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Studies on Novel Bacterial Translocase I Inhibitors, A-500359s

I. Taxonomy, Fermentation, Isolation, Physico-chemical Properties and Structure Elucidation of A-500359 A, C, D and G

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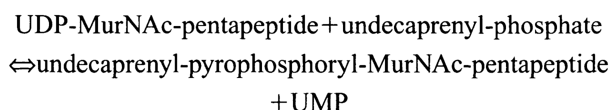
In the course of our screening for bacterial phospho-*N*-acetylmuramyl-pentapeptide-translocase (translocase I: EC 2.7.8.13) inhibitors, we found inhibitory activity in the cultured broth of the strain identified as *Streptomyces griseus* SANK 60196. The strain produced capuramycin and four novel capuramycin derivatives designated as A-500359 A, C, D and G. Purification and structural analysis were performed, and the structures of A-500359 A, C, D and G were elucidated as 6‴-methylcapuramycin, 3′-demethyl-6‴-methylcapuramycin, 2″-deoxy-6‴-methylcapuramycin and 3′-demethylcapuramycin, respectively.

Enzymes responsible for the synthesis of the peptidoglycan component of the bacterial cell wall are well precedented targets for antibiotics. Among these enzymes, bacterial phospho-*N*-acetylmuramyl-pentapeptide-translocase (translocase I) catalyzes the first step in the lipid cycle of peptidoglycan biosynthesis as follows:



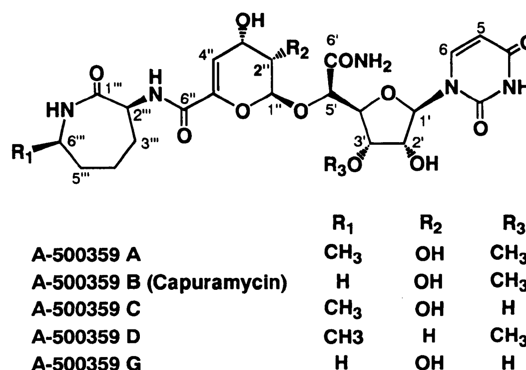
UDP-MurNAc-pentapeptide+undecaprenyl-phosphate
⇔undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide
+UMP

Thus, selective toxicity was expected to translocase I inhibitors. There are some compounds reported as translocase I inhibitors[1)], such as mureidomycins[2)], pacidamycins[3)], tunicamycin[4)], liposidomycins[5)] and napsamycins[6)]. But none of these are in clinical use and tunicamycin possesses other inhibiting activity to a process of mammalian dolichyl-pyrophosphate-*N*-acetylglucosamine biosynthesis[7)].

In the course of our screening for bacterial translocase I specific inhibitors, we found inhibitory activity in the cultured broth of *Streptomyces griseus* SANK 60196. The strain produced capuramycin[8,9)] and four novel capuramycin derivatives designated as A-500359 A, C, D and G (Fig. 1).

In this paper we describe the taxonomy and fermentation of the producing microorganism, Strain SANK 60196, and the isolation, physico-chemical properties and structure

Fig. 1. Structures of A-500359 A, C, D, G and capuramycin.



	R_1	R_2	R_3
A-500359 A	CH_3	OH	CH_3
A-500359 B (Capuramycin)	H	OH	CH_3
A-500359 C	CH_3	OH	H
A-500359 D	CH3	H	CH_3
A-500359 G	H	OH	H

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elucidation of A-500359 A, C, D and G, derivatives of capuramycin.

Results and Discussion

Taxonomy of Strain SANK 60196

Strain SANK 60196 grew well on both organic and synthetic media. The cultural characteristics on various agar media at 28°C for 14 days are presented in Table 1. Physiological properties of the strain are summarized in Table 2. Detection of LL-diaminopimelic acid in the whole-cell hydrolysates of the culture indicated that this strain had type I cell walls. Based on the taxonomic properties and a direct comparison of strain SANK 60196 and the type strain of *Streptomyces griseus* ATCC 23345, the strain SANK 60196 was identified as *Streptomyces griseus*. The strain SANK 60196 has been deposited in the National

Table 1. Cultural characteristics of strain A-500359 and *Streptomyces griseus* ATCC 23345.

Agar media		Strain A-500359	*S. griseus* ATCC 23345
Yeast extract-malt extract	G[a]:	Abundant, flat, yellowish brown	Abundant, flat, pale yellowish brown
(ISP No. 2)	AM[a]:	Abundant, velvety, pale brown	Abundant, velvety, yellowish gray
	R[a]:	Yellowish brown	Light brown
	SP[a]:	Yellowish brown	Yellowish brown
Oatmeal (ISP No. 3)	G:	Abundant, flat, yellowish brown	Abundant, flat, light olive gray
	AM:	Abundant, velvety, pale yellowish orange	Abundant, velvety, yellowish gray
	R:	Dull yellow	Yellowish gray
	SP:	None	None
Inorganic salts-starch	G:	Good, flat, yellowish brown	Abundant, flat, pale yellow
(ISP No. 4)	AM:	Abundant, velvety, yellowish gray	Good, velvety, pale yellowish orange
	R:	Yellowish brown	Yellowish gray
	SP:	None	None
Glycerol-asparagine	G:	Abundant, flat, pale yellowish brown	Abundant, flat, pale yellowish brown
(ISP No. 5)	AM:	Abundant, velvety, yellowish gray	Abundant, velvety, yellowish gray
	R:	Pale yellowish brown	Yellowish gray
	SP:	None	Yellowish gray
Peptone-yeast extract-iron	G:	Abundant, flat, pale olive	Abundant, flat, light olive gray
(ISP No. 6)	AM:	Poor, velvety, yellowish gray	None
	R:	Yellowish gray	Light olive gray
	SP:	None	Pale yellowish brown
Tyrosine (ISP No. 7)	G:	Good, flat, grayish yellow brown	Abundant, flat, yellowish brown
	AM:	Abundant, velvety, light olive gray	Abundant, velvety, yellowish gray
	R:	Yellowish brown	Dark brown
	SP:	Grayish yellow brown	Dark brown
Sucrose-nitrate	G:	Moderate, flat, pale yellow	Good, flat, yellowish gray
	AM:	Abundant, velvety, light olive gray	Moderate, velvety, yellowish gray
	R:	Dull yellow	Yellowish gray
	SP:	Pale yellow	None
Glucose-asparagine	G:	Good, flat, pale yellow	Abundant, flat, yellowish gray
	AM:	Moderate, velvety, yellowish gray	None
	R:	Yellowish gray	Yellowish gray
	SP:	None	None
Nutrient (Difco)	G:	Good, flat, pale yellowish brown	Good, flat, yellowish gray
	AM:	Good, velvety, yellowish gray	Poor, velvety, white
	R:	Yellowish gray	Yellowish gray
	SP:	None	None
Potato extract-carrot extract	G:	Moderate, flat, yellowish gray	Good, flat, yellowish gray
	AM:	Moderate, velvety, yellowish gray	Moderate, velvety, white
	R:	Yellowish gray	Yellowish gray
	SP:	Yellowish gray	None
Water	G:	Poor, flat, yellowish gray	Poor, flat, yellowish gray
	AM:	Poor, velvety, yellowish gray	Poor, velvety, white
	R:	Yellowish gray	Yellowish gray
	SP:	Pale yellow	None

[a]G: Growth, AM: Aerial mycelium, R: Reverse, SP: Soluble pigment

Table 2. Physiological properties of strain A-500359 and *S. griseus* ATCC 23345.

	Strain A-500359	*S. griseus* ATCC 23345
Hydrolysis of starch	+	+
Liquifaction of gelatin	+	+
Reduction of nitrate	+	+
Coagulation of milk	−	−
Peptonization of milk	+	+
Production of melanoid pigment	+	+
Decomposition of		
casein	+	+
tyrosine	+	+
xanthine	−	−
Growth temperature	7～35℃	8～40℃
Optimum growth temperature	18～30℃	14～27℃
Sodium chloride tolerance	10%	7%
Utilization of		
D-glucose	+	+
L-arabinose	−	−
D-xylose	+	+
inositol	−	−
D-mannitol	+	+
D-fructose	+	+
L-rhamnose	−	−
sucrose	−	−
raffinose	−	−

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Fermentation of Strain SANK 60196

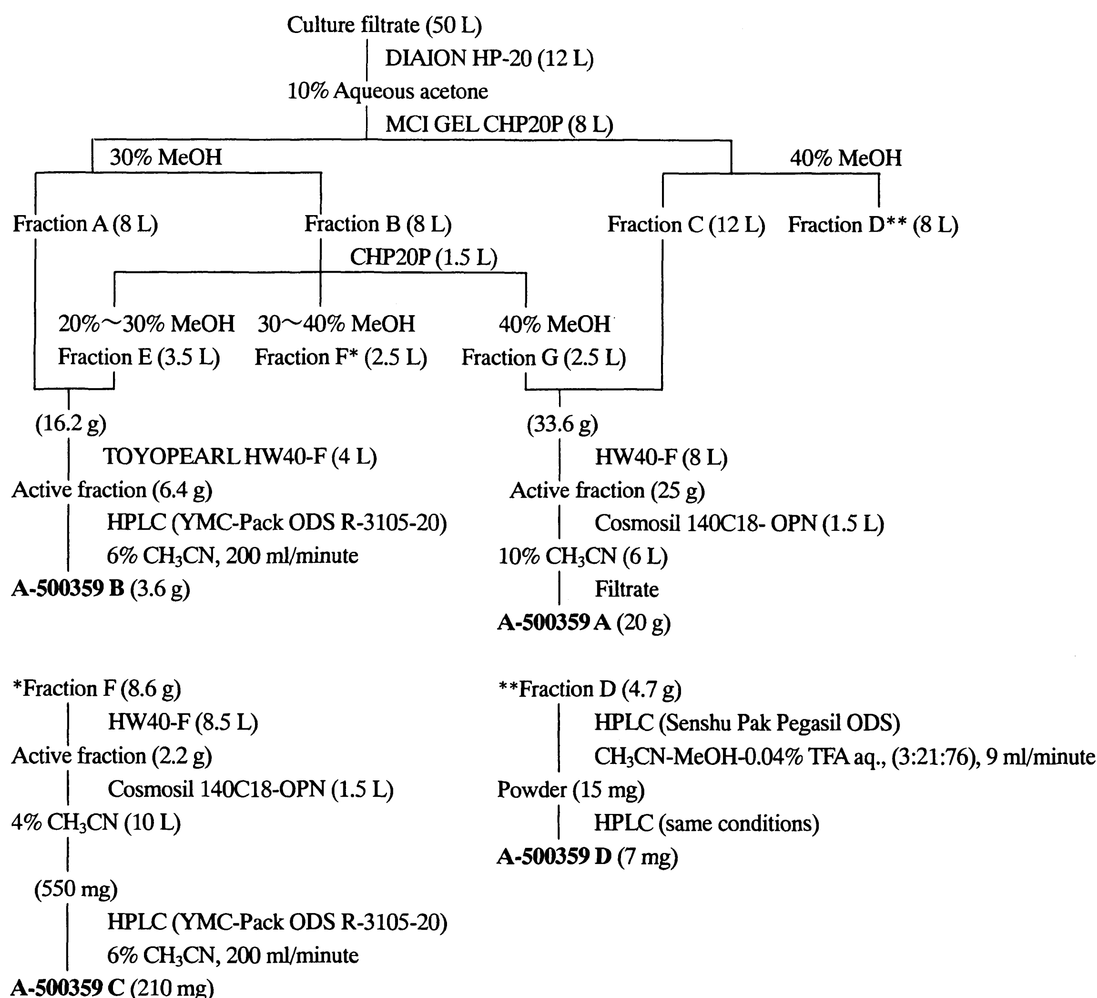
Production of A-500359 A, B, C and D: Four loopfuls of spores from a culture of strain SANK 60196 were inoculated into each of four 2-liter Erlenmeyer flasks which each contained 400 ml of seed medium consisting of maltose 3%, meat extract (Kyokuto) 0.5%, Polypepton (Nihon Seiyaku) 0.5%, NaCl 0.5% and $CaCO_3$ 0.3%, pH 7.4. The inoculated flasks were incubated on a rotary shaker (210 rpm) at 28°C for 5 days. Then a 300-ml aliquot of the culture was transferred into each of four 30-liter jar fermentors each containing 15 liters of sterilized main culture medium consisting of glucose 3%, meat extract 0.5%, Polypepton 0.5%, NaCl 0.5%, $CoCl_2 \cdot 6H_2O$ 0.005%, $CaCO_3$ 0.3% and CB442 (NOF Co., Ltd.) 0.005%, pH 7.4. Fermentation was carried out at 28°C for 8 days with an air-flow rate of 15 liters/minute and an agitation rate of 150 rpm.

Production of A-500359 G: Four loopfuls of spores from a culture of strain SANK 60196 were inoculated into each of three 2-liter Erlenmeyer flasks which each contained 500 ml of seed medium consisting of maltose 3%, meat extract 0.5%, Polypepton 0.5%, NaCl 0.5%, $CaCO_3$ 0.3% and CB442 0.005%, pH 7.4. The inoculated flasks were incubated on a rotary shaker (210 rpm) at 23°C for 5 days. A 450-ml aliquot of the culture was transferred into each of two 30-liter jar fermentors each containing 15 liters of sterilized main culture medium consisting of maltose 3%, yeast extract (Difco) 0.5%, meat extract 0.5%, Polypepton 0.5%, NaCl 0.5%, $CoCl_2 \cdot 6H_2O$ 0.05%, $CaCO_3$ 0.3% and CB442 0.005%, pH 7.4. One day after commencement of cultivation at 23°C, filter-sterilized *S*-(2-aminoethyl)-L-cysteine hydrochloride solution was added to give a final concentration of 8 mM, and cultivation was then carried out for 7 days with an air-flow rate of 15 liters/minute and an agitation rate of 150 rpm. The addition of the *S*-(2-aminoethyl)-L-cysteine hydrochloride solution in the culture is described in detail in a following report[10].

Isolation of A-500359 A, B, C and D

The isolation procedure for A-500359 A, B, C and D is shown schematically in Fig. 2. Fifty-liters of the culture

Fig. 2. Purification procedure of A-500359 A, B, C and D.



filtrate were adsorbed onto a DIAION HP-20 column (12 liters, Mitsubishi Chemical Corporation). The column was washed with water and the active substance was eluted with 10% aqueous acetone (50 liters). The eluate was concentrated *in vacuo* to remove the acetone and adsorbed onto a MCI GEL CHP20P column (8 liters, Mitsubishi Chemical Corporation). The column was washed successively with 10% and 20% aqueous MeOH, followed by stepwise elution of the active substances with 30% aqueous MeOH (16 liters) and 40% aqueous MeOH (24 liters) to give 4 fractions (fractions A~D). Each fraction was concentrated *in vacuo* to remove the MeOH.

After fraction B was adsorbed again onto a MCI GEL CHP20P column (1.5 liters), the column was washed with 10% aqueous MeOH (3 liters) and the active substance was eluted stepwise with 20%, 30% and 40% aqueous MeOH (3 liters each) to give 3 fractions (fractions E~G). Each fraction was concentrated *in vacuo* to remove the MeOH.

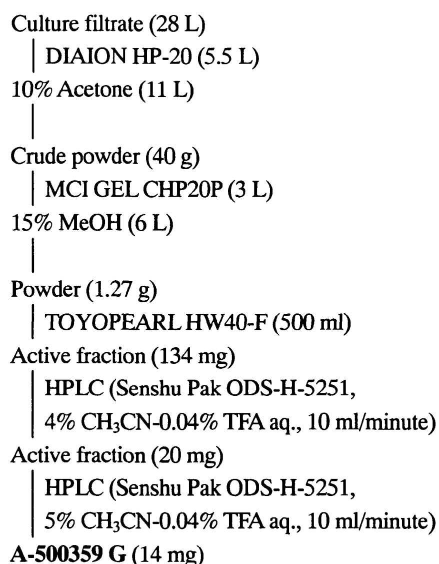
Fractions A and E were combined and lyophilized to give a crude powder (16.2 g). The powder was dissolved in 250 ml of water and applied on a TOYOPEARL HW40-F column (4 liters, TOSOH Corporation). After the column was developed with water, active fractions were collected, concentrated *in vacuo* and lyophilized to give a crude powder (6.4 g). The powder was dissolved in water and further purified by preparative HPLC using a YMC-Pack ODS column (R-3105-20, 100 i.d.×500 mm; YMC Co., Ltd.) with 6% aqueous acetonitrile as a mobile phase (flow rate, 200 ml/minute). Active fractions were collected, evaporated *in vacuo* to remove the acetonitrile, and lyophilized to obtain A-500359 B as a colorless powder (3.6 g).

Fractions C and G were combined and lyophilized to give a crude powder (33.6 g). The powder was dissolved in 450 ml of water and applied on a TOYOPEARL HW40-F column (4 liters). After the column was developed with water, active fractions were collected, concentrated *in vacuo* and lyophilized to give a crude powder (25 g). The powder was dissolved in 300 ml of water and was charged on a Cosmosil 140C18-OPN column (1.5 liters, Nacalai Tesque). After the column was washed successively with water and 1% aqueous acetonitrile, the active substance was eluted with 10% aqueous acetonitrile (6 liters). The eluate was concentrated *in vacuo*, filtered to remove insoluble materials and lyophilized to give A-500359 A as a colorless powder (20 g).

Fraction F was lyophilized to give a crude powder (8.6 g). The powder was dissolved in 500 ml of water and applied on a TOYOPEARL HW40-F column (4 liters). After the column was developed with water, active fractions were collected, concentrated and lyophilized to give a crude powder (2.2 g). The powder was dissolved in 150 ml of water and was charged on a Cosmosil 140C18-OPN column (1.5 liters). The column was washed successively with water, 0.5% aqueous acetonitrile, 1% aqueous acetonitrile and 2% aqueous acetonitrile. The active substance was finally eluted with 4% aqueous acetonitrile (10 liters), and the eluate was concentrated *in vacuo* and lyophilized to give a crude powder (550 mg). The powder was dissolved in 80 ml of water, and was further purified by preparative HPLC using a YMC-Pack ODS column (R-3105-20, 100 i.d.×500 mm) with 6% aqueous acetonitrile as a mobile phase (flow rate, 200 ml/minute). A-500359 C was thus obtained as a colorless powder (210 mg).

Fraction D was lyophilized to give a crude powder (4.7 g). The powder (800 mg) was dissolved in 10 ml of water and a 500 μl portion was injected into an HPLC column (Senshu Pak Pegasil ODS, 20 i.d.×250 mm, Senshu Scientific; mobile phase: acetonitrile - MeOH - 0.04% aqueous trifluoroacetic acid [3 : 21 : 76]; flow rate: 9 ml/minute). The same preparation was repeated 20 times and active fractions were collected, concentrated and lyophilized to give a powder (15 mg). The powder was chromatographed again on the same HPLC column, and then the pure fraction was concentrated and lyophilized, whereby 7 mg of A-500359 D was obtained in a pure form.

Fig. 3. Purification procedure of A-500359 G.



Isolation of A-500359 G

The isolation procedure for A-500359 G is shown schematically in Fig. 3. Twenty-eight liters of the culture filtrate were adsorbed onto a DIAION HP-20 column (5.5 liters). The column was washed with water and the active substance was eluted with 10% aqueous acetone (11 liters). The eluate was concentrated *in vacuo* to remove the acetone and adsorbed onto a MCI GEL CHP20P column (3 liters). After the column was washed successively with water, 5% aqueous MeOH and 10% aqueous MeOH, the active substance was eluted with 15% aqueous MeOH (6 liters). The eluate was concentrated *in vacuo* and lyophilized to give a crude powder (1.27 g). The powder was dissolved in water and applied on a TOYOPEARL HW40-F column (500 ml). After the column was developed with water, active fractions were collected, concentrated and lyophilized to give a crude powder (134 mg). The powder was dissolved in 3 ml of water and a 750 μl portion was injected into an HPLC column (Senshu Pak ODS-H-5251, 20 i.d.×250 mm, Senshu Scientific; mobile phase: acetonitrile - 0.04% aqueous trifluoroacetic acid [4 : 96]; flow rate: 10 ml/minute). The above procedure was repeated 3 times. Active fractions were collected, concentrated and lyophilized to give a powder (20 mg). The powder was dissolved in 1.6 ml of water and further purified by the same HPLC column (mobile phase: acetonitrile - 0.04% aqueous trifluoroacetic acid [5 : 95]; flow rate: 10 ml/minute) in 2 preparations. Active fractions were combined, concentrated and lyophilized, whereby 14 mg of A-500359 G was obtained in a pure form.

Table 3. Physico-chemical properties of A-500359 A, B, C, D and G.

	A-500359 A	A-500359 B	A-500359 C	A-500359 D	A-500359 G
Appearance	Colorless powder	Colorless powder	Colorless powder	Colorless powder	Colorless powder
$[\alpha]_D^{20}$	+ 94.7°	+97°	+ 89°	+ 68°	+ 110°
	(*c* 1.00, MeOH)	(*c* 0.20, H_2O)	(*c* 0.44, H_2O)	(*c* 0.69, H_2O)	(*c* 0.72, H_2O)
FAB-MS (*m/z*)	584 $(M+H)^+$	570 $(M+H)^+$	570 $(M+H)^+$	568 $(M+H)^+$	556 $(M+H)^+$
HR-FAB-MS (*m/z*)	for $C_{24}H_{34}N_5O_{12}$	for $C_{23}H_{32}N_5O_{12}$	for $C_{23}H_{32}N_5O_{12}$	for $C_{24}H_{34}N_5O_{11}$	for $C_{22}H_{30}N_5O_{12}$
Calcd.:	584.2204	570.2048	570.2048	568.2255	556.1891
Found:	584.2189	570.2028	570.2034	568.2239	556.1891
Molecular formula	$C_{24}H_{33}N_5O_{12}$	$C_{23}H_{31}N_5O_{12}$	$C_{23}H_{31}N_5O_{12}$	$C_{24}H_{33}N_5O_{11}$	$C_{22}H_{29}N_5O_{12}$
UV $\lambda_{max}^{H_2O}$nm (ε)	257 (10,300)	257 (9,600)	257 (10,700)	244 (10,000)	257 (10,000)

Physico-chemical Properties of A-500359 A, B, C, D and G

Physico-chemical properties of A-500359 A, B, C, D and G are summarized in Table 3. The molecular formula of A-500359 A, B, C, D and G were determined by high resolution FAB-MS to be $C_{24}H_{33}N_5O_{12}$, $C_{23}H_{31}N_5O_{12}$, $C_{23}H_{31}N_5O_{12}$, $C_{24}H_{33}N_5O_{11}$ and $C_{22}H_{29}N_5O_{12}$, respectively. Based on the data from ^1H NMR, ^{13}C NMR (Table 4) and UV absorption spectra, A-500359 B was identified as capuramycin[7]. Because of the similarity of the physico-chemical properties of the others to capuramycin (Table 3), it was considered that A-500359 A, C, D and G were capuramycin-related compounds.

Elucidation of the Planar Structures

In order to elucidate the planar structure of A-500359 A, we performed a comparison study of the NMR spectra with those of capuramycin. The appearance of a doublet methyl signal (δ 1.22) and the change of methylene signals (δ 3.24 and 3.33) to methine (δ 3.58) was characteristic of the ^1H NMR spectrum of A-500359 A. Precise assignment of ^1H NMR and ^{13}C NMR signals by the analysis of DQF COSY and HMQC spectra with the assistance of HMBC spectrum revealed that the planar structure of A-500359 A was elucidated as 6‴-methylcapuramycin.

The planar structure of A-500359 C was also elucidated by a comparison study with A-500359 A. Disappearance of the 3′-attached *O*-methyl signal (δ 3.44) was typical of the ^1H NMR spectrum of A-500359 C. Accordingly, the planar structure of A-500359 C was elucidated as 3′-demethyl-6‴-methylcapuramycin.

In the case of A-500359 D, disappearance of the *O*-methine (δ 3.96) proton assigned as the 2″ position and observation of extra methylene proton signals (δ 1.88 and 2.33) was characteristic of A-500359 D. The planar structure of A-500359 D was thus elucidated to be 2′-deoxy-6‴-methylcapuramycin.

The planar structure of A-500359 G was elucidated as 3′-demethylcapuramycin because of the disappearance of the 3′-attached *O*-methyl signal (δ 3.43) in the ^1H NMR spectrum.

Determination of the Absolute Stereochemistry of A-500359 A

Since a suitable crystal of either A-500359 A or its derivatives for X-ray analysis had not been previously obtained, its absolute configuration was elucidated with the following strategy. The A-500359 A molecule was divided into two partial structures, A and B (Fig. 4). If the absolute stereochemistry of each unit is determined, the whole stereochemistry can be elucidated.

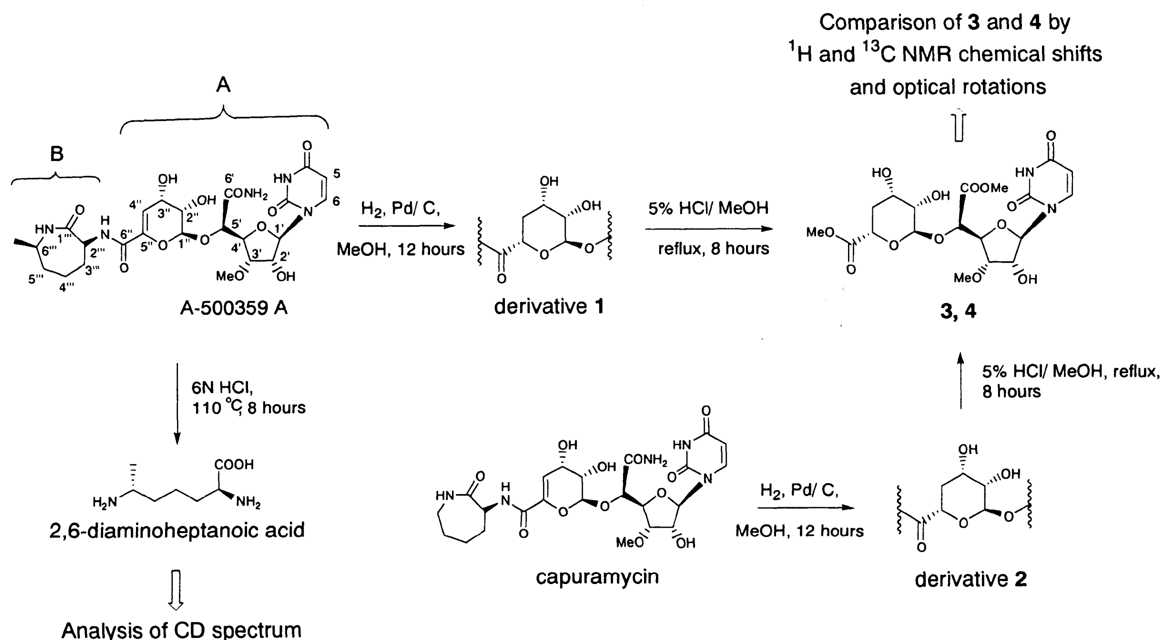
The whole carbon skeleton except for the methyl carbon connecting to the 6‴ position of the aminocaprolactam moiety of A-500359 A was identical with capuramycin whose absolute structure has been already reported[8]. Therefore, we chemically converted A-500359 A and capuramycin into the same compound, and compared their NMR and CD spectra. Because of the stabilization of the

Table 4. ^{1}H and ^{13}C signal assignments of A-500359 A, C, D and G.

	A-500359 A		A-500359 C		A-500359 D		A-500359 G	
Position	δ_C [a]	δ_H [a]	δ_C [b]	δ_H [c]	δ_C [d]	δ_H [c]	δ_C [b]	δ_H [c]
2	152.4		151.6		152.4		152.2	
4	166.1		166.4		166.3		166.9	
5	102.9	5.72 (1H, d, 8.1)	101.9	5.84 (1H, d, 8.1)	103.0	5.64 (1H, d, 8.1)	102.7	5.82 (1H, d, 8.2)
6	142.0	7.91 (1H, d, 8.1)	141.0	7.72 (1H, d, 8.1)	142.0	7.77 (1H, d, 8.1)	141.8	7.71 (1H, d, 8.2)
1'	90.4	5.88 (1H, d, 5.2)	89.7	5.77 (1H, d, 3.3)	90.7	5.73 (1H, d, 4.8)	90.5	5.75 (1H, d, 3.2)
2'	74.6	4.30 (1H, t, 5.2)	73.8	4.18 (1H, dd, 3.3, 5.2)	74.7	4.08 (1H, t, 4.8)	74.5	4.17 (1H, dd, 3.2, 5.0)
3'	81.1	3.86 (1H, br. t, 4.6)	69.4	4.11 (1H, dd, 5.2, 6.3)	80.9	3.57 (1H, t, 4.7)	70.2	4.10 (1H, dd, 5.0, 6.5)
3'-OCH$_3$	58.8	3.44 (3H, s)			58.8	3.24 (3H, s)		
4'	83.6	4.58 (1H, dd, 2.0, 4.3)	83.1	4.43 (1H, dd, 2.1, 6.3)	83.8	4.37 (1H, m)	83.9	4.41 (1H, dd, 2.1, 6.5)
5'	79.2	4.67 (1H, d, 2.0)	76.7	4.76 (1H, d, 2.1)	77.7	4.50 (1H, d, 2.0)	77.5	4.78 (1H) [e]
6'-CONH$_2$	173.5		173.5		173.6		174.3	
1"	101.3	5.23 (1H, d, 5.8)	100.1	5.36 (1H, d, 4.0)	99.5	5.30 (1H, br. s)	100.9	5.35 (1H, d, 4.1)
2"	68.8	3.96 (1H, ddd, 0.7, 4.5, 5.7)	65.3	4.15 (1H, ddd, 1.4, 4.2, 4.3)	35.8	1.88 (1H, m), 2.33 (1H, m)	66.1	4.14 (1H, dt, 1.5, 4.4)
3"	63.6	4.37 (1H, t, 4.1)	61.9	4.49 (1H, dd, 3.0, 4.4)	60.7	4.40 (1H, m)	62.7	4.47 (1H, dd, 2.9, 4.4)
4"	109.3	6.02 (1H, br. dd, 0.7, 3.9)	109.1	5.98 (1H, br. dd, 1.3, 3.0)	112.3	5.97 (1H, d, 2.4)	109.9	5.97 (1H, dd, 1.5, 2.9)
5"	144.4		141.8		144.1		142.7	
6"	161.9		161.7		162.4		162.6	
1'''	175.3		175.8		175.5		177.6	
2'''	53.5	4.56 (1H, dd, 2.0, 11.9)	52.6	4.62 (1H, dd, 1.7, 10.8)	53.6	4.46 (1H, br. d, 10.7)	53.3	4.61 (1H, dd, 1.8, 11.4)
3'''	32.1	1.49 (1H, m), 2.01 (1H, m)	29.4	1.62 (1H, m), 1.90 (1H, m)	32.3	1.40 (1H, m), 1.90 (1H, m)	30.5	1.65 (1H, m), 1.92 (1H, m)
4'''	28.4	1.87 (1H, m), 1.92 (1H, m)	26.8	1.72 (1H, m), 1.92 (1H, m)	28.6	1.80 (1H, m), 1.88 (1H, m)	28.2	1.71 (1H, m), 1.98 (1H, m)
5'''	37.9	1.29 (1H, m), 1.78 (1H, m)	35.4	1.29 (1H, m), 1.75 (1H, m)	38.0	1.17 (1H, m), 1.67 (1H, m)	28.4	1.37 (1H, m), 1.79 (1H, m)
6'''	50.1	3.58 (1H, m)	48.9	3.65(1H, m)	50.2	3.50 (1H, m)	42.2	3.29 (1H, m), 3.36 (1H, m)
6'''-CH$_3$	22.2	1.22 (3H, d, 6.7)	21.0	1.20 (3H, d, 6.7)	22.3	1.12 (3H, d, 8.1)		

Number of protons, multiplicity and coupling constants are shown in parentheses.
[a]Observed in deuterated methanol with tetramethylsilane as an internal reference (δ 0.0).
[b]Observed in deuterium oxide with 1, 4- dioxane as an internal reference (δ 67.4).
[c]Observed in deuterium oxide with HDO as an internal reference (δ 4.75).
[d]Observed in deuterated methanol with deuterated methanol as an internal reference (δ 49.2).
[e]Signal overlapped the HDO signal

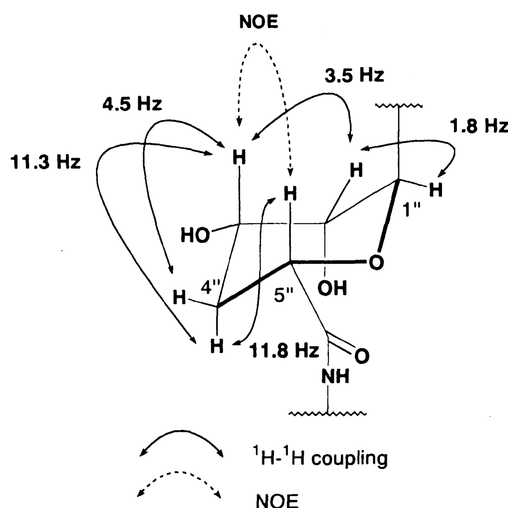
Fig. 4. Chemical conversion of A-500359 A and capuramycin.



peptide bond between unit A and B with a conjugated 4″ double bond, we could not obtain simple de-caprolactam derivatives from either A-500359 A or capuramycin by normal acid hydrolysis. Then, catalytic hydrogenation of A-500359 A and capuramycin was performed to give dihydro derivatives **1** and **2**, respectively (Fig. 4). Since the stereochemistry of C-5″ in the resulting 4″-deoxyhexuronic acid moiety in derivatives **1** and **2** converged to a sole configuration, the stereochemistry of the C-5″ position was elucidated by the adoption of the Karplus rule to the observed coupling constants as well as the NOE correlations shown in Fig. 5. Derivatives **1** and **2** were then hydrolyzed with HCl-MeOH to obtain methyl ester derivatives **3** and **4**, respectively (Fig. 4). The obtained derivatives **3** and **4** were identical based on the data from ^{1}NMR and their optical rotations (**3**: $[\alpha]_D^{26}=+65°$ [c 0.39, MeOH], **4**: $[\alpha]_D^{26}=+75°$ [c 0.76, MeOH]). Therefore, the absolute stereochemistry of the partial structure A of A-500359 A shown in Fig. 4 was identical to that of capuramycin.

The relative configuration of the partial structure B was determined by the observation of NOE between H-2‴ and H-6‴. In order to determine the absolute configurations of the two chiral carbons on the aminocaprolactam ring, we measured the CD spectrum of 2,6-diaminoheptanoic acid derived from A-500359 A by acid hydrolysis. The 2,6-diaminoheptanoic acid showed a positive Cotton effect under both acid and neutral conditions (pH 1.2: λ 208 nm, $\Delta\varepsilon$ +0.39, pH 7.0: λ 202 nm, $\Delta\varepsilon$ +0.40). The result clearly indicated that the stereochemistry of the α position was equivalent to an L-amino acid. Therefore, the absolute stereochemistry of C-2‴ and C-6‴ of unit B was determined as *R* and *S*, respectively.

Fig. 5. Coupling constants and NOE correlation observed for derivative **1**.



Based on the data obtained above, the absolute stereochemistry of A-500359 A was determined as shown in Fig. 4. Since the stereochemistry of all the chiral carbons of A-500359 A except for C-6‴ was identical to that of capuramycin, it is suggested that capuramycin might be a precursor of A-500359 A in regard to its biosynthesis, as described in the following paper[14].

Experimental

General Experimental Procedures

The various NMR spectra were obtained on a Brucker AMX 360 or AVANCE 500 spectrometer. Tetramethylsilane (δ 0.0), 1,4-dioxane (δ 67.4), HDO (δ 4.75) and deuterated methanol (δ 49.2) were used as internal references. FAB-MS spectra were recorded on a JEOL JMS-700QQ mass spectrometer. TOF-MS spectra were obtained on a Micromass LCT mass spectrometer equipped with an ESI ion source. Optical rotations were recorded on a JASCO DIP-370 spectropolarimeter. CD spectra were obtained with a JASCO J-720W spectropolarimeter. UV spectra were obtained on a Shimadzu UV-265FW spectrometer.

Taxonomy of the Producing Organism

The producing organism, strain SANK 60196, was isolated from a soil sample collected at Makabe, Ibaraki prefecture, Japan. The methods and media described by the International Streptomyces Project (ISP)[11] and WAKSMAN[12] were used to determine the morphological characterizations and the physiological properties of the producing organism. The cell walls and whole-cell hydrolysates were analyzed by the methods of HASEGAWA *et al.*[13]

Acid Hydrolysis of A-500359 A

A-500359 A (100 mg) was dissolved in 3 ml of 6 N HCl, and hydrolyzed at 110°C for 8 hours in a sealed ampule. After chilling, the solution was concentrated *in vacuo*, and applied onto a Sephadex G-10 column (20 ml, Pharmacia Fine Chemical). The column was developed with acetonitrile - water - acetic acid (3 : 1 : 1) to give crude 2,6-diaminoheptanoic acid. Then the material was further purified by preparative HPLC using a Capcell pak C-18 UG-120 column (i.d. 20×250 mm, SHISEIDO) with 0.01% aqueous trifluoroacetic acid (flow rate: 10 ml/minute) and the eluate (Rt=18.6 minutes) was lyophilized to give

14.0 mg of pure 2,6-diaminoheptanoic acid.

2,6-Diaminoheptanoic acid: HR-TOF-MS (ESI, positive): *m/z* 161.1291 $(M+H)^+$ (calcd. for $C_7H_{17}N_2O_2$, 161.1290). ^1H NMR (D_2O, 500 MHz): δ 3.77 (1H, t, J=6.3 Hz, 2-H), 3.16 (1H, m, 6-H), 1.74 (2H, m, 3-H), 1.42 (1H, m, 5a-H), 1.50 (1H, m, 5b-H), 1.29 (2H, m, 4-H), 1.07 (3H, d, J=6.6 Hz, 7-H). ^{13}C NMR (D_2O, 125 MHz): δ 172.8 (C-1), 53.3 (C-2), 47.5 (C-6), 33.5 (C-5), 29.7 (C-3), 20.6 (C-4), 17.5 (C-7).

Catalytic Hydrogenation of A-500359 A

A-500359 A (100 mg) was dissolved in 10 ml of MeOH, followed by addition of 0.5 mg of 10% Pd-C. Then the mixture was shaken for 12 hours at room temperature under hydrogen atmosphere. After filtration, the solution was concentrated *in vacuo*, and applied to an HPLC column (Capcell pak C-18 UG-120, i.d. 20×250 mm, mobile phase A: 0.01% aqueous trifluoroacetic acid, mobile phase B: 90% aqueous acetonitrile, B [%]: 0~30/20 minutes, flow rate: 10 ml/minute). Then the eluate (Rt=13.9 minutes) was concentrated *in vacuo* and lyophilized to give the dihydroderivative of A-500359 A (**1**, 50.6 mg).

Derivative **1**: HR-TOF-MS (ESI, positive): *m/z* 608.2207 $(M+Na)^+$ (calcd. for $C_{24}H_{35}N_5O_{12}Na$, 608.2180). ^1H NMR (CD_3OD, 500 MHz): δ 7.82 (1H, d, J=8.1 Hz, 6-H), 5.78 (1H, d, J=3.5 Hz, 1′-H), 5.68 (1H, d, J=8.1 Hz, 5-H), 5.02 (1H, d, J=1.8 Hz, 1″-H), 4.52 (1H, dd, J=1.5, 11.2 Hz, 2‴-H), 4.41 (1H, d, J=4.2 Hz, 5′-H), 4.36 (1H, dd, J=3.5, 5.2 Hz, 2′-H), 4.33 (1H, dd, J=4.2, 5.6 Hz, 4′-H), 4.23 (1H, dd, J=2.3, 11.8 Hz, 5″-H), 4.00 (1H, ddd, J=3.5, 4.3, 11.3 Hz, 3″-H), 3.93 (1H, dd, J=1.8, 3.5 Hz, 2″-H), 3.89 (1H, dd, J=5.2, 5.6 Hz, 3′-H), 3.40 (3H, s, 3′-OCH_3), 3.23 (1H, m, 6‴-H), 2.04 (1H, m, 4″b-H), 1.87 (1H, m, 4″a-H), 1.96 (1H, m, 4‴a-H), 1.93 (1H, m, 3‴a-H), 1.87 (1H, m, 4‴b-H), 1.79 (1H, m, 5‴a-H), 1.55 (1H, m, 3‴b-H), 1.29 (1H, m, 5‴b-H), 1.23 (3H, d, J=6.6 Hz, 6‴-CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 175.8 (C-1‴), 173.6 (C-6′), 172.0 (C-6″), 166.3 (C-4), 152.3 (C-2), 142.3 (C-6), 102.8 (C-5), 101.7 (C-1″), 92.1 (C-1′), 83.4 (C-4′), 79.9 (C-3′), 77.0 (C-5′), 73.9 (C-2′), 71.2 (C-5″), 69.7 (C-2″), 66.6 (C-3″), 58.5 (3′-OCH_3), 53.2 (C-2‴), 50.2 (C-6‴), 37.9 (C-5‴), 32.3 (C-3‴), 28.6 (C-4‴), 22.3 (6‴-CH_3). $[\alpha]_D^{26}$=+47.0° (*c* 0.32, MeOH).

Catalytic Hydrogenation of Capuramycin

Capuramycin (100 mg) was dissolved in 10 ml of MeOH, followed by addition of 0.5 mg of 10% Pd-C. Then the mixture was shaken for 12 hours at room temperature under hydrogen atmosphere. After filtration, the solution was concentrated *in vacuo*, and applied to an HPLC column (Capcell pak C-18 UG-120, i.d. 20×250 mm, mobile phase A: 0.01% aqueous trifluoroacetic acid, mobile phase B: 90% aqueous acetonitrile, B[%]: 0~30/15 minutes, flow rate: 10 ml/minute). Then the eluate (Rt=11.4 minutes) was concentrated *in vacuo* and lyophilized to give the dihydroderivative of capuramycin (**2**, 60.2 mg).

Derivative **2**: HR-TOF-MS (ESI, positive): *m/z* 594.2025 $(M+Na)^+$ (calcd. for $C_{23}H_{33}N_5O_{12}Na$, 594.2023). ^1H NMR (CD_3OD, 500 MHz): δ 7.83 (1H, d, J=8.1 Hz, 6-H), 5.78 (1H, d, J=3.3 Hz, 1′-H), 5.68 (1H, d, J=8.1 Hz, 5-H), 5.03 (1H, d, J=1.8 Hz, 1″-H), 4.52 (1H, dd, J=1.5, 11.4 Hz, 2‴-H), 4.41 (1H, d, J=4.1 Hz, 5′-H), 4.34 (1H, dd, J=4.1, 5.6 Hz, 4′-H), 4.33 (1H, dd, J=3.3, 5.6 Hz, 2′-H), 4.25 (1H, dd, J=2.2, 11.7 Hz, 5″-H), 4.00 (1H, ddd, J=3.5, 4.5, 11.5 Hz, 3″-H), 3.94 (1H, dd, J=1.8, 3.5 Hz, 2″-H), 3.89 (1H, t, J=5.6 Hz, 3′-H), 3.40 (3H, s, 3′-OCH_3), 3.23 (1H, m, 6‴a-H), 3.33 (1H, m, 6‴b-H), 2.05 (1H, m, 4″b-H), 1.85 (1H, m, 4″a-H), 1.98 (1H, m, 4‴a-H), 1.94 (1H, m, 3‴a-H), 1.84 (1H, m, 5‴a-H), 1.83 (1H, m, 4‴b-H), 1.56 (1H, m, 3‴b-H), 1.36 (1H, m, 5‴b-H). ^{13}C NMR (CD_3OD, 125 MHz): δ 176.8 (C-1‴), 173.6 (C-6′), 172.0 (C-6″), 166.3 (C-4), 152.3 (C-2), 142.2 (C-6), 102.8 (C-5), 101.7 (C-1″), 92.0 (C-1′), 83.4 (C-4′), 79.9 (C-3′), 77.0 (C-5′), 73.8 (C-2′), 71.2 (C-5″), 69.6 (C-2″), 66.6 (C-3″), 58.5 (3′-OCH_3), 53.1 (C-2‴), 42.7 (C-6‴), 30.0 (C-5‴), 32.6 (C-3‴), 29.2 (C-4‴). $[\alpha]_D^{26}$=+60.0° (*c* 1.22, MeOH).

Methanolysis of Derivatives 1 and 2

Derivative **1** (40 mg) and derivative **2** (80 mg) were each dissolved in 5 ml of 5% HCl/MeOH, and refluxed for 8 hours. After chilling, each solution was concentrated *in vacuo*, and applied to an HPLC column (Capcell pak C-18 UG-120, i.d. 20×250 mm, mobile phase: acetonitrile-0.01% aqueous trifluoroacetic acid [12:88], flow rate: 10 ml/minute). The eluate (Rt=15.3 minutes) was concentrated *in vacuo*, lyophilized to give the methyl ester of derivative **1** (**3**, 20.3 mg) or that of derivative **2** (**4**, 60.9 mg).

3 and **4**: HR-TOF-MS (ESI, positive): *m/z* 513.1331 $(M+Na)^+$ (calcd. for $C_{19}H_{26}N_2O_{13}Na$, 513.3333). ^1H NMR (CD_3OD, 500 MHz): δ 7.90 (H-6, 1H, d, J=8.1 Hz), 5.80 (H-1′, 1H, d, J=3.1 Hz), 5.74 (H-5, 1H, d, J=8.1 Hz), 5.11 (H-1″, 1H, d, J=1.9 Hz), 4.62 (H-5′, 1H, d, J=2.6 Hz), 4.52 (H-4′, 1H, dd, J=2.6, 5.4 Hz), 4.36 (H-2′, 1H, dd, J=3.1, 5.4 Hz), 4.35 (H-5″, 1H, m), 3.96 (H-3″, 1H, m), 3.89 (H-3′, 1H, t, J=5.4 Hz), 3.83 (H-2″, 1H, m), 3.81 (6′-OCH_3, 3H, s), 3.75 (6″-OCH_3, 3H, s), 3.44 (3′-OCH_3, 3H, s), 2.00 (H-4″, 2H, m). ^{13}C NMR (CD_3OD, 125 MHz): δ 172.7 (C-6′), 171.2 (C-6″), 166.3 (C-4), 152.3 (C-2), 141.9 (C-6), 102.7 (C-5), 92.0 (C-1″), 92.0 (C-1′), 83.3 (C-4′), 75.0 (C-5′),

74.3 (C-2′), 70.4 (C-5″), 70.0 (C-2″), 67.0 (C-3″), 66.8 (C-3′), 58.6 (C-3′OCH_3), 53.2 (C-6′OCH_3), 52.9 (C-6″OCH_3).

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