

PS-8, A MINOR CARBAPENEM
ANTIBIOTIC

Sir:

During the isolation and purification of PS-5,¹⁾ PS-6 and PS-7²⁾ (PS-group of carbapenem compounds) from the culture broths of *Streptomyces cremeus* subsp. *auratilis* A 271, we have discovered a new minor component designated PS-8. Same as other carbapenem antibiotics, PS-8 possesses an excellent antimicrobial activity against Gram-positive and Gram-negative bacteria as well as a potent β -lactamase-inhibitory property.

A spore suspension of *Streptomyces cremeus* subsp. *auratilis* A271 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium SE-4 [0.3% beef extract (Difco), 0.5% Bacto-tryptone (Difco), 0.5% defatted soybean meal, 0.1% glucose, 2.4% soluble starch, 0.5% yeast extract and 0.4% calcium carbonate in tap water; pH 7.5 before autoclaving]. After cultivation for 48 hours on a rotary shaker, 100 ml of the seed culture was transferred into a 30-liter jar fermentor containing 15 liters of medium SE-4. The jar fermentor was operated at 28°C for 24 hours (agitation rate 200 rpm; aeration rate 7.5 liters per minute). One liter of the growth was used to inoculate a 200-liter stainless steel tank fermentor containing 100 liters of production medium AGB-7 (3.0% maltose, 2.0% dry yeast, 0.3% defatted soybean meal, 0.1% peptone, 0.5% sodium chloride, 0.05% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, 0.4% $CaCO_3$ and 0.00013% $CoCl_2 \cdot 6H_2O$ in tap water; pH 7.0). The fermentation of carbapenems was carried out at 28°C for 72 hours using an agitation rate of 100 rpm and an aeration rate of 50 liters per minute. The time course of production of carbapenem compounds was followed in the total antibacterial activity against *Comamonas terrigena* B-996 by the disc-agar diffusion assay method. Under usual fermentation conditions, the strain produced about 30~40 $\mu g/ml$ of carbapenem compounds expressed in PS-5 equivalents. The stimulatory effect of valine, leucine or isoleucine on the preferential formation of PS-8 was observed at 0.1%, as is the case in the production of PS-6 and PS-7.²⁾

As at an initial phase of the purification work PS-8 was assumed to have the unsaturated C-3 side chain based on its chromatographic behavior (see Fig. 3), methods which had been effective for

the separation of PS-7 (with the unsaturated C-3 side chain) from PS-5 and PS-6 (with the saturated one) were suitably combined for isolation and purification of PS-8. The culture filtrate (90 liters) was charged on a column (200 \times 900 mm) of Diaion PA306 (Cl^- form) and the column was washed with 30 liters of 1/100M phosphate buffer, pH 8.0, containing 3% sodium chloride. Carbapenem compounds including PS-8 was eluted with 50% methanol containing 3% sodium chloride. Antimicrobially active fractions were collected and combined. After removal of the solvent by evaporation, the aqueous solution was successively subjected to column chromatography on Diaion HP20, QAE-Sephadex (Cl^- form), Sephadex G-10 and Diaion HP20AG. During the column chromatographic work, antimicrobially active fractions were routinely monitored for the location of PS-8 among other carbapenem components by descending paper chromatography and ion exchange paper chromatography. Fractions of PS-8 collected from the Diaion HP20AG column were combined and lyophilized to give 2.0 mg of a white powder of PS-8 sodium salt.

The sodium salt of PS-8 was freely soluble in water and substantially insoluble in acetone, ethyl acetate and benzene. It turned yellow around 148°C and gradually decomposed above 160°C. UV absorption $\lambda_{max}^{H_2O}$ 223, 308 nm; IR spectrum (KBr) (Fig. 1); 1H NMR data (100 MHz in D_2O) δ 0.94 [3H, d, $(CH_3)_2-CH-$], δ 0.98 [3H, d, $(CH_3)_2-CH-$], δ 2.0 (3H, s, CH_3-CO-), δ 6.02 (1H, d, $-CH=CH-$), δ 7.14 (1H, d, $-CH=CH-$); molecular weight (m/z) 324 (M^+ of PS-8 methyl ester). Chromatographic and electrophoretic data of PS-8 sodium salt are as follows: Rf (thin-layer chromatography with Chromatogram Sheet 3254 Cellulose®) 0.80 in *n*-butanol - isopropanol - water (7: 7: 6); 0.67 in *n*-butanol - ethanol - water (4: 1: 2); and 0.79 in *n*-propanol -

Fig. 1. Infrared spectrum of PS-8 sodium salt (KBr).

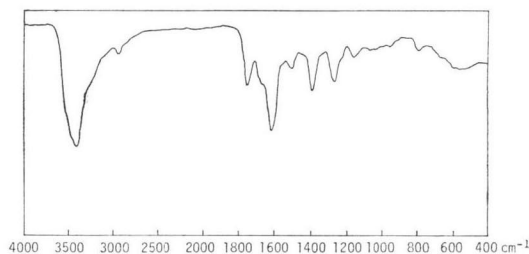
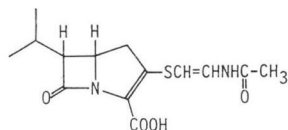


Fig. 2. Structure of PS-8.



water (8:2); Rf(descending paper chromatography with Toyo filter paper No. 50) 0.51 in acetonitrile - 0.1 M tris-HCl, pH 7.5 - 0.1 M ethylenediaminetetraacetate, pH 7.5 (120:30:1); Rf (ion exchange paper chromatography with DEAE-cellulose paper) 0.50 in 1/20 M phosphate buffer, pH 8.0; Rm against bromthymol blue [high voltage paper electrophoresis at 75 V/cm for 20 minutes in Veronal buffer, pH 8.6 ($I=0.05$)] 0.82.

The physicochemical properties of PS-8 described above are interpreted to indicate that PS-8 has the chemical structure shown in Fig. 2. In addition to PS-5,¹⁾ PS-6, PS-7²⁾ and PS-8, the following carbapenem compounds were isolated from the culture broths of *Streptomyces cremeus* subsp. *auratilis* A271 and identified by physicochemical and chromatographic comparison as indicated: PS-3A (epithienamycin C³⁾), PS-3B (epithienamycin A³⁾), PS-4A (epithienamycin D³⁾), PS-4B (epithienamycin B³⁾), PS-E₁ (MM 17880⁴⁾), PS-E₂ (MM 13902⁵⁾). These carbapenem components could clearly be analyzed by the combination of paper chromatography with ion exchange paper chromatography (Fig. 3).

Table 1. Antimicrobial activity of PS-8 (MIC in $\mu\text{g/ml}$).

	PS-8	Cefazolin
<i>Staphylococcus aureus</i> FDA 209P	1.56	0.24
<i>Streptococcus pneumoniae</i> Type III	0.19	0.13
<i>Alcaligenes faecalis</i> B326	0.78	6.25
<i>Comamonas terrigena</i> B996	0.10	0.05
<i>Citrobacter freundii</i> E9*	6.25	100
<i>Enterobacter cloacae</i> E16*	6.25	100

Inoculum size: 10^8 cells/ml.

* β -lactamase producer.

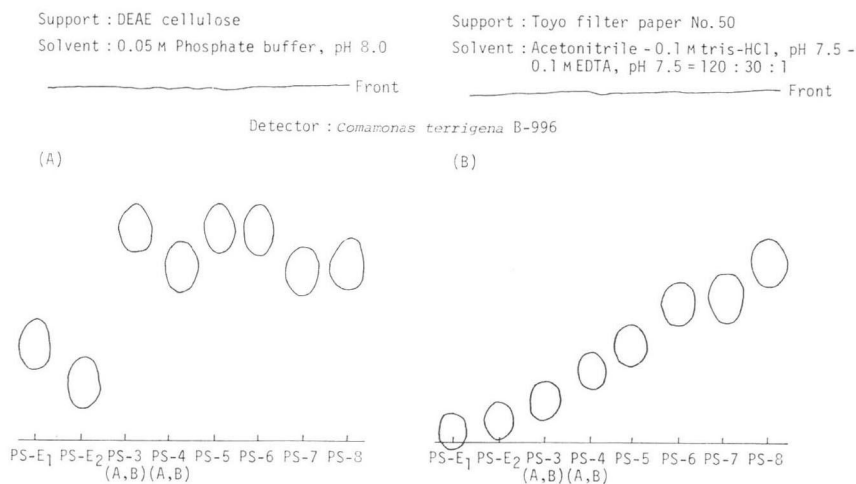
The antimicrobial spectrum of PS-8 was determined by the broth dilution method and the results are summarized in Table 1. PS-8 was found to have a more potent inhibitory activity against β -lactamase of *Citrobacter freundii* GN 346 than PS-6.

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Fig. 3. Chromatographic data of the PS group of carbapenem compounds.



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