CARPETIMYCINS A AND B, NEW β-LACTAM ANTIBIOTICS

Sir:

Carpetimycins A and B, two new carbapenem antibiotics related to the thienamycin[1,2], olivanic acid derivatives[3~5] and PS-5[6] have been found in the culture filtrate of *Streptomyces* sp. KC-6643. The strain was cultured in an Erlenmeyer flask which contained 100 ml of a medium composed of 3.6% starch, 2.2% soybean meal, 1.5% cotton seed oil, 0.62% $Na_2HPO_4 \cdot 12H_2O$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$ and 0.0005% $CoCl_2 \cdot 6H_2O$, on a rotary shaker at 29°C for 72 hours. Five hundred ml of the culture broth was inoculated into 100 liters of the same medium in a 200-liter fermentor.

Fermentation was carried out at 29°C under aeration at 100 liters/minute, agitation of 240 rpm and inner pressure of 0.5 kg/cm^2. The 144-hour cultured broth was filtered with Dicalite (Dicalite Orient Co., Ltd., Japan) as filter aid. The antibiotics in the filtrate (200 liters) were adsorbed on a column of Diaion PA-306 (Mitsubishi Kasei Kogyo Co., Ltd.) and then eluted with 20% NaCl. The active fractions were desalted on a Diaion HP-20 (Mitsubishi Kasei Kogyo Co., Ltd.) column. The active eluate was charged on a column of Amberlite IRA-458. Carpetimycins A and B were eluted with 0.9% and 20% NaCl, respectively. The fraction containing carpetimycin A was subjected to successive column chromatography with QAE-Sephadex A-25, Diaion HP-20 and Sephadex G-10. Lyophilization of active fractions from the last column gave a crude powder of carpetimycin A (230 mg, 200 mcg/mg).

The fraction containing carpetimycin B was extracted with 1% trioctylmethylammonium chloride in dichloromethane, followed by back extraction with 3% sodium iodide. The sodium iodide extract was subjected to succesive column chromatography using DEAE-Sephadex A-25, Diaion HP-20 and Sephadex G-10. Lyophilization of active fractions from the last column gave a crude powder of carpetimycin B (400 mg, 600 mcg/mg). Further purification of the crude powders was accomplished by HPLC (Bondapak C_{18}/Porasil B, Waters Assoc.) to give pure carpetimycins A (39 mg) and B (210 mg) as the sodium salts.

Carpetimycin A sodium salt is a colorless solid melting above 145°C with decomposition. $[\alpha]_D^{24}$ −27° (*c* 1.7, water). The antibiotic shows UV (H_2O): λ_{max} 240 nm ($E^{1\%}_{1cm}$ 369) and 288 nm ($E^{1\%}_{1cm}$ 300), IR (KBr): 1770, 1695, 1625 and 1265 cm^{-1}, ^{1}H-NMR (D_2O): δ 1.83 (3H s, CH_3–C–), 1.91 (3H s, CH_3–C–), 2.65 (3H s, CH_3–CO–), 3.54 (1H dd, J=11, 17 Hz), 4.32 (1H, d, J=5.5 Hz), 4.43 (1H dd, J=8, 17 Hz), 4.98 (1H m), 6.92 (1H d, J=14 Hz), 8.10 (1H d, J=14 Hz). FD Mass: 357 (MH^+ of methylester). Molecular ion peak of mass spectrum, the ^{13}C-NMR data and the analytical data for carpetimycin A agreed with the molecular formula $C_{14}H_{18}N_2O_6S$.

Carpetimycin B disodium salt is a colorless solid melting above 130°C with decomposition. $[\alpha]_D^{24}$ −145° (*c* 1, water). The antibiotic shows UV (H_2O): λ_{max} 240 nm ($E^{1\%}_{1cm}$ 357) and 285 nm ($E^{1\%}_{1cm}$ 305), IR (KBr): 1770, 1695, 1625, 1270~1220 and 1050 cm^{-1}, ^{1}H-NMR (D_2O): δ 2.15 (3H s, CH_3–C–), 2.23 (3H s, CH_3–C–), 2.65 (3H s, CH_3–CO–), 3.62 (1H dd, J=11, 18 Hz), 4.42

Table 1. Antibacterial activity of carpetimycins A and B.

Organisms	MIC (μg/ml)	
	Carpetimycin A	Carpetimycin B
Staphylococcus aureus 209P JC-1	0.39	6.25
Bacillus subtilis ATCC 6633	0.2	6.25
Escherichia coli NIHJ JC-2	0.05	1.56
Escherichia coli ML 1410 R EC-1*	0.2	3.13
Klebsiella pneumoniae PCI-602	0.2	6.25
Kiebsiella pneumoniae 25*	0.78	6.25
Proteus vulgaris IID 874	0.39	12.5
Proteus vulgaris 69*	0.78	25
Proteus morganii IFO 3168	0.39	12.5
Proteus morganii 41*	1.56	25
Citrobacter freundii 24*	1.56	12.5
Enterobacter cloacae IID 977	0.78	6.25
Enterobacter cloacae 3*	3.13	12.5
Serratia marcescens NHL	0.2	6.25
Serratia marcescens 4*	3.13	25
Pseudomonas aeruginosa NCTC 10490	6.25	25

Tests were conducted in Brain Heart Infusion agar inoculated with 10^6 cells per ml.

* β-lactamase producing organism

Table 2. β-Lactamase inhibitory activity of carpetimycins A and B.

	I_{50} (ng/ml)	
	Escherichia coli ML 1410 R EC-1 (PCase)	*Proteus vulgaris* 69 (CSase)
Substrate	PCG	CER
Carpetimycin A	0.64	0.52
Carpetimycin B	0.21	0.74

Inhibitor was incubated with enzyme at 30°C for 5 minutes prior to addition of substrate (100 μM final concentration). The hydrolysis of PCG was assayed by a modification of the NOVIK microiodometric method and that of CER was measured by direct UV spectrophotometry.

(1H dd, J=8, 18 Hz), 4.50 (1H d, J=5.5 Hz), 5.04 (1H m), 6.95 (1H d, J=14 Hz), 8.13 (1H d, J=14 Hz). The ^{13}C-NMR data and the analytical data for carpetimycin B agreed with the molecular formula $C_{14}H_{18}N_2O_9S_2$. By high-voltage paper electrophoresis with 3,000 V for 20 minutes in 1/30 M phosphate buffer (pH 8.0), carpetimycins A and B move to the anode with *Rm* (relative mobility to penicillin N) 1.0 and 2.4, respectively. Carpetimycins A and B can be separated by thin-layer chromatography on cellulose F_{254} (E. Merck, Art 5574) developed with 1-butanol - 2-propanol - water (7: 7: 6) (Rf 0.60 for A and 0.40 for B).

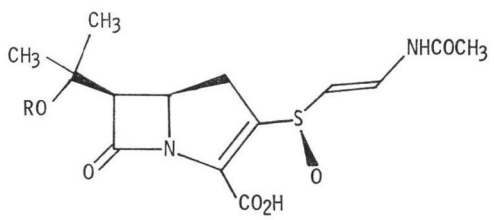
The antibiotics are more stable than olivanic acid derivatives[7]. In McIlvains sodium phosphate-citric acid buffer (pH 7.0) at 25°C the half-lives of carpetimycins A and B are 187 and 170 hours, respectively.

Carpetimycins A and B have strong activity against Gram-positive and Gram-negative bacteria, including β-lactamase producing strains, as shown in Table 1. The activity of carpetimycin A is 8~32 times greater than that of B. They also show inhibitory activity against β-lactamase, as shown in Table 2.

The structures of carpetimycins A and B were elucidated by their spectral data and conversion of carpetimycin B into A. The final structure determination including stereochemistry was accomplished by X-ray crystallographic analysis* of the carpetimycin A *p*-bromobenzyl ester as shown in Fig. 1.

The structure elucidation of carpetimycins A and B will be described in due course.

Fig. 1. Chemical structures of carpetimycins A and B.



	R
Carpetimycin A	H
B	SO_3H

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