STRUCTURES AND ABSOLUTE CONFIGURATIONS OF CARPETIMYCINS A AND B

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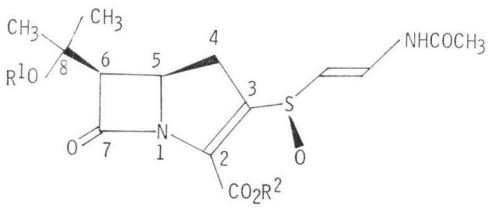
The structures and stereochemistries of carpetimycins A (**1**) and B (**2**) have been determined as shown below.

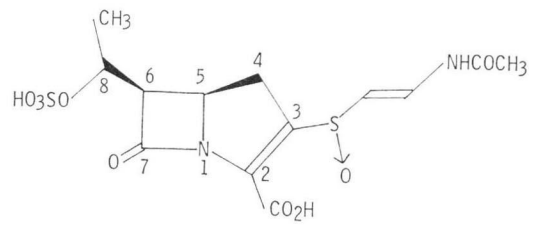
Carpetimycins A and B, two new β-lactam antibiotics related to thienamycin[1], epithienamycins[2], olivanic acids[3~5] and PS-5[6], were found in the culture filtrate of *Streptomyces* sp. KC-6643. These antibiotics have strong activity against Gram-positive and Gram-negative bacteria including β-lactamase producing strains. Although the carbapenem antibiotics have been reported to be unstable substances, carpetimycins A and B are more stable than olivanic acids.

In a previous paper[7], we presented the isolation, characterization, biological properties and structures of these antibiotics.

In this paper, we describe the structure elucidation including stereochemistry of carpetimycins A and B on the basis of chemical studies and X-ray analysis.



(**1**) R^1=H, R^2=H
(**2**) R^1=SO_3H, R^2=H
(**3**) R^1=H, R^2=CH_3
(**4**) R^1=H, R^2=$CH_2C_6H_4(NO_2)$-*p*
(**5**) R^1=SO_3H, R^2=$CH_2C_6H_4(NO_2)$-*p*
(**6**) R^1=H, R^2=$CH_2C_6H_4Br$-*p*



MM 4550 (MC696-SY2-A)

Structures and Absolute Configurations

Carpetimycins A (**1**) and B (**2**) were isolated as freeze-dried sodium salts. The UV spectrum of carpetimycin A showed two maxima at $\lambda_{max}^{H_2O}$ 240 nm ($E_{1cm}^{1\%}$ 369) and 288 nm ($E_{1cm}^{1\%}$ 300). The IR band

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Table 1. Chemical shifts and coupling constants of ^1H NMR spectra of carpetimycins A (**1**) and B (**2**) in D_2O.

Proton	**1**		**2**	
	ppm	*J* (Hz)	ppm	*J* (Hz)
8-CH_3	1.83		2.15	
8-CH_3	1.91		2.23	
6-CH	4.32	5.5	4.50	5.5
5-CH	4.98	5.5, 8, 11	5.04	5.5, 8, 11
4-CH_2	3.54	11, 17	3.62	11, 18
	4.43	8, 17	4.42	8, 18
N-$COCH_3$	2.65		2.65	
N-CH=	6.92	14	6.95	14
S-CH=	8.10	14	8.13	14

Me_4Si as external standard.

at 1770 cm^{-1} in the spectrum of **1** was attributed to a β-lactam carbonyl group. The CD spectrum of **1** showed at $[\theta]_{nm}^{H_2O}$ 210 (-1.90×10^4), 235 ($+7.22\times10^4$), 260 (-6.27×10^4) and 300 (-3.17×10^4). It was suggested from the above data that **1** had the same chromophore as MM 4550[8] (MC696-SY2-A)[8] having a sulfoxide function. The molecular formula of **1** was established as $C_{14}H_{18}N_2O_6S$ from the FD mass spectrum of the methyl ester [(**3**), MH^+ 357], the elemenal analysis and the ^{13}C NMR spectrum. Instead of a doublet methyl signal in the ^1H NMR spectrum of MM 4550, two singlet methyl signals were observed in that of **1** (Table 1). The orientation of H-5 and H-6 in **1** was determined to be *cis* virtue of the observed coupling constants ($J_{5,6}$=5.5 Hz). The ^{13}C NMR spectrum (Table 2) of **1** revealed fourteen carbons, and indicated the presence of a tetra substituted carbon-carbon double bond (C-2–C-3) and a tetra substituted carbon (C-8, δ 71.8). The C-8 carbon in MM 4550 was observed as a tri substituted carbon. Esterification of **1** with *p*-nitrobenzyl bromide in dimethylformamide afforded the *p*-nitrobenzyl ester (**4**). The ^1H NMR spectrum of **4** in DMF-d_7 showed an exchangeable hydroxy proton (δ 4.97). The *R* configuration of C-5 was probable as in all other known naturally occurring β-lactam antibiotics[5] and confirmed by X-ray crystallographic analysis as described below.

Table 2. ^{13}C Chemical shifts (ppm) of carpetimycins A (**1**) and B (**2**) in D_2O.

Carbon	**1**	**2**
C=O	179.6 (s)	178.0 (s)
C=O	173.6 (s)	173.7 (s)
C=O	166.5 (s)	166.3 (s)
2-C=	141.5 (s)	140.5 (s)
3-C=	138.0 (s)	139.6 (s)
NHCH=	134.9 (d)	134.9 (d)
S-CH=	112.7 (d)	113.0 (d)
C-O	71.8 (s)	85.0 (s)
5-CH	63.8 (d)	64.0 (d)
6-CH	55.1 (d)	55.0 (d)
4-CH_2	29.4 (t)	29.7 (t)
CH_3-CO	29.6 (q)	26.2 (q)
CH_3	28.0 (q)	25.1 (q)
CH_3 C-	23.5 (q)	23.6 (q)

Me_4Si as external standard.

Thus, the structure of **1** was proposed as (5*R*,6*R*)-3-[(*E*)-2-acetamidoethenylsulfinyl]-6-(2-hydroxy-2-propyl)-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid.

The structure of carpetimycin B (**2**) was suggested to be similar to **1** from its spectral data. The UV and CD spectra of **2** showed $\lambda_{max}^{H_2O}$ 240 nm ($E_{1cm}^{1\%}$ 357) and 285 nm ($E_{1cm}^{1\%}$ 305), and $[\theta]_{nm}^{H_2O}$ 210 (-2.35

$\times 10^4$), 235 ($+7.14\times 10^4$), 261 (-5.85×10^4) and 300 (-3.78×10^4), respectively. The ^{1}H NMR and ^{13}C NMR signals of **2** were listed in Tables 1 and 2. The ^{13}C NMR data and the elemental analysis for **2** agreed with the molecular formula $C_{14}H_{18}N_2O_9S_2$. The IR spectrum of **2** indicated the presence of sulfate (1270~1220 and 1050 cm^{-1}, KBr), which was not observed in that of **1**. The exchangeable hydroxy proton was not observed in the ^{1}H NMR spectrum of *p*-nitrobenzyl ester (**5**) of **2** in DMF-d_7. In the ^{13}C NMR spectra (Table 2), the most significant difference between **1** and **2** was the marked deshielding of the C-8 carbon resonance from 85.0 ppm in the latter to 71.8 ppm in the former. It was apparent from the above evidence that the hydroxy function of **1** was absent and replaced by a sulfate moiety. This was confirmed by the fact that the mild acid hydrolysis of **2** afforded **1** and sulfate ion.

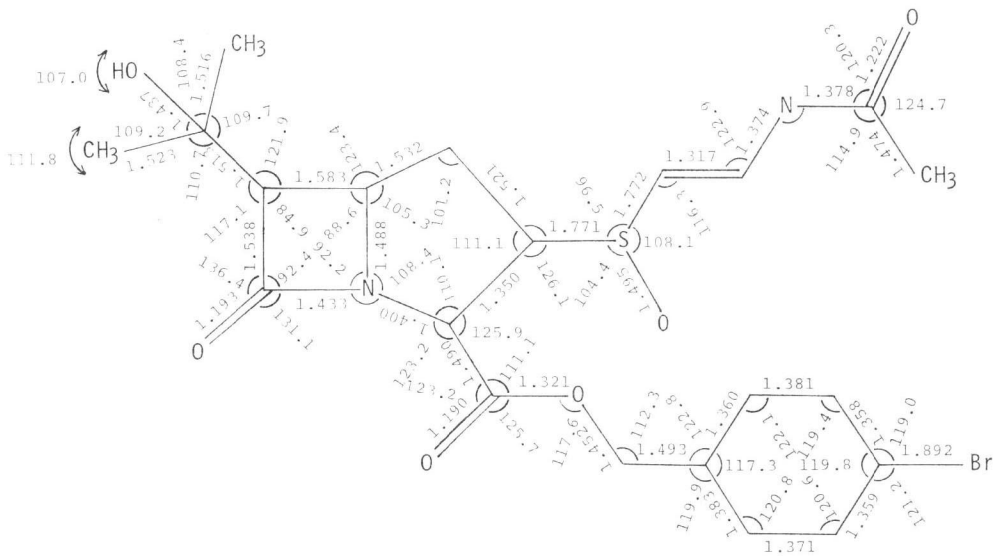
Thus, the structure of **2** was proposed as (5*R*,6*R*)-3-[(*E*)-2-acetamidoethenylsulfinyl]-6-(2-hydroxy-sulfonyloxy-2-propyl)-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid. Lately, the similar structures to **1** and **2** have been reported by other workers as C-19393 H_2 and S_2[9].

The complete structures of **1** and **2** were determined from the X-ray crystallographic analysis of *p*-bromobenzyl ester of **1**.

Carpetimycin A *p*-bromobenzyl ester (**6**) was prepared and crystallized from acetone - *n*-hexane to afford colorless needles. Crystal data: $C_{21}H_{23}N_2O_6SBr$ (MW=511.39), orthorhombic, space group $P_{2_12_12_1}$, a=16.901 (4), b=13.359 (3), c=10.001 (2) Å, V=2258.0 Å^3, $D_{calcd.}$=1.504 gcm^{-3}, $D_{obsd.}$=1.52 gcm^{-3} (in CCl_4 - *n*-hexane solution), Z=4.

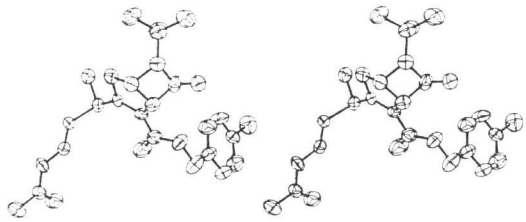
Cell constants and intensity data were determined on a SYNTEX $P\bar{1}$ computer-controlled fourcircle diffractometer with graphite monochromated Mo Kα radiation (λ=0.71069 Å) using a crystal with approximate dimensions of 0.15×0.2×0.45 mm. A total of 2296 unique intensities were collected by $2\theta-\theta$ scanning technique with 2θ less than 50.0°, among which 1420 with $I>2\sigma(I)$ were regarded to be observed. Two reference reflections were monitored every 58 reflections and their intensities decreased by 22% at the end of data collection. After usual decay correction, the intensities were further corrected for Lorenz and Polarization factors, but not for absorption and extinction.

Fig. 1. Bond angles and bond distances in carpetimycin A *p*-bromobenzyl ester (**6**).



The structure was solved by heavy-atom method and block-diagonal least-squares refinement with anisotropic non-hydrogen and isotropic hydrogen atoms other than those of hydroxy and amide groups for the observed reflections converged the R-factor to the final value of 0.047. The atomic scattering factors were taken from International Tables for X-ray Crystallography[10]. The structure of antipode of **6** was refined to give the R-factor value of 0.067.

Fig. 2. Stereoscopic view of the single molecule of **6**.



The bond lengths and the bond angles for non-hydrogen atoms are shown in Fig. 1. The stereoscopic view[11] of **6** is shown in Fig. 2.

Thus, the structures and stereochemistries of carpetimycins A and B have been determined to be (5*R*,6*R*)-3-[(*E*)-2-acetamidoethenyl-(*R*)-sulfinyl]-6-(2-hydroxy-2-propyl)-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid and (5*R*,6*R*)-3-[(*E*)-2-acetamidoethenyl-(*R*)-sulfinyl]-6-(2-hydroxysulfonyloxy-2-propyl)-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid, respectively. It is the first establishment among natural carbapenem antibiotics that the configuration of sulfoxide has been determined.

The sum of three nitrogen bond angles ($\sum_N$) and the distance (D) of the nitrogen from the plane of the attached three carbon atoms were calculated from the available X-ray data of **6**. The derivative of **1** with $\sum_N=326.3°$ and $D=0.51$ Å is the most folded fused β-lactam ring system, similar to the thienamycin derivative[1] with $\sum_N=325.9°$ and $D=0.49$ Å. It seems that the high chemical and biological reactivities of carpetimycins are partly based on its geometry.

Experimental

IR spectra were obtained with a JASCO-403G spectrophotometer. UV spectra were recorded with a Hitachi 200-20 spectrophotometer. ORD and CD spectra were obtained with a JASCO J-20 and a JASCO J-500C with DP-500 instruments, respectively. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. ^{1}H NMR and ^{13}C NMR spectra were measured at 100 MHz on a JEOL FX-100 spectrometer with Me_4Si as internal standard. FD mass spectra were obtained with a JEOL JMS 01 SG-2 spectrometer.

All the calculations of X-ray analysis were performed on a FACOM M-190 computer at the Computer Center of Kyushu University using the UNICS II programs[12].

Carpetimycin A Methyl Ester (**3**)

To a solution of **1** (5 mg) in dry dimethylformamide (1 ml), methyl iodide (0.06 ml) was added. The solution was stirred for 3 hours at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was chromatographed on two prewashed silica gel HF_{254} plates (E. Merck, 20×20×0.05 cm) in methylene chloride - methanol (9: 1). The zone at Rf 0.34 corresponding to **3** was collected and eluted with the same solvent to afford pure **3** (3.6 mg): mp 143~144°C; $[\alpha]_D^{24.5}$ −96.0° (*c* 1, CH_2Cl_2); IR (KBr) 1780, 1715, 1620, 1500, 1435, 1370, 1330, 1260 cm^{-1}; UV (MeOH) 244 (ε 17,700), 299 (11,000) nm; ^{1}H NMR ($CDCl_3$) δ 1.29, 1.49 (each 3H, s, C(CH_3)-CH_3), 2.11 (3H, s, $COCH_3$), 2.98 (1H, dd, J=15.0, 23.0 Hz, H-4), 3.55 (1H, d, J=5.7 Hz, H-6), 3.87 (3H, s, OCH_3), 4.25 (2H, m, H-4, H-5), 6.27 (1H, d, J=13.6 Hz, SO–CH=CH), 7.56 (1H, dd, J=10.8, 13.6 Hz, CH=CH–NH), 8.86 (1H, br-d, J=10.8 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 23.3, 28.1 (q, C(CH_3)-CH_3), 29.7 (q, $COCH_3$), 29.7 (t, C-4), 52.9 (q, OCH_3), 53.9 (d, C-6), 63.7 (d, C-5), 70.6 (s, C-8), 111.2 (d, SO–CH=CH), 131.7 (s, C-3), 134.0 (d, CH=CH–NH), 148.2 (s, C-2), 160.2, 169.1, 175.7 (s, C=O); FD mass m/z 357 (MH^+), 340, 308.

Anal. Calcd. for $C_{15}H_{20}N_2O_6S$: C, 50.55; H, 5.66; N, 7.86; S, 9.00.
Found: C, 50.61; H, 5.69; N, 7.82; S, 8.92.

CD (*c* 0.1, MeOH) [θ] 215 (-3.476×10^4), 244 ($+4.757\times10^4$), 265 (-1.427×10^4), 298 (-2.671×10^4) nm; ORD (*c* 0.1, MeOH) [ϕ] 231 (-3.549×10^2), 255 ($+5.122\times10^2$), 280 ($+2.415\times10^2$), 328 (-1.208×10^2) nm.

Carpetimycin A *p*-Nitrobenzyl Ester (**4**)

A solution of **1** (5 mg) and *p*-nitrobenzyl bromide (7 mg) in dry dimethylformamide (1 ml) was stirred at room temperature for 3 hours. The reaction mixture was evaporated to dryness under reduced pressure. Preparative chromatography of the evaporation residue on two prewashed silica gel HF_{254} plates in methylene chloride - methanol (9: 1) gave **4** (3 mg, Rf 0.38): IR (KBr) 1785, 1710, 1620, 1520, 1365, 1345, 1320, 1250 cm^{-1}; UV (MeOH) 250 (ε 17,900), 305 (9,400) nm; ^1H NMR (DMF-d_7) δ 1.28, 1.45 (each 3H, s, $C(CH_3)CH_3$), 2.06 (3H, s, $COCH_3$), 4.97 (1H, s, OH), 5.40, 5.59 (each 1H, d, J=13.6 Hz, CH_2Ar), 6.34 (1H, d, J=14.1 Hz, SO–C*H*=CH), 7.48 (1H, dd, J=10.5, 14.1 Hz, CH=C*H*–NH), 7.48, 8.29 (each 2H, d, J=8.8 Hz, Ar*H*), 10.58 (1H, br-d, J=10.5 Hz, NH).

Carpetimycin B *p*-Nitrobenzyl Ester (**5**)

Under the same conditions as described above, **5** [3.5 mg, Rf 0.32, CH_2Cl_2 - MeOH (4: 1)] was prepared by treatment of **2** (5 mg) with *p*-nitrobenzyl bromide (5 mg): IR (KBr) 1790, 1720, 1620, 1510, 1420, 1370, 1350, 1320, 1280~1210 (br) cm^{-1}; UV (MeOH) 251 (ε 19,200), 300 (11,400) nm; ^1H NMR (DMF-d_7) δ 1.58, 1.60 (each 3H, s, $C(CH_3)CH_3$), 2.06 (3H, s, $COCH_3$), 5.40, 5.58 (each 1H, d, J=13.5 Hz, CH_2Ar), 6.37 (1H, d, J=13.6 Hz, SO–C*H*=CH), 7.48 (1H, dd, J=10.6, 13.6 Hz, CH=C*H*–NH), 7.84, 8.28 (each 2H, d, J=8.4 Hz, Ar*H*), 10.61 (1H, br-d, J=10.6 Hz, NH).

Carpetimycin A *p*-Bromobenzyl Ester (**6**)

Under the same conditions as prepared **4**, **6** (10 mg, Rf 0.62) was obtained by treatment of **1** (20 mg) with *p*-bromobenzyl bromide (40 mg). The resulting solid was crystallized from acetone - *n*-hexane to give **6** which was recrystallized from the same solvent system to obtain sturdy and well-formed needles for X-ray analysis: mp 149~149.5°C; IR (KBr) 1785, 1710, 1620 cm^{-1}; UV (MeOH) 228 (ε 19,500), 245 (16,400), 303 (9,800) nm.

Anal. Calcd. for $C_{21}H_{23}N_2O_6SBr$: C, 49.32; H, 4.53; N, 5.48; S, 6.27.
Found: C, 49.39; H, 4.55; N, 5.44; S, 6.21.

Hydrolysis of Carpetimycin B (**2**)

A solution of **2** (80 mg) in 2 ml of 0.01 M acetate buffer pH 6.0 was heated at 60°C for 2 hours. The hydrolysate was purified by semipreparative HPLC using a Bondapak C_{18}/Porasil B column (Waters Assoc., 0.8×120 cm) with 2% MeOH in 0.05 M phosphate buffer pH 6.8. The active eluate was desalted with Diaion HP-20 resin (Mitsubishi Kasei Kogyo). The desalted eluate was freeze-dried to give **1** (5 mg), which was identical with the authentic product obtained from the fermentation in physico-chemical and biological properties.

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