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

III. Deaminocaprolactam Derivatives of Capuramycin: A-500359 E, F, H, M-1 and M-2

YASUNORI MURAMATSU, SHUNICHI MIYAKOSHI, YASUMASA OGAWA, TAKASHI OHNUKI[†], MICHIKO MIYAZAWA ISHII, MASATOSHI ARAI, TOSHIO TAKATSU[†,*] and MASATOSHI INUKAI

Lead Discovery Laboratories, Sankyo Co., Ltd.,
[†] Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd.,
1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

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Novel derivatives of capuramycin were obtained when 10 mM of 2-aminoethyl-L-cysteine (AEC), an inhibitor of aspartokinase, was added to the culture of *Streptomyces griseus* SANK 60196, the producer of A-500359. They were purified from the culture filtrate and their chemical structures were elucidated as a deaminocaprolactam derivative of capuramycin designated as A-500359 F, A-500359 E, a methyl ester of A-500359 F, and A-500359 H, a 3′-demethyl derivative of A-500359 F. Two other compounds, A-500359 M-1 and A-500359 M-2, were purified from the same medium and their structures were elucidated. A-500359 E, F, H, M-1 and M-2 inhibited bacterial translocase I with an IC_{50} of 0.027 μM, 1.1 μM, 0.008 μM, 0.058 μM and 0.010 μM, respectively. A-500359 E, M-1 and M-2 inhibited the growth of mycobacteria as well.

A-500359 A, C, D and G were discovered in the culture broth of *Streptomyces griseus* SANK 60196.[1,2] These compounds and capuramycin, a structurally related antibiotic, as well as tunicamycin, liposidomycins and mureidomycins were found to inhibit bacterial translocase I.[2,3] Moreover, A-500359 A and capuramycin specifically inhibited the growth of mycobacteria. Therefore, the A-500359 derivative may be a useful antibiotic for mycobacterial diseases. Based on this result, the removal of the aminocaprolactam moiety from the original compounds widens the range of possible chemical modifications. It is well known that the aminocaprolactam moiety of A-500359 derivatives is derived from L-lysine or 2,6-diaminopimelic acid *via* a biosynthetic pathway. 2-Aminoethyl-L-cysteine (AEC) is known as a specific inhibitor of aspartokinase which is involved in the so-called "lysine-diaminopimelic acid pathway" of *Corynebacterium* and actinomycete. Since the producing strain was identified as *Streptomyces griseus*, which is also an actinomycete, the pathway was considered to be the same. Thus, the addition of AEC to the culture was expected to block the lysine biosynthetic pathway of the producing strain to produce deaminocaprolactam derivatives.

We report here the novel metabolites, A-500359 E, F, H, M-1 and M-2 (Fig. 1), obtained by adding AEC to the culture of *Streptomyces griseus* SANK 60196. The isolation, structure elucidation, and biological activities of these derivatives are described.

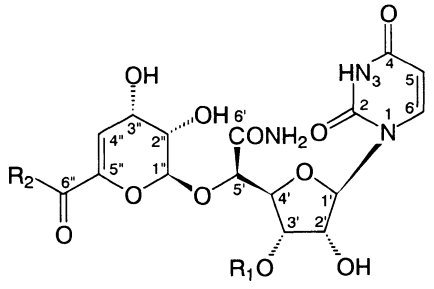
Materials and Methods

Microorganism and Culture Conditions

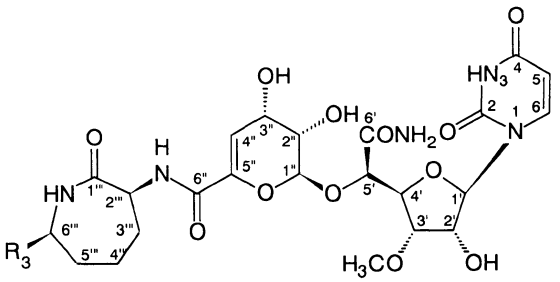
Streptomyces griseus SANK 60196, the producer strain of A-500359, was cultured on a YM agar slant, containing 1.0% glucose, 0.5% Polypepton (Nihon Seiyaku), 0.3% yeast extract (Difco), and 0.3% malt extract (Difco), at 23°C for 14 days. Four loopfuls of spores were inoculated

* Corresponding author: taka@shina.sankyo.co.jp

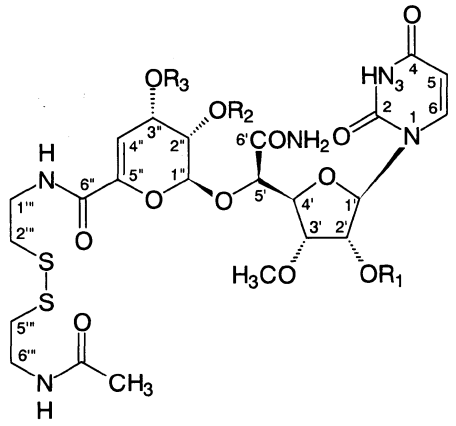
Fig. 1. Structures of A-500359 components.



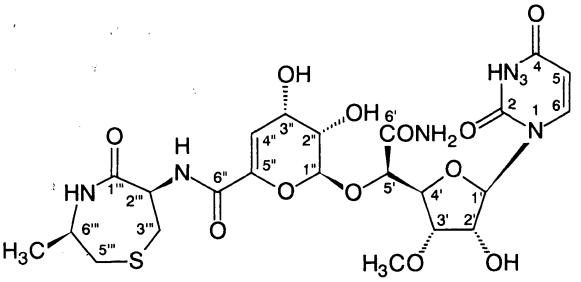
	R_1	R_2
A-500359 E	CH_3	OCH_3
A-500359 F	CH_3	OH
A-500359 F amide	CH_3	NH_2
A-500359 H	H	OH



	R_1	R_2	R_3
A-500359 A	CH_3	OH	CH_3
A-500359 B (Capuramycin)	CH_3	OH	H



	R_1	R_2	R_3
A-500359 M-1	H	H	H
A-500359 M-1 triacetete	$COCH_3$	$COCH_3$	$COCH_3$



A-500359 M-2

into 500 ml of a medium containing 3.0% maltose, 0.5% yeast extract (Difco), 0.5% meat extract (Kyokuto), 0.5% Polypepton (Nihon Shinyaku), 0.5% NaCl, 0.3% $CaCO_3$ (pH 7.4) in a 2-liter Erlenmeyer flask and cultured in a rotary shaker at 23°C and 210 rpm for 3 days. The seed culture was transferred at 3% (v/v) into each of two 30-liter jar fermentors containing 15 liters of the same sterilized medium. At the beginning of the production culture and after 6 hours of cultivation at 23°C with an aeration of 15 liters/minute (1.0 vvm) and an agitation of 300 rpm, a filter-sterile AEC solution was separately added to the culture at a final concentration of 5 mM and the cultivation was then carried out under the same conditions for 6 days.

Isolation of A-500359 E, F, H, M-1 and M-2

As shown in Fig. 2, A-500359 E, F, H, M-1 and M-2 were purified from the culture broth by column chromatography using Diaion HP-20, PA316, CHP-20P and Toyopearl HW-40F as well as by preparative reverse-phase HPLC.

Alkaline Hydrolysis of A-500359E

A-500359 E was hydrolyzed with 0.5 N ammonia aqueous solution at room temperature for 3 hours and purified by reverse-phase preparative HPLC (Senshupak ODS-H-5251, 0.04% trifluoroacetic acid, 10 ml/minute).

Acetylation of A-500359 M-1

In order to observe amide protons, A-500359 M-1 was acetylated as follows. First, 100 ml of acetic anhydride was added to a 1-ml pyridine solution of A-500359 M-1 (10.7 mg/ml) and the mixture was incubated overnight at room temperature. A-500359 M-1 triacetate (6.4 mg) was purified by preparative HPLC (SenshuPak ODS-H-2151, 22% CH_3CN - 0.04% trifluoroacetic acid, 10 ml/minute).

Evaluation of Biological Activities

Inhibitory activities of A-500359 E, F, H, M-1 and M-2 against bacterial translocase I were measured as described by MURAMATSU *et al.*[2] *In vitro* antimicrobial activities of these derivatives were determined by the validated methods.[4]

Results

Effects of AEC Addition on the Metabolites of A-500359

Five peaks with similar UV spectra to those of capuramycin newly appeared on the HPLC chromatogram when AEC was added to the culture. On the other hand, the productivity for A-500359 A and capuramycin decreased after the addition of AEC to the culture (data not shown).

Isolation of A-500359 E, F, H, M-1 and M-2

500359 E, F, H, M-1 and M-2 were isolated from the culture broth as shown in Fig. 2. First, 30 liters of the culture broth was filtered with Celite 545 (Celite Co.) and the filtrate was charged onto a Diaion HP-20 (Mitsubishi Chemical Industry) column (6 liters). The column was washed with 6 liters of deionized water. The adsorbed substances were eluted with 10% aqueous acetone and fractionated into four 6-liters portions. The eluted fraction #1 and #2 were combined and further purified by chromatography with a Diaion CHP-20P column (2 liters) and a Toyopearl HW-40F (1 liter) column, followed by preparative HPLC with YMC-pack ODS-1050-20-SR, to give A-500359 E (87 mg).

Fraction #3 and #4 were combined and charged onto a Diaion CHP-20P (1 liter) and eluted with 0.5 liters of 30% methanol, followed by Toyopearl HW-40F column chromatography and preparative HPLC using Senshupak ODS-H-5251, to obtain A-500359 M-1 (110 mg) and A-500359 M-2 (190 mg).

The unadsorbed fraction after Diaion HP-20 column chromatography was further purified by chromatography with a Diaion PA316 (Cl^-, 8.5 liters), and CHP-20P (2 liters, 1 liter) column and by preparative HPLC (Senshupak ODS-H-5251), to give A-500359 F (173 mg) and A-500359 H (96 mg).

Structural Elucidation of A-500359 E, F and H

Molecular formulas of A-500359 E, F and H were determined as shown in Table 1 by high-resolution FAB-MS. In the ^1H NMR spectrum of A-500359 E in DMSO-d_6, a singlet signal of the methyl group (δ 3.72) was observed instead of a disappearance of those of the signals for the aminocaprolactam ring. These aminocaprolactam-derived signals also disappeared in the ^1H NMR spectra of A-500359 F and H. In addition, the signal for the 3′-methyl group was absent in the spectrum of A-500359 H. Based on further NMR analysis with DQF-COSY, HMQC and HMBC, the structures of A-500359 E, F and H were elucidated as shown in Fig. 1.

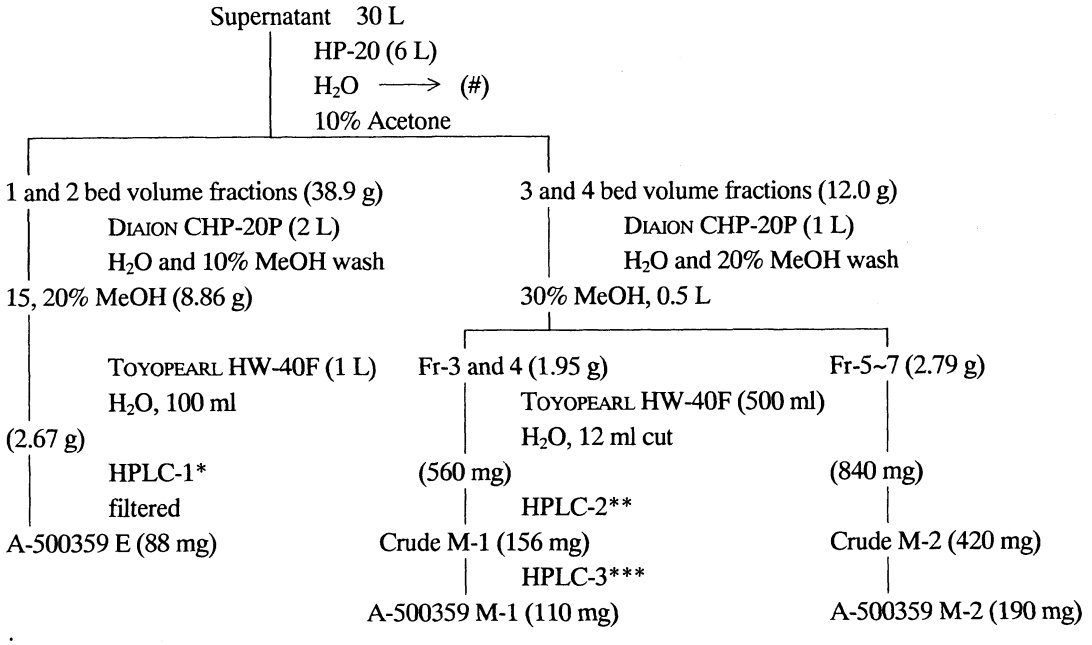
Alkaline Hydrolysis of A-500359E

In order to prepare A-500539 F from A-500359 E, 73 mg of A-500359 E was hydrolyzed and purified as described in Materials and Methods, to obtain 50 mg of A-500359 F and 14 mg of A-500359 F amide as a by-product.

Structural Elucidation of A-500359 M-1

The molecular formula of A-500359 M-1 was determined by high-resolution FAB-MS as $C_{23}H_{33}N_5O_{12}S_2$. In addition, the results of the high-resolution FAB-MS of A-500359 M-1 triacetate was consistent with the presence of two sulfur atoms in the A-500359 M-1 molecule. The NMR spectra of A-500359 M-1 indicated that the core structure was the same as A-500359 F and the existence of a partial structure of acetylcystamine was suggested. Observed C-H long-range coupling between H-1‴ (δ 3.62) and C-6″ (δ 163.7) revealed that one 2-aminoethanethiol moiety was connected to C-6″, and the couplings between H-6‴ (δ 3.48) and 6‴-acetyl carbonyl (δ 175.1), and 6‴-acetyl methyl (δ 1.97) and 6‴-acetyl carbonyl (δ 175.1) showed that the acetyl group was attached to the other 2-aminoethanethiol moiety. Although no direct information about the correlation between 2‴ and 5‴ position was obtained from the NMR analysis, the incorporation of three acetyl moieties into 2′, 2″ and 3″-OH residues by the acetylation (data not shown) well confirmed that two 2-aminoethanethiol moieties were bound to each other *via* a disulfide bond to form acetylcystamine moiety. Thus the

Fig. 2. Purification procedure of A-500359 E, F, H, M-1 and M-2.



*: YMC-Pack ODS-1050-20-SR, 4% CH_3CN-0.04% TFA, 208 ml/min., UV 210 nm
**: Senshupak ODS-H-5251, 4% CH_3CN-10% CH_3OH-0.04% TFA, 10 ml/min., UV 210 nm
***: Senshupak ODS-H-5251, 7% CH_3CN-0.04% TFA, 10 ml/min., UV 210 nm

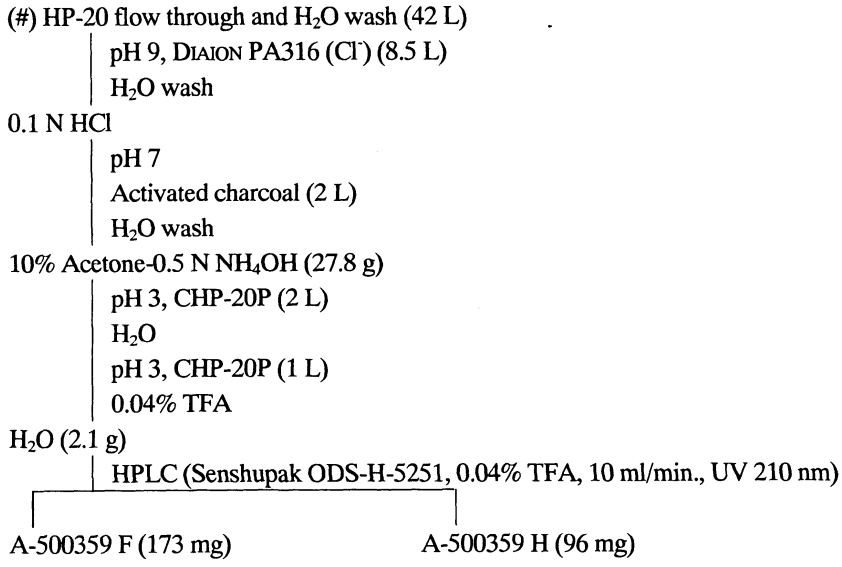


Table 1. Physico-chemical properties of A-500359 E, F, G, H, M-1, M-1 triacetate, M-2 and F amide.

	A-500359 E	A-500359 F	A-500359 F amide	A-500359 H
Appearance	colorless powder	colorless powder	colorless powder	colorless powder
$[\alpha]_D^{20}$	+115° (*c* 0.28 H_2O)	+111° (*c* 0.41 H_2O)	+119° (*c* 0.87 H_2O)	+115° (*c* 0.33 H_2O)
FAB-MS (*m/z*)	474 $(M+H)^+$	460 $(M+H)^+$	459 $(M+H)^+$	446 $(M+H)^+$
HR-FAB-MS (*m/z*)	for $C_{18}H_{24}N_3O_{12}$	for $C_{17}H_{22}N_3O_{12}$	for $C_{17}H_{23}N_4O_{11}$	for $C_{16}H_{20}N_3O_{12}$
Calcd.:	474.1359	460.1203	459.1364	446.1047
Found:	474.1349	460.1201	459.1328	446.1025
Molecular formula	$C_{18}H_{23}N_3O_{12}$	$C_{17}H_{21}N_3O_{12}$	$C_{17}H_{22}N_4O_{11}$	$C_{16}H_{19}N_3O_{12}$
UV $\lambda_{max}^{H_2O}$nm (ε)	251 (10,000)	262 (7,000)	258 (7,500)	262 (7,400)
IR ν_{max} (KBr) cm^{-1}	3410, 2955, 1683, 1464, 1441, 1396, 1309, 1267, 1206, 1138, 1115, 1088, 1062, 1023, 992, 884, 854, 817, 769, 722, 701, 668, 559, 568, 552	3391, 2941, 1684, 1466, 1400, 1333, 1269, 1205, 1137, 1115, 1062, 1020, 978, 884, 853, 818, 780, 722, 669, 600, 570, 554, 421	3339, 2943, 1686, 1598, 1495, 1402, 1337, 1272, 1205, 1136, 1115, 1060, 1019, 977, 884, 850, 819, 778, 721, 569, 570, 553, 421	3361, 2934, 1683, 1467, 1403, 1336, 1270, 1206, 1114, 1090, 1058, 1021, 979, 884, 855, 818, 778, 722, 669, 624, 605, 567, 554, 462, 421

	A-500359 M-1	A-500359 M-1 triacetate	A-500359 M-2
Appearance	colorless powder	colorless powder	colorless powder
$[\alpha]_D^{20}$	+85° (*c* 0.93 H_2O)	NT	+58° (*c* 0.39 H_2O)
FAB-MS (*m/z*)	658 $(M+Na)^+$	762 $(M+H)^+$	602 $(M+H)^+$
HR-FAB-MS (*m/z*)	for $C_{23}H_{33}N_5O_{12}S_2Na$	for $C_{29}H_{40}N_5O_{15}S_2$	for $C_{23}H_{32}N_5O_{12}S$
Calcd.:	658.1465	762.1963	602.1769
Found:	658.1503	762.1942	602.1779
Molecular formula	$C_{23}H_{33}N_5O_{12}S_2$	$C_{29}H_{39}N_5O_{15}S_2$	$C_{23}H_{31}N_5O_{12}S$
UV $\lambda_{max}^{H_2O}$nm (ε)	233 (12,200) 265 (9,140)	NT	244 (14,000)
IR ν_{max} (KBr) cm^{-1}	3332, 2936, 1684, 1534, 1464, 1409, 1392, 1331, 1268, 1205, 1187, 1138, 1119, 1096, 1059, 1021, 990, 883, 819, 767, 721, 668, 597, 569, 421	NT	3990, 2937, 1683, 1510, 1461, 1432, 1411, 1344, 1268, 1206, 1179, 1135, 1071, 1023, 994, 898, 836, 803, 768, 722, 624, 597, 554

NT : not tested

Table 2. NMR spectral data of A-500359 E, F, F amide, H, M-1 and M-2 in D_2O.

	E[a)]		F		F amide		H	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	150.3		152.1		152.1		152.2	
4	163.1		167.0		167.0		167.0	
5	101.2	5.61 (1H, d, J=8.1	102.7	5.90 (1H, d, J=8.1	102.7	5.85 (1H, d, J=8.1	102.9	5.89 (d, J=8.2 Hz)
6	139.8	7.74 (1H, d, J=8.1	141.9	7.75 (1H, d, J=8.1	141.9	7.71 (1H, d, J=8.1	141.9	7.75 (d, J=8.2 Hz)
1'	89.0	5.61 (1H, d, J=3.3	91.2	5.77 (1H, d, J=3.5	91.3	5.71 (1H, d, J=3.2	90.3	5.80 (d, J=4.0 Hz)
2'	72.1	4.10 (1H, m)	72.7	4.38 (1H, 1H, dd, J=3.5. 5.1 Hz)	72.6	4.35 (1H, dd, J=3.2, 5.0 Hz)	74.6	4.18c)
2'-OH		5.54 (1H, d, J=5.9						
3'	78.2	3.52 (1H, dd, J=4.5, 6.1 Hz)	78.8	3.79 (1H, dd, J=5.1, 6.4 Hz)	78.7	3.67 (1H, dd, J=5.0, 6.8 Hz)	70.3	4.13 (br. t, J=5.4 Hz)
3'-OCH3	57.3	3.24 (3H, s)	58.6	3.37 (3H,s)	58.6	3.30 (3H, s)		
4'	81.3	4.33 (1H, dd, J=2.0, 6.1 Hz)	82.4	4.48 (1H, dd, J=2.4, 6.4 Hz)	82.3	4.43 (1H, dd, J=2.3, 6.8 Hz)	84.2	4.43 (dd, J=2.5, 5.8 Hz)
5'	75.4	4.29 (1H, d, J=2.0	76.3	4.69 (1H, d, J=2.4	75.7	4.66 (1H, d, J=2.3	77.0	4.72 (d, J=2.5 Hz)
6'-CONH2	170.1	7.56 (1H, br. s), 7.69 (1H, br. s)	173.9				174.2	
1"	99.2	5.05 (1H, d, J=3.9 Hz)	100.0	5.32 (1H, dd, J=0.6, 3.4 Hz)	99.8	5.35 (1H, d, J=2.9 Hz)	100.3	5.31 (d, J=4.0 Hz)
2"	64.9	3.98 (1H, m)	65.5	4.17 (1H, ddd, J=1.6. 3.4. 4.6 Hz)	65.3	4.17 (1H, ddd, J=1.8. 2.9. 4.4 Hz)	65.8	4.16c)
2"-OH		5.45 (1H, d, J=4.2						
3"	61.5	4.25 (1H, m)	62.7	4.49 (1H, ddd, J=0.6. 2.7. 4.6 Hz)	62.7	4.45 (1H, dd, J=2.4, 4.4 Hz)	62.8	4.48 (dd, J=2.9, 4.7 Hz)
3"-OH		5.16 (1H, d, J=6.8						
4"	114.2	5.93 (1H, dd, J=1.3, 2.9 Hz)	114.8	6.11 (1H, dd, J=1.6, 2.7 Hz)	110.8	5.97 (1H, dd, J=1.8, 2.4 Hz)	113.9	6.12 (br.dd, J=1.4, 2.9 Hz)
5"	139.2		140.7		142.3		141.2	
6"	161.8		165.4		166.0		165.9	
6"-CH3	52.0	3.72 (3H, s)						

a) in DMSO-d_6

structure of A-500359 M-1 was elucidated as shown in Fig. 1.

Structural Elucidation of A-500359 M-2

The molecular formula of A-500359 M-2 was determined by high-resolution FAB-MS as $C_{23}H_{31}N_5O_{12}S$. Based on the NMR spectrum, A-500359 M-2 was elucidated to have a cyclized component of methylated AEC instead of the aminocaprolactam of capuramycin. The stereochemical configuration of this 7-membered ring was determined to be the same as that of methylaminocaprolactam of A-500359 A based on the NOESY spectrum (data not shown), *i.e.* key NOE was observed between H-2‴ (δ 5.02) and H-6‴ (δ 3.98).

Biological Activities of A-500359 E, F, H, M-1 and M-2

The antimicrobial activities of aminocaprolactam-free derivatives of A-500359 are shown in Tables 4 and 5 as well as their activities of translocase I inhibition. A-500359 E, F, F amide, M-1 and M-2 inhibited bacterial translocase I with an IC_{50} value of 0.027 μM, 1.1 μM, 0.19 μM, 8 μM, 0.010 μM and 0.058 μM, respectively. A-500359 E and F showed 2.7 to 110 times weaker activities than that of A-500359 A. A-500359 E and M-1 specifically inhibited the growth of mycobacteria. A-500359 M-2 showed a broader antibacterial spectrum than that of A-500359 A, although its level of enzyme inhibition activity was almost the same as that of A-500359 A. On the other hand, the other derivatives showed no antibacterial activity (Tables 4, 5).

Table 3. NMR spectral data of A-500359 M-1 and M-2 in D_2O.

	M-1		M-2	
	^{13}C	^{1}H	^{13}C	^{1}H
2	152.1		152.1	
4	167.0		166.9	
5	102.7	5.88 (1H, d, J=8.3 Hz)	102.7	5.85 (1H, d, J=8.1 Hz)
6	141.9	7.76 (1H, d, J=8.3 Hz)	141.9	7.74 (1H, d, J=8.1 Hz)
1'	91.2	5.75 (1H, d, J=3.0 Hz)	91.1	5.75 (1H, d, J=3.2 Hz)
2'	72.6	4.38 (1H, dd, J=3.0, 5.0 Hz)	72.7	4.38 (1H, dd, J=3.2, 5.0 Hz)
3'	78.7	3.71 (1H, dd, J=5.0, 6.8 Hz)	78.9	3.73 (1H, dd, J=5.0, 6.5 Hz)
3'-OCH_3	58.7	3.32 (3H, s)	58.6	3.28 (3H, s)
4'	82.3	4.45 (1H, dd, J=2.4, 6.8 Hz)	82.4	4.47 (1H, dd, J=2.6, 6.5 Hz)
5'	75.8	4.67 (1H, d, J=2.4 Hz)	76.5	4.73 (1H, d, J=2.6 Hz)
6'-$CONH_2$	173.9		173.8	
1"	99.9	5.39 (1H, d, J=3.0 Hz)	100.3	5.39 (1H, d, J=3.5 Hz)
2"	65.4	4.20 (1H, ddd, J=1.8, 3.0, 4.4	65.7	4.19 (1H, ddd, J=1.5, 3.5, 4.4
3"	62.7	4.50 (1H, dd, J=2.4, 4.4 Hz)	62.7	4.50 (1H, dd, J=2.6, 4.4 Hz)
4"	110.2	5.98 (1H, dd, J=1.8, 2.4 Hz)	110.6	6.03 (1H, dd, J=1.5, 2.6 Hz)
5"	142.4		142.3	
6"	163.7		162.3	
1'"	39.0	3.62 (2H, m)c)	174.5	
2'"	37.4	2.89 (2H, t, J=6.2 Hz)	55.9	5.02 (1H, dd, J=2.9, 8.8 Hz)
3'"			30.0	2.76 (1H, dd, J=2.9, 14.4 Hz), 2.84 (1H, dd, J=8.8, 14.4 Hz)
5'"	37.4	2.84 (2H, t, J=6.2 Hz)	36.3	2.63 (2H, m)
6'"	38.9	3.48 (2H, t, J=6.2 Hz)	53.2	3.98 (1H, m)
6'"-acetyl-CO	175.1			
6'"-acetyl-CH_3	22.7	1.97 (3H, s)		
6'"-CH_3			21.3	1.30 (3H, d, J=6.8 Hz)

Discussion

A-500359 F, an aminocaprolactam-free intermediate metabolite of capuramycin, and its derivatives were obtained by adding an inhibitor of L-lysine biosynthesis to the culture of A-500359 producer strain. This result indicates that the producing strain uses a so-called lysine-diaminopimelic acid pathway to synthesize the aminocaprolactam moiety of A-500359 derivatives. Among them, A-500359 E and F are useful as leading compounds for chemical derivatization. The production of A-500359 A and B was not completely suppressed by the addition of AEC. This is probably because L-lysine or its precursor was present among the ingredients in the production medium or because the aspartokinase was not inhibited completely under these conditions.

Although the removal of the aminocaprolactam ring of capuramycin or substitution of the ring to a methyl group causes a drastic loss of inhibitory activity, it is to note that they still possess some activity; *i.e.* this moiety is not absolutely required for the inhibitory activity. Production of A-500359 M-1 obtained only by the addition of AEC to the medium. However, the origin of the cystamine moiety, whether it came from AEC or not is uncertain because the formation mechanism of the cystamine moiety from AEC has not been elucidated yet.

Furthermore, the accumulation of A-500359 M-2 suggested that AEC was cyclized, methylated and incorporated into the structure of A-500359. It is interesting to note that the same position, C-6‴, was methylated in the case of A-500359 M-2 as in A-500359 A.

Table 4. Antimicrobial spectra and translocase I inhibitory activities of A-500359 E, F, G, H, M-1, M-2, F amide and A.

Test organisms	Diameter of inhibition zone (mm)*						
	E	F	H	M-1	M-2	F amide	A
Staphylococcus aureus FDA 209P	0	0	0	0	0	0	0
Enterococcus faecalis S-299	0	0	0	0	0	0	0
Bacillus subtilis PCI 219	0	0	0	0	14	0	0
Mycobacterium smegmatis ATCC 607	A12	0	0	B12	A30	B12	28
Escherichia coli NIHJ JC-2	0	0	0	0	0	0	0
Klebsiella pneumoniae PCI 602	0	0	0	A11	15	0	0
Proteus vulgaris OX 19	0	0	0	0	0	0	0
Pseudomonas aeruginosa 1046	0	0	0	0	0	0	0
Mucor hiemalis	0	0	0	0	0	0	0
Aspergillus niger	0	0	0	0	0	0	0
Trichophyton mentagrophytes	0	0	0	0	0	0	0
Candida albicans YU 1200	0	0	0	0	0	0	0
IC_{50} (ng/ml) (non RI method)	27	1100	8000	58	10	190	10

*: Each sample concentration was adjusted to 1 mg/ml with water.

Table 5. Antimicrobial activities of A-500359 E, F, G, H, M-1, M-2, F amide and A.

Test organisms	MIC (μg/ml)						
	E	F	H	M-1	M-2	F amide	A
Staphylococcus aureus 209P	>100	>100	>100	>100	>100	>100	>100
Bacillus subtilis ATCC 6633	>100	>100	>100	>100	50	>100	>100
Mycobacterium smegmatis SANK 75075	>100	>100	>100	>100	25	>100	6.2
Escherichia coli NIHJ JC-2	>100	>100	>100	>100	>100	>100	>100
Shigella flexneri IID 642	>100	>100	>100	>100	>100	>100	>100
Candida albicans	>100	>100	>100	>100	>100	>100	>100
Trichophyton mentagrophytes	>100	>100	>100	>100	>100	>100	>100
Trichophyton interdigitale	>100	>100	>100	>100	>100	>100	>100
Trichophyton rubrum	>100	>100	>100	>100	>100	>100	>100

This result suggests that the origin of C-7‴ methyl carbon of A-500359 A is not from the carbonyl group of 2,6-diaminopimelic acid, and that 2,6-diaminopimelic acid is decarboxylated to L-lysine. And after that, the methylation occurred to form A-500359 A. Further study of the biosynthesis of A-500359A will be described in another report. In this study, the possibility of discovering novel derivatives by the addition of metabolite analogs to the culture of a producer strain is suggested, and the improvement of enzyme inhibition and antibacterial activities by using this method was demonstrated.

References

1) MURAMATSU, Y.; A. MURAMATSU, T. OHNUKI, M. MIYAZAWA ISHII, M. KIZUKA, R. ENOKITA, S. TSUTSUMI, M. ARAI, Y. OGAWA, T. SUZUKI, T. TAKATSU & M. INUKAI: 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. J. Antibiotics 56: 243~252, 2003

2) MURAMATSU, Y.; M. MIYAZAWA ISHII & M. INUKAI: Studies on novel bacterial translocase I inhibitors, A-500359s. II. Biological activities of A-500359 A, C, D and G. J. Antibiotics 56: 253~258, 2003

3) BRANDISH, P. E.; K. KIMURA, M. INUKAI, R. SOUTHGATE, J. T. LONSDALE & T. D. H. BUGG: Modes of action of tunicamycin, liposidomycin B and mureidomycin A: Inhibition of phospho-*N*-acetylmuramyl-pentapeptide translocase from *Escherichia coli*. Antimicrob. Agents Chemother. 40: 1640~1644, 1996

4) National Committee for Clinical Laboratory Standards: Standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, NCCLS, Villanova, 1988