MYCINAMICINS, NEW MACROLIDE ANTIBIOTICS. I

TAXONOMY, PRODUCTION, ISOLATION, CHARACTERIZATION AND PROPERTIES

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Mycinamicins, novel macrolide antibiotics were obtained from the culture broth of *Micromonospora griseorubida* sp. nov. Isolation of five components, mycinamicins I, II, III, IV and V, was accomplished by silica gel adsorption or partition chromatography. Mycinamicin I and II exhibit a strong UV absorption peak at 218 nm and have a shoulder at 240 nm. Mycinamicin III, IV and V show strong UV absorption peaks at 215 nm and around 280 nm. From their physicochemical and biological properties, the mycinamicins are classified as new macrolide antibiotics.

In the continuing search by our laboratory, for new antibiotics a novel group of antibiotics, the mycinamicins, was isolated from the culture broth of *Micromonospora griseorubida* sp. nov. The mycinamicins were found to consist of five components. Their physicochemical properties together with other detailed chemical studies show that the mycinamicins are new basic macrolide antibiotics. This paper deals with description of the producing microorganism and the isolation and preliminary characterization of the mycinamicins.

Taxonomy of Producing Organism

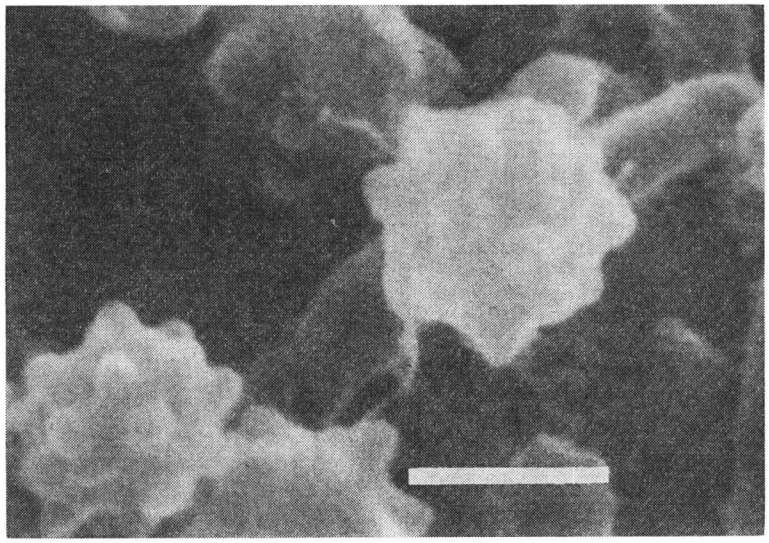
Strain A11725 was isolated from a soil sample collected in Unazuki-cho, Shimohirakawa-gun, Toyama Prefecture, Japan.

Most of the taxonomic studies of the culture were carried out in accordance with methods adopted by the International Streptomyces Project (ISP)[1]. Additional media recommended by WAKSMAN[2] were also used. The various media were inoculated with washed mycelial suspensions from a broth culture grown in shake flasks at 30°C for 96 hours in a liquid medium (1.0% dextrin, 1.0% glucose, 0.5% yeast extract, 0.5% Polypeptone and 0.1% $CaCO_3$). For whole-cell analysis, the methods used were those of BECKER *et al.*[3] for 2,6-diaminopimelic acid (DAP) and those of LECHEVALIER[4] for sugars. The mycelium was cultivated for 5 days at 30°C in the liquid medium (described above with the exception of $CaCO_3$).

Morphological Characters

The mycelial colonies were colored grayish red to reddish brown when grown on sucrose nitrate agar, inorganic salts-starch agar or other media. These pigments behaved as pH indicators, being yellow orange in the acid range and grayish red in the basic range. The spore layer was black when present and waxy to dry rather than moist or viscoid. Usually no aerial mycelium was formed, but occassionally it formed in a rudimentary non-sporulating form. Microscopic observation showed that the hyphae of the substrate mycelium were well developed, straight to wavy and monopodialy branched.

Fig. 1. Scanning electron micrograph of spores of strain A11725 (oat meal agar, the bar represents 1 μ).



The hyphae had a diameter of about 0.6~0.8 μ and did not fragment into bacillary or coccoid elements. The spore was formed singly on the substrate mycelium, usually on short sporophores but sometimes sessile. Electron microscopy revealed that the mature spores were about 1.0~1.5 μ in diameter and spherical or oval in shape with a rough surface (blunt spines)[5] (Fig. 1).

Whole-cell Analysis

Whole-cell DAP analysis revealed hydroxy-DAP as major constituent and meso-DAP as minor constituent. Whole-cell sugar analysis revealed xylose and arabinose.

Cultural Characters

The cultural characters of strain A11725 shown in Table 1 were observed after 20 days of incubation at 30°C on the designated media. The number in parentheses corresponds to the hue number used in "Color Harmony Mannual[6]".

Physiological Characters

The physiological characters of strain A11725 are shown in Table 2, and the properties of carbohydrate utilization are shown in Table 3. The test for the utilization of carbohydrate was performed using a basal medium consisting of 0.5% yeast extract and 1.5% agar because the growth of the strain was not enough for a visual estimate on the ISP medium[1]. The temperature range for growth was observed on the ISP medium 2[1] for 2 weeks and decomposition of cellulose was observed after one month. Sodium chloride tolerance and nitrate reduction were determined on the media described by LUEDEMANN[7].

The microscopical and cultural studies and whole-cell analysis of strain A11725 indicate that this isolate belongs to the genus *Micromonospora* OERSKOV 1923.

From the results of these studies, the main characters of strain A11725 were summarized as follows: The spore surface had blunt spines; the spore layer was black in color; the color of the substrate mycelium was grayish red to reddish brown; the soluble pigment was grayish red on some media and these grayish red to reddish brown pigments were pH indicators, being yellow orange in the acid range and grayish red in the basic range.

Table 1. Cultural characters of strain A11725.

Medium	Growth	Color of substrate mycelium*	Spore layer*	Rudimentary aerial mycelium*	Soluble pigment*
Sucrose nitrate agar (WAKSMAN No. 1)	good	Cedar (61e) to Brick Red (6ng)	none	poor; Flesh Pink (5ca) to Dusty Peach (5ec)	Dusty Coral (6gc) to Redwood (6ie)
Glucose-asparagine agar (WAKSMAN No. 2)	trace to poor	Nude Tan (4gc)	none	none	none
Glycerol-asparagine agar (ISP medium 5)	trace	Nude Tan (4gc) to Bisque (4ec)	none	none	none
Inorganic salts-starch agar (ISP medium 4)	moderate to good	Brick Red (5ng)	moderate; Lamp Black (p), waxy to dry	none	none
Tyrosine agar (ISP medium 7)	trace	Bisque (3ec) to Beige (3gc)	trace; Lamp Black (p),	none	none
Oatmeal agar (ISP medium 3)	good to moderate	Brick Red (5ng) to Copper Brown (5pi)	good; Lamp Black (p), waxy to dry	none	Copper Tan (5ie) around the colonies
Yeast extract-malt extract agar (ISP medium 2)	good	Light Rose Brown (71g) to Rose Brown (7ni)	trace; Lamp Black (p)	none	Cedar (61e) slightly
Glucose-yeast extract agar (WAKSMAN No. 29)	moderate	Cocoa Brown (51g) to Dark Redwood (61g)	poor to trace; Lamp Black (p)	trace; Shell Pink (5ba)	none
Nutrient agar (WAKSMAN No. 14)	trace	colorless to Light Tan (3gc)	none	none	none
BENNETT's agar	moderate to good	Light Rose Brown (71g) to Rose Brown (7ni)	moderate; Lamp Black (p), waxy to dry	none	Light Rose Brown (71g) to Rose Brown (7ni) around the colonies
EMERSON's agar	good	Cedar (61e to $6^1/_2$1e)	none	none	none
Starch-NZ·Amine type A-yeast extract agar (ATCC No. 172)**	good	Dark Wine (8pi) to Mauve Wine (8ni)	good; Lamp Black (p), waxy to dry	none	Old Wine (8ng)
1% Glucose-3% NZ·Amine type A agar	moderate to poor	Cedar (61e) to Rust Tan (51e)	none	none	none
Glucose-peptone agar (WAKSMAN No. 12)	moderate	Rose Brown (7ni)	moderate to poor; Lamp Black (p), waxy to dry	none	Old Wine (7ng)
Potato plug	none to trace				
Potato plug+$CaCO_3$	good	Dark Rose Brown (7pn)	moderate; Lamp Black (p), waxy to dry	none	Dark Rose Brown (7pn) slightly
Peptone-yeast extract iron agar	trace to poor	Light Tan (3gc)	trace; Lamp Black (p)	none	none

* Color designation from Color Harmony Manual[6].

** The American Type Culture Collection, Catalogue of Strains 18th ed. p. 142, 1968.

Table 2. Physiological characters of strain A11725.

Liquefaction of gelatin	positive
Hydrolysis of starch	positive
Peptonization of milk	positive
Coagulation of milk	positive
Formation of melanoid pigment	negative
Sodium chloride tolerance:	
0%	good
1.5%	moderate to good
3.0% or more	no growth
Decomposition of cellulose	negative
Reduction of nitrate	negative
Temperature range	15~45°C

Table 3. Carbohydrate utilization of strain A11725.

Response	Carbohydrate
Positive	D-Arabinose, D-glucose, D-fructose, D-mannose, sucrose, trehalose, soluble starch
Doubtful	L-Arabinose, D-cellobiose, D-ribose
Negative	D-Galactose, β-lactose, melezitose, α-melibiose, raffinose, L-rhamnose, L-sorbose, D-xylose, glycerol, salicin, dulcitol, *i*-inositol, D-mannitol, D-sorbitol, cellulose

When the characteristics of strain A11725 were compared in detail with those of *Micromonospora* species described previously, the following five species and one subspecies resembled the newly isolated strain: 1. *Micromonospora rosaria*[8]; 2. *Micromonospora lilacina*[9]; 3. *Micromonospora rubra*[9]; 4. *Micromonospora roseopurpurea*[10]; 5. *Micromonospora echinospora* subsp. *echinospora*[5,11]; and 6. *Micromonospora echinospora* subsp. *ferruginea*[5,11].

Tables 4 and 5 show a comparison of various characters of strain A11725 with the above five species and one subspecies of *Micromonospora*.

M. rosaria was observed to produce a wine red to reddish brown soluble pigment in EMERSON's agar, nutrient agar and inorganic salts-starch agar, to yield moderate growth on 4% NaCl medium and to utilize D-galactose, β-lactose, L-rhamnose, D-xylose and D-mannitol, while strain A11725 showed no soluble pigment in EMERSON's agar, nutrient agar and inorganic salts-starch agar, no growth on 4% NaCl medium and no utilization of D-galactose, β-lactose, L-rhamnose, D-xylose and D-mannitol.

M. lilacina was observed to produce no spore layer, have moderate growth on a potato plug, to reduce nitrate and to utilize D-galactose, β-lactose, raffinose, L-rhamnose, D-xylose, glycerol, *i*-inositol and D-mannitol, while strain A11725 showed a black spore layer, no growth on a potato plug, no reduction of nitrate, and no utilization of D-galactose, β-lactose, raffinose, L-rhamnose, D-xylose, glycerol, *i*-inositol and D-mannitol. *M. lilacina* differed in characters of substrate mycelium pigment from strain A11725.

M. rubra was observed to produce no black spore layer, have poor growth on 4% NaCl medium, moderate growth on 3% NaCl medium and good growth on a potato plug, to reduce nitrate and to utilize D-galactose, α-melibiose, raffinose, L-rhamnose, D-xylose, glycerol, *i*-inositol and D-mannitol, while strain A11725 showed the black spore layer, no growth on 3% and 4% NaCl media and a potato plug, no reduction of nitrate and no utilization of D-galactose, α-melibiose, raffinose, L-rhamnose, D-xylose, glycerol, *i*-inositol and D-mannitol.

M. roseopurpurea was observed to produce a moist to viscoid spore layer, to reduce nitrate, to decompose cellulose and to utilize D-galactose, L-rhamnose, D-xylose and D-mannitol, while strain A11725 showed the waxy to dry spore layer, no reduction of nitrate, no decomposition of cellulose and no utilization of D-galactose, L-rhamnose, D-xylose and D-mannitol.

M. echinospora subsp. *echinospora* and *M. echinospora* subsp. *ferruginea* resemble strain A11725 closely, but some differences could be seen. *M. echinospora* subsp. *echinospora* and *M. echinospora*

Table 4. Comparison of characters of strain A11725 with *M. rosaria*, *M. lilacina*, *M. rubra*, *M. roseopurpurea*, *M. echinospora* subsp. *echinospora* and *M. echinospora* subsp. *ferruginea*.

Characters	Strain A11725	*M. rosaria*	*M. lilacina*	*M. rubra*	*M. roseopurpurea*	*M. echinospora* subsp. *echinospora*	*M. echinospora* subsp. *ferruginea*
Spore layer	waxy to dry	waxy to dry*	not formed	not formed	moist to viscoid	waxy to dry	waxy to dry
Characters of substrate mycelium							
pH<7	yellow orange	**	orange	yellow	**	red	red
pH>7	grayish red	**	violet	red	**	blue	blue
Soluble pigment							
EMERSON's agar	none	wine red	**	**	slightly darken	none	none
Nutrient agar	none	reddish brown	**	**	**	none	none
Inorganic salts-starch agar	none	wine red	**	**	**	none	none
NaCl tolerance: 0 %	good	good*	good*	good*	**	good	good
1.5%	moderate to good	good*	moderate*	good*	**	moderate	moderate
3.0%	no growth	moderate to good*	no growth*	moderate*	**	poor	poor
4.0%	no growth	moderate*	no growth*	poor*	**	no growth	no growth
Reduction of nitrate	negative	negative	positive	positive	positive	variable	negative
Acid tolerance							
potato plug	no growth	no growth*	poor to moderate*	good*	no growth	no growth	no growth
potato plug+$CaCO_3$	good	good*	good	good*	moderate	good	good
Decomposition of cellulose	negative	positive, slowly	negative	negative	positive	**	**

* The data were obtained from comparative experiments with type strains (*i.e. M. rosaria* NRRL3718, *M. lilacina* ATCC 27030 and *M. rubra* ATCC 27031).

** No data was obtained.

Table 5. Comparison of carbohydrate utilization pattern of strain A11725 with *M. rosaria*, *M. lilacina*, *M. rubra*, *M. roseopurpurea*, *M. echinospora* subsp. *echinospora* and *M. echinospora* subsp. *ferruginea*.

Carbohydrate	Strain A11725	*M. rosaria**	*M. lilacina**	*M. rubra**	*M. roseopurpurea*	*M. echinospora* subsp. *echinospora*	*M. echinospora* subsp. *ferruginea*
D-Arabinose	+	+	+	+	+	−	−
L-Arabinose	±	+	±	+	+	+	+
D-Cellobiose	±	+	+	+	**	+	+
D-Galactose	−	+	+	+	+	±	±
β-Lactose	−	+	+	−	±	±	±
D-Fructose	+	+	+	+	+	±	±
α-Melibiose	−	−	+	+	−	−	−
Raffinose	−	−	+	+	−	−	−
L-Rhamnose	−	+	+	+	+	+	+
D-Ribose	±	+	+	+	±	−	+
D-Xylose	−	+	+	+	+	+	−
Glycerol	−	−	+	+	−	−	−
i-Inositol	−	−	+	+	−	−	−
D-Mannitol	−	+	+	+	+	−	−

* The data were obtained from comparative experiments with type strains (*i.e. M. rosaria* NRRL 3718, *M. lilacina* ATCC 27030 and *M. rubra* ATCC 27031).

** No data was obtained.

subsp. *ferruginea* differed from strain A11725 with respect to substrate mycelial pigmentation. *M. echinospora* subsp. *echinospora* was observed to utilize L-rhamnose, D-cellobiose and D-xylose well, to utilize D-galactose, β-lactose and D-fructose slightly and not to utilize D-arabinose, while strain A11725 showed good utilization of D-arabinose and D-fructose, slight utilization of D-cellobiose and no utilization of D-galactose, L-rhamnose and D-xylose. *M. echinospora* subsp. *echinospora* did not utilize D-ribose and *M. echinospora* subsp. *ferruginea* utilized D-ribose well, while strain A11725 utilized D-ribose only slightly.

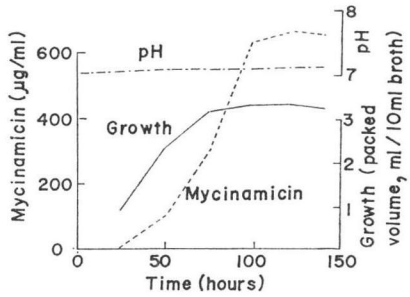
Considering the above mentioned data, strain A11725 was not identical with the previously described five species and one subspecies of *Micromonospora*. Therefore, it is proposed that strain A11725 is a new species of *Micromonospora* and should be designated as *Micromonospora griseorubida* sp. nov. MUTO *et* SATOI because of the production of the grayish red substrate mycelial pigment. The type strain is designated as A11725, and has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaragi, Japan and at the United States Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois where it has been assigned accession numbers FERM-P No. 4488 and NRRL 11452, respectively. Etymology: *M. L. griseus*, gray: *L. rubidus* red: *griseorubida*, grayish red.

Production and Isolation

The producing organism, *Micromonospora griseorubida*, was grown in submerged culture in a 30-liter jar fermentor at 30°C in a medium containing 5% glucose, 2% Pharma-media, 0.5% corn steep liquor, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$ and 0.5% $CaCO_3$ (pH 7.0). The fermentation time course is shown in Fig. 2. The yield of the antibiotic was determined by the conventional paper disk-agar diffusion assay method, using *Bacillus subtilis* PCI219 as a test organism. In the submerged fermenta-

tion culture under these conditions, the production of the mycinamicin complex after about 120 hours fermentation was determined to be 650 mcg/ml as mycinamicin II.

Fig. 2. Time course of mycinamicin fermentation.



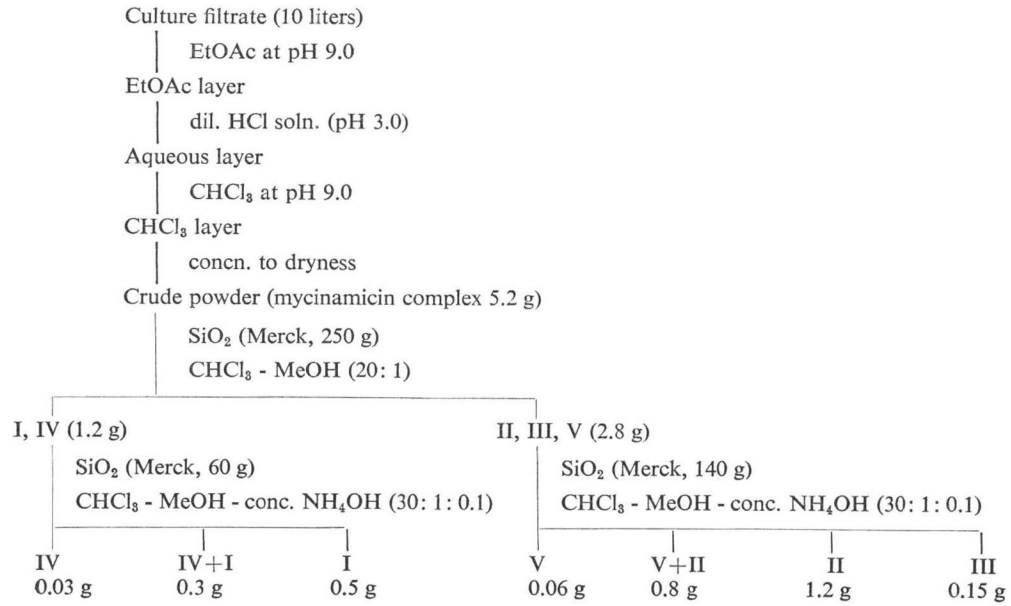
The isolation of the mycinamicin complex was accomplished using the general procedure for basic macrolide antibiotics as shown in Chart 1. The mycinamicin complex was extracted from the culture filtrate of *Micromonospora griseorubida* with ethyl acetate at pH 9.0 and transferred to a dilute hydrochloric acid solution (pH 3.0). The acidic aqueous layer was extracted with chloroform at pH 9.0 and this organic extract was concentrated to afford the mycinamicin complex as a crude powder. Thus, 5.2 g of the crude powder was obtained from 10 liters of the culture filtrate. Fractionation of the antibiotic complex into five components was accomplished by silica gel adsorption or partition chromatography as follows:

The crude mycinamicin complex was dissolved in a small amount of chloroform and chromatographed on a column of silica gel using the solvent system of $CHCl_3$ - MeOH (20: 1) or $CHCl_3$ - MeOH - 28% ammonia (30: 1: 0.1). Each fraction eluated was monitored by TLC on a silica gel GF_{254} plate with a solvent system of $CHCl_3$ - MeOH - 28% ammonia (15: 1: 0.1), detection was done by spraying with 50% H_2SO_4 followed by heating. Five components were detected. By repeated crystallization from acetone - *n*-hexane, mycinamicins IV and V were obtained as colorless crystalline substances. The other components were obtained as white amorphous powders.

Physicochemical Properties

Each mycinamicin component was basic in nature and soluble in methanol, ethanol, acetone,

Chart 1. Purification of mycinamicin.



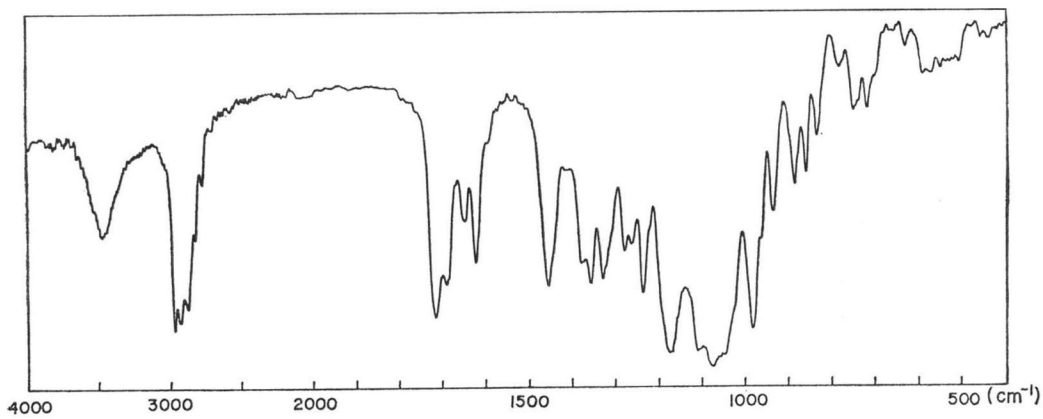
ethyl acetate, benzene and acidic water, but barely soluble or insoluble in *n*-hexane, petroleum ether and water. Each gave the same color reactions: Positive DRAGENDORFF, anthrone and sulfuric acid reactions; negative ninhydrin, biuret, MILLON and EHRLICH reactions. The physicochemical properties of the five mycinamicin components are summarized in Table 6. The IR spectrum of mycinamicin I (Fig. 3) showed the presence of hydroxyl (3470 cm^{-1}) and conjugated carbonyl (1715, 1685 cm^{-1}) functions and also double bonds (1645, 1620 cm^{-1}). The UV absorption maximum at 218 nm (log ε, 4.37) and a shoulder at 240 nm (log ε, 4.11) (Fig. 4) suggested the presence of an α,β-unsaturated lactone and an γ,δ-epoxy-α,β-enone chromophores. The IR and UV spectra of mycinamicin II (Fig. 5) were quite similar to those of mycinamicin I. The IR spectrum of mycinamicin III (Fig. 6) displayed the presence of hydroxy (3460 cm^{-1}), α,β-unsaturated lactone (1710, 1645 cm^{-1}) and $\alpha,\beta,\gamma,\delta$-dienone (1675, 1625, 1590 cm^{-1}) chromophores. The presence of these functions was supported by UV

Table 6. Physicochemical properties of mycinamicins.

Mycinamicin	I	II	III	IV	V
Formula	$C_{37}H_{61}NO_{12}$	$C_{37}H_{61}NO_{13}$	$C_{36}H_{59}NO_{11}$	$C_{37}H_{61}NO_{11}$	$C_{37}H_{61}NO_{12}$
M.W. (mass)	711	727	681	695	711
M.P. (°C)	103~107	102~106	99~102	174~176	148~150
$[\alpha]_D^{25}$ (*c* 1.0, MeOH)	−40.0°	−31.0°	−2.3°	+2.7°	+18.7°
UV λ_{max}^{MeOH} nm (log ε)	218 (4.37) 240 (sh., 4.11)	218 (4.37) 240 (sh., 4.09)	215 (4.32) 281.5 (4.33)	215 (4.32) 281.5 (4.33)	215 (4.32) 280 (4.33)
Analysis (%)	Found C: 62.34 H: 9.25 N: 1.96 Calcd. C: 62.43 H: 8.64 N: 1.97	Found C: 60.57 H: 8.95 N: 1.96 Calcd. C: 61.05 H: 8.44 N: 1.92	Found C: 63.25 H: 9.01 N: 2.10 Calcd. C: 63.41 H: 8.72 N: 2.05	Found C: 64.05 H: 9.10 N: 2.04 Calcd. C: 63.86 H: 8.84 N: 2.01	Found C: 62.21 H: 8.82 N: 1.94 Calcd. C: 62.43 H: 8.64 N: 1.97
Rf value*	0.72	0.56	0.44	0.74	0.59

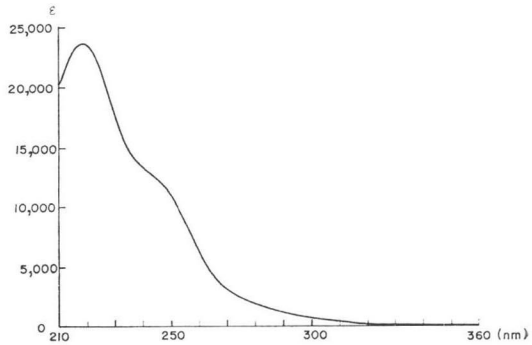
* Silica gel sheet, developed by lower phase of $CHCl_3$ - MeOH - 7% NH_4OH (40 : 12 : 20).

Fig. 3. IR spectrum of mycinamicin I in KBr disk.



absorption maxima at 215 nm (log ε, 4.32) and 281.5 nm (log ε, 4.33) (Fig. 7). The IR and UV spectra of mycinamicin IV (Fig. 8) and V (Fig. 9) were similar to those of mycinamicin III.

Fig. 4. UV spectrum of mycinamicin I in methanol.



Biological Properties

The antimicrobial activities of the mycinamicins are shown in Tables 7 and 8, in comparison with erythromycin and leucomycin A_3. Of the mycinamicin components, mycinamicin I showed the highest antibacterial activity against Gram-positive bacteria. The antibacterial activities of mycinamicins I and IV against macrolide-sensitive strains of *Staphylococcus aureus*, were superior to that of erythromycin. With an inoculum size of 1×10^6 cells/ml (Table 8), the mycinamicins showed activities against *S. aureus* 0116 and *S. aureus* 0119, both of which are macrolide-resistant strains of group A. They were not active against Gram-negative bacteria except *Haemophilus*

Fig. 5. IR spectrum of mycinamicin II in KBr disk.

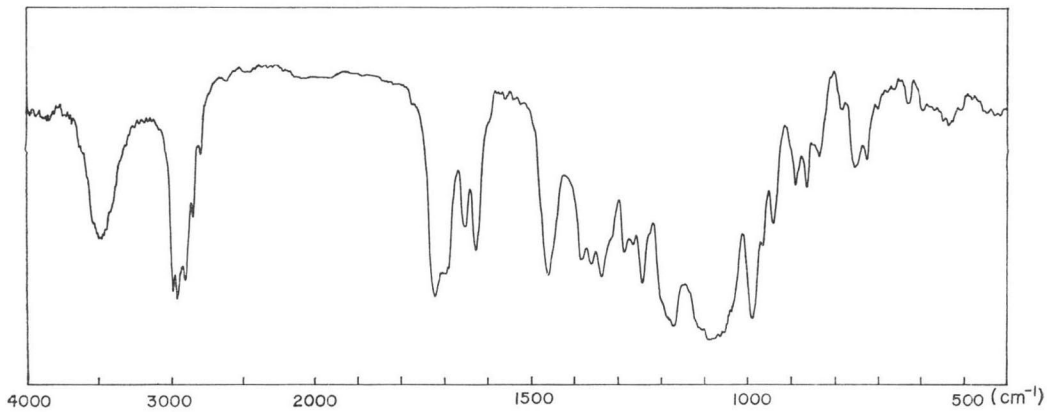
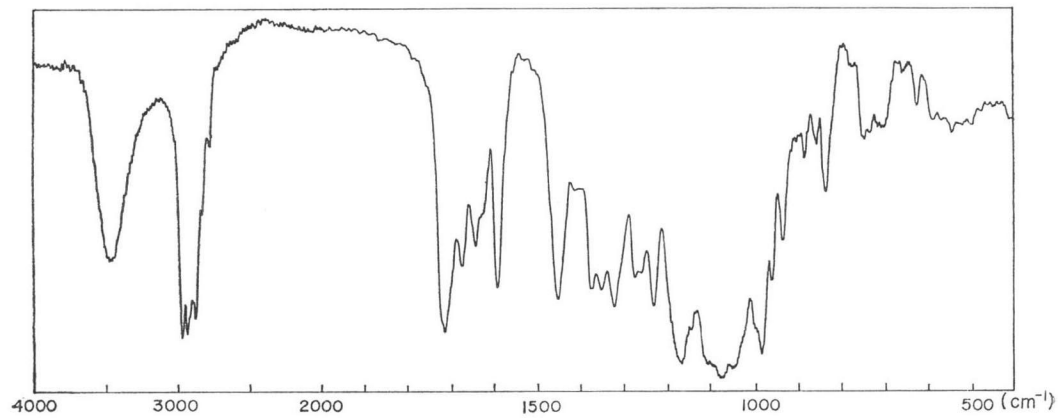


Fig. 6. IR spectrum of mycinamicin III in KBr disk.

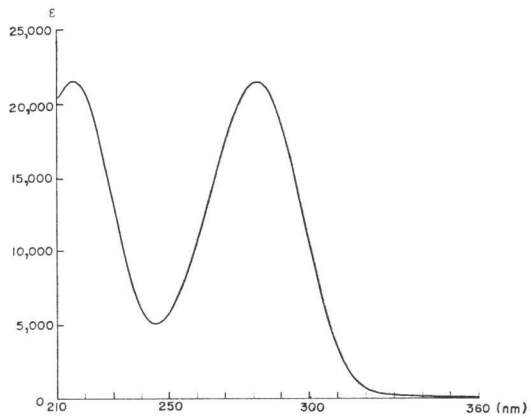


influenzae 1346.

The acute toxicities of mycinamicins I and II were determined with *ddY* male mice using intraperitoneal, subcutaneous and oral routes of administration, and the results are shown in Table 9.

The absorption of mycinamicins I and II was studied in dogs after single oral dose of 500 mg/dog which approximated 50 mg/kg. As shown in Fig. 10, the serum level of each antibiotic reached about 15 mcg/ml after about 2 hours.

Fig. 7. UV spectrum of mycinamicin III in methanol.



Discussion

The physicochemical and biological properties described above indicate that the mycinamicins may be basic macrolide antibiotics. The UV spectra of mycinamicins I and II were similar to those

Fig. 8. IR spectrum of mycinamicin IV in KBr disk.

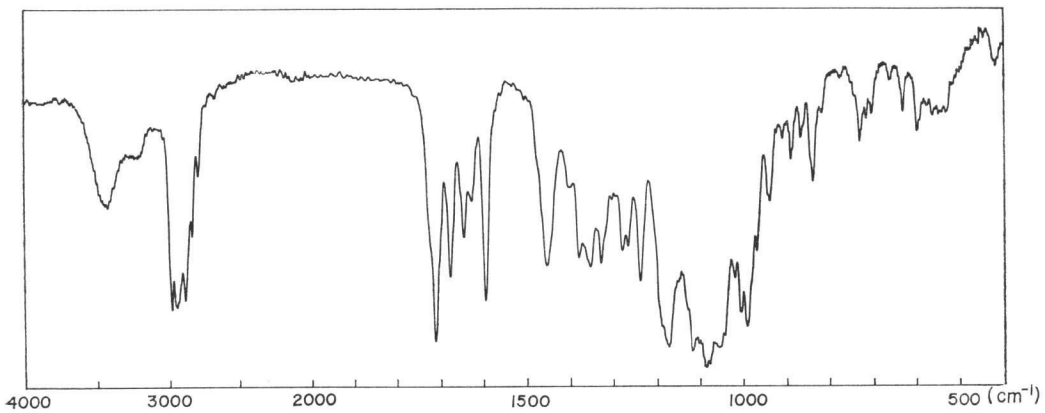


Fig. 9. IR spectrum of mycinamicin V in KBr disk.

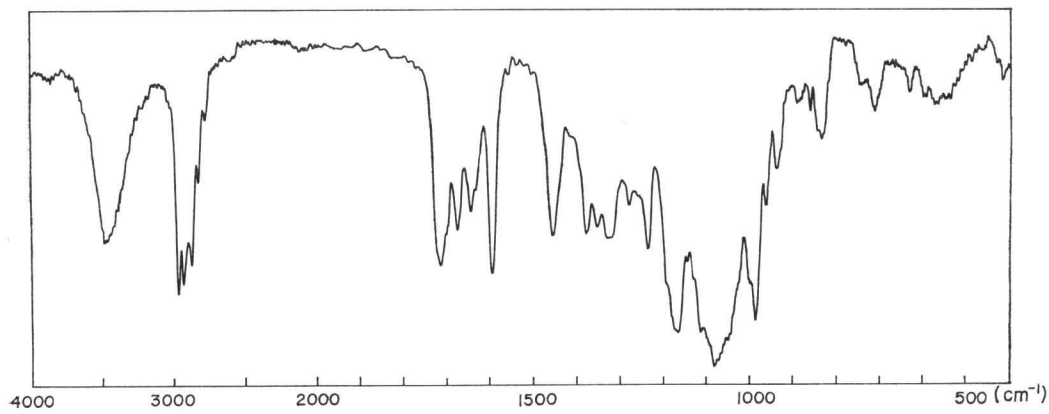


Table 7. Antibacterial spectra of mycinamicins, erythromycin (EM) and leucomycin A_3 (LMA_3).

Organism	Medium*	MIC (mcg/ml)						
		I	II	III	IV	V	EM	LMA_3
1 *Staph. aureus* ATCC 6538P	1	0.1	0.4	0.2	0.1	0.4	0.2	3.2
2 *Staph. aureus* MS 353	1	0.1	0.4	0.4	0.1	0.8	0.2	1.6
3 *Staph. aureus* MS 353 C-36 (Mac^r C)	1	0.1	0.2	0.2	0.1	0.2	>100	1.6
4 *Staph. aureus* 0126 (Mac^r B)	1	0.2	0.4	0.4	0.4	0.8	>100	1.6
5 *Staph. aureus* MS 353 AO (Mac^r A)	1	>100	>100	>100	>100	>100	>100	>100
6 *Staph. aureus* 0116 (")	1	>100	>100	>100	>100	>100	>100	>100
7 *Staph. aureus* 0119 (")	1	>100	>100	>100	>100	>100	>100	>100
8 *Staph. aureus* 0127 (")	1	>100	>100	>100	>100	>100	>100	>100
9 *Staph. epidermidis* sp-al-1	1	<0.05	0.1	0.1	<0.05	0.2	0.1	1.6
10 *Strept. pyogenes* N.Y. 5	1	<0.05	<0.05	0.1	<0.05	0.2	<0.05	0.2
11 *Strept. pyogenes* 1022 (Mac^r)	1	>100	>100	>100	>100	>100	>100	>100
12 *S. pneumoniae* NCTC 7465 (type 1)	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
13 *Sarcina lutea* ATCC 9341	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
14 *Micrococcus flavus* ATCC 10240	1	<0.05	0.1	<0.05	<0.05	0.1	0.1	0.1
15 *Corynebact. diphtheriae* P.W. 8	1	0.8	0.8	3.2	1.6	6.3	<0.05	<0.05
16 *Bac. subtilis* ATCC 6633	1	0.4	0.4	1.6	0.4	1.6	0.1	0.4
17 *E. coli* NIHJ JC-2	1	>100	>100	>100	>100	>100	>100	>100
18 *Salm. enteritidis* Gaertner	1	>100	>100	>100	>100	>100	>100	>100
19 *Shigella flexneri* type 3a	1	100	100	>100	>100	>100	25	>100
20 *Ps. aeruginosae* IAM 1095	1	>100	>100	>100	>100	>100	>100	>100
21 *Haemophilus influenzae* 1346	2	1.6	3.2	3.2	1.6	3.2	1.6	12.5

Inoculum size: 1×10^8 cells/ml

* Medium 1: Heart infusion agar
Medium 2: HIA supplemented with 2% Bacto-Fildes enrichment (Difco)

of neutral macrolide antibiotics such as chalcomycin[12] and neutramycin[13]. Among the known basic macrolide antibiotics, none showed UV spectra similar to that of mycinamicin I or II. Mycinamicins III, IV and V showed strong UV absorption peaks at 215 nm and around 280 nm. There are no known macrolide antibiotics which exhibit similar UV spectra. Thus mycinamicins III, IV and V are easily distinguishable from other basic macrolide antibiotics. The mycinamicins are also distinguished from other macrolide antibiotics by their IR spectra, molecular weights, molecular formulae, melting points and specific rotations as shown in Table 6.

Detailed chemical studies of the mycinamicins to be reported elsewhere[14,15] have confirmed the possibility, shown from these properties, that they are basic macrolide antibiotics. The structures determined separately for mycinamicins I, II, IV and V are shown as Fig. 11.

From the studies of cross-resistance to the macrolide antibiotics, clinical isolates of *Staphylococci* was classified into three groups, namely groups A, B and C. The group A strains were resistant to all of the macrolide antibiotics at high levels and the resistance was considered to be constitutive. The group B and C strains were resistant to both erythromycin and oleandomycin or only erythromycin, respectively, and these resistances were inducible. In the group B strains erythromycin and oleandomycin were active inducers, while in the C group only erythromycin was active. As reported here, mycinamicin showed strong activity on sensitive *Staphylococci* such as ATCC 6538P and MS 353 and

Table 8. Antibacterial spectra of mycinamicins, erythromycin (EM) and leucomycin A_8 (LMA_8).

Organism	Medium*	MIC (mcg/ml)						
		I	II	III	IV	V	EM	LMA_8
1 *Staph. aureus* ATCC 6538P	1	<0.05	0.1	0.1	<0.05	0.1	<0.05	0.8
2 *Staph. aureus* MS 353	1	<0.05	0.1	0.1	<0.05	0.1	0.1	0.8
3 *Staph. aureus* MS 353 C-36 (Mac^r C)	1	<0.05	0.1	<0.05	<0.05	0.1	1.6	0.4
4 *Staph. aureus* 0126 (Mac^r B)	1	0.1	0.2	0.2	0.1	0.2	>100	0.8
5 *Staph. aureus* MS 353 AO (Mac^r A)	1	100	>100	>100	100	>100	>100	>100
6 *Staph. aureus* 0116 (Mac^r A)	1	0.8	6.3	50	1.6	25	>100	>100
7 *Staph. aureus* 0119 (")	1	50	50	>100	25	>100	>100	>100
8 *Staph. aureus* 0127 (")	1	>100	>100	>100	>100	>100	>100	>100
9 *Staph. epidermidis* sp-al-1	1	<0.05	0.1	0.1	<0.05	0.1	<0.05	0.8
10 *Strept. pyogenes* N.Y. 5	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.1
11 *Strept. pyogenes* 1022 (Mac^r)	1	25	100	>100	>100	>100	>100	>100
12 *S. pneumoniae* NCTC 7465 (type 1)	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
13 *Sarcina lutea* ATCC 9341	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
14 *Micrococcus flavus* ATCC 10240	1	<0.05	0.2	<0.05	<0.05	0.1	<0.05	0.1
15 *Corynebact. diphtheriae* P.W. 8	1	0.1	0.4	0.8	0.1	0.8	<0.05	<0.05
16 *Bac. subtilis* ATCC 6633	1	0.1	0.2	0.8	0.1	0.8	<0.05	0.4
17 *E. coli* NIHJ JC-2	1	>100	100	>100	>100	>100	100	>100
18 *Salm. enteritidis* Gaertner	1	>100	>100	100	>100	>100	100	>100
19 *Shigella flexneri* type 3a	1	50	25	12.5	100	100	12.5	100
20 *P. aeruginosae* IAM 1095	1	>100	>100	>100	>100	>100	>100	>100
21 *Haemophilus influenzae* 1346	2	0.8	1.6	1.6	0.8	1.6	0.8	6.3

Inoculum size: 1×10^6 cells/ml

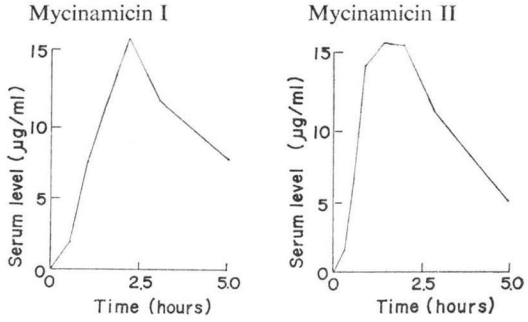
* Medium 1: Heart infusion agar
Medium 2: HIA supplemented with 2% Bacto-Fildes enrichment (Difco)

Table 9. Acute toxicity in male mice.

	LD_{50} (mg/kg)		
	i.p.	s.c.	p.o.
I	177	310	>1,000
II	363	465	>1,000

strains of groups B and C. This fact indicates that mycinamicin is not an active inducer for resistance in groups B and C strains. In addition, mycinamicin was moderately effective against some group A strains (MS 353 AO, 0116 and 0119) at low inoculation levels. The reason why mycinamicin acts on such group A strains but not on another group A strain (0127) is not clear.

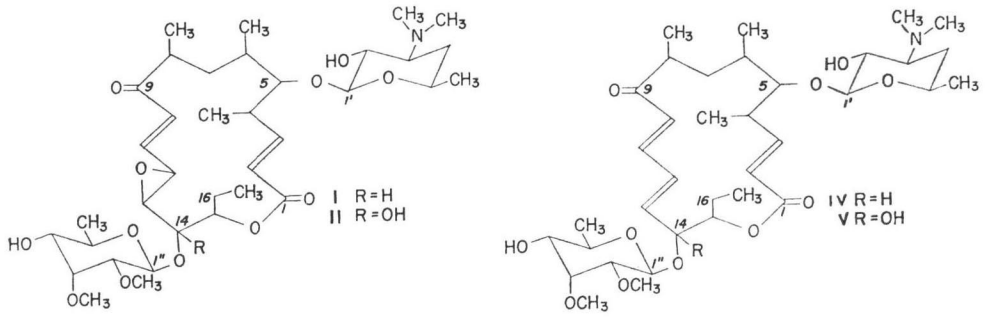
Fig. 10. Oral absorption in dogs after single dose (50 mg/kg).



Drug was administered in gelatin capsules.

The serum levels of mycinamicins I and II are the average values of two series experiments, and the concentration of the drugs in the serum were calculated by the paper disk agar method using *Sarcina lutea* ATCC 9341 as a test organism.

Fig. 11.



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