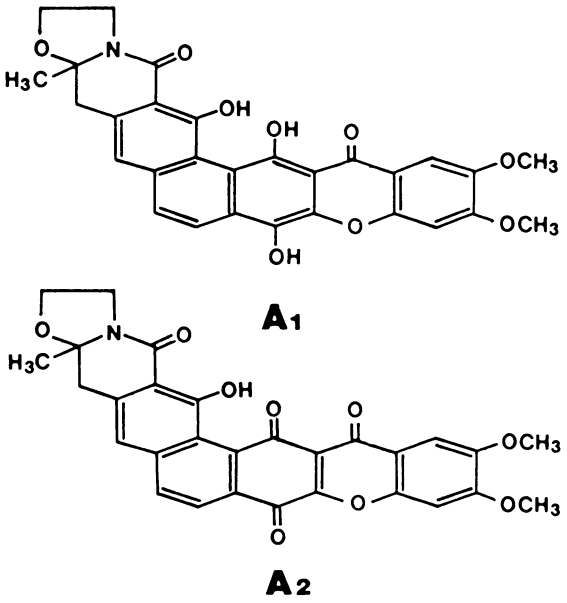


FIG. 15. Structure of cervinomycin.

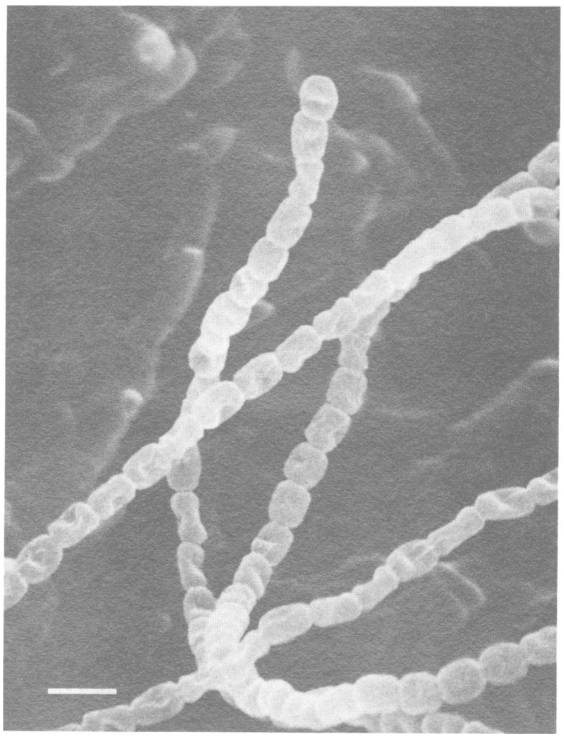


FIG. 16. *S. cervinus*. Bar, 1 μm.

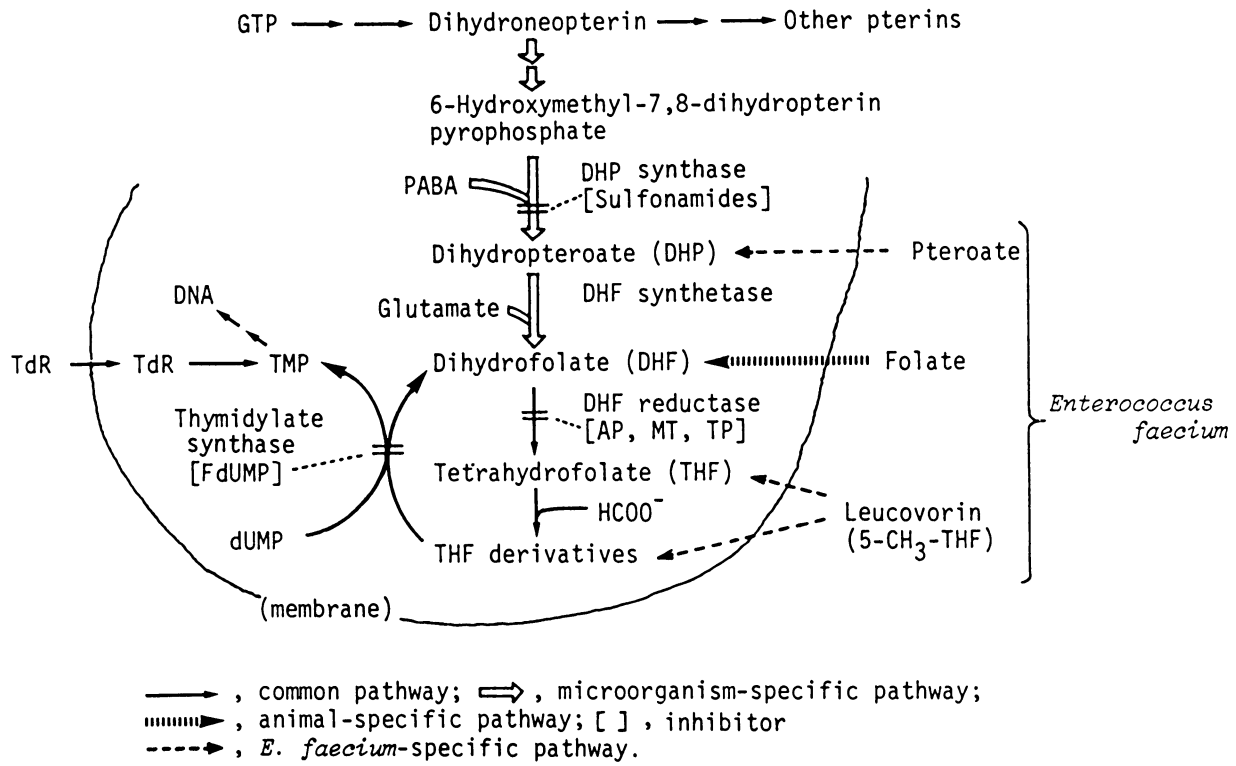


FIG. 17. Folate metabolism and inhibitors. GTP, Guanosine triphosphate; PABA, p-aminobenzoic acid; TdR, thymidine; TMP, thymidine monophosphate; dUMP, deoxyuridine monophosphate.

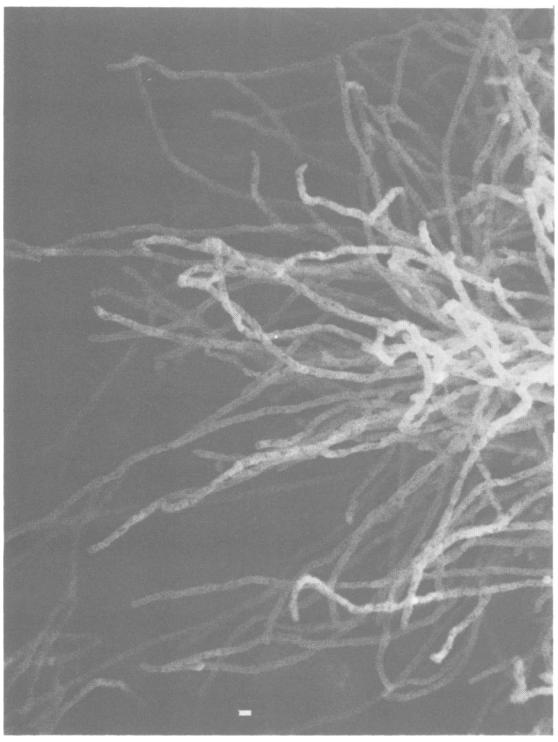


FIG. 18. *Streptomyces* sp. strain OM-704. Bar, 1 μm.

prevent the inhibition. Kanumatsuchi is the name of a soil material collected in the Kanuma district of Japan. Kanumatsuchi is often used in bonsai making because it absorbs phosphate well and releases phosphate gradually to the culture medium of a miniature tree which is called a "bonsai."

BASIC AND APPLIED RESEARCH

The most important thing in the screening for new drugs is that the researchers fully understand that screening is not

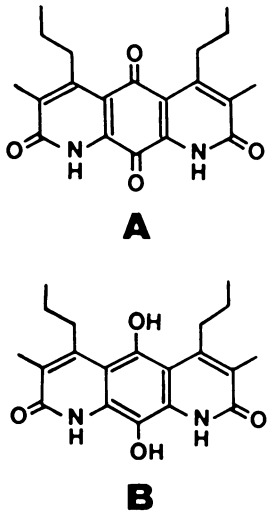


FIG. 19. Structure of diazaquinomycin.

TABLE 5. Taxonomic comparison of *Kitasatosporia* with related genera of the order *Actinomycetales*

Genus	Cell wall					Sporulation of AM[a]	Fragmentation of VM[a]
	Dpm		Gly	Ara	Gal		
	meso	LL					
Kitasatosporia	+	+	+	−	+	+	−
Streptomyces	−	+	+	−	−	+	−
Nocardioides	−	+	+	−	−	+	+
Actinomadura	+	−	−	−	−	+	−
Nocardiopsis	+	−	−	−	−	+	+
Pseudonocardia	+	−	−	+	+	+	+/−
Nocardia	+	−	−	+	+	−/+	+

[a] AM, Aerial mycelium; VM, vegetative mycelium.

just routine work. Thus each one of our group has a basic research assignment such as taxonomy, structure determination, mode of action, biosynthesis, or chemical synthesis, as well as participation in screening work. It is important for each researcher to enjoy being a pioneer in an undeveloped field, through the study of one's own new antibiotics, and at the same time to keep a challenging spirit to improve one's research capabilities. Although these may be merits in a computerized screening system, I am rather prudent about introducing a computer only for convenience, because it would make screening routine work.

Mode of Action of Cerulenin

Referring to basic and applied studies of cerulenin, I would like to describe how one substance expanded the scope of research and led us to the discovery of new substances.

TABLE 6. Enhancement of antibiotic production by NH_4^+- and PO_4^{3-}-trapping agents

Trapping agent	Amt added (%)	Antibiotic	Maximum antibiotic titer (μg/ml)	
			No addition	Addition
NH_4^+				
Magnesium phosphate (MgP)	1.0	Leucomycin	700	3,800
Sodium phosphotungstate	0.5	Spiramycin	150	450
Natural zeolite (ZL)	1.0	Tylosin	59	149
MgP	1.0	Cephalosporin	400	1,600[a]
MgP	1.0	Dihydrostreptomycin	55	145
NH_4^+-saturated ZL	0.2	Nanaomycin	85	750
ZL	1.0	Cerulenin	40	280
PO_4^{3-}				
Kanumatsuchi	0.5	Tylosin	70	130
Kanumatsuchi	0.5	Nanaomycin	110	560
Kanumatsuchi	0.5	Candicidin	180	530

[a] Shen et al. (107).

After cerulenin was isolated from the culture broth of *Cephalosporium caerulens* (Fig. 34) (1, 103), some attempts were made to develop this antibiotic into a chemotherapeutic agent for dermatomycosis. Unfortunately, it was unsuccessful because of its inflammatory side effect on the skin. Later, recollecting that this antibiotic has very broad antibacterial and antifungal spectra, we studied its mode of action to clarify why the antibiotic shows such a broad antimicrobial

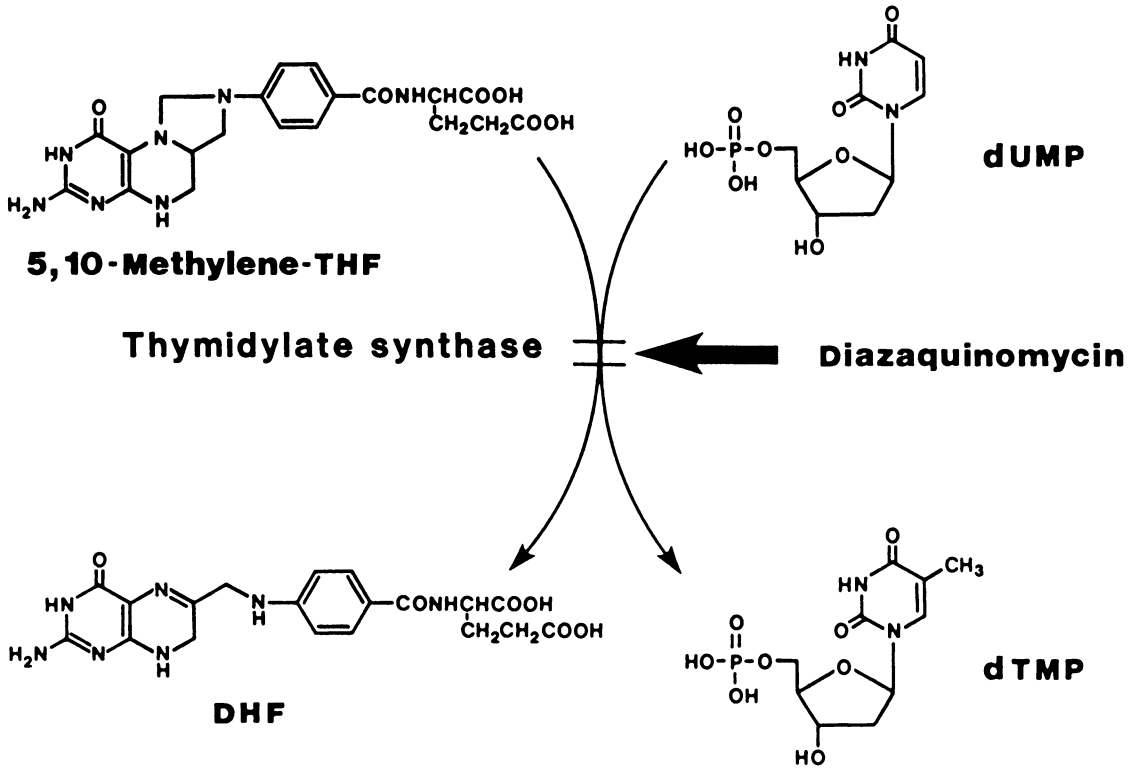


FIG. 20. Site of action of diazaquinomycin. THF, Trihydrofolate; DHF, dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate.

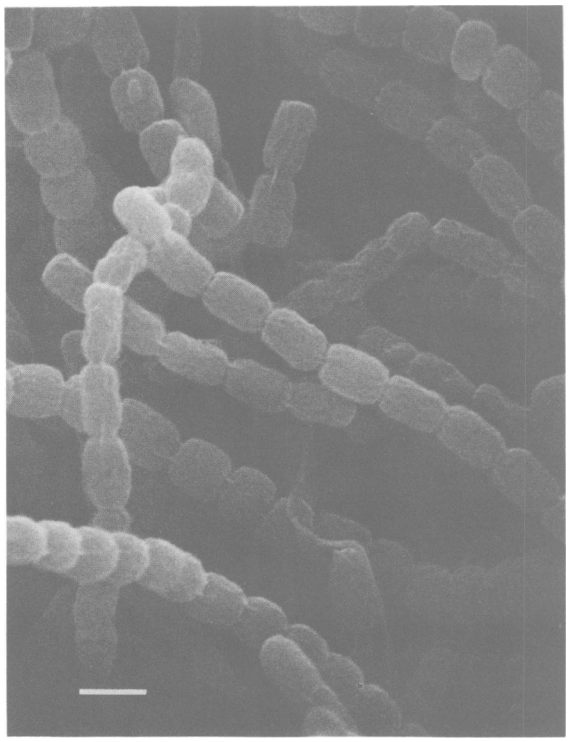


FIG. 21. *K. phosalacinea*. Bar, 1 μm.

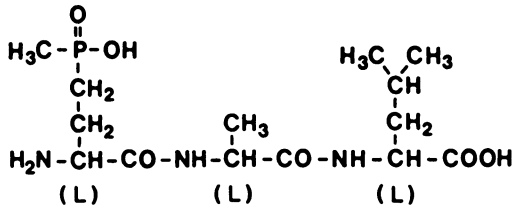


FIG. 22. Structure of phosalacine (L-phosphinothricyl-L-alanyl-L-leucine.

activity (51). Cerulenin was found to be the first specific inhibitor of fatty acid biosynthesis.

Since then, many researchers, including K. Bloch of Harvard University, have collaborated with me in showing that cerulenin inhibits the condensation of acyl-acyl carrier protein and malonyl-acyl carrier protein, an important stage in fatty acid biosynthesis (Fig. 35). The alkylation of the condensing enzyme with cerulenin, accompanied by the SN_2 type of epoxide opening reaction, results in the loss of enzyme activity (Fig. 36) (10, 122). Recently, our studies on the self-resistance mechanism of the cerulenin producer *C. caerulens* revealed that one of the active sites, the cysteine

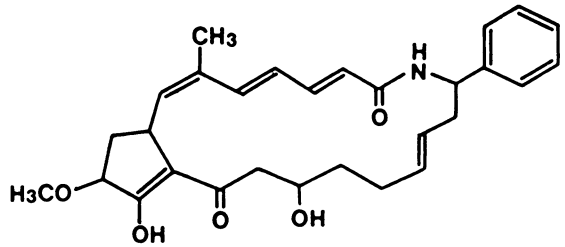


FIG. 23. Structure of hitachimycin (stubomycin).

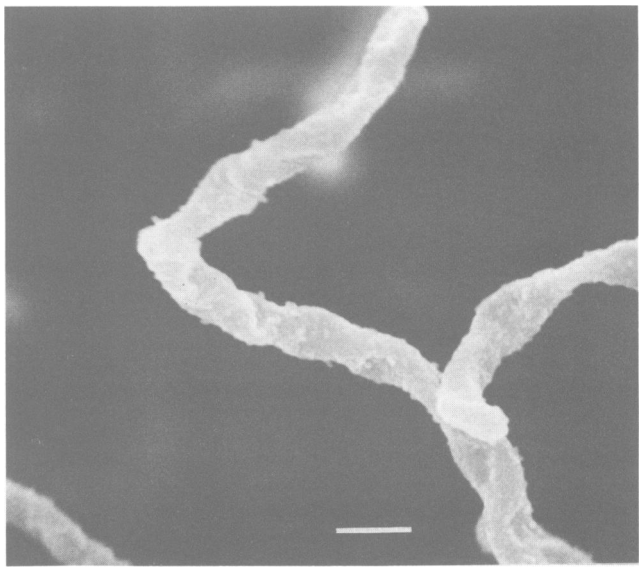


FIG. 24. *Streptomyces* sp. strain KG-2245. Bar, 1 μm.

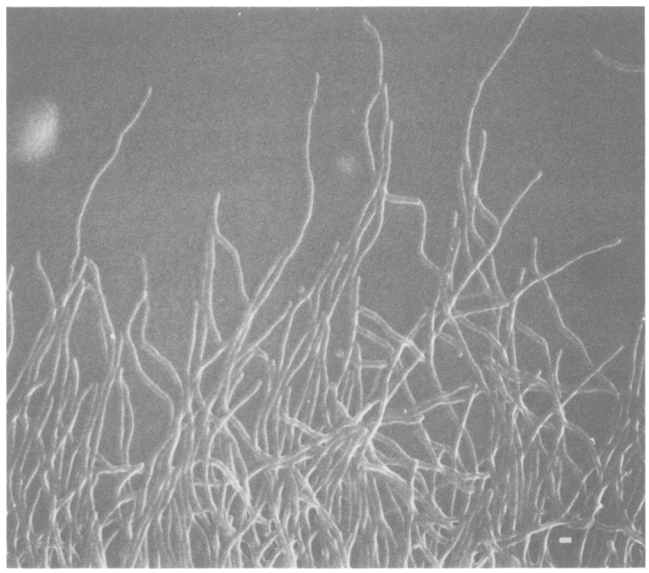


FIG. 25. *Actinomycetales* strain KM-4927. Bar, 1 μm.

residue of the fatty acid synthase from this fungus, contains more polar amino acids such as glutamine and serine than those from sensitive eucaryotes such as *Saccharomyces cerevisiae* (31).

Today, cerulenin is used as a biochemical reagent by many

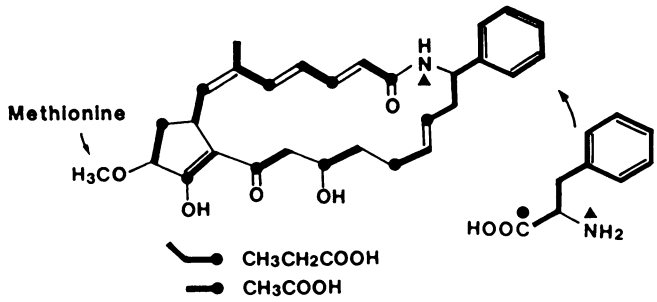


FIG. 26. Biosynthetic origins of hitachimycin.

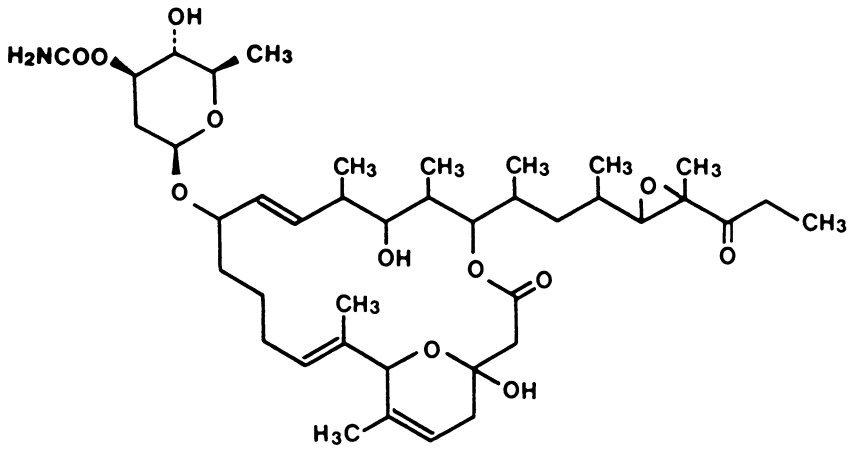


FIG. 27. Structure of irumamycin.

researchers and has contributed greatly to progress in the biochemical field.

Cerulenin as a Tool for New Hybrid Antibiotics

Further studies on the mechanism of cerulenin action showed that it inhibited biosynthesis of polyketide compounds. We now know that cerulenin inhibits the biosynthesis of polyketide antibiotics such as tetracycline, macrolides, and isochromanequinone antibiotics. Figure 37 shows the biosynthesis of nanaomycin and the site of action of cerulenin. Taking advantage of this mechanism of action, we have obtained a number of hybrid antibiotics (80, 99, 100).

We tried to replace the aglycone of spiramycin with that of tylosin by a hybrid biosynthesis technique (Fig. 38) (80). Cerulenin was added to the culture of the spiramycin producer *S. ambofaciens*. A low concentration of cerulenin was selected so as to inhibit the biosynthesis of the aglycone moiety of spiramycin but not to inhibit the growth of the organism. Then protylonolide, the lactone part of tylosin (which was obtained from a mutant of the tylosin-producing strain *S. fradiae*) was added to yield a new hybrid antibiotic named chimeramycin. Here, we need two microorganisms, *S. ambofaciens* KA-448 and *S. fradiae* KA-427-261, to obtain the new hybrid antibiotic (Fig. 39).

Recently, we were approached by D. A. Hopwood of John Innes Institute and H. Floss of Ohio State University to conduct coresearch work. Our joint study resulted in another new hybrid antibiotic, mederrhodin A (Fig. 40) (18). A gene for the enzyme from *S. coelicolor* which hydroxylates the sixth position of an actinorrhodin precursor was taken, ligated to plasmid vector PIJ922, and transformed into the medermycin-producing strain *Streptomyces* sp. strain AM-7161. One of the transformants, with plasmid PIJ2315 carrying the gene for the hydroxylating enzyme, produced a new hydroxylated medermycin named mederrhodin. Mederrhodin is the first antibiotic obtained by recombinant deoxyribonucleic acid technology thus far.

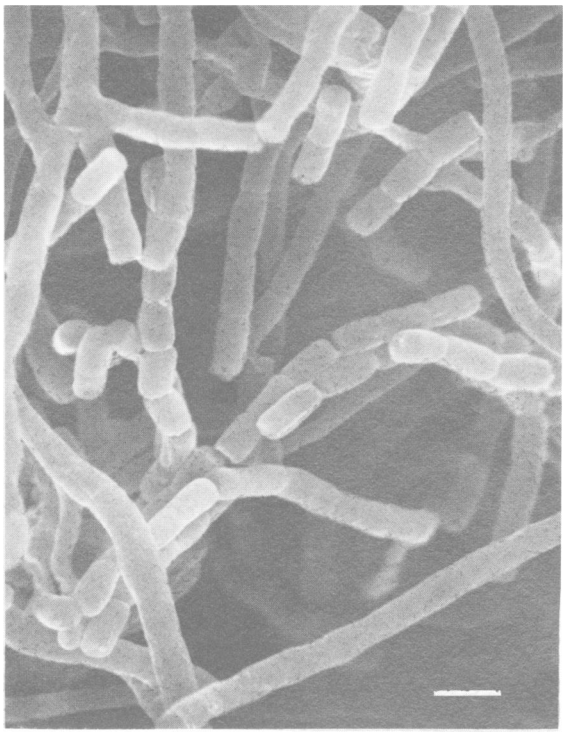


FIG. 28. *S. subflavus* subsp. *irumaensis*. Bar, 1 μm.

GOOD HUMAN RELATIONS IN RESEARCH WORK

The next point is somewhat different from the scientific matters which I have discussed above. However, I believe my achievements would not have been possible without

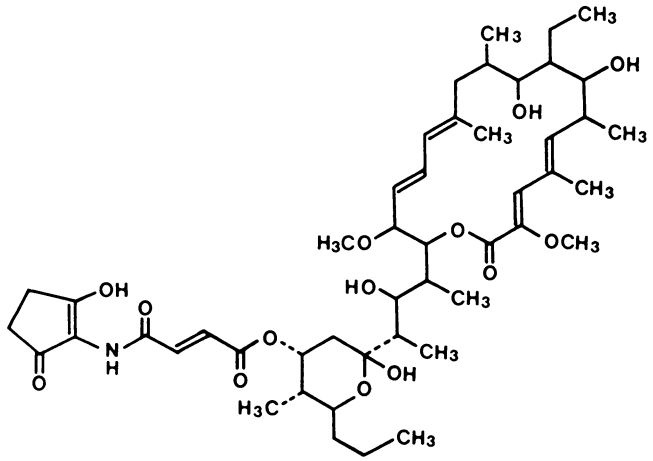


FIG. 29. Structure of virustomycin.

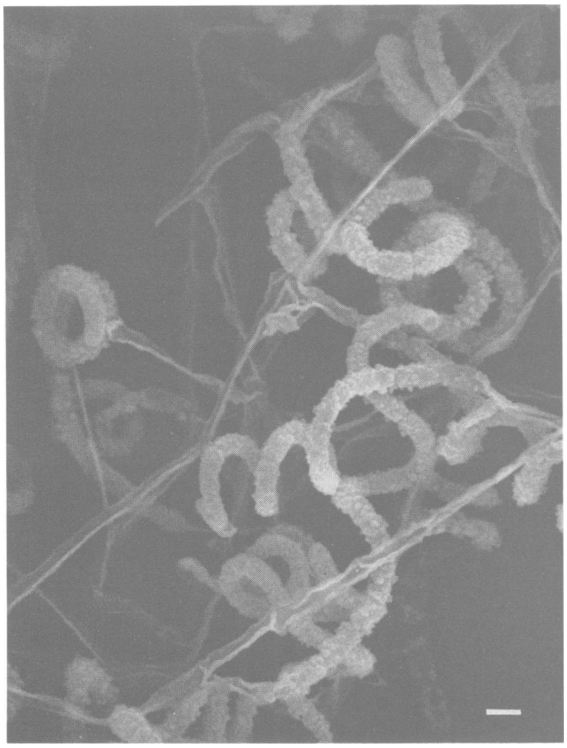


FIG. 30. *Streptomyces* sp. strain AM-2604.

good human relationships. To discover new substances, we need cooperation with many researchers in various fields. I feel that the screening research must be conducted under such circumstances where researchers respect and encourage one another. In our research group at Kitasato, a great effort has been made toward establishing a close relation among chemists, biochemists, microbiologists, and pharmacologists. We exchange opinions, learn techniques from other researchers, and discuss matters with many outside specialists. Such close communication is very important for all of us when we are involved in screening work.

Discovery of Salvarsan and Avermectin

I would like to recall the great international cooperation in the finding of salvarsan, one of the earliest chemotherapeutics (8). Salvarsan is known as a specific chemotherapeutic for syphilis. The discovery of salvarsan in 1909 at George Speyer House in Germany, the first institute for experimental chemotherapy, resulted from international cooperation

TABLE 7. In vitro activity[a] of anthelmintics against *C. elegans*

Anthelmintic(s)	Activity (ppm)
Bephenium	250
Thiophanate, uredofos, methyridine, diethylcarbamazine	100
Morantel, pyrantel, oxantel, rafoxanide, Vydate[b]	50
Oxfendazole, phenothiazine, pyruvinum nitroscanate, Temik,[b] Yaltux,[b] Nemacur[b]	10
Albendazole, fenbendazole, cambendazole, thiabendazole, parbendazole, oxibendazole, mebendazole, levamisole, stilbazium	1
Avermectin B_{1a}	0.1

[a] Simpkin et al. (108).
[b] Plant nematicides.

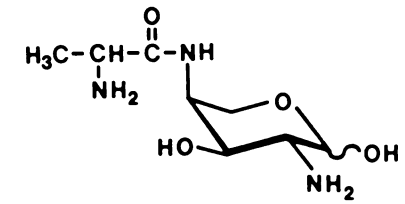


FIG. 31. Structure of prumycin.

between Paul Ehrlich and Sahachiro Hata from the Kitasato Institute. Shibasaburo Kitasato, the founder of the Kitasato Institute, and Paul Ehrlich were old friends, having worked together in their youth in Robert Koch's laboratory. Kitasato sent his assistant, Hata, to his friend, Ehrlich. Ehrlich taught Hata experimental chemotherapy, and Hata instructed Ehrlich's colleagues in the technique of experimental transmission of syphilis in the rabbit. Without either scientist, salvarsan would have never been discovered.

For myself, my research work on antibiotics began 20 years ago under the direction of Toju Hata (Sahachiro Hata's son-in-law), who is the discoverer of the leucomycins and mitomycins. Having friendly relationships with many researchers at home and abroad, I have always been enthusiastic in my research work. I am happy that many of my achievements were accomplished by collaboration with researchers from all over the world. I would like to describe the discovery of avermectin, which is an epoch-making broad-spectrum anthelmintic discovered and developed in cooperation with research groups in the Merck Sharp & Dohme Research Laboratories.

From 1971 to 1973, I conducted research at Wesleyan University in Middletown, Conn., as a visiting professor. I was invited by Max Tishler via an introduction by the late Yukimasa Yagisawa, the former executive director of the Japanese Antibiotic Research Association. Through Max Tishler, I was introduced to H. Boyd Woodruff, David Hendlin, Edward Stapley, and other researchers of the

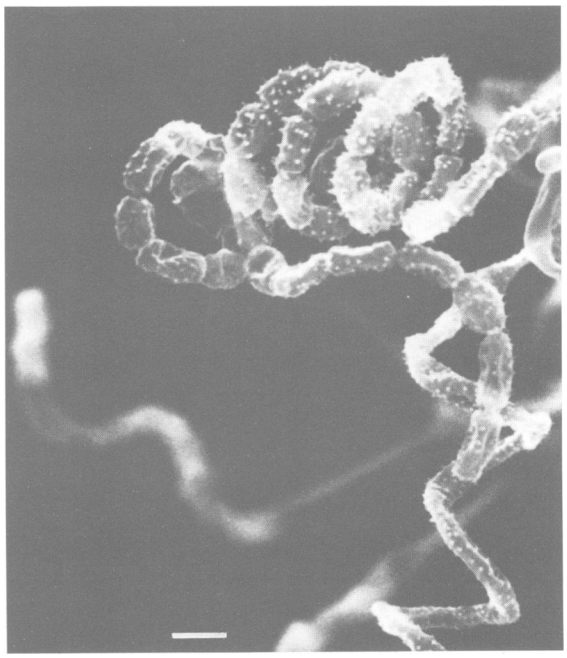


FIG. 32. *S. kagawaensis*. Bar, 1 μm.

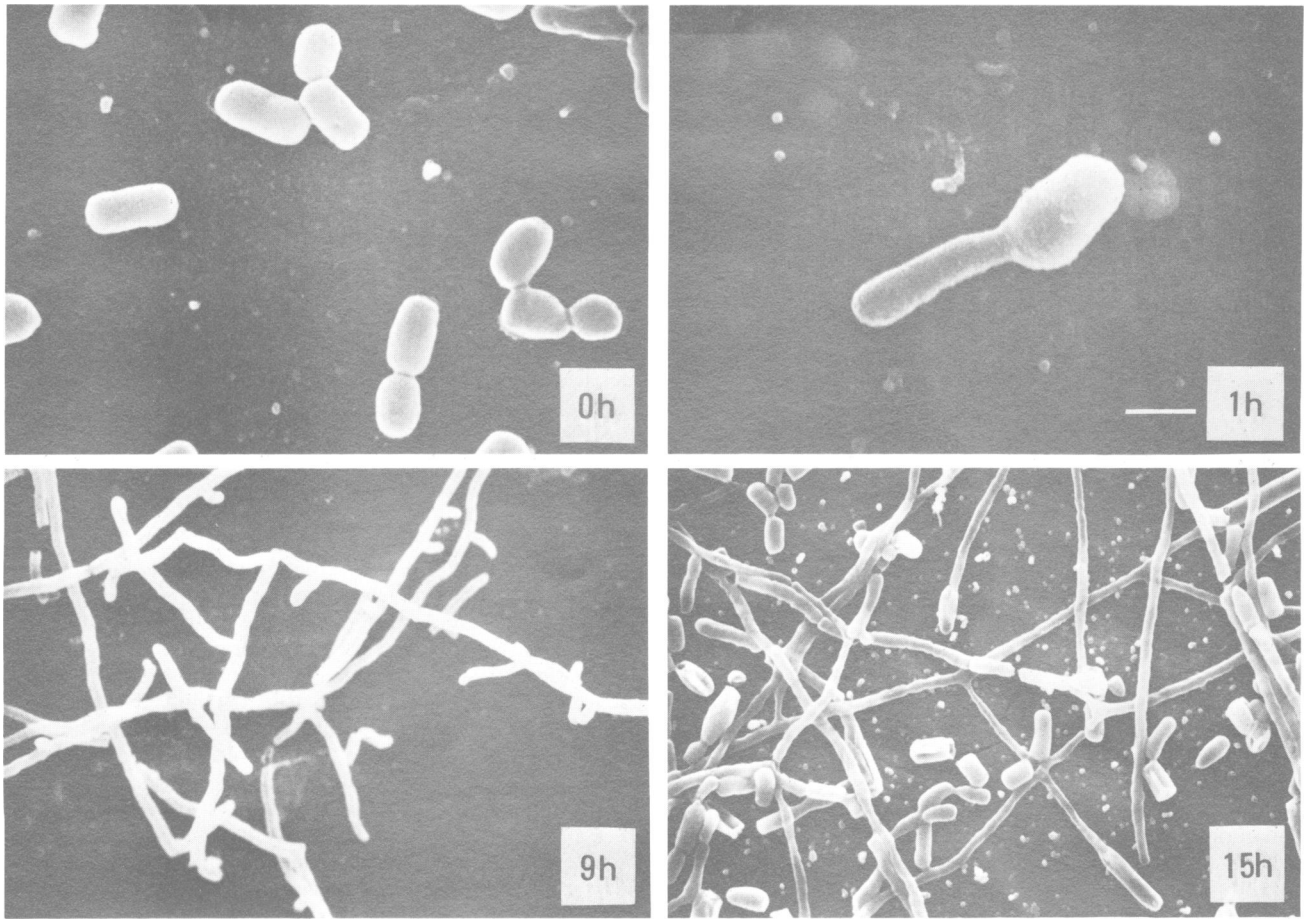


FIG. 33. Morphological change in *K. setae* in submerged culture. Bar, 1 μm.

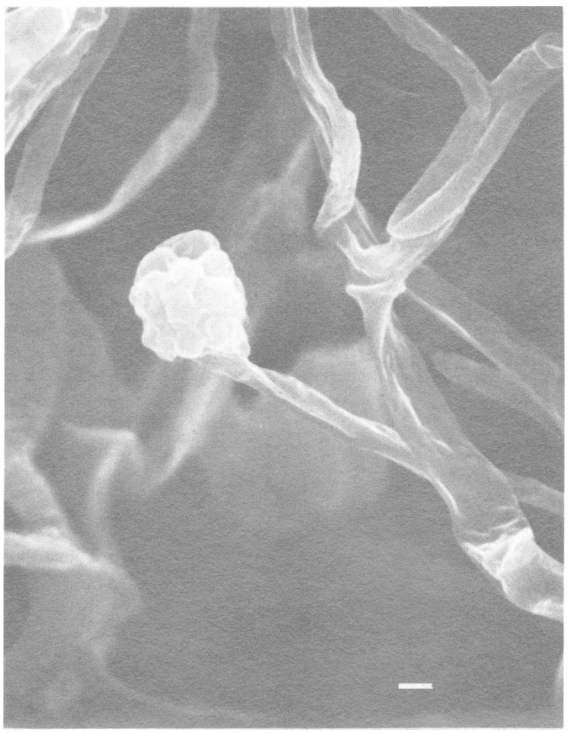


FIG. 34. *Cephalosporium caerulens*. Bar, 1 μm.

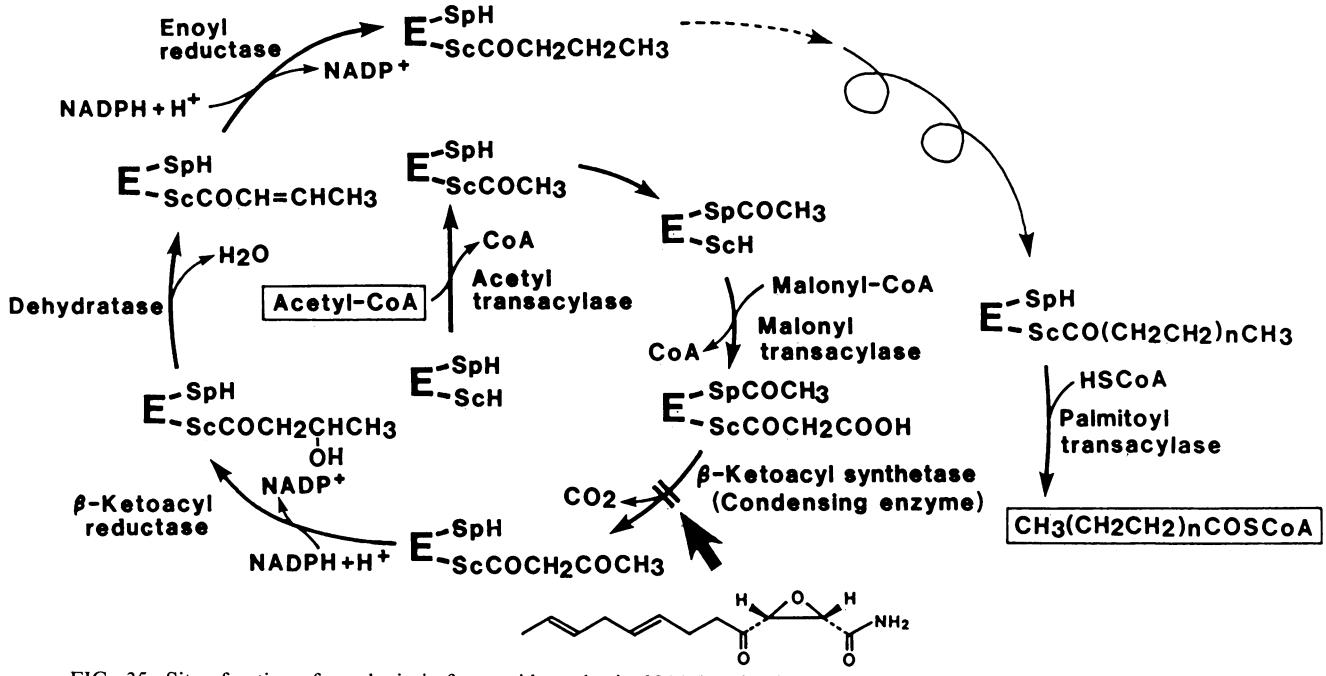
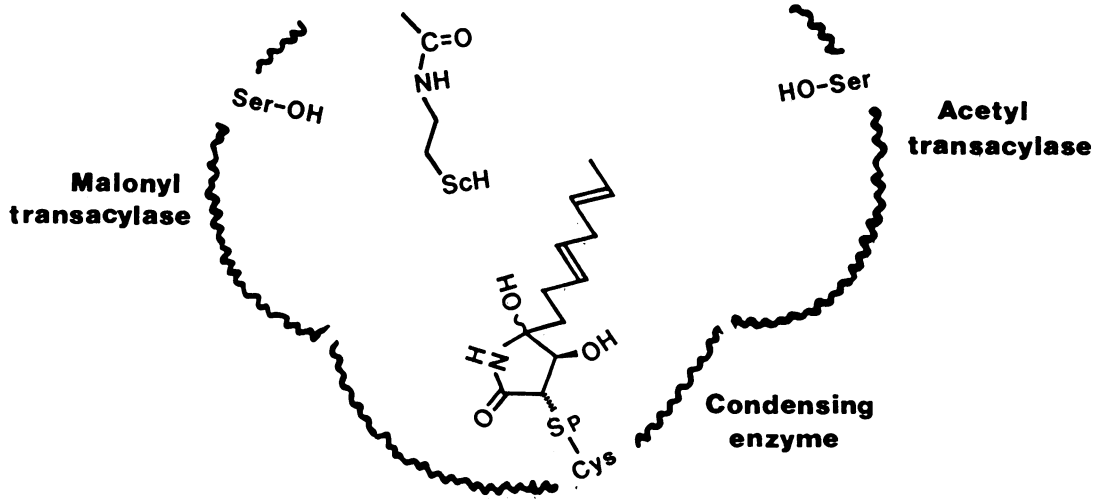


FIG. 35. Site of action of cerulenin in fatty acid synthesis. NADP, Nicotinamide adenine dinucleotide; CoA, coenzyme A.



Thr-Pro-Val-Gly-Ala-Cys : S. cerevisiae Lynen et al. (35)

Tyr-Gln-Val-Gln-Ser-Cys-Pro-Ile-Leu-Glu-Gly-Lys : C. caerulens

FIG. 36. Hypothetical model of interaction between cerulenin and fatty acid synthetase.

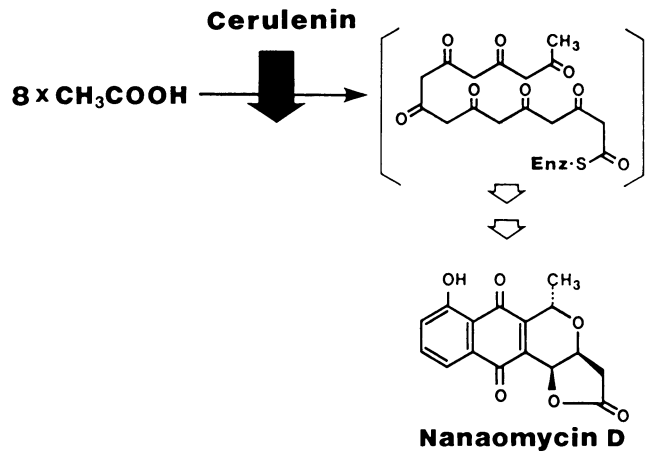


FIG. 37. Inhibition of nanaomycin biosynthesis by cerulenin.

Merck Sharp & Dohme Co. in Rahway, N.J. Immediately after returning to the Kitasato Institute, we started research work in cooperation with the Merck Sharp & Dohme research group. This coresearch has been very important and fruitful, because it led us to the discovery of avermectin (3). Without such a close relationship which linked the researchers on both sides of the Pacific, the discovery of avermectin would have been impossible.

I would like to stress here again that behind an important discovery of a new substance, there is always a good human relationship. I am strongly convinced that such a relation is one of the major factors in the successful discovery and development of an excellent drug.

Avermectins

The producing organism of avermectin, *S. avermitilis* (Fig. 41), was isolated in our laboratory and its excellent activity was found at the Merck Sharp & Dohme laboratory in a screening program using mice infected with *Nematospiroides dubius* (3). The place where we collected the soil sample containing the novel actinomycete strain was close to the Kawana Golf Club in Shizuoka Prefecture, Japan. Among many active components (Fig. 42), avermectins B_{1a} and B_{1b} showed the highest activity. They were chemically reduced to dihydro derivatives with low toxicity. A mixture of dihydro derivatives was named ivermectin and is now being applied as an anthelmintic and an anti-mite drug for livestock. Recent clinical evaluation of ivermectin has suggested a great efficacy in the control of onchocerciasis in humans (2, 13, 36). Research of the Merck Veterinary Institute has shown efficacy of ivermectin against endo- and exoparasites in horses, cattle, swine, and sheep (7). Table 7 shows how small a quantity of this antibiotic is effective. At a concentration of 1/1,000 that of diethylcarbamide, avermectin has a lethal effect on *Caenorhabditis elegans*, a soil nematode, in vitro.

The mode of action of avermectin has been studied by many researchers (9). Avermectin shows antiparasite activity against levamisole-resistant *C. elegans*. This indicates that avermectin is not a nicotinic agonist. The inhibitory action of avermectin on the neuron in *Ascaris suum* is reversed by picrotoxin, an antagonist of γ-aminobutyric acid. These experimental data suggest that avermectin stimulates chloride ion conductance to block neuromuscular transmission (117).

PERSPECTIVE

Recently it has been said that discovery of new antibiotics is becoming more and more difficult. However, the number of newly discovered antibiotics is increasing year by year. I believe this tendency will continue. From our past experience, we can see that one-third of the soil isolates tested produce antimicrobial substances. This productivity of antibiotics was observed by using two to three culture media under conventional incubation conditions. I hope that our further studies on fermentation physiology will allow us to discover the conditions which allow nonproducing strains to become producing strains.

More than 6,000 antibiotics have been discovered so far. As I earlier explained the differing physiological activities of erythromycin, it should be very interesting to study activities other than antibacterial activities. It will be possible to discover biologically active substances other than antibacterial agents only if new screening systems are established. For the future, I feel that establishing new screening systems

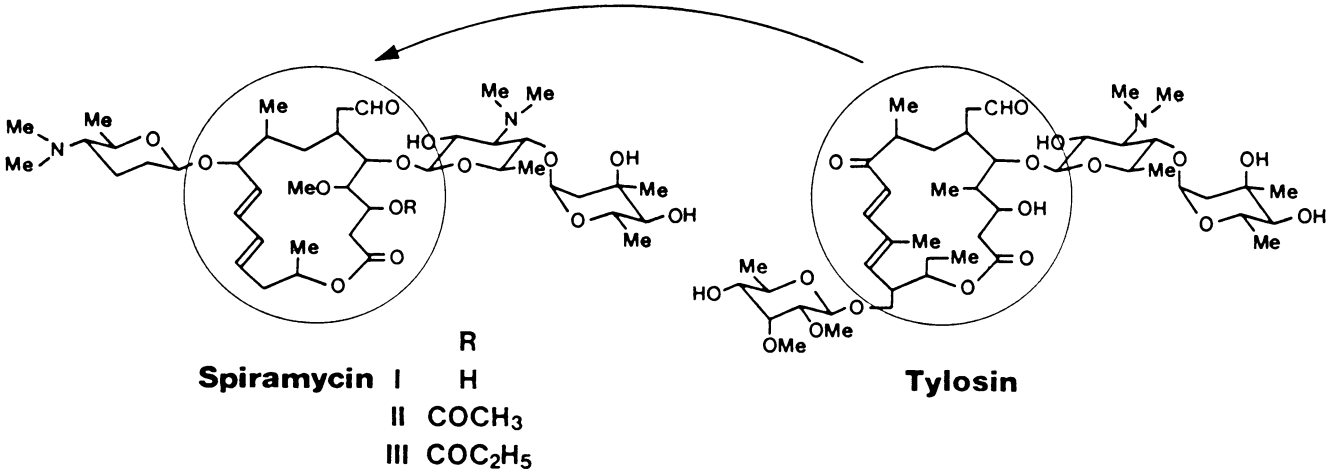


FIG. 38. Structures of spiramycins and tylosin. The aglycone of spiramycin was replaced with that of tylosin by the hybrid biosynthesis technique.

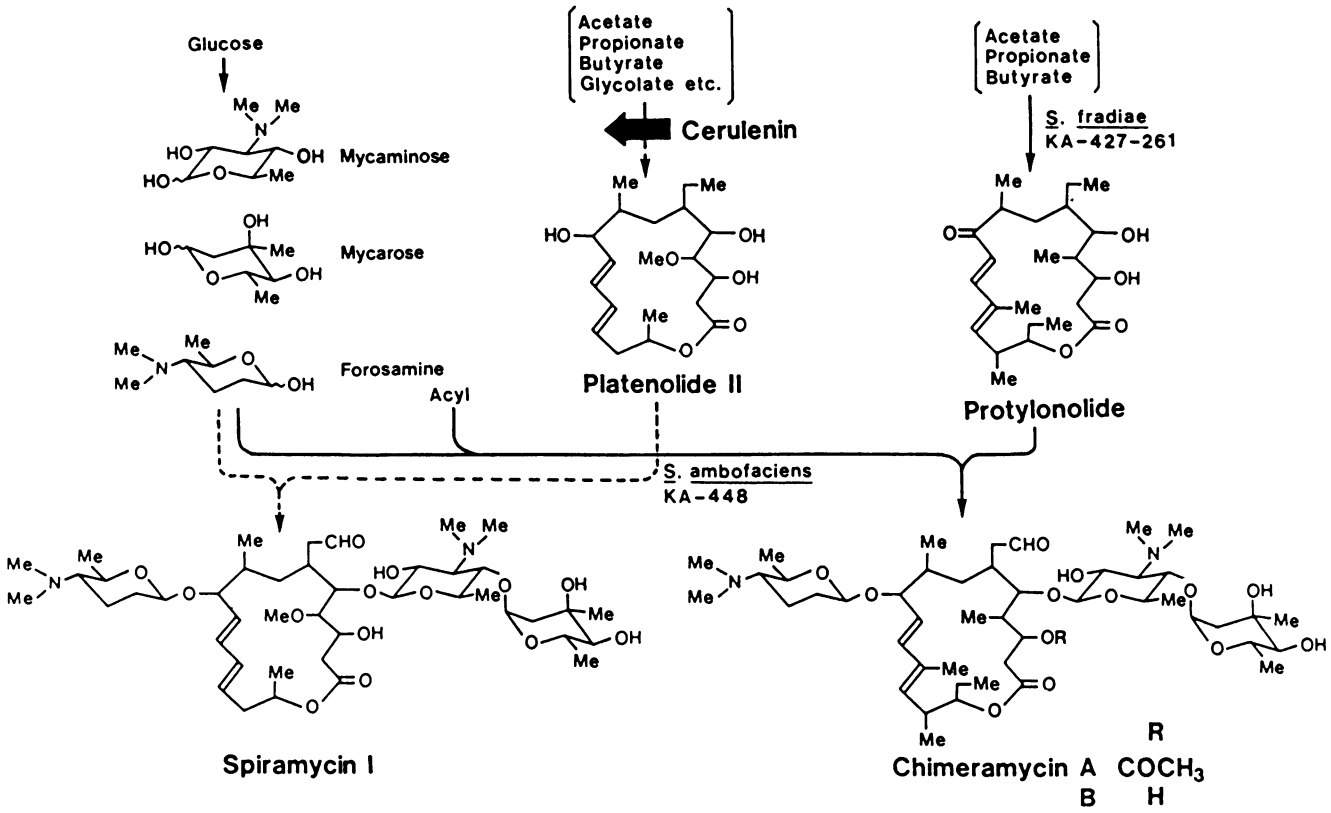


FIG. 39. Hybrid biosynthesis of chimeramycins.

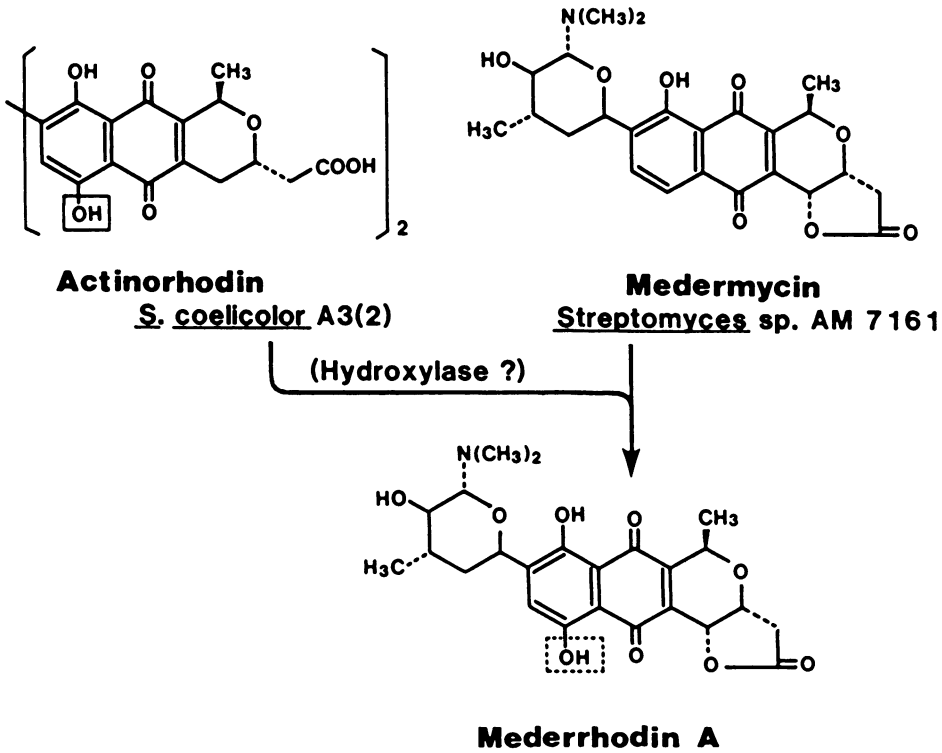


FIG. 40. Hybrid biosynthesis of mederrhodin A.

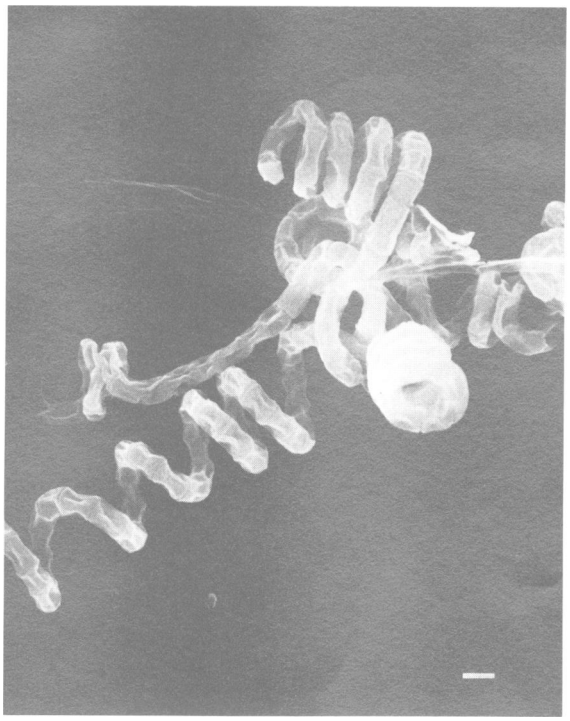


FIG. 41. *S. avermitilis*. Bar, 1 μm.

by using living cells of higher forms such as nematoda and animal cells is most interesting. Many more biochemists and physiologists are needed to take part in the screening for physiologically active substance from microorganisms.

The development of technology during the past decade is remarkable, e.g., high-pressure liquid chromatography for extraction and purification and nuclear magnetic resonance spectrometry for structure elucidation. These techniques have made it possible to isolate and analyze easily a very small quantity of an active substance. If we apply the most advanced technology, i.e., gene manipulation techniques, to microorganisms, there will be an infinite possibility of microorganisms producing useful substances.

Human beings earnestly desire medicines effective against many serious diseases such as rheumatism, heart disease, and hypertension, as well as antimicrobial agents effective against fungi, many kinds of antibiotic-resistant bacteria, and parasites. I am determined to make every effort to find new drugs from microorganisms, because I believe such research work is my social responsibility as a member of society. I truly believe it will be beneficial for the future of society.

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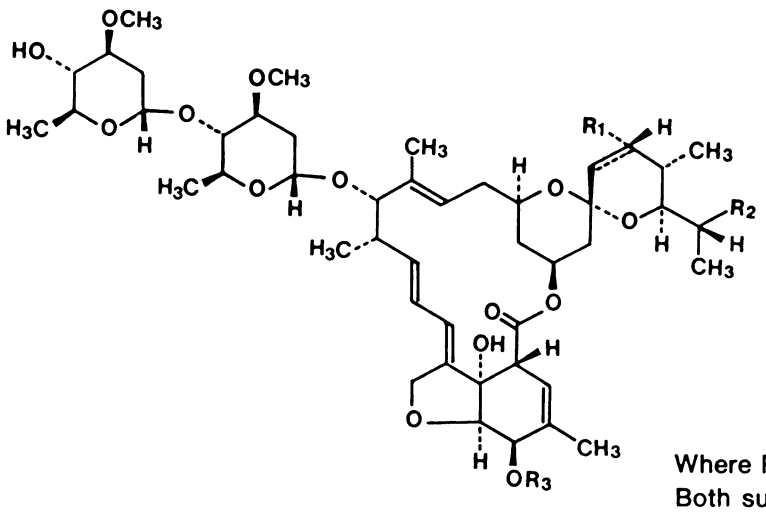

	R_1	R_2	R_3
A 1a		C_2H_5	CH_3
A 1b		CH_3	CH_3
A 2a	OH	C_2H_5	CH_3
A 2b	OH	CH_3	CH_3
B 1a		C_2H_5	H
B 1b		CH_3	H
B 2a	OH	C_2H_5	H
B 2b	OH	CH_3	H

Where R_1 is absent, the double bond (⋯) is present.
Both sugars are α-L-oleandrose.

FIG. 42. Structure of avermectin.

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