STUDIES ON ANTIBIOTIC SF-837, A NEW ANTIBIOTIC. III
ISOLATION AND PROPERTIES OF MINOR COMPONENTS

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Three new antibiotics were isolated as minor components from the culture broth of *Streptomyces mycarofaciens*, and named as SF-837 A_2, A_3 and A_4. They are basic macrolides and have the molecular formulae of $C_{42}H_{69}NO_{15}$, $C_{41}H_{65}NO_{15}$ and $C_{42}H_{67}NO_{15}$, respectively. SF-837 A_2 showed a UV maximum at 232 mμ, and gave on hydrolysis propionic acid and *n*-butyric acid, one mole each, suggesting to be a homologue of SF-837. SF-837 A_3 and A_4 exhibited a UV maximum at 280 mμ, and liberated two moles of propionic acid and a pair of one propionic acid and one *n*-butyric acid in their respective hydrolyzates. Their antibacterial activities were analogous to that of SF-837. The physico-chemical characterization of three components established their novelties.

In the previous papers[1,2], we reported a new macrolide antibiotic SF-837, which is a main product of *Streptomyces mycarofaciens* nov. sp. Further investigation of bioactive metabolites of this strain led to the isolation of three minor components of macrolide nature, and they are named as SF-837 A_2, A_3 and A_4 in order of increasing mobility on alumina TLC. In this paper, we describe the isolation and characterization of antibiotics SF-837 A_2, A_3 and A_4.

Isolation

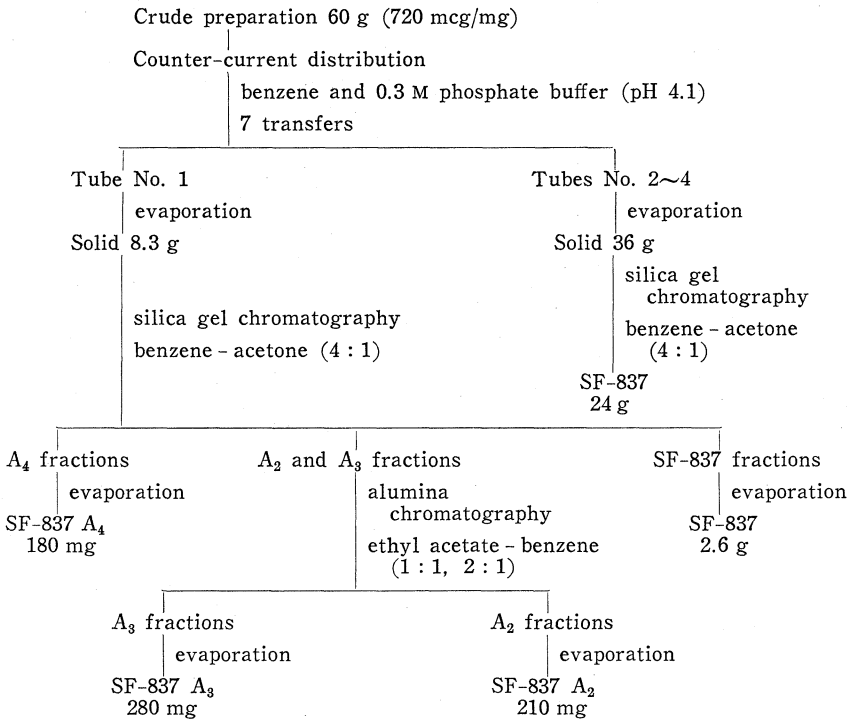
A crude preparation of SF-837 complex was obtained from fermentation broth by the procedure employed for the isolation of SF-837. The filtered broth (150 liters) of *Streptomyces mycarofaciens* was extracted at pH 8 with ethyl acetate (50 liters). Antibiotics in the ethyl acetate layer were extracted with water (20 liters) at pH 2, and then re-extracted with ethyl acetate (10 liters) at pH 8. Decolorization of the final ethyl acetate extract by active carbon, and evaporation of solvent gave a solid which was dissolved in benzene (300 ml). The benzene solution was evaporated to dryness to afford a crude preparation (62 g, 720 mcg/mg).

TLC of this crude preparation revealed, in addition to a main spot due to SF-837,

Table 1. Rf Values of antibiotic SF-837 A_2, A_3 and A_4 on thin-layer chromatograms

	Solvent system	SF-837 A_2	SF-837 A_3	SF-837 A_4	SF-837
Silica gel TLC	Benzene - acetone (2 : 1)	0.51	0.50	0.55	0.45
Alumina TLC	Ethyl acetate - benzene (2 : 1)	0.40	0.45	0.52	0.34

Chart 1. Separation and purification of antibiotic SF-837 A_2, A_3 and A_4

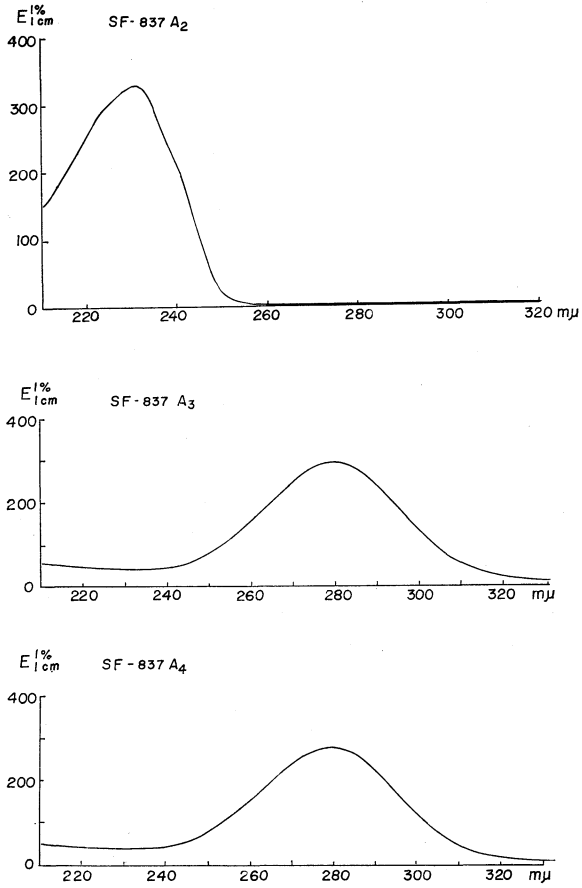


three minor spots of Rf values higher than that of SF-837. Rf Values of three minor components (SF-837 A_2, A_3 and A_4) are shown in Table 1 together with those of SF-837.

In order to remove SF-837 that was a main component of this crude mixture, the complex (60 g) was subjected to a counter-current distribution using a solvent system of benzene and 0.3 M phosphate buffer of pH 4.1. Two liters of each solvent were used, and the displacement of the buffer layer was effected 7 times. Most of SF-837 was transferred into Nos. 2~4 tubes, while SF-837 A_2, A_3 and A_4 remained in the No. 1 tube. Evaporation of the No. 1 fraction gave a white powder (8.3 g), from which the individual components, SF-837 A_2, A_3 and A_4, were isolated by column chromatography over silica gel (700 ml) developing with benzene - acetone (4 : 1). Effluents were collected in 50 ml fractions. SF-837 A_4 was eluted first in fractions Nos. 25~28, and evaporation of the solvent gave 180 mg of the pure antibiotic as a white powder. SF-837 A_2 and A_3 were found in fractions Nos. 36~52 as a mixture accompanied with a small amount of SF-837. Evaporation of solvent gave 2.1 g of the mixture. From fractions Nos. 64~86 were obtained 2.3 g of SF-837. More of SF-837 (24 g) was recovered from Nos. 2~4 tubes of the counter-current distribution after chromatographic purification over silica gel.

A mixture of SF-837 A_2 and A_3 (2.0 g) described above was dissolved in benzene (10 ml), and applied to an alumina column (200 ml), which was developed with a solvent mixture of ethyl acetate - benzene (1 : 1). Effluents were taken in fractions of each 20 ml. Evaporation of fractions Nos. 52~76 yielded pure SF-837 A_3 as a

Fig. 1. UV spectra of antibiotic SF-837 A_2, A_3 and A_4 (in ethanol)



white powder (280 mg). After fraction No. 90, the developer was switched to ethyl acetate - benzene (2 : 1), and evaporation of fractions Nos. 115~132 gave a white powder of SF-837 A_2 (210 mg).

Separation procedure of each component is summarized in Chart 1.

Properties

Antibiotics SF-837 A_2, A_3 and A_4 have solubilities similar to that of SF-837, *i.e.*, they are soluble in methanol, ethanol, acetone, chloroform, ethyl acetate, butyl acetate, benzene, ethyl ether and acidic water, but almost insoluble in *n*-hexane, petroleum ether and water.

Table 2 summarizes the physical and chemical properties of SF-837 A_2, A_3 and A_4. These three components showed similar melting points, pKa′ and elemental analyses.

It was found, however, that the UV spectrum of SF-837 A_2 was considerably different from those of SF-837 A_3 and A_4. As seen in Fig. 1, SF-837 A_2 shows a strong maximum at 232 mμ, as SF-837 does, whereas SF-837 A_3 and A_4 exhibits a strong maximum at 280 mμ, *ca.* 50 mμ being red-shifted.

The difference between SF-837 A_2 and the other two components were further

Table 2. Physical and chemical properties of antibiotic SF-837 A_2, A_3 and A_4

	SF-837 A_2	SF-837 A_3	SF-837 A_4
Melting point	125~128°C	122~125°C	120~122°C
UV max.	232 mμ ($E^{1\%}_{1cm}$ 320)	280 mμ ($E^{1\%}_{1cm}$ 295)	280 mμ ($E^{1\%}_{1cm}$ 285)
Rotation $[\alpha]^{22}_{D}$	−68° (*c* 1, EtOH)	−44° (*c* 1, EtOH)	−40° (*c* 1, EtOH)
pKa′ (50 % aqueous EtOH)	6.9	7.0	7.0
Molecular weight (Mass spectrometry)	827	811	825
Molecular formula	$C_{42}H_{69}NO_{15}$	$C_{41}H_{65}NO_{15}$	$C_{42}H_{67}NO_{15}$
Found	C 60.58, H 8.85, N 1.72 %	C 60.53, H 8.23, N 1.87 %	C 60.53, H 8.52, N 1.73 %
Calcd.	C 60.94, H 8.38, N 1.69 %	C 60.67, H 8.01, N 1.73 %	C 61.09, H 8.12, N 1.70 %

Fig. 2. IR spectra of antibiotic SF-837 A_2, A_3 and A_4 (KBr pellets)

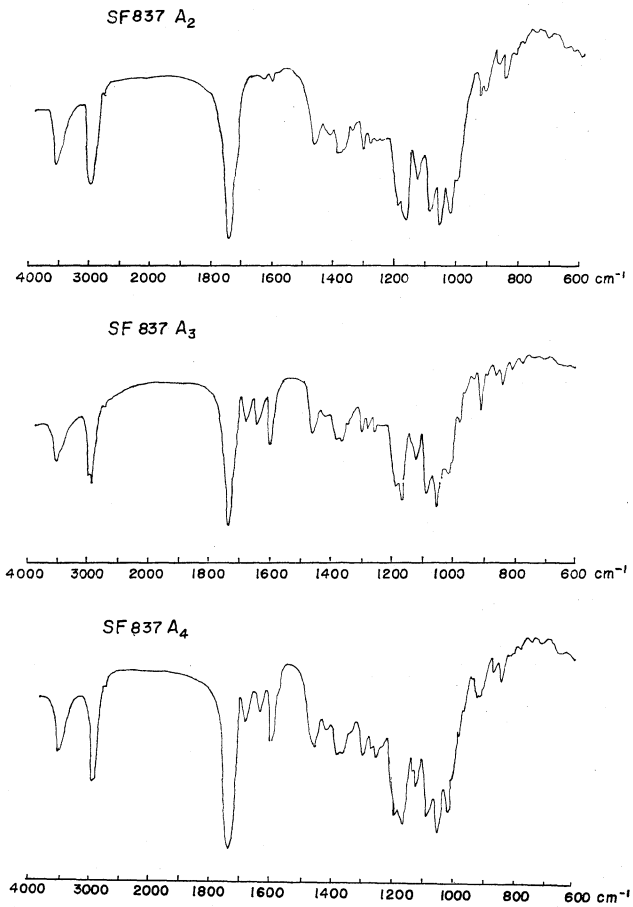


Fig. 3. Gas-liquid chromatograms* of alkaline hydrolysate** of antibiotic SF-837 A_2 (——) and standard fatty acids (------).

1. acetic acid
2. propionic acid
3. isobutyric acid
4. *n*-butyric acid
5. isovaleric acid

* GLC was conducted with a Hewlett-Packard Model Gas Chromatograph with flame ionization detector and with a U-shaped glass column (0.4 ×180 cm) packed with Chromosorb 101 (60/80 mesh, Johns-Manville, USA). The carried gas was helium at a flow rate of 40 ml/min. Oven temperature was 200℃.

** A solution of antibiotic SF-837 A_2 (5 mg) in 0.1 N ethanolic sodium hydroxide (0.5 ml) was heated at 75℃ for 40 minutes and evaporated to dryness. The residue was dissolved in 2 M phosphoric acid (0.1 ml) and 1 μl of this solution was analyzed by GLC.
Sodium acetate and sodium isobutyrate were used as the internal standard.

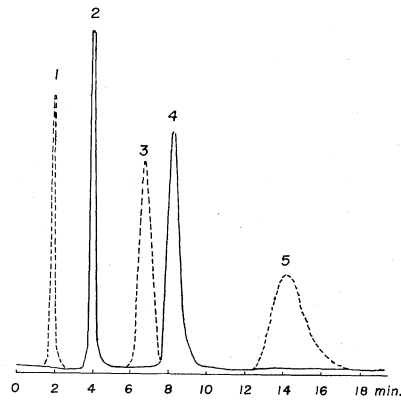


Table 3. Physical and chemical properties of acetyl derivatives of antibiotic SF-837 A_2, A_3 and A_4

	Diacetyl SF-837 A_2	Monoacetyl SF-837 A_3	Monoacetyl SF-837 A_4
Appearance	needles	plates	plates
Melting point	130～134℃	182～185℃	166～168℃
Molecular weight (Mass spectrometry)	911	853	867
Molecular formula	$C_{46}H_{73}NO_{17}$	$C_{43}H_{67}NO_{16}$	$C_{44}H_{69}NO_{16}$
Found	C 60.18, H 7.65, N 1.62 %	C 60.72, H 8.23, N 1.68 %	C 60.52, H 7.73, N 1.57 %
Calcd.	C 60.57, H 8.06, N 1.53 %	C 60.47, H 7.90, N 1.64 %	C 60.88, H 8.01, N 1.61 %

recognized in the IR spectra illustrated in Fig. 2. The IR bands characteristic of SF-837 A_3 and A_4 were found at 1600 cm^{-1}, 1640 cm^{-1} and 1680 cm^{-1}. These bands are not present in the spectrum of either SF-837 A_2 or SF-837.

Acetylation of SF-837 A_2 with acetic anhydride in pyridine at room temperature gave the crystalline di-O-acetyl derivative, whose physico-chemical properties are

Table 4. Antibacterial spectra of antibiotic SF-837 A_2, A_3 and A_4 by broth dilution method

Test organisms	M.I.C. (mcg/ml)				Medium
	SF-837	A_2	A_3	A_4	
Staphylococcus aureus FDA 209P	0.39	0.39	0.39	0.39	1
〃 〃 〃 penicillin-R	0.78	0.39	0.39	0.39	1
〃 〃 〃 streptomycin-, and A-249 substance-R	0.39	0.39	0.39	0.78	1
〃 〃 〃 novobiocin-R	3.125	3.125	3.125	3.125	1
〃 〃 〃 kanamycin-R	1.56	0.78	0.78	0.78	1
〃 〃 Smith	0.19	0.09	0.19	0.19	1
〃 〃 Terajima	0.78	0.39	0.78	0.78	1
〃 〃 streptomycin-, tetracycline- and penicillin-R	1.56	0.78	1.56	1.56	1
Bacillus subtilis ATCC 6633	0.39	0.19	0.39	0.39	1
Sarcina lutea	0.09	0.05	0.09	0.09	1
Mycobacterium smegmatis ATCC 607	25.0	12.5	25.0	25.0	2

Medium: 1=Bouillon, 2=Glycerine bouillon

given in Table 3. UV and IR spectra of diacetyl SF-837 A_2 are also close to those of diacetyl SF-837.

Alkaline hydrolysis of SF-837 A_2 with 0.1 N ethanolic sodium hydroxide followed by GLC as described in the preceding paper[1], showed liberation of one mole of propionic acid and one mole of *n*-butyric acid (Fig. 3). It was suggested from these data, that SF-837 A_2 might be a homologue of SF-837, with a *n*-butyryl group in place of a propionyl group. This was confirmed by the structural study[3].

SF-837 A_3 and A_4 seemed to be closely related compounds, since they showed almost identical UV and IR spectra. Comparison of molecular weights and molecular formulae of both compounds revealed that SF-837 A_4 contained one more methylene (CH_2) in its molecule than SF-837 A_3. GLC Determination of fatty acids liberated by alkaline hydrolysis indicated that SF-837 A_3 contained two propionyl groups, and that SF-837 A_4 contained one propionyl group and one *n*-butyryl group. Thus, the difference between the two components (A_3 and A_4) could be ascribable to the difference of acyl groups on the side chain.

Acetylation of both components with acetic anhydride in pyridine gave the respective crystalline monoacetates, in contrast to SF-837 and SF-837 A_2 that afforded diacetates under the same condition. The physicochemical properties of these monoacetates are listed in Table 3.

As evidenced by comparison of molecular formulae, SF-837 A_3 has two hydrogens less than SF-837, and therefore, is assumed to be a dehydrogenated product of SF-837. Actually, SF-837 was converted into SF-837 A_3 by a manganese dioxide oxidation as will be reported in a subsequent paper[3]. Similarly, SF-837 A_4 has two hydrogens less than SF-837 A_2 and is correlated with SF-837 A_2 by converting the latter into the former by a manganese dioxide oxidation[3].

The antimicrobial spectra of antibiotics SF-837 A_2, A_3 and A_4 are identical with that of SF-837, and they are equally active as shown in Table 4. Oral

administration of 5,000 mg/kg of each component to mice caused no death, indicating low toxicities.

Discussion

It is apparent from the data presented above that SF-837 A_2, A_3 and A_4 are basic macrolides. SF-837 A_2 shows a strong UV maximum at 232 mμ, and therefore belongs to subgroup c[1] that includes the leucomycins[4,5,6,7], josamycin[8], the spiramycins[9], the tertiomycins[10,11] and SF-837[1].

Table 5 indicates comparative Rf values obtained from TLC developed under the same condition of SF-837 A_2 and other macrolides. Among them, SF-837 A_2 showed the highest Rf value, thereby being differentiated from other macrolides. Furthermore, there has been no report on the isolation of a macrolide bearing a pair of one propionyl group and one *n*-butyryl group in its molecule.

SF-837 A_3 and A_4 exhibit a strong UV band at 280 mμ, and therefore belong to subgroup b[1], like carbomycin B[12], niddamycin[13], tylosin[14], relomycin[15] and macrocin[16].

Table 5. Comparison of Rf values of antibiotic SF-837 A_2 and related macrolides on alumina thin layer chromatogram

Solvent: ethyl acetate

Macrolide	Rf	Macrolide	Rf
SF-837 A_2	0.84	Leucomycin A_5	0.05
SF-837	0.78	〃 A_7	0.01
Josamycin	0.65	〃 A_9	0.02
Leucomycin A_3	0.65	Tertiomycin A	0.61
〃 A_4	0.60	〃 B	0.64
〃 A_6	0.57	Spiramycin I	0.05
〃 A_8	0.53	〃 II	0.12
〃 A_1	0.08	〃 III	0.18

Fig. 4. Comparison of antibiotic SF-837 A_3 and A_4 with other related macrolides on thin-layer chromatograms.

A: alumina TLC developed with ethyl acetate - benzene (2 : 1)
B: silica gel TLC developed with benzene - acetone (2 : 1)

CMB: Carbomycin B
NM: Niddamycin
TS: Tylosin

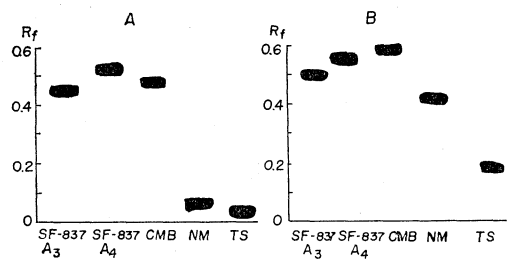


Table 6 shows the physico-chemical properties of known macrolides belonging to subgroup b. With regard to molecular weights and molecular formulae, SF-837 A_3 and A_4 are clearly different from tylosin, relomycin and macrocin. In this respect, carbomycin B and niddamycin are more closely related to SF-837 A_3 and A_4.

Differentiation of antibiotic SF-837 A_3 ane A_4 from carbomycin B and niddamycin was accomplished with TLC as shown in Fig. 4. Moreover, comparison of volatile carboxylic acids obtained on hydrolysis clearly shows that neither carbomycin B nor niddamycin should give propionic acid and *n*-butyric acid as SF-837 A_3 and A_4 did.

Table 6. Properties of known macrolides having a UV maximum at *ca.* 280 mμ

Antibiotic	M. P. (°C)	UV_{max} (mμ)	Molecular formula	M.W.	$[\alpha]_D$	Acyl group on side chain
Carbomycin B	141~144	278 ($E^{1\%}_{1cm}$ 276)	$C_{42}H_{67}O_{15}N$	825	−35	acetyl and isovaleryl groups
Niddamycin	132~134	279 ($E^{1\%}_{1cm}$ 275)	$C_{40}H_{65}O_{14}N$	783	−43	isovaleryl group
Tylosin	128~132	284 ($E^{1\%}_{1cm}$ 245)	$C_{45}H_{77}O_{17}N$	907	−46	——
Relomycin	172~175	282 ($E^{1\%}_{1cm}$ 245)	$C_{45}H_{79}O_{17}N$	909	−44	——
Macrocin	134~136	283 ($E^{1\%}_{1cm}$ 244)	$C_{46}H_{79}O_{17}N$	921	−52.5	——

Thus, the novelties of these three macrolides (A_2, A_3 and A_4) were established. Further support of this came from structural studies, which are the subject of a subsequent paper[3].

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References

1) Tsuruoka, T.; T. Shomura, N. Ezaki, H. Watanabe, E. Akita, S. Inouye & T. Niida: Studies on antibiotic SF-837, a new antibiotic. I. The producing microorganism and isolation and characterization of the antibiotic. J. Antibiotics 24 : 452～459, 1971
2) Inouye, S.; T. Tsuruoka, T. Shomura, S. Omoto & T. Niida: Studies on antibiotic SF-837, a new antibiotic. II. Chemical structure of antibiotic SF-837. J. Antibiotics 24 : 460～475, 1971
3) Tsuruoka, T.; S. Inouye, T. Shomura, N. Ezaki & T. Niida: Studies on antibiotic SF-837, a new antibiotic. IV. Structures of antibiotic SF-837 A_2, A_3 and A_4. J. Antibiotics 24 : 1971 (in press)
4) Watanabe, T.; H. Nishida, J. Abe & K. Satake: Studies on leucomycin. III. Isolation and properties of six antibacterial components in leucomycin complex. Bull. Chem. Soc. Japan 33 : 1105～1108, 1960
5) Hata, T.; S. Ōmura, A. Matsumae, M. Katagiri & Y. Sano: Leucomycin A_3, a new antibiotic from *Streptomyces kitasatoensis*. Antimicr. Agents & Chemoth. -1966 : 631～636, 1967
6) Ōmura, S.; M. Katagiri & T. Hata: The chemistry of leucomycins. IV. Structure of leucomycin A_1. J. Antibiotics 21 : 199～203, 1968
7) Ōmura, S.; M. Katagiri & T. Hata: The structures of leucomycin A_4, A_5, A_6, A_7, A_8 and A_9. J. Antibiotics, Ser. A 20 : 234～235, 1967
8) Osono, T.; Y. Oka, S. Watanabe, Y. Numazaki, K. Moriyama, H. Ishida, K. Suzaki, Y. Okami & H. Umezawa: A new antibiotic, josamycin. I. Isolation and physico-chemical characteristics. J. Antibiotics, Ser. A 20 : 174～180, 1967
9) Paul, R. & S. Tchelitcheff: Structure de la spiramycine. I. Étude des produits de dégradation, caractérisation du mycarose. Bull. Soc. Chim. France 1957 : 443～447, 1957
10) Ōsato, T.; M. Ueda, S. Fukuyama, K. Yagishita, Y. Okami & H. Umezawa: Production of tertiomycin (a new antibiotic substance), azomycin and eurocidin by *S. eurocidicus*. J. Antibiotics, Ser. A 8 : 105～109, 1955
11) Ōsato, T.; K. Yagishita & H. Umezawa: On tertiomycin B produced by *Streptomyces eurocidicus*. J. Antibiotics, Ser. A 8 : 161～163, 1955
12) Hochstein, F. A. & K. Murai: Magnamycin B, a second antibiotic from *Streptomyces halstedii*. J. Am. Chem. Soc. 76 : 5080～5083, 1954
13) Huber, G.; K. H. Wallhäusser, L. Fries, A. Steigler & H. L. Weidenmüller: Niddamycin, ein neues Makrolide-Antibioticum. Arzneimittel Forsch. 12 : 1191～1195, 1962
14) Hamill, R. L.; M. E. Haney, Jr., M. Stamper & P. F. Wiley: Tylosin, a new antibiotic. II. Isolation, properties and preparation of desmycosin, a microbiologically active degradation product. Antibiot. & Chemoth. 11 : 328～334, 1961
15) Whaley, H. A.; E. L. Patterson, A. C. Dornbush, E. J. Backus & N. Bohonos: Isolation and characterization of relomycin, a new antibiotic. Antimicr. Agents & Chemoth. -1963 : 45～48, 1963
16) Hamill, R. L. & W. M. Stark: Macrocin, a new antibiotic, and lactenocin, an active degradation product. J. Antibiotics, Ser. A 17 : 133～139, 1964