FORTIMICINS A AND B, NEW AMINOGLYCOSIDE ANTIBIOTICS

I. PRODUCING ORGANISM, FERMENTATION AND BIOLOGICAL PROPERTIES OF FORTIMICINS

TAKASHI NARA, MITSUYOSHI YAMAMOTO, ISAO KAWAMOTO, KENICHIRO TAKAYAMA, RYO OKACHI, SEIGO TAKASAWA, TOMOYASU SATO and SEIJI SATO

Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
3-6-6, Asahimachi, Machidashi, Tokyo, Japan

(Received for publication April 13, 1977)

A culture of *Micromonospora* species MK-70 was found to produce two new antibiotics, fortimicins A and B. Antibacterial and paper chromatographic data on an eluate from IRC-50 treatment of fermentation beers indicated that fortimicins A and B are new antibiotics with broad-spectrum, basic and water-soluble properties. Fortimicin A exhibited potent, unique, broad-spectrum antibacterial activity against Gram-positive and negative bacteria both *in vitro* and *in vivo*, while fortimicin B was only weakly active.

In the course of screening for new antibiotics, a complex of antibacterial antibiotics was obtained from the culture broth of *Micromonospora* sp. MK-70. This antibiotic complex XK-70 contained two main components designated as XK-70-1 and XK-70-A, both of which exhibited broad-spectrum antibacterial activities. Subsequent studies showed that the two antibiotics are new aminoglycosides, as reported both in the present report and in other papers describing isolation, characterization and structure determination[1,2]. Therefore we designated XK-70-1 and XK-70-A as fortimicin A and fortimicin B, respectively. This paper deals with the producing organism, fermentation and the biological properties of fortimicins A and B.

1. Producing Organism

The producing organism MK-70 was isolated from a soil in Hiroshima City, Hiroshima Prefecture, Japan.

This organism has been deposited at the American Type Culture Collection, Rockville, Maryland, U.S.A., and has been assigned accession number 21819.

The culture on ISP No. 2 medium (yeast extract 0.4%, malt extract 1%, glucose 0.4% and agar 2%) shows growth typical of the genus *Micromonospora*.

Macroscopically, well-developed colonies appear after a few days incubation at 30°C. Colonies are dark yellow to light wheat turning to olive or dark green with formation of spores. There are no aerial mycelia. Sporulation requires about 2 weeks incubation at 30°C.

Microscopically, the mycelium is long, branched and about 0.5 μ in diameter. The spores are sessile or born singly on short sporophores from substrate mycelia, about 1.0 μ in diameter, ovoid to spherical in shape, and bluntly spiny-surfaced (Plates 1 and 2).

Besides the strain MK-70, several other strains capable of producing fortimicins were isolated from soils widely distributed in the world. They were from soils in Hiroshima Prefecture, Japan, Ibaragi Prefecture, Japan, North Chicago, U.S.A. and West Virginia, U.S.A. All of them belong to the genus

Plate 1. Electron micrograph of *Micromonospora* sp. MK-70

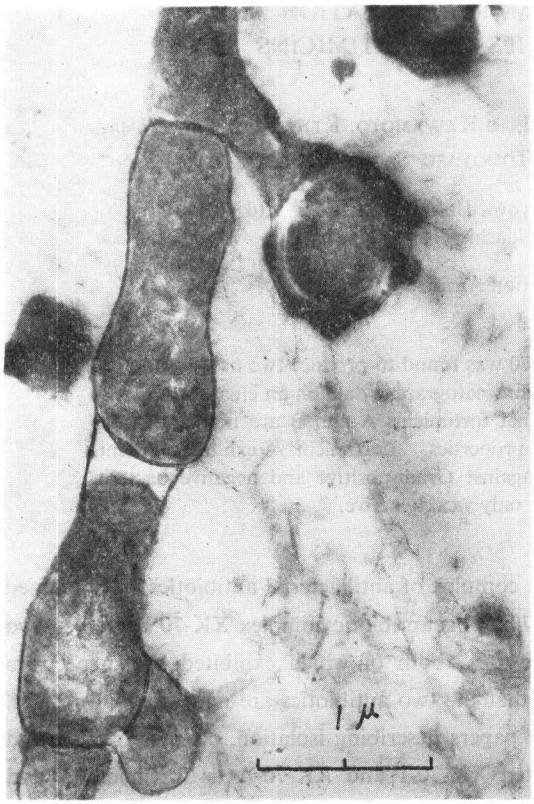
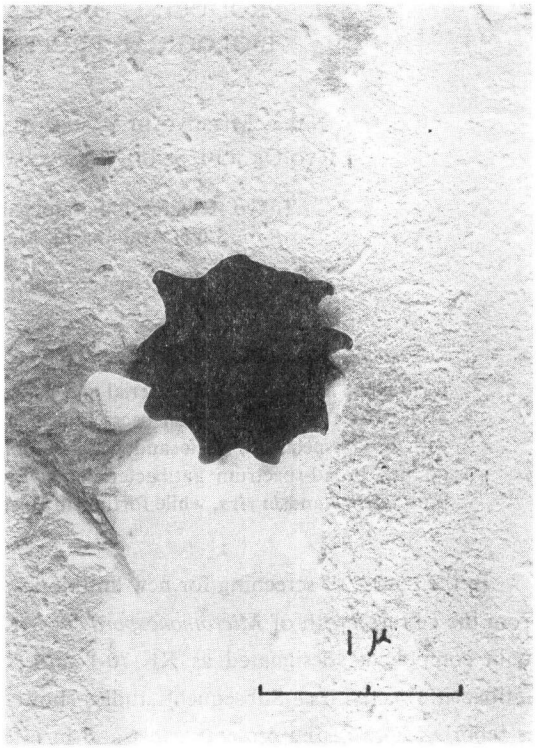


Plate 2. Electron micrograph of *Micromonospora* sp. MK-70 spore.



Micromonospora. Further taxonomical details on MK-70 and these other strains will be reported elsewhere.

2. Fermentative Production

Because of its low productivity, we undertook mutation work to obtain a high-yield mutant from MK-70. A mutant designated G-518 was obtained by UV irradiation and used in the following investigation.

The composition of seed medium was: Soluble starch 2%, glucose 0.5%, Polypepton (casein hydrolysate) 0.5%, Polypepton-S (soy bean hydrolysate) 0.5%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.1% in deionized water. The pH was 7.2 before autoclaving. The composition of fermentation medium initially used was: Soluble starch 3%, Ebios (dried yeast) 3%, K_2HPO_4 0.3%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.2% and $CaCO_3$ 0.1% in deionized water, pH 7.2 before autoclaving. All media were sterilized by autoclaving at 120°C for 15 minutes. The inoculum from the G-518 strain was prepared by cultivation in the above seed medium, 30 ml per 250-ml Erlenmeyer flask. After 3 days on a rotary shaker at 30°C, the inoculum, 10% by volume, was transferred to 250-ml Erlenmeyer flasks containing 30 ml of the fermentation medium. Fermentation was carried out at 30°C for 4~7 days with agitation at 230 r.p.m.

Every day during the fermentation period, two or three flasks were removed from a shaker for

Table 1. Effects of carbon sources on the formation of fortimicins.

Carbon[a] source	pH	Growth[b] (%)	Fortimicins[c] (mcg/ml)
Sol. starch	6.7	32	150
Dextrin	6.8	30	90
D-Raffinose	6.9	8	<30
Sucrose	7.1	9	<30
D-Maltose	6.9	8	<30
D-Galactose	6.8	9	<30
D-Glucose	6.8	8	<30
D-Mannose	6.8	9	<30
D-Arabinose	7.2	9	<30
Mannitol	7.2	9	<30
Lard oil	6.8	18	<30
Soybean oil	6.9	10	<30

a) Ebios was used as nitrogen source.
b) Packed cell volume
c) Agar diffusion assay

Table 2. Effects of nitrogen sources on the formation of fortimicins

Nitrogen[a] source	pH	Growth (%)	Fortimicins (mcg/ml)
Polypepton-S	7.0	30	110
Polypepton	7.2	14	40
NZ-amine A	8.6	8	<30
Bacto-tryptone	8.2	8	<30
Ebios	7.1	40	130
Yeast extract	7.6	10	50
Malt extract	6.4	2	<30
Soybean meal	7.0	11	<30
Casamino acid	8.8	4	<30
Corn steep liq.	8.0	19	40

a) Soluble starch was used as carbon source.

Table 3. Combination effect of Polypepton-S with Ebios on production of fortimicins

Nitrogen source added*	Fermentation time**	pH	Growth	Yield (mcg/ml)
none	5 days	6.9	55%	170
Polypepton-S				
0.5%	4	6.6	50	250
1.0%	4	6.6	50	230
2.0%	4	7.5	60	230

* Each concentration of Polypepton-S indicated above was added to the basal fermentation medium (soluble starch 4%, Ebios 4%, K_2HPO_4 0.3%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.2% and $CaCO_3$ 0.1%).
** The same as Table 2.

assay. The yield figures in Tables 1~4 are average values of data obtained from these two or three flasks. Growth was determined by measurement of volume ratio of mycelia in the broth centrifuged at 2,500 r.p.m. for 10 minutes. The antibiotic activity of beer supernatant was measured by the agar diffusion assay using *Escherichia coli* KY 4271 as test organism and a nearly pure fortimicin A sample as assay standard.

Effect of Carbon and Nitrogen Sources

Among various carbon sources, soluble starch was most effective for growth and antibiotic production, followed by dextrin (Table 1). Other sugars and oils were quite inferior to these two carbohydrates. Among the nitrogen sources tested, Ebios (dried yeast prepared by Ebios Yakuhin Kogyo Co., Ltd., Japan) and Polypepton-S (soy bean hydrolysate prepared by Daigo Eiyo Kagaku Co., Ltd., Japan) showed the best effect on growth and antibiotic production, as shown in Table 2. A combination of these two nitrogen sources resulted in higher yields than those in case of single additions (Table 3). Several other kinds of protein hydrolysate were tested, but none of them showed more stimulatory effect than Polypepton-S. Based on these data, a fermentation medium consisting of soluble starch 4%, Ebios 4% and Polypepton-S 0.5% was selected.

Effect of Metal Ions

Following the study on carbon and nitrogen sources, the influence of metal ions in the fermentation medium was investigated. All of them showed some effect on antibiotic production, and their optimum concentrations were found out as follows: NaCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.2%, K_2HPO_4 0.2% and $CaCO_3$ 0.1% (Table 4).

Fermentation Time Course

Table 4. Effects of metal ions on the formation of fortimicins

Metals	%	pH	Growth (%)	Fortimicins (mcg/ml)
$CaCO_3$	0	7.5	69	135
	0.05	7.3	65	160
	0.1	7.0	60	200
	0.2	7.3	58	185
$MgSO_4 \cdot 7H_2O$	0	6.4	59	160
	0.05	6.5	57	210
	0.2	6.6	55	230
	0.5	6.5	53	200
NaCl	0	7.4	70	160
	0.05	7.2	68	200
	0.2	6.7	60	220
	0.5	6.5	62	170
KH_2PO_4 + K_2HPO_4	0 + 0	7.2	45	200
	0 + 0.05	7.2	48	240
	0 + 0.2	7.2	52	270
	0 + 0.4	6.9	55	240
	0.1 + 0.3	6.8	52	235

Basal medium: Sol. starch 4%, Ebios 4%, Polypepton-S 0.5%, K_2HPO_4 0.3%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.2%, $CaCO_3$ 0.1%.

Table 5. Antibacterial spectrum of fortimicins in IRC-50 eluate

Test organisms	Dilution units	
	Assay at pH 8.0	Assay at pH 7.0
Bacillus subtilis KY 4273	2,500	320
Staphylococcus aureus KY 4279	1,280 (2,500)	160 (320)
Streptococcus faecalis KY 4280	< 10	< 10
Escherichia coli KY 4271	640 (2,500)	80 (160)
E. coli KY 8310	80 (160)	10
Klebsiella pneumoniae KY 4275	640 (1,280)	80 (160)
Pseudomonas aeruginosa KY 4276	10 (20)	< 10
Proteus vulgaris KY 4227	320 (640)	40 (80)
Shigella sonnei KY 4281	160 (320)	10 (20)
Salmonella typhosa KY 4278	640 (1,280)	80 (160)

Fig. 1. Time course of fermentation

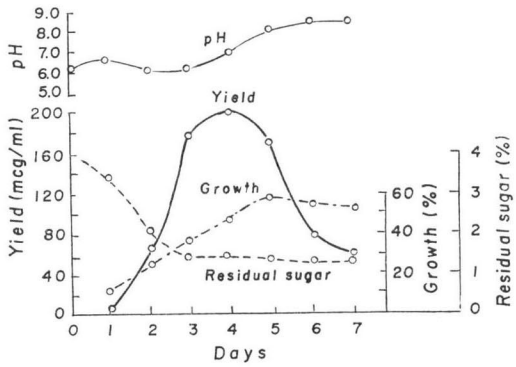


Fig. 2. Paper chromatography

Paper: Whatman #1 Developed ascendingly for 20 hours at 28°C.

Solvent: Lower phase of $CHCl_3$ - CH_3OH - NH_4OH (2: 1: 1, v/v)

Bioautographed on *E. coli*

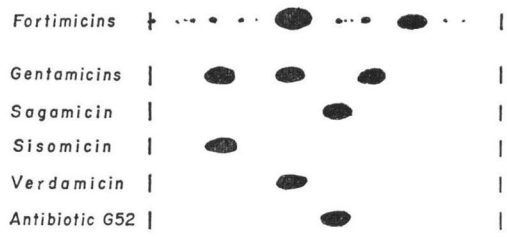


Table 6. Paper chromatography of an IRC-50 eluate

Solvents	Rf*	Development (hours)
20% (w/v) Ammonium chloride	0.98	3
Water-saturated *n*-butanol	0.02	15
n-Butanol - acetic acid - water (3: 1: 1)	0.10	15
Water-saturated ethylacetate	0.00	3
Water-saturated *n*-butanol containing 2% (w/v) of *p*-toluene sulfonic acid and 2% (w/v) of piperidine	0.03	15

* Bioautographed on *B. subtilis*

Based on the above data, the following fermentation medium was established: Soluble starch 4%, Ebios 4%, Polypepton-S 0.5%, NaCl 0.2%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.2% and $CaCO_3$ 0.1% in deionized water, adjusted to pH 7.2 before autoclaving. A fermentation time course in this medium is shown in Fig. 1. The consumption of soluble starch stopped on the 4th day with more than 1% of it remaining in the medium. Growth reached its maximum on the 5th day and antibiotic activity showed its peak on the 4th day just before the growth maximum. On the 4th day, pH started to rise

and on the next day it was beyond 8.0. Fortimicin A is unstable under alkaline conditions, losing glycine and changing thereby to fortimicin B[1,2]. Fortimicin B is only weakly active as compared with fortimicin A, as reported later. This may be the cause of a sudden decrease of total antibiotic activity after 4th day, when the pH of the fermented broth turned to the alkaline side. On days 5, 6 and 7, the fortimicin A concentration continued to decrease and that of B increased as observed by paper chromatography in the system used for the separation of the gentamicin C complex[3], followed by bioautography. Further studies on these phenomena will be reported elsewhere.

3. Antibacterial Properties of Eluate from IRC 50

Fermentation beers of *Micromonospora* species MK-70 in Erlenmeyer flasks were collected and adjusted to pH 7.5 with dil. HCl. After removing the mycelium by filtration, the filtrate was passed through IRC 50 (NH_4^+). Fortimicins thereby adsorbed to the resin were eluted with 0.5 N NH_4OH[1]. The eluate was concentrated *in vacuo* and its antibacterial properties were studied. The antibacterial spectrum of the eluate is shown in Table 5. The spectra show a broad-range activity against various Gram-positive and negative bacteria. These activities were enhanced when bioassayed at pH 8.0 rather than at pH 7.0 or less. This is in agreement with the basic property of the fortimicins.

As shown in Table 6, paper chromatograms obtained with five solvent systems indicated that the antibiotics are water-soluble and insoluble in organic solvent. A paper chromatographic system used for the separation of the gentamicin C

Table 7. Antibacterial *in vitro* activities of fortimicins A and B

Test organism	M.I.C. (mcg/ml)	
	FM-A	FM-B
Bacillus subtilis KY4273	0.02	104
Staphylococcus aureus ATCC 6538P	0.04	6.6
Streptococcus faecalis ATCC 10541	10	>200
Escherichia coli ATCC 26	0.16	13
E. coli KY8310*	0.13	52
Klebsiella pneumoniae ATCC 10031	0.08	26
Pseudomonas aeruginosa KY4276	5	>200
Proteus vulgaris ATCC 6897	0.16	26
Shigella sonnei ATCC 9290	0.3	52
Salmonella typhosa ATCC 9992	0.08	13

* Resistant to chloramphenicol, gentamicin, kanamycin, streptomycin, paromomycin, nebramycin, tobramycin, spectinomycin and tetracycline.

Table 8. Comparative antibacterial activities of fortimicin A and other aminoglycosides

Test organisms	M.I.C. (mcg/ml)				
	FM-A	KM	SGM	TBM	GM
Staphylococcus aureus ATCC 6538P	0.04	0.08	0.016	0.008	0.02
Escherichia coli ATCC 26	0.16	0.32	0.04	0.04	0.04
Klebsiella pneumoniae ATCC 10031	0.08	0.08	0.02	—	0.02
Proteus inconstans ATCC 9886	0.16	0.32	0.08	0.08	0.08
P. mirabilis Finland 9	0.64	0.32	0.16	—	0.16
P. morganii Jenkins	0.32	0.16	0.08	—	0.08
P. rettgeri Booth	0.16	0.16	0.32	—	0.32
Providencia stuartii ATCC 25826	0.16	0.32	1.3	0.32	1.3
Pseudomonas aeruginosa KY 4276	10	20	1.3	0.64	1.3
Salmonella typhosa ATCC 9992	0.16	0.16	0.04	0.04	0.04
Serratia marcescens 177VA	0.08	1.28	0.02	—	0.04
Shigella sonnei ATCC 9290	0.32	0.32	0.08	0.08	0.08

FM-A; Fortimicin A, KM; Kanamycin A, SGM; Sagamicin, TBM; Tobramycin, GM; Gentamicin.

Table 9. *In vitro* activities of fortimicin A and other aminoglycoside antibiotics against resistant organisms

Test organisms	Inactivating enzymes	M.I.C. (mcg/ml)			
		FM-A	KM	SGM	TBM
Escherichia coli ATCC 26	—	0.16	0.32	0.26	0.065
E. coli KY 8302	APH(3′)-I	0.16	>20.8	0.52	0.13
E. coli KY 8321	APH(3′)-II ANT(2″)	0.08	>20.8	1.04	0.52
E. coli KY 8327	ANT(2″)	0.32	20.8	8.34	2.08
E. coli KY 8332	AAC(6′)-I	0.04	1.3	0.016	0.032
E. coli KY 8348	AAC(3)-I	>20.8	0.04	4.2	0.008
E. coli KY 8349	APH(3′)-I	0.08	>20.8	0.04	0.04
Providencia sp. 164	AAC(2′)-I	0.63	>20.8	8.3	1.25
Serratia marcescens POE 1065	AAC(6′)	0.32	>20.8	0.32	8.3
Pseudomonas aeruginosa KY 8511	AAC(3)-I	>100	>100	100	0.78
P. aeruginosa KY 8563	AAC(3)-II	50	>100	>100	>100

components[3] gave the result shown in Fig. 2. The eluate from IRC 50 resin contained two major components and several minor components. Details of the minor components will be reported elsewhere. Fortimicin B is not identical with any component of the gentamicins. Though fortimicin A showed the same Rf value as gentamicin C_2 in the above system, it was distinct from gentamicin C_2 in some other chromatographic systems, as reported in a subsequent paper[1].

Table 10. Comparative *in vivo* activities of fortimicin A, kanamycin and sagamicin.

Infectious organisms	Challenge dose (cells/mouse, ip)	PD_{50} (mg/kg, sc)		
		FM-A*	KM-A	SGM
S. aureus Smith	2×10^4	3.7	4.5	1.2
E. coli Juhl	2×10^6	5.8	6.7	1.7

* LD_{50} of fortimicin A sulfate (mg/kg) iv, mice 380; sc, mice 400 (BEHRENS-KÄRBER method)

These results suggest that fortimicins A and B are probably new aminoglycoside antibiotics. More detailed comparisons with known aminoglycosides will be reported in the succeeding report[1].

4. *In vitro* and *In vivo* Activities of Fortimicins A and B

The next three tables (Tables 7~9) show the *in vitro* antibacterial activities of fortimicins. These bioassay results were obtained at pH 8 in order to increase sensitivities.

As shown in Table 7, fortimicin A exhibits a potent, broad-spectrum antibacterial activity against Gram-positive and negative bacteria, while fortimicin B is rather weakly active. As shown in the succeeding paper[2], fortimicin A is 4-N-glycyl fortimicin B. Therefore it is interesting that the removal of glycine from fortimicin A reduced antibiotic activity markedly.

Table 8 shows comparative data with four other aminoglycosides, kanamycin, sagamicin[4,5,6], tobramycin and gentamicin. Although fortimicin A is less active than sagamicin and tobramycin, it is equal or slightly superior to kanamycin in activity. It is interesting to note that, although fortimicin A is structurally a disaccharide, it is comparable to or a little superior to the trisaccharide kanamycin.

Table 9 compares the activities of fortimicin A, kanamycin, sagamicin, and tobramycin *in vitro* against resistant organisms known to possess various aminoglycoside-inactivating enzymes. Fortimicin was found to be active against organisms possessing aminoglycoside 3′-phosphotransferases I and II [APH (3′)-I and APH (3′)-II], aminoglycoside 2″-nucleotidyltransferase [ANT(2″)][7], aminoglycoside 6′-N-acetyltransferase [AAC(6′)] and aminoglycoside 2′-N-acetyltransferase [AAC(2′)-I].

Because fortimicin A, like sagamicin and tobramycin, has no 3′-hydroxyl group, it would be expected to be active against *E. coli* KY 8302, 8321, and 8349 possessing APH(3′)-I and APH(3′)-II. Furthermore, lacking a sugar corresponding to the 3-aminoglucose of tobramycin and the garosamine of sagamicin, fortimicin A should be active against *E. coli* KY 8327 possessing ANT(2″).

Somewhat unexpectedly in view of its 6′-amino group, fortimicin A is highly active against organisms possessing AAC(6′), such as *E. coli* KY 8332 and *Serratia marcescens* POE 1065. The latter strain is quite resistant to kanamycin and tobramycin. Also unexpectedly, fortimicin A is quite active against *Providencia* sp. 164, a strain which possesses AAC(2′)-I and is quite resistant to kanamycin and sagamicin.

However it is of extreme interest that *E. coli* KY 8348 possessing aminoglycoside 3-N-acetyltransferase [AAC(3)][8] is resistant to fortimicin A, as it is to sagamicin, while kanamycin and tobramycin are active against this organism. This enzyme has been reported to inactivate only the gentamicin-sagamicin-sisomicin group antibiotics and seldomycin factor 5[9,10,11,12], but not DKB(3′,4′-dideoxykanamycin B), tobramycin and amikacin. Since fortimicin A is structurally quite different from the gentamicin-sagamicin-sisomicin group, it is interesting to see that the aminoglycoside 3-N-acetyltransferase [AAC(3)] so far thought to have a very high specificity for substrates seems to inactivate both fortimicin A and seldomycin factor 5 which are structurally quite different from the gentamicins and from each other*.

Results of the *in vivo* activity tests and the acute toxicity test of fortimicin A are shown in Table 10. Against *Staphylococcus aureus* Smith and *E. coli* Juhl, fortimicin A is comparable to kanamycin in activity but is less active than sagamicin.

In view of its antibacterial activity *in vitro* and *in vivo* and its low acute toxicity, fortimicin A may become a medically useful antibiotic.

Acknowledgements

The authors are very grateful to Dr. SINCLAIR and his associates of Abbott Laboratories, U.S.A. for their kind advice and encouragement. We are also thankful to Dr. G. H. WAGMAN of Schering Corp., U.S.A. for the gifts of gentamicin components as reference antibiotics.

References

1) OKACHI, R.; S. TAKASAWA, T. SATO, S. SATO, M. YAMAMOTO, I. KAWAMOTO & T. NARA: Fortimicins A and B, new aminoglycoside antibiotics. II. Isolation, physico-chemical and chromatographic properties. J. Antibiotics 30: 541～551, 1977
2) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DEVAULT, A. C. SINCLAIR, E. E. FAGER & L. A. MITSCHER: Fortimicins A and B, new aminoglycoside antibiotics. III. Structural identification. J. Antibiotics 30: 552～563, 1977
3) WAGMAN, G. H.; E. M. ODEN & M. J. WEINSTEIN: Differential chromatographic bioassay for the gentamicin complex. Appl. Microbiol. 16: 624～627, 1968
4) OKACHI, R.; I. KAWAMOTO, S. TAKASAWA, M. YAMAMOTO, S. SATO, T. SATO & T. NARA: A new antibiotic XK-62-2 (Sagamicin). I. Isolation, physicochemical and antimicrobial properties. J. Antibiotics 27: 793～800, 1974
5) NAFA, T.; I. KAWAMOTO, R. OKACHI, S. TAKASAWA, M. YAMAMOTO, S. SATO, T. SATO & A. MORIKAWA: New antibiotic XK-62-2 (Sagamicin). II. Taxonomy of the producing organism, fermentative production and characterization of sagamicin. J. Antibiotics 28: 21～28, 1975

* Incidentally, our recent studies showed that the aminoglycoside 3-N-acetyltransferase of *E. coli* KY 8348 acetylates 3- amino of deoxystreptamine in seldomycin factor 5.[13]

6) EGAN, R. S.; R. L. DEVAULT, S. L. MUELLER, M. I. LEVENBERG, A. C. SINCLAIR & R. S. STANASZEK: A new antibiotic XK-62-2. III. The structure of XK-62-2, a new gentamicin C complex antibiotic. J. Antibiotics 28: 29~34, 1975
7) BENVENISTE, R. & J. DAVIES: R-Factor mediated gentamicin resistance: A new enzyme which modifies aminoglycoside antibiotics. FEBS Letters 14: 293~296, 1971
8) BRZEZINSKA, M.; R. BENVENISTE, J. DAVIES, P. J. L. DANIELS & J. WEINSTEIN: Gentamicin resistance in strains of *Pseudomonas aeruginosa* mediated by enzymatic N-acetylation of the deoxystreptamine moiety. Biochem. 11: 761~766, 1972
9) NARA, T.; M. YAMAMOTO, S. TAKASAWA, S. SATO, T. SATO, I. KAWAMOTO, R. OKACHI, I. TAKAHASHI & A. MORIKAWA: A new aminoglycoside antibiotic complex—The seldomycins. I. Taxonomy, fermentation and antibacterial properties. J. Antibiotics 30: 17~24, 1977
10) SATO, S.; S. TAKASAWA, T. SATO, M. YAMAMOTO, R. OKACHI, I. KAWAMOTO, T. IIDA, A. MORIKAWA & T. NARA: A new aminoglycoside antibiotic complex—The seldomycins. II. Isolation, physicochemical and chromatographic properties. J. Antibiotics 30: 25~30, 1977
11) EGAN, R. S.; A. C. SINCLAIR, R. L. DEVAULT, J. B. MCAPLINE, S. L. MUELLER, P. C. GOODLEY, R. S. STANASZEK, M. CIROVIC, R. J. MAURITZ, L. A. MITSCHER, K. SHIRAHATA, S. SATO & T. IIDA: A new aminoglycoside antibiotic complex—The seldomycins. III. The structures of seldomycin factor 1 and 2. J. Antibiotics 30: 31~38, 1977
12) MCALPINE, J. B.; A. C. SINCLAIR, R. S. EGAN, R. L. DEVAULT, R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, P. C. GOODLEY, L. A. MITSCHER, K. SHIRAHATA, H. MATSUSHIMA, S. SATO & T. IIDA: A new aminoglycoside antibiotic complex—The seldomycins. IV. The structure of seldomycin factor 5. J. Antibiotics 30: 39~49, 1977
13) In preparation