# STUDIES ON A NEW AMINO ACID ANTIBIOTIC, AMICLENOMYCIN

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(Received for publication March 7, 1974)

A new amino acid antibiotic, amiclenomycin is produced by *Streptomyces lavendulae* subsp. *amiclenomycini* OKAMI. The chemical structure of amiclenomycin is L-2-amino-4-(4′-amino-2′,5′-cyclohexadienyl)-butyric acid. The antibiotic inhibits growth of mycobacteria and this activity is reversed by biotin and desthiobiotin. α-Methyl-desthiobiotin is coproduced along with amiclenomycin.

In the course of searching for antimicrobial agents, a new amino acid antibiotic named amiclenomycin was discovered in culture filtrates of a streptomycete, strain MD580-S1. The results of taxonomic studies, indicate the producing organism is a new subspecies for which the name *Streptomyces lavendulae* subsp. *amiclenomycini* OKAMI is proposed. The antibiotic inhibits growth of mycobacteria, including antibiotic-resistant mycobacteria.

This paper deals with the taxonomy of the new subspecies as well as the production, isolation, characterization, structure and biological properties of amiclenomycin.

## Taxonomy

Among isolates of streptomycetes obtained from a soil sample, one isolate, MD580-S1, exhibited antibacterial activity and possessed the following characteristics on various media. The color designations followed are those of the Japan color standard[1] and are indicated by "(Color phase)".

(1) Microscopic characteristics:

Branched substrate mycelium develops aerial mycelia, about 1 μ in width with tips that are flexuous to loosely coiled with less than 5 turns, as shown in Plate 1. Under an electron microscope, the spores showed smooth surfaces without spiny or hairy structures. The spores were cylindrical and measured 0.3~0.5×0.8~1.0 μ. Typical chains of spores are shown in Plate 2, with more than 10 spores observed in a chain. The morphology of the spore chains places the organism in Sections *Retinaculiaperti* or *Spirales*. Special organs such as sclerotia were not observed.

(2) Characteristics on various media:

1) On sucrose-nitrate-agar plate incubated at 27°C: Colorless to faint yellowish, fair growth, thin, white to sparse, grayish-white aerial mycelium. Almost no diffusible pigment was formed.

2) On glucose-asparagine-agar plate incubated at 27°C: Faint yellowish to pale yellowish-brown growth; aerial mycelium of light brownish-gray color (Color phase, 5-8). Diffusible pigment of pale yellowish-brown color may be produced.

3) On glycerol-asparagine-agar plate (ISP* medium No. 5) incubated at 27°C: Essentially the same characteristics as those on glucose-asparagine agar except there was sparse formation of aerial mycelium.

4) On inorganic salts-starch-agar plate (ISP medium No. 4) incubated at 27°C: Excellent

* International *Streptomyces* Project.

growth of pale yellowish-brown color (Color phase, 7-6); abundant aerial mycelium of light brownish gray (Color phase, 5-8) to light gray color. No diffusible pigment was observed. Medium to strong hydrolysis of starch (13~18 mm width around the growth) was detected by the KI-starch reaction.

**5)** On tyrosine-agar plate (ISP medium No. 7) incubated at 27°C: Good growth of brownish-gray color; abundant aerial mycelium of grayish-white to light gray color. Diffusible pigment of light brownish-gray color (unlike melanin, *i.e.*, probably a negative melanin reaction).

**6)** On nutrient agar plate incubated at 27°C: Fair growth of light brownish color; no aerial mycelium formed; produced diffusible pigment of a very light brownish color.

**7)** On yeast-malt-agar plate (ISP medium No. 2) incubated at 27°C: Good growth of pale yellowish-brown to yellowish-brown color (Color phase, 5-7); formed abundant aerial mycelium of light gray color (Color phase, colorless-2). Pale yellowish-brown (Color phase, 7-4) diffusible pigment was formed.

**8)** On oatmeal-agar plate (ISP medium No. 3) incubated at 27°C; Fair growth of pale yellowish brown color (Color phase, 7-5); thin aerial mycelium of light gray color (Color phase, 7-4) and produced diffusible pigment of pale yellowish brown color.

**9)** On glucose-peptone-gelatin medium incubated at 20°C and 27°C: At 20°C, good growth of brownish color; grayish-white aerial mycelium; and blackish-brown diffusible pigment. Gelatin was not liquefied. At 27°C, good growth of brownish color; formed no aerial mycelium and produced a blackish-brown diffusible pigment. Although gelatin was liquefied slightly, the strain was considered to be negative for that characteristic.

**10)** On skimmed milk incubated at 27°C and 37°C: Faint growth of pale yellowish color on surface of milk; no aerial mycelium; none to small amount of slight yellowish pigment. Slight coagulation and peptonization slowly occurred at both 27°C and 37°C.

**11)** On peptone-yeast-iron agar plate (ISP medium No. 6) incubated at 27°C: Fair growth of pale yellowish-brown color (Color phase, 7-4); no aerial mycelium; diffusible pigment of pale yellowish-brown color (doubtful or negative melanin reaction).

**12)** Utilization of carbohydrates for growth on PRIDHAM-GOTTLIEB's basal medium (ISP medium No. 9) incubated at 27°C:

| Carbohydrate added | Growth* | Carbohydrate added | Growth* |
|---|---|---|---|
| None | − | Meso-inositol | − |
| L-Arabinose | − | L-rhamnose | −/± |
| D-Xylose | − | Raffinose | −/± |
| D-Glucose | ++ | D-Mannitol | − |
| D-Fructose | − | Cellulose powder | − |
| Sucrose | −/± | | |

*−: no growth, ±: doubtful growth, ++: good growth

**13)** Optimum temperature for growth on maltose-yeast extract agar (maltose 1 %, yeast extract 0.4 %, agar 1.7 %, pH 7.0~7.2 before sterilization):

| Temperature (°C) | 4 | 8 | 15 | 20 | 24 | 27 | 32 | 37 | 42 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|
| Growth* | − | − | − | ++ | ++ | +++ | ++ | + | ± | − |

*−: no growth, ±: faint growth, +: fair growth, ++: good growth, +++: excellent growth

Summarizing the above, strain MD580-S1 belongs to genus *Streptomyces* and forms branched aerial mycelium without whorls. Spore chains are flexuous and coiled with more than 10 spores per chain. The spore surfaces are smooth with no spiny or hairy structures and the spore shape is cylindrical. Growth is pale yellowish-brown and develops aerial mycelium of white to brownish gray color on various media. Almost no aerial mycelium is formed on nutrient agar, glucose-

Plate 1. Photomicrograph of strain MD 580-S1 (×150) on inorganic salts-starch agar after 7 days at 27°C.

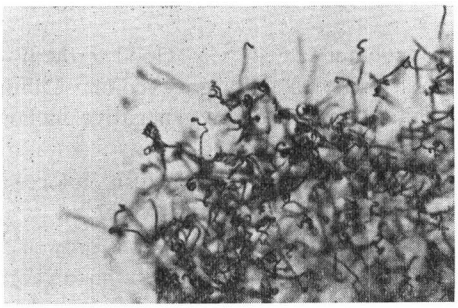
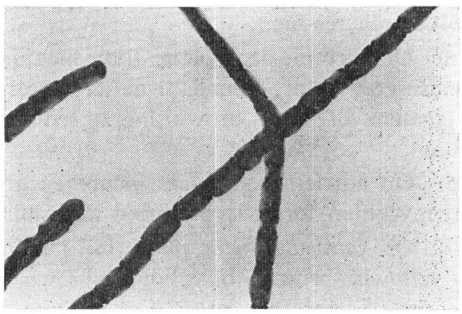


Plate 2. Electronmicrograph of strain MD 580-S1 (×5,000) on inorganic salts-starch agar after 7 days at 27°C.



peptonegelatin medium and skimmed milk medium. Blackish-brown pigment is produced in glucosepeptone-gelatin medium, whereas slightly yellowish-brown pigment is produced with tyrosine agar, nutrient agar or peptone-yeast-iron agar, conventionally used for the detection of melanin-pigment formation. Medium to strong hydrolysis of starch occurs. Proteolytic activity on skimmed milk and gelatin is weak. Only glucose is utilized for growth with PRIDHAM-GOTTLIEB's basal medium, while L-arabinose, D-xylose, D-fructose, sucrose, inositol, L-rhamnose, raffinose, D-mannitol and cellulose are not. Among known species of *Streptomyces*, *Streptomyces lavendulae* (WAKSMAN and CURTIS 1916) WAKSMAN and HENRICI 1948[2,3] has many features in common with strain MD580-S1, *i.e.*, tips of aerial mycelia being flexous or curled with less than 5 turns (cf. p. 41 and p. 61 in Reference 4), spores of cylindrical shape with sharp angle (cf. Plate in p. 137 of Reference 5) and no (or poor) utilization of L-arabinose, D-xylose, D-fructose, sucrose, inositol, rhamnose, raffinose and D-mannitol. Good utilization of D-glucose on PRIDHAM-GOTTLIEB's basal medium is observed. (cf. References 5, 6, 7 and 8). The following differences, however, are noted in features between *S. lavendulae* and strain MD580-S1: strain MD580-S1 does not elicit a clearly recognizable melanin pigment on melanin test media but it does produce a blackish-brown pigment on glucose-gelatin medium. In contrast, *S. lavendulae* produces melanin-pigment with proteinous media according to the original description (cf. p. 126 in Reference 2) and according to the data of HÜTTER (cf. p. 60 in Reference 4); strain MD580-S1 forms aerial mycelium of white to brownish-gray color, whereas *S. lavendulae* produces that of gray color with lavender or pink tinge according to the original description. However, it should be noted that the color of aerial mycelium of *S. lavendulae*, strain IMRU 3440 (ISP 5069) was categorized in the Red (or Gray) series depending on the observer (cf. p. 138 in Reference 5). Finally, amiclenomycin, a new antibiotic produced by strain MD580-S1, never has been reported as a metabolite of any strain of *S. lavendulae*. Regarding the production of $\alpha$-methyldesthiobiotin by strain MD580-S1, *Streptomyces lydicus* DEBOER, DIETZ, SILVER and SAVAGE 1956[9] was stated to produce a related substance, $\alpha$-methylbiotin. However, utilization of many sugars (except rhamnose) by *S. lydicus* differentiates it from strain MD58-S1 which is incapable of using many sugars except glucose. Other antibiotics (streptolydigin and lydimycin) which were stated to be produced by *S. lydicus* are not detectable in preparations of strain MD580-S1.

Thus, strain MD580-S1 is closely related to *S. lavendulae* (WAKSMAN and CURTIS 1916)

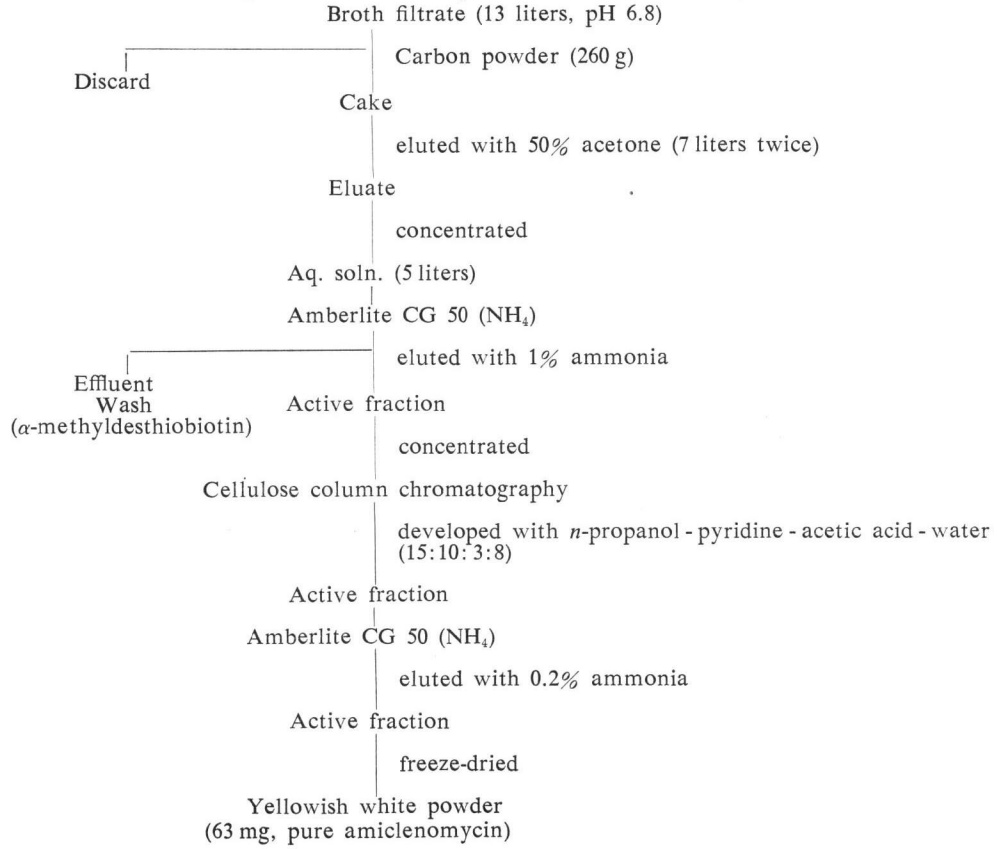
WAKSMAN and HENRICI 1948, but is distinguishable from that species in lack of chromogenicity (negative melanin reaction). Therefore, we propose the name *S. lavendulae* subsp. *amiclenomycini* OKAMI for strain MD580-S1. Type: Strain MD580-S1 was deposited in the collection of the Institute of Microbial Chemistry, Tokyo, Japan as IMC MD580-S1. The subspecific epithet is derived from the name of the antibiotic produced.

## Isolation and Characterization

Strain MD580-S1 was inoculated into a medium containing 2.0 % corn starch (w/v), 2.0 % corn gluten meal, 1.0 % glucose, 1.0 % corn steep liquor, 0.25 % $NH_4Cl$, 0.3 % NaCl, 0.6 % $CaCO_3$ (pH 6.2 before sterilization) and cultured on a reciprocal shaker (8 cm amplitudes 120 strokes/min.) at 27°C for 40 hours. The culture (1.7 % v.) was transferred into the same medium in a jar fermentor and cultured at 27°C for 4 days. The production of amiclenomycin was followed by a cylinder plate assay method using *Mycobacterium smegmatis* ATCC 607 as the test organism.

Strain MD580-S1 was found to produce simultaneously another antibacterial substance which inhibited growth of *Mycobacterium smegmatis* ATCC 607. This second active principle was identified as $\alpha$-methyldesthiobiotin.[14] The activities of both antibiotics were measured separately after extraction of $\alpha$-methyldesthiobiotin with *n*-butanol at pH 2. Amiclenomycin

Fig. 1. Isolation and purification of amiclenomycin.



was isolated by treatment with carbon and Amberlite CG 50 resin followed by cellulose column chromatography, as shown in Fig. 1.

Amiclenomycin is a yellowish-white powder melting at 215~216°C with decomposition, $[\alpha]_D^{25}+10.9°$(*c*. 0.3, water), titration equivalent 210 (in 0.1 N HCl with 1 N NaOH). The empirical formula, $C_{10}H_{16}N_2O_2$ (mol. wt. 196) is derived from the elemental analysis of the crystalline monosulfate (mp 178~181°C, dec.). Amiclenomycin has no UV absorption maximum except end absorption. The IR and NMR spectra are shown in Figs. 2 and 3, respectively. The antibiotic gives positive ninhydrin, potassium permanganate and RYDON-SMITH reactions, but negative biuret, MOLISCH, LEMIEUX, ferric chloride, MILLON and SAKAGUCHI reactions. It is soluble in water and methanol, but almost insoluble in ethanol, ethyl acetate, chloroform and ethyl ether. Under high voltage paper electrophoresis (3,000 V, 20 minutes) in formic acid - acetic acid - water (25:75:900, v/v), it moves to the cathode: Rm is 1.33 in relation to 1.0 of alanine. On thin-layer chromatography (TLC) using Cellulose F (product

Fig. 2. IR spectrum of amiclenomycin (KBr).

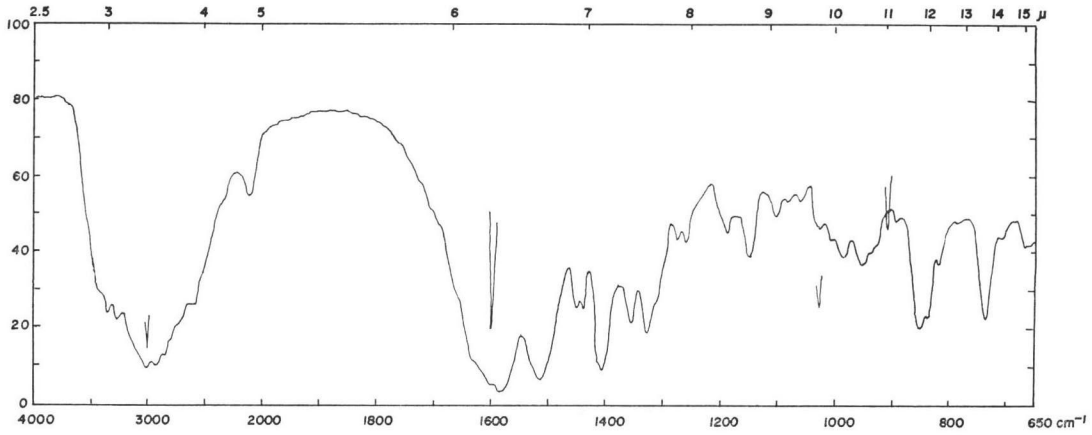
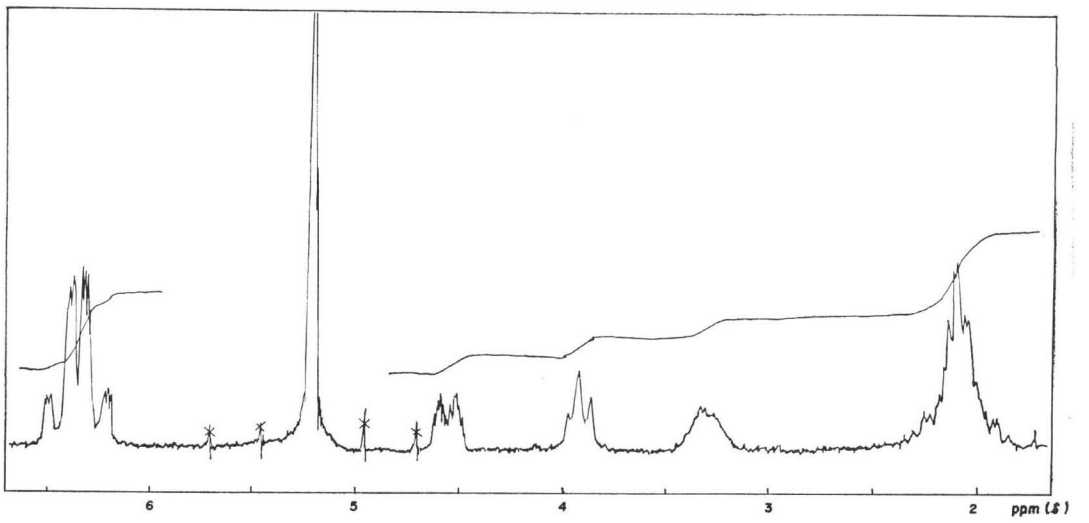



Fig. 3. NMR spectrum of amiclenomycin (100 MHz, in $D_2O$).

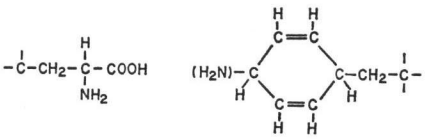


of E. Merck Co.), it gives a single spot at Rf 0.45 with *n*-propanol-pyridine-acetic acid-water (15:10:3:12) and Rf 0.03 with *n*-butanol-acetic acid-water (2:1:1). Amiclenomycin is unstable in alkaline solution but stable in neutral and acidic solution.

## Structure of Amiclenomycin

The molecular formula of amiclenomycin, $C_{10}H_{16}N_2O_2$ is supported by the C-13 NMR spectrum in $D_2O$ (dioxane as internal reference, $\delta$ 67.4 from TMS) showing the presence of 10 carbons. The chemical shifts are as follows: $\delta$ (ppm) 181.8, 135.0 (two equivalent olefinic carbons), 126.3 (two equivalent olefinic carbons), 56.1, 44.9, 34.8, 29.5, 29.3. The high resolution mass spectrum gives a peak at *m/e* 179.0965 (calcd. for $C_{10}H_{13}NO_2$, 179.0946, $M^+-NH_3$). The potentiometric titration indicates the presence of one acidic function of pKa′ 2.0 and two basic functions of pKa′ 8.7 and 9.9. The presence of a carboxylic group is shown by the bands at 1580 and 1405 $cm^{-1}$ and that of the monosulfate at 1730 $cm^{-1}$ in IR spectra (KBr). The treatment of amiclenomycin with acetic anhydride in methanol afforded N, N′-diacetylamiclenomycin methyl ester (**I**). The NMR spectrum of **I** shows signals at $\delta$ 3.71 ($CO\text{-}OCH_3$), $\delta$ 1.94 ($N\text{-}CO\text{-}CH_3$) and $\delta$ 1.98 ($N\text{-}CO\text{-}CH_3$). The mass spectrum of **I** shows the parent peak at *m/e* 294 and an intense peak at *m/e* 292 ($M^+\text{-}H_2$).

The NMR spectrum of amiclenomycin in $D_2O$ (100 MHz, TMS as external reference) shows signals at $\delta$ 2.06 (2H, m), 2.19 (2H, m), 3.30 (1H, m), 3.93 (1H, t, J=6), 4.56 (1H, m) and 6.36 (4H, m). Irradiation at $\delta$ 2.19 causes the collapse of the signal at $\delta$ 3.93 to a singlet. Irradiation at $\delta$ 2.06 collapses the signal at $\delta$ 3.30 to a broad doublet (J=7.5). The simultaneous irradiation at $\delta$ 3.30 and 4.56 causes a change of the complexed signal at $\delta$ 6.36 to $A_2B_2$ quartet pattern (J=10). A long range coupling (J=7.5) is shown between the signals at $\delta$ 3.30 and 4.56 by the irradiation at $\delta$ 2.06 or 6.36. These findings on the intact molecule can be summarized in the following structures:



The treatment of amiclenomycin with Pd-charcoal in methanol under reflux for several hours followed by acetylation with acetic anhydride in methanol afforded a mixture of methyl 2-acetamido-4-phenylbutyrate (**II**) and methyl 2-acetamido-4-(4′-acetamidophenyl) butyrate (**III**). **II** and **III** have the UV spectra of alkyl- and *p*-aminoalkyl-benzene derivatives, respectively. The molecular formula of **III** was confirmed by high resolution mass spectrum: *m/e* 292.1445; calcd. for $C_{15}H_{20}N_2O_4$, 292.1422. The NMR spectrum of **III** in $CDCl_3$ shows two N-acetyl groups ($\delta$ 2.00 and 2.14) and characteristic $A_2B_2$ type aromatic protons (4H, $\delta$ 7.25, J=9). As for the side chain part, two methylene protons at $\delta$ 2.10 and 2.62 are coupled with each other (J=7.5) and the methine proton at $\delta$ 4.65 is coupled with the methylene at $\delta$ 2.10. These results indicate the aromatization of the cyclohexa-2′,5′-diene ring of amiclenomycin to **III** and the substitutions at 1′, 4′ of the cyclohexadiene ring. Hydrolysis of **II** with 6 N HCl gave a crystalline compound ($C_{10}H_{13}NO_2$, mp 310~313°C, dec.), which was identical with L-$\gamma$-phenylbutyrine[11] in all respects including optical rotation ($[\alpha]_D^{25}$ +53°). From this result, the configuration at $\alpha$-carbon of amiclenomycin corresponds to

L-series.

As described above, the methine protons of amiclenomycin at $\delta$ 3.30 and 4.56 are coupled with each other and the value (J=7.5) of this long range coupling constant is consistent with that reported in the spectrum of a *trans*-1,4-dihydrobenzene derivative in boat form.[12] But the stereochemistry of the cyclohexadiene ring still remains in doubt. From all the information mentioned above, the structure of L-2-amino-4-(4′-amino-2′, 5′-cyclohexadienyl)-butyric acid can be assigned to amiclenomycin. This structure suggests a close relation to stravidine.[13]

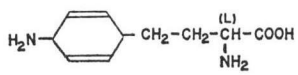



Table 1. Minimum inhibitory concentration of amiclenomycin against mycobacteria.

| Organism | M.I.C. (mcg/ml) |
|---|---|
| *Mycobacterium smegmatis* ATCC 607 | 6.25 |
| ,, paromomycin-r.* | 6.25 |
| ,, capreomycin-r.* | 6.25 |
| ,, viomycin-r.* | 6.25 |
| ,, staphylomycin-r.* | 6.25 |
| ,, kanamycin-r-* | 6.25 |
| ,, streptomycin-r.* | 6.25 |
| *Mycobacterium phlei* | 6.25 |
| *Mycobacterium tuberculosis* $H_{37}Rv$ | 3.1** |
| ,, kanamycin-r. | 6.3** |
| ,, ,, #890 | 6.3** |

Medium: nutrient agar +1% glycerine
*; resistance acquired *in vitro*
**; KIRCHNERS' semisolid medium with 10% horse serum

## Biological Properties of Amiclenomycin

The antimicrobial spectrum of amiclenomycin tested by the agar dilution method is shown in Table 1. It inhibits mycobacteria including resistant tubercle bacilli, but not other bacteria or fungi. The activity of amiclenomycin (10~1,000 mcg/ml) is reversed in the presence of biotin or DL-desthiobiotin at the concentrations of 0.01 or 0.1 mcg/ml respectively. Biotin at lower than 0.001 mcg/ml and DL-desthiobiotin at lower than 0.01 mcg/ml showed no reversion of the activity of amiclenomycin (10~1,000 mcg/ml). Intravenous injection of amiclenomycin to mice at a dose of 1,000 mg/kg caused no death.

Detailed biological properties against mycobacteria *in vitro* and *in vivo* will be presented elsewhere.

Actithiazic acid,[10] $\alpha$-dehydrobiotin,[14] $\alpha$-methylbiotin[15] and $\alpha$-methyldesthiobiotin[15] are naturally occurring antimetabolites of biotin. L-1,4-Cyclohexadiene-1-alanine[16,17] which is structurally related to amiclenomycin has been reported in culture broths of streptomycetes. As shown by the structure, amiclenomycin was first isolated during the present study.

## Experimental

Isolation of amiclenomycin: Strain MD580-S1 was inoculated into 125 ml of medium* in 500-ml Sakaguchi flasks. Cultures were incubated on a reciprocal shaker (8-cm amplitude, 120 strokes/min.) at 27°C for 40 hours at which time 250 ml of the culture was transferred into 15 liters of the same medium in 30-liter jar fermentor. Cultures were incubated with agitation at 250 rpm and aerated at 15 liters/min. at 27°C for 4 days.

Fermented broth (15 liters) was filtered and the filtrate (13 liters, pH 6.8) was treated with active carbon (260 g) to adsorb the active substances. The carbon cake was washed with water (5 liters) and eluted with 50 % aqueous acetone (7 liters, twice). The eluate was then

* as described under Isolation and characterization.

concentrated to 5 liters by evaporating off the acetone and applied to a column of Amberlite CG 50 ($NH_4^+$, 5×40 cm). The effluent contained α-methyldesthiobiotin. After washing with water (3 liters), amiclenomycin was eluted from the column with 1.0 % ammonia and the active eluate (700 ml) was concentrated to dryness (oily, 288 mg) under reduced pressure. The oily substance was applied to a cellulose column (2.5×30 cm), for chromatography and developed with *n*-propanol - pyridine - acetic acid - water (15:10:3:8). The active eluate (100 ml) was concentrated and adsorbed on Amberlite CG 50 column ($NH_4^+$, 2×40 cm). The column was eluted with 0.2 % ammonia and the active eluate (240 ml) was freeze-dried to give a yellowish-white powder of pure amiclenomycin (63 mg).

Crystalline amiclenomycin monosulfate: To a solution of amiclenomycin (30 mg) in a small volume of diluted sulfuric acid, acetone was added to make the solution cloudy. After standing overnight in a refrigerator, colorless needles were obtained (15 mg), mp 178~181°C, dec.. Anal. calcd. for $C_{10}H_{16}N_2O_2 \cdot H_2SO_4$: C 40.81, H 6.16, N 9.52, O 32.61, S 10.89. Found: C 40.10, H 6.17, N 9.74, O 32.12*, S 10.87. The monosulfate showed the same Rf values as amiclenomycin on cellulose TLC. The activity of the monosulfate indicated a purity of 700 mcg/mg compared with amiclenomycin.

N, N'-Diacetylamiclenomycin methyl ester (**I**): To a solution of amiclenomycin (20 mg) in 10 ml of methanol, 2 ml of acetic anhydride was added. After standing overnight at room temperature, the reaction mixture was concentrated to dryness. The preparation was purified by preparative silica gel TLC (benzene-methanol, 9:1) to obtain a colorless oil (7 mg); $\lambda_{max}^{MeOH}$ end absorption: $\delta$ 1.94 (3H, s), 1.98 (3H, s), 3.71 (3H, s) (100 MHz, $d_4$-methanol); *m/e* 294 ($M^+$), 292, 251, 236, 136.

Methyl 2-acetamido-4-phenylbutyrate (**II**) and methyl 2-acetamido-4-(4'-acetamidophenyl) butyrate (**III**): To a solution of amiclenomycin (152 mg) in 30 ml methanol, 10 % Pd-charcoal (100 mg) was added and the mixture was refluxed for 2.5 hours. The Pd-charcoal was removed by filtration and washed with hot methanol (30 ml, twice). The filtrate and washings were combined and concentrated to obtain a colorless powder. The powder was acetylated with 4 ml of acetic anhydride in 20 ml of methanol overnight at room temperature to give a colorless oil (171 mg). The oil was separated into **II** (Rf 0.58 on silica gel TLC developing with ethyl acetate) and **III** (Rf 0.20) by column chromatography (1×30 cm) of silicic acid (Mallinckrodt, CC-4) developing with a mixture of benzene and ethyl acetate (1:1, v/v).

The eluate (5 ml) containing **II** was concentrated to afford 63 mg of a colorless oil. $\lambda_{max}^{MeOH}$ 260 nm ($E_{1cm}^{1\%}$ 10); $\delta$ 1.96 (3H, s, $-CO-CH_3$), 2.15 (2H, m, $-\underset{|}{C}-CH_2-C-$), 2.68 (2H, m, $\phi-CH_2-C-$), 3.70 (3H, s, $-O-CH_3$), 4.7 (1H, m, $-\underset{|}{CH}-$), 6.15 (1H, broad m, $-NH-$), 7.2 (5H, s, $\phi-C-$) (60 MHz, $CDCl_3$); *m/e* 235($M^+$), 204, 176, 131, 105, 99, 91.

The eluate (40 ml) containing **III** was concentrated to afford 20 mg of a colorless oil. $\lambda_{max}^{MeOH}$ 246, 286 (sh) nm (similar to the absorption of N-acetyl-*p*-toluidine**, $\lambda_{max}^{MeOH}$ 246, 287 (sh) nm); $\delta$ 2.0 (3H, s, $-CO-CH_3$), 2.14 (3H, s, $-CO-CH_3$), 2.10 (2H, m, $-\underset{|}{C}-CH_2-C-$), 2.62 (2H, t, J=7.5, $\phi-CH_2-C-$), 3.72 (3H, s, $-O-CH_3$), 4.65 (1H, m, $-\underset{|}{CH}-$), 6.14 (1H, m, $-\underset{|}{C}-NH-C-$), 7.25 (4H, $A_2B_2$ quartet, J=9, p-disubstituted benzene), 7.51 (1H, m, $\phi$-NH—C—) (100 MHz, $CDCl_3$); *m/e* 292.1445 (calcd. for $C_{15}H_{20}N_2O_4$, 292.1422, $M^+$), 261, 233, 203, 162, 148.

L-γ-Phenylbutyrine from **II**: A solution of **II** (30 mg) in 6 N HCl (10 ml) was refluxed for 3 hours. The reaction mixture was concentrated with addition of water. The residue was crystallized from water to give colorless crystals (14 mg, mp 310~313°C, dec.) $[\alpha]_D^{22}$ +53° (*c* 0.15, 1 N HCl). Anal. clacd. for $C_{10}H_{13}NO_2$: C 67.02, H 7.31, N 7.81. Found: C 66.88, H 7.30, N 7.48. It was identical with authentic L-γ-phenylbutyrine in all respects.

* The value obtained by direct analysis.

** This compound was synthesized from *p*-toluidine.

Acknowledgement

The authors express their appreciation to Dr. M. OHNO, Toray Industries Inc., for his helpful advice and also to Dr. K. OGATA, Kyoto University, for a generous gift of DL-desthiobiotin.

## References

1) Japan Color Standard Laboratory (Nihon Shikisai Kenkyusho): Guide to color standard. Japan Color Standard Co. (Nihon Shikisai Sha), Tokyo, Japan, 1954
2) WAKSMAN, S.A. & R.E. CURTIS: The actinomyces of the soil. Soil Sci. 1: 99~134, 1966
3) WAKSMAN, S.A. & A.T. HENRICI: Family Actinomycetaceae BUCHANAN and Family *Streptomycetaceae* WAKSMAN and HENRICI. pp. 892~980. In R.S. BREED, E.G.D. MURRAY & A.P. HITCHENS (ed.), BERGEY's Manual of Determinative Bacteriology, 6th ed., The Williams & Wilkins Co., Baltimore, 1948
4) HÜTTER, R.: Systematik der Streptomyceten unter besonderer Berücksichitigung der von ihnen gebildeten Antibiotica. S. Karger AG., Basel, p. 382, 1967
5) SHIRLING, E.B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. Intl. J. Syst. Bact. 18: 69~189, 1968
6) PRIDHAM, T.G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bact. 56: 107, 1948
7) OKAMI, Y.: Taxonomic study of antibiotic actinomycetes belonging to *Streptomycetaceae* found in Japan. Thesis, Hokkaido Univ., Japan, p. 70, 1952
8) OKAMI, Y.: A study for classification of *Streptomyces*. On the *S. lavendulae* group with reference to its immunological properties. Giorn. Microbiol. 2: 63~75, 1956
9) DEBOER, C.; A. DIETZ, W.S. SILVER & G.M. SAVAGE: Streptolydigin, a new antimicrobial antibiotic. I. Biologic studies of streptolydigin. Antibiot. Annual 1955/1956: 886~892, 1956
10) GRUNDY, W.E.; A.L. WHITEMAN, E.G. RDZOK, E.J. RDZOK, M.E. HANES & J.C. SYLVESTER: Actithiazic acid. I. Microbiological studies. Antibiot. & Chemoth. 2: 399~408, 1952
11) DU VIGNEAUD, V. & O.J. IRISH: The role of the acetyl derivative as an intermediary stage in the biological synthesis of amino acids from keto acids. J. Biol. Chem. 122: 349~370, 1938
12) DURHAN, L.J. & J. STUDEBAKER: Long-range coupling in the proton magnetic resonance spectra of 1,4-dihydrobenzene. J.C.S. Chem. Comm. 1965: 456~457, 1965
13) BAGGALEY, K.H.; B. BLESSINGTON, C.P. FALSHAW, W.D. OLLIS, L. CHAIET, & F.J. WOLF: The constitution of stravidin, a novel microbiological product. J.C.S. Chem. Comm. 1969: 101~102, 1969
14) HANKA, L.J.; M.E. BERGY & R.B. KELLY: Naturally occurring antimetabolite antibiotic related to biotin. Science 154: 1667~1668, 1966
15) MARTIN, D.G.; L.J. HANKA & L.M. REINEKE: New antimetabolite antibiotics related to biotin: $\alpha$-methyldethiobiotin and $\alpha$-methylbiotin. Tetrahedron Letters 1971: 3791~3794, 1971
16) YAMASHITA, T.; N. MIYAIRI, K. KUNUGITA, K. SHIMIZU & H. SAKAI: L-1,4-Cyclohexadiene-1-alanine, and antagonist of phenylalanine, from *Streptomyces*. J. Antibiotics 23: 537~541, 1970
17) SCANNELL, J.P.; D.L. PRUESS, T.C. DEMNY, T. WILLIAMS & A. STEMPEL: L-3-(2, 5-Dihydrophenyl) alanine, an antimetabolite of L-phenylalanine produced by a streptomycete. J. Antibiotics 23: 618~619, 1970