ASPARENOMYCINS A, B AND C, NEW CARBAPENEM ANTIBIOTICS

III. STRUCTURES

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The structures of asparenomycins were elucidated as new carbapenem antibiotics having a hydroxyisopropylidene group on the β-lactam ring. The stereochemistry was unequivocally confirmed by X-ray analysis and chemical degradation.

Asparenomycins A, B and C, produced by *Streptomyces tokunonensis* sp. nov. as well as by *Streptomyces argenteolus*, were isolated and characterized as water-soluble acidic antibiotics presumably having new carbapenem structures from their physicochemical properties and bioassay data[1,2]. This paper is concerned with the elucidation of the structures of these antibiotics.

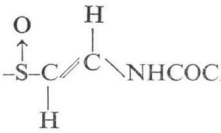
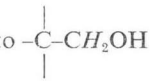
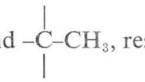
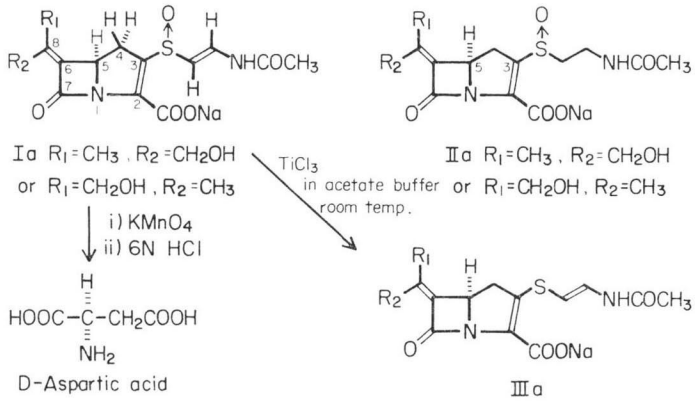
Asparenomycin A (ASM A) is the main component of the antibiotic mixture, the assumption that ASM A is likely to be a carbapenem antibiotic was supported by the ^{1}H NMR data shown in Table 1. When compared to the ^{1}H signals of MM 4550[3~5], the presence of a  group in ASM A is evident. The ^{1}H signals at 3.16 ppm (2H) and at 5.01 ppm (1H) showing AA'X type are attributed to the 4-CH_2 and the 5-CH of the carbapenem ring system. Differing from MM 4550, ASM A lacks the ^{1}H signal due to 6-CH. Further, ASM A has two characteristic ^{1}H signals at 4.26 ppm (2H, singlet) and 1.99 ppm (3H, singlet) ascribable to  and  , respectively. Assuming the

Table 1. ^{1}H NMR data[a] of ASM A, B and C in D_2O at room temperature (100 MHz, δ ppm from internal DSS, J (Hz)).

Assignment	A	B	C
8-CH_3	1.99 (3H, s)	1.98 (3H, s)	1.97 (3H, s)
N-Ac	2.12 (3H, s)	1.98 (3H, s)	2.05 (3H, s)
4-CH_2	~3.16 (2H, d-like)	~3.14 (2H, d-like)	~3.1 (2H, dd-like)
8-CH_2OH	4.26 (2H, s)	4.26 (2H, s)	4.20 (2H, s)
5-CH	5.01 (t-like)	5.03 (t-like)	~4.83 (m)
S-CH=	6.34 (d, J=14.0)	—	6.01 (d, J=13.7)
N-CH=	7.53 (d, J=14.0)	—	7.11 (d, J=13.7)
S-CH_2	—	3.59[b] (2H, m)	—
N-CH_2	—	3.22[b] (2H, m)	—

[a] ^{1}H FT NMR spectra were recorded on a Varian XL-100-12A spectrometer.
[b] Tentatively assigned and may be interchanged.

Fig. 1.



carbapenem structure of ASM A, the two groups should be included in the substituent at C(6). Since the UV spectrum of ASM A[2] is also significantly different from that of MM 4550, the chromophores of both compounds should differ at C(6). The ^{13}C NMR data (Table 2) of ASM A demonstrated the existence of an additional tetrasubstituted double bond, >C(6)=C(8)<, besides >C(2)=C(3)< of the carbapenem ring. From these NMR evidences the structure Ia is assigned to ASM A.

Table 2. ^{13}C NMR data[a] of ASM A.

Assignment	δ ppm	Assignment	δ ppm
8-CH_3	15.9 (q)	N–CH=	134.7 (d)
N–CO–CH_3	23.0 (q)	3->C=	135.6 (s)[b]
4-CH_2	32.5 (t)	2->C=	143.2 (s)
5-CH	60.4 (d)	8->C=	150.3 (s)
8-CH_2OH	64.5 (t)	7-C=O	166.7 (s)
S–CH=	111.7 (d)	N–*C*O–CH_3	173.2 (s)
6->C=	134.2 (s)[b]	COOH	174.6 (s)

a) Spectrum was recorded with a Varian XL-100-12A spectrometer in D_2O at 5°C using CH_3CN as an internal reference. δ: Calculated by assuming δ (CH_3CN)=1.7 ppm from DSS.

b) Tentative assignments.

Esterification of ASM A with methyl iodide gave the corresponding methyl ester, the FD-MS spectrum of which showed the peak 354 *m/z* (M^+ for methyl ester of $C_{14}H_{16}O_6N_2S$) agreeable with the above structure. On acetylation with acetic anhydride - pyridine the methyl ester yielded an acetate. The ^1H NMR of the acetate clearly proved the introduction of a methyl ester and an *O*-acetyl group to the ASM A, and the existence of the COOH and the CH_2OH functions was established. Further, the spin-spin coupling between vicinal protons was confirmed by spin-decoupling experiments on this acetate.

In order to ascertain the configuration at C(5), ASM A was oxidized with potassium permanganate and successively hydrolyzed with 6 N HCl to give D-aspartic acid. This experiments demonstrated not only the *R*-configuration at C(5) but also the presence of the double bond between C(6) and C(8).

Asparenomycin B (ASM B) is a minor antibiotic having a different chromophore from that of ASM A[2]. The ^1H NMR data of ASM B (see Table 1) clearly indicates that ASM B has no vinyl protons but four methylene protons at 3.59 ppm (2H, m) and 3.22 ppm (2H, m), and the other signals are essentially correlative to the respective signals of ASM A. Accordingly, it is easily assumed that ASM B only differs from ASM A in the side-chain at C(3) and presumably has a saturated substituent, an *N*-acetyl cysteamine type, at this position. However, as the chemical shifts of the two methylene groups of this side-chain showed a remarkable down-field shift in comparison with those of the *N*-acetyl cysteamine side-

chain of known carbapenem antibiotics, the unprecedented sulfoxide structure of this saturated side-chain is preferable to the sulfide. In order to confirm by FD-MS, ASM B was converted to *p*-nitrobenzyl ester and successively to its acetate. Both derivatives gave the molecular peaks corresponding to respective sulfoxide structures though the peaks were extremely unstable on heating, and ASM B was deduced to be structure IIa.

Table 3. Retention-times (minute) of asparenomycins on HPLC[a].

	ASM A	ASM B	ASM C
Nucleosil-7-C_{18} (4.0ϕ×200 mm, 1.5 ml/minute)	13.6[b]	10.4[b]	18.2[c]
Nucleosil-5-C_8 (4.0ϕ×200 mm, 1.0 ml/minute)	9.7[b]	7.4[b]	12.6[c]

a) Recorded on a Waters Model 6000A.
b) 0.05 M Phosphate buffer (pH 7.0).
c) 0.05 M Phosphate buffer (pH 7.0) in 5% MeOH.

Asparenomycin C (ASM C), the least abundant component of the three, was isolated from the less hydrophilic fraction of the metabolites[2]. From its biological character it was easily assumed that ASM C would also have a similar structure to asparenomycins. Examination of the reverse-phase HPLC of the known carbapenem antibiotics suggests that the sulfoxide structure significantly contributes to the hydrophilic character. Taking account of the retention-times of asparenomycins (Table 3), it seemed that ASM C might have a sulfide structure correlative to ASM A.

In order to confirm the above speculation ASM A was treated with titanium trichloride in acetate-buffer. ASM A was immediately deoxygenated in a good yield, and the ^{1}H NMR data of the product (Table 1), which showed the up-field shift of the vinyl protons and of 5-CH comparable to $\Delta\delta$ found between MM 13902 and its sulfoxide, MM 4550[3~5], corroborated the anticipated structure IIIa (Fig. 1). The HPLC of this product, as expected, was identical with that of ASM C under several conditions and the antibacterial characters of both compounds were also identical so far as examined. Thus, ASM C was proved to be deoxy ASM A (structure IIIa).

The final problem of the structures of asparenomycins is the determination of the geometry at C(8) and the configuration of the sulfoxide. For this purpose the preparation of a crystalline derivative available to X-ray analysis was intended. Esterification of ASM A with *p*-nitrobenzyl bromide afforded a crystalline ester, however, the ester gave only fine needles from all recrystallization solvents examined. The *p*-nitrobenzyl ester was converted to its acetate with acetic anhydride. The product was confirmed to be the desired acetate, $C_{23}H_{23}N_3O_9S$, by ^{1}H NMR and FD-MS, and was recrystallized from tetrahydrofuran-ether to give crystals suitable for X-ray analysis.

Crystal data of the product were: orthorhombic; space group $P2_12_12_1$; a=19.249(2), b=25.631(1), c=4.952(1) Å; Z=4. Intensity data were collected by ω-2θ scan on a Rigaku diffractometer with graphite-monochromated Cu $K\alpha$ radiation and a crystal of dimensions 0.3×0.3×0.3 mm. Intensities were measured in the range $\theta \leqq 70°$ with variable scan range $(1.2+0.2 \tan\theta)°$ and a constant scan speed of 0.05° s^{-1}. The 2598 independent intensities were corrected for Lorentz and polarization factors, but not for absorption effects.

The structure was solved by use of the program MULTAN 78[9]. In a difference electron density map calculated after block-diagonal least-squares refinement, all the hydrogen atoms were located. Successive refinement of the positional and anisotropic thermal parameters of the non-hydrogen atoms gave an R value ($\Sigma|\Delta F|/\Sigma|F_0|$) of 0.036 for 2372 reflections. The weighting scheme used was $w=1/\sigma^2(F_0)$ for $|F_c| \geqq \sigma(F_0)$ and $w=0$ for $|F_c| < \sigma(F_0)$ or $|\Delta F| \geqq 3\sigma(F_0)$. $\sigma(F_0)$ was estimated as $\sigma(F_0)=[\sigma_1{}^2(F_0)+0.00066|F_0|^2]^{1/2}$, where $\sigma_1(F_0)$ is the standard deviation due to counting errors. Final positional

Table 4. Fractional coordinates ($\times 10^4$) and equivalent isotropic temperature factors B_{eq} ($Å^2 \times 10^2$) for the acetate with estimated standard deviations.

The atom-numbering scheme is shown in Fig. 2.

$B_{eq}=4/3(\beta_{11}a^2+\beta_{22}b^2+\beta_{33}c^2+2\beta_{12}ab\cos\gamma+2\beta_{13}ac\cos\beta+2\beta_{23}bc\cos\alpha)$.

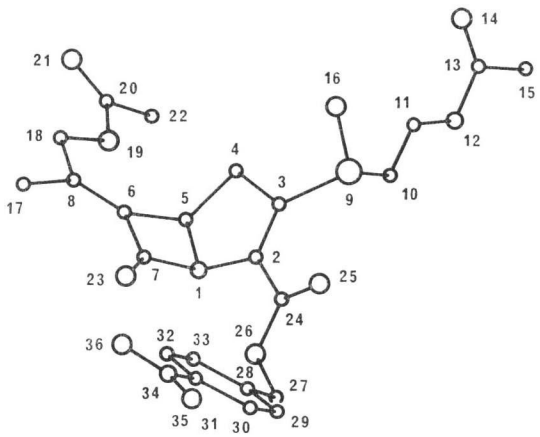
	x	y	z	B_{eq}		x	y	z	B_{eq}
N (1)	4145 (1)	2738 (1)	6689 (5)	333 (5)	O (19)	2741 (1)	1868 (1)	1681 (5)	492 (6)
C (2)	4878 (1)	2695 (1)	6449 (5)	293 (5)	C (20)	2520 (2)	1434 (1)	460 (8)	512 (9)
C (3)	5052 (1)	2313 (1)	4738 (5)	295 (6)	O (21)	2209 (1)	1435 (1)	−1644 (6)	642 (8)
C (4)	4423 (1)	2063 (1)	3392 (6)	353 (6)	C (22)	2698 (3)	964 (1)	2111 (11)	819 (15)
C (5)	3813 (1)	2312 (1)	4976 (6)	350 (6)	O (23)	3836 (1)	3599 (1)	5313 (6)	507 (6)
C (6)	3375 (1)	2738 (1)	3690 (7)	358 (6)	C (24)	5341 (1)	3060 (1)	7943 (5)	313 (6)
C (7)	3776 (1)	3135 (1)	5227 (7)	380 (7)	O (25)	5930 (1)	3151 (1)	7318 (4)	396 (5)
C (8)	2869 (1)	2792 (1)	1888 (6)	384 (7)	O (26)	5004 (1)	3282 (1)	10004 (4)	373 (5)
S (9)	5920 (1)	2150 (1)	3852 (2)	322 (1)	C (27)	5411 (2)	3654 (1)	11550 (6)	430 (8)
C (10)	5943 (2)	1509 (1)	5197 (6)	354 (6)	C (28)	5464 (2)	4175 (1)	10180 (6)	372 (7)
C (11)	6127 (1)	1129 (1)	3557 (6)	332 (6)	C (29)	6010 (2)	4502 (1)	10921 (9)	562 (10)
N (12)	6281 (1)	621 (1)	4346 (5)	360 (6)	C (30)	6052 (2)	5002 (1)	9846 (9)	613 (11)
C (13)	6481 (1)	251 (1)	2506 (7)	370 (6)	C (31)	5563 (2)	5159 (1)	7968 (7)	447 (8)
O (14)	6491 (1)	348 (1)	77 (5)	466 (5)	C (32)	5037 (2)	4838 (1)	7169 (9)	498 (9)
C (15)	6681 (2)	−268 (1)	3645 (8)	496 (9)	C (33)	4987 (2)	4341 (1)	8299 (8)	456 (8)
O (16)	5914 (1)	2096 (1)	861 (4)	428 (5)	N (34)	5613 (2)	5683 (1)	6803 (8)	630 (10)
C (17)	2616 (2)	3323 (1)	1020 (9)	508 (9)	O (35)	6055 (2)	5977 (1)	7665 (8)	913 (12)
C (18)	2507 (2)	2353 (1)	530 (7)	429 (8)	O (36)	5207 (2)	5805 (1)	5018 (10)	1041 (13)

parameters and equivalent isotropic temperature factors of the non-hydrogen atoms are listed in Table 4.

Since the *R* configuration at the C(5) atom has been confirmed by chemical degradation as mentioned above, the absolute configuration of the molecule is shown in Fig. 2. It indicates that the S(9) atom adopts the *R* configuration as carpetimycins[7], and that the bond of >C(6)=C(8)< exhibits the *E* geometry.

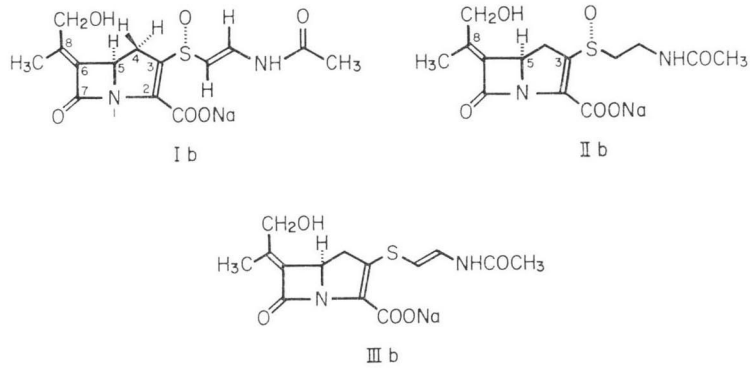
The N(1) atom deviates from the plane of C(2), C(5) and C(7) by 0.538(3) Å. The bond lengths in the β-lactam amide group [N(1)−C(7) = 1.437(4); C(7)=O(23) = 1.196(5) Å] differ from those in the exocyclic amide group [N(12)−C(13) = 1.370(4); C(13)=O(14) = 1.228(4) Å]. These reveal that the non-planarity of the β-lactam N atom reduces the normal amide resonance, $O=C-N< \leftrightarrow \bar{O}-C=\overset{+}{N}<$, as pointed out for β-lactam antibiotics[8].

Fig. 2. Perspective view of the molecule of acetylasparenomycin A *p*-nitrobenzyl ester with the atom-numbering scheme.



Thus, the structures of ASM A and ASM C which had been correlated to ASM A were unequivocally determined to be Ib and IIIb (Fig. 3), respectively. While there are no further evidences which ascertain the stereochemistry of ASM B, but the *E* geometry at C(8) is reasonable because of the ^1H

Fig. 3. Structures of asparenomycins.



chemical shifts, δ C(8)–CH_3 and δ C(8)–CH_2–OH, exactly corresponded to those of ASM A. Further, since both ASM A and B are the products of the same streptomycete strain under the same fermentation condition, it is likely from biogenetic point of view that ASM B also has the configurations at C(5) and S atom same to those of ASM A and corresponds to the dihydro ASM A (IIb in Fig. 3).

The *E* geometry of the hydroxyisopropylidene group is compatible with the following data. In acidic solution ASM A lost its biological activity and gave a product lacking the carbonyl absorption band in the range of 1700~1800 cm^{-1}. Paper electrophoresis of the product exhibited the formation of an additional carboxyl group but the product was negative to ninhydrin. From these evidences and the ^1H NMR data listed in Table 5, the structure of the product was concluded to be diacid IV, a mixture of diastereoisomers concerning the stereochemistry at C(3) (Fig. 4). This structure is comparable with the hydrolyzed product of MC696-SY2-A (MM 4550)[5]. The fact that IV was not a lactone but a dicarboxylic acid is agreeable with the *E* geometry of the double bond.

In relation to the above result, an interesting metabolite was isolated from the fermentation broth. The substance was found as a HPLC peak which also disappeared by the pretreating with hydroxylamine as other carbapenem compounds[2], however the substance showed no biological activity. The structure was deduced to be the lactone V shown in Fig. 4 by IR (1735 cm^{-1}, α,β-unsaturated γ-

Table 5. ^1H NMR data of IV and V (Na salts) in D_2O at 100 MHz (δ ppm from internal DSS).

Assignment	IV	V
8-CH_3	1.85 (s)	2.15 (s)
N–Ac	2.16 (s) (2.17)*	2.15 (s)
4-CH_2	2.0~2.9 (m)	2.42 (m), J_{gem}=14.0 2.75 (m)
8-CH_2–O	4.24 (s) (4.23)*	4.91 (s)
5-CH	4.6~5.4 (m)	5.05 (m)
3-CH		5.22 (m)
S–CH=	6.11 (d), J=14.0 (6.22)*	6.16 (d), J=14.0
N–CH=	7.50 (d), J=14.0 (7.61)*	7.52 (d), J=14.0

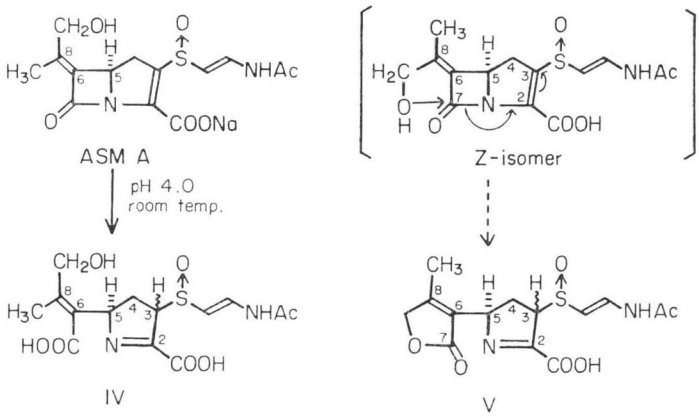
* Separate chemical shifts due to minor diastereomer are shown in parentheses.

Table 6. ^{13}C NMR data of V (Na salt) in D_2O at 25.2 MHz.

Assignment	δ ppm	Assignment	δ ppm
8-CH_3	12.6 (q)	6-C=	124.7 (s)
$NCOCH_3$	23.1 (q)	N–CH=	135.5 (d)
4-CH_2	28.4 (t)	8-C=	165.9 (s)
5-CH*	66.9 (d)	2-C=	168.5 (s)
3-CH*	71.1 (d)	7-CO	170.6 (s)
8-CH_2–O	74.7 (t)	N–$COCH_3$	173.2 (s)
S–CH=	108.6 (d)	COOH	176.6 (s)

δ: ppm from DSS (internal CH_3CN, δ=1.7)
* Tentative assignments.

Fig. 4.



lactone) and the ^{1}H and ^{13}C NMR data confirmed by spin-decoupling experiments (see Tables 5 and 6). Though the metabolite V is not a mixture of diastereomers, the configuration at C(3) is uncertain. The configurations of C(5) and the sulfoxide are presumably *R* as other metabolites.

In connection with the formation of IV from ASM A, it is easily assumed that the lactone V should originate from the potential *Z* isomer of ASM A probably due to the intramolecular interaction between the C(8)–CH_2OH and the β-lactam functions. Accordingly, the isolation of V reveals that the unique 6-(*E*)-hydroxyisopropylidene group of the asparenomycins is synthesized biologically not in overwhelming selectivity but the less stable *Z* isomer co-produced is degraded quickly to the inactive compound V in the fermentation medium.

Experimental

Methyl Ester of ASM A

To a solution of 10 mg ASM A (Na salt) in 1 ml of DMF was added 0.2 ml of methyliodide, and the solution was kept at 4°C for 16 hours. After evaporation of the solvent, the residue was separated by TLC on silica gel ($CHCl_3$ - MeOH=1:1) to give 5 mg of methyl ester as a pale yellow powder ($C_{15}H_{18}O_6N_2S$). FD-MS: *m/z* 354 (M^+).

O-Acetyl ASM A Methyl Ester

The above methyl ester was acetylated with Ac_2O/pyridine at −20°C for 16 hours. The product was purified by preparative TLC on silica gel ($CHCl_3$ - MeOH=9:1). ^{1}H NMR $\delta_{TMS}^{CDCl_3}$ ppm, *J* (Hz): 2.10, 2.12, 2.13 (each 3H, s, C(8)–CH_3, *O*–Ac, *N*–Ac), 3.15 (1H, m, $J_{5,4a}$=11.1, $J_{4a,b}$=18.6, 4-CHa), 3.32 (1H, m, $J_{5,4b}$=7.0, $J_{4a,b}$=18.6, 4-CHb), 3.89 (3H, s, $COOCH_3$), 4.55 and 4.72 (each 1H, ABq, J_{gem}=16.0, C(8)–CH_2–OAc), 4.91 (1H, m, $J_{5,4a}$=11.1, $J_{5,4b}$=7.0, 5-CH), 6.33 (1H, d, *J*=13.8, S–CH=), 7.48 (1H, d.d, *J*=13.8, *J*=10.0, NH–*CH*=), 8.64 (disappeared by the addition of D_2O, 1H, d, *J*=10.0, NH).

Oxidative Degradation of ASM A

To a solution of 1 mg ASM A in 0.2 ml of 0.01 M phosphate buffer (pH 7.0) was added 5 mg of $KMnO_4$ at 0°C and the mixture was allowed to stand at 4°C for 16 hours. After decomposition of the excess $KMnO_4$ with MeOH, the reaction mixture was centrifuged to remove precipitates and the solution was evaporated *in vacuo*. The residue was hydrolyzed with 0.2 ml of 6 N HCl at 110°C for 2 hours. Examination with an amino acid analyzer showed that this solution contained about 0.7 μ moles of aspartic

acid. The product was separated and purified by TLC on a precoated cellulose plate (*n*-BuOH - AcOH - H_2O=3: 1: 1). According to the previously published procedure[7] the aspartic acid was L-leucylated and analyzed by HPLC to prove that the product is L-leucyl-D-aspartic acid.

Derivatives of ASM B

On treatment with *p*-nitrobenzyl bromide in DMF, ASM B afforded *p*-nitrobenzyl ester ($C_{21}H_{23}O_8N_3S$), FD-MS: *m/z* 477 $(M)^+$. Acetylation of the *p*-nitrobenzyl ester with Ac_2O/pyridine gave corresponding acetate ($C_{23}H_{25}O_9N_3S$), FD-MS: *m/z* 519 $(M)^+$. ^1H NMR $\delta^{CDCl_3}_{TMS}$ ppm, *J* (Hz); 2.01 (3H, s, NAc), 2.13 (3H, s, C(8)–CH_3), 2.15 (3H, s, OAc), 3.1~3.4 (4H, m, 4-CH_2 and N–CH_2), 3.76 (2H, m, O↑S–CH_2), 4.68 (2H, ABq, J_{gem}=16.0 C(8)–CH_2OAc), 4.98 (1H, m, 5-CH), 5.32 and 5.48 (each 1H, ABq, J_{gem}=13.6, –CH_2–ϕ), 7.81 and 8.29 (each 2H, ABq, *J*(apparent)=9.0, aromatic H).

ASM C from ASM A

ASM A (10 mg) was dissolved in 1.5 ml of 0.5 M acetate buffer (pH 7.3). With stirring under N_2 atmosphere, a solution of 17 mg $TiCl_3$ (×4 equiv.) in 17 ml of the same buffer was added to the above solution at room temperature. After 5 minutes the excess of $TiCl_3$ was decomposed with air and the reaction mixture was applied on a 4 ml of Diaion HP-20AG (Mitsubishi-Kasei Co.) column which had been conditioned with a solution of 5% NaCl in 0.05 M phosphate buffer (pH 7.0). The column was washed with 60 ml of the same buffer solution, connected to a 18 ml of HP-20AG column and eluted with water. The fractions including the product were combined (45 ml) and freeze-dried to afford 4.5 mg of the deoxygenated compound (47%). UV λ_{max} nm: 234, 296. ^1H NMR: see Table 1. CD $\lambda[\theta]$: 425 (0), 350 (−2540), 332 (−2190), 279 (−10600), 263 (0), 258 (+3030), 252 (0), 238 (−10900), 224 (0), 216.5 (+8200), 211 (+6060), 200 (+23300).

Esterification of this product with *p*-nitrobenzyl bromide gave corresponding *p*-nitrobenzyl ester ($C_{21}H_{21}O_7N_3S$), FD-MS: *m/z* 459 $(M)^+$.

This deoxy ASM A was identified with natural ASM C by HPLC on Nucleosil-7-C_{18} and on Nucleosil-5-C_8 (Macherey-Nagel Co.) under varied eluting conditions. Further, both compounds showed equal antibacterial activity and inhibition against β-lactamase.

Crystalline Derivative of ASM A

To a solution of 10 mg ASM A in 1.5 ml of DMF was added 25 mg of *p*-nitrobenzyl bromide and the solution was shaken for 2 hours at 15°C. The solvent was distilled off *in vacuo* and the residue was extracted with acetonitrile. After evaporation of the solvent the residue was loaded on a Lobar column (size A LiChroprep Si60, E. Merck) and eluted with $CHCl_3$ - MeOH (8: 2) to give 7 mg of *p*-nitrobenzyl ester as fine needles.

The ester (7 mg) was dissolved in 300 μl pyridine and 5 μl Ac_2O was added to the solution. After shaking 2 hours the solution was evaporated and the residue was chromatographed on a Lobar column (size A LiChroprep Si60) using $CHCl_3$ - MeOH (9: 1). The pure fractions gave 7 mg of the acetate, which was recrystallized from THF-ether as colorless prisms, mp 165~165.5° (decomp.), ($C_{23}H_{23}O_9N_3S$) FD-MS: *m/z* 518 $(M+1)^+$. ^1H NMR $\delta^{CDCl_3}_{TMS}$ ppm, *J* (Hz): 2.13 (9H, s, *N*–Ac, *O*–Ac, C(8)–CH_3), 3.11 (1H, m, $J_{5,4a}$=11.0, $J_{4a,b}$=18.8, 4-CHa), 3.36 (1H, m, $J_{5,4b}$=7.4, $J_{4a,b}$=18.8, 4-CHb), 4.58 and 4.71 (each 1H, ABq, *J*=16.1, C(8)–CH_2OAc), 4.90 (1H, m, 5-CH), 5.32 and 5.47 (each 1H, ABq, *J*=13.6, O↑–$CH_2\phi$), 6.32 (1H, d, *J*=13.6, S–*CH*=), 7.44 (1H, d, *J*=13.6, N–*CH*=), 7.67 and 8.25 (each 2H, ABq, *J*(apparent)=8.8, aromatic H).

Hydrolysis of ASM A

ASM A (40 mg) was dissolved in water and the pH of the solution was adjusted to 4.0. After standing at room temperature for 6 hours, the solution was neutralized and evaporated *in vacuo*. The residue was purified by TLC on cellulose chromatogram sheet (Eastman-Kodak Co.) with 70% CH_3CN (visualized by UV). The product was extracted with 50% MeOH and lyophilized to give 23 mg of IV as colorless powder. UV λ_{max} nm (ε): 249 (18200). ^1H NMR see Table 5.

Isolation of Lactone V

The fraction including carpetimycin A and lactone V was separated from ASM C fraction by column chromatography on Diaion HP-20 AG with 0.02 M phosphate buffer (pH 7.0). The fraction was desalted on HP-20 and freeze-dried. Compound V was separated from carpetimycin A by HPLC on a LiChroprep RP-18 (20 mmϕ × 500 mm, Merck & Co.) using 0.01 M phosphate buffer (pH 7.0). The fractions including V, checked by analytical HPLC, were combined, desalted on HP-20 and freeze-dried to give pure sodium salt of V. UV λ_{max} nm (ε): 218 (15400), 252 (12300).

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