A NEW CARBAPENAM NO. 17927 D SUBSTANCE

Sir:

A new carbapenam compound No. 17927 D[1] was obtained as the sodium salt (**1**) from the culture filtrates of three strains of streptomycetes, isolated from soil samples and this compound was also detected in the culture filtrates of other known carbapenem antibiotics producers such as *Streptomyces fulvoviridis* ATCC 15863[2], *S. olivaceus* ATCC 21379[2] and ATCC 31126[2], *S. argenteolus* ATCC 11009[2] and *S. flavogriseus* NRRL 8139 and NRRL 8140[3].

Taxonomic studies of these three soil isolates revealed that their morphological, physiological and cultural characteristics were very closely related to each other and they might belong to almost the same species of streptomycete. Among known species of *Streptomyces*, *S. fulvoviridis* was selected as the most closely related species. The results of simultaneous cultivation of *S. fulvoviridis* ISP 5210 with these strains indicated their identity in various characteristics and the new strains were named *S. fulvoviridis* SANK 61278, SANK 60183 and SANK 60283, respectively.

Fermentation of *S. fulvoviridis* SANK 61278 was carried out in two 600-liter fermentors, each containing 300 liters of medium composed of soluble starch 3.0%, soy bean meal 2.0%, Fermamine 1.0%, stearyl monoglyceride 1.0%, KH_2PO_4 0.2%, $CoCl_2 \cdot 6H_2O$ 0.001% and Disfoam CB-442 0.02% (pH 7.0 before sterilization), at 24°C for 139 hours with agitation of 220~360 rpm and aeration of 300 liters/minute.

Table 1. Physico-chemical properties of sodium salt (**1**) and *p*-nitrobenzyl ester (**2**) of No. 17927 D.

		1	**2**
Nature		Colorless, amorphous powder	Colorless crystals
$[\alpha]_D^{20}$			+4.5° (*c* 1.79, $CHCl_3$ - 10% MeOH)
Elemental analysis (%)	Found;	C 43.53, H 5.91, N 7.28, S 8.66, Ash 11.86	
	Calcd. for	$C_{13}H_{19}N_2O_5SNa \cdot H_2O$; C 43.82, H 5.89, N 7.86, S 8.98	
Molecular formula		$C_{13}H_{19}N_2O_5SNa$	$C_{20}H_{25}N_3O_7S$
Molecular weight		338	452 $(M+H)^+$
UV max.		End absorption	
IR (KBr)		1750, 1660, 1610 cm^{-1}	1760, 1750, 1610 cm^{-1}

Fig. 1. ^1H NMR spectrum of sodium salt of No. 17927 D (**1**) in D_2O.

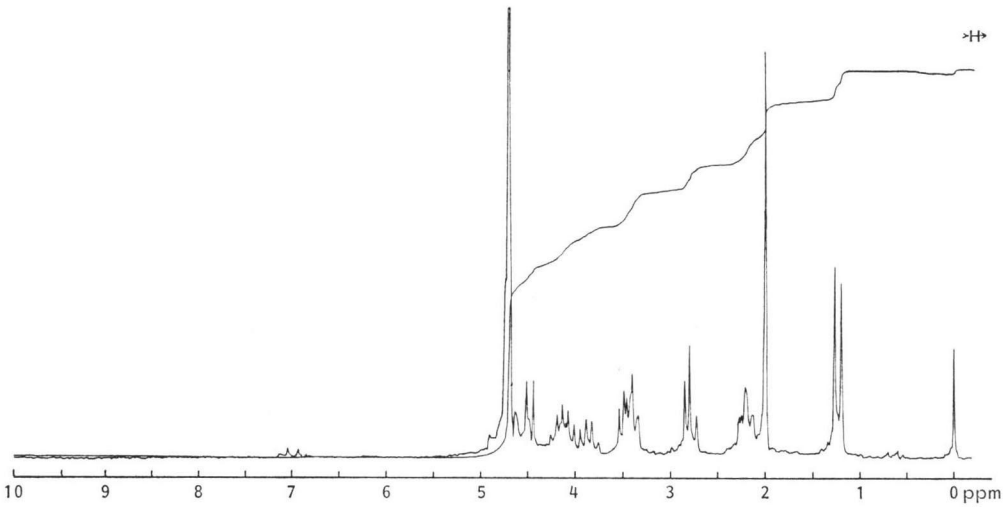


Fig. 2. IR spectrum of *p*-nitrobenzyl ester of No. 17927 D (**2**) in KBr.

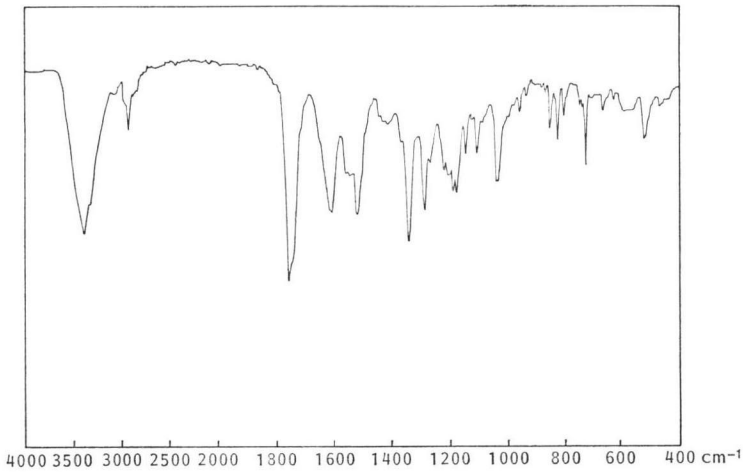
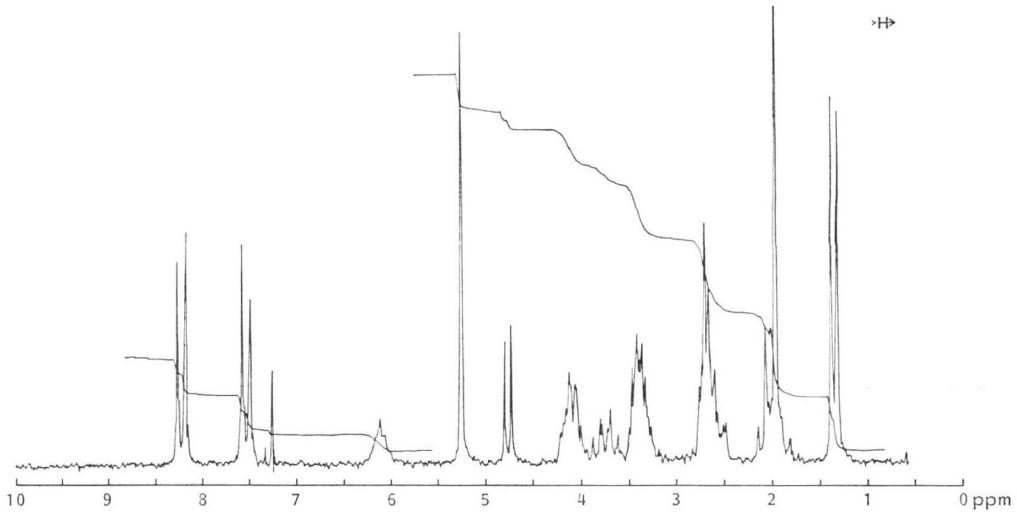


Fig. 3. ^{1}H NMR spectrum of *p*-nitrobenzyl ester of No. 17927 D (**2**) in $CDCl_3$.



The cultured broth (600 liters) was filtered by addition of 600 liters of cold deionized water and 60 kg of Celite. The filtrate was adjusted to pH 7.5 and passed through a column of 220 liters of Diaion HP-20. **1** was eluted with cold deionized water, together with No. 17927 A_1 and A_2 (identified as epithienamycins A and B, respectively) which were simultaneously produced by this strain. **1** in the concentrated aqueous eluate (50 liters) was adsorbed on a column of 23 liters of DEAE-Sephadex A-25 equilibrated with 0.01 M phosphate buffer pH 7.5 and developed and eluted with the same buffer. The fraction containing **1** and No. 17927 A_1 was purified by repeated column chromatography on Diaion HP-20 and Diaion HP-20AG to yield 17 g of lyophilized brownish powder. The powder thus obtained (300 mg) was dissolved in 2 ml of dimethylformamide and reacted with *p*-nitrobenzylbromide at room temperature for 1 hour under continuous stirring. From the reaction mixture, *p*-nitrobenzyl esters of **1** (**2**) and No. 17927 A_1 were separated each other by column chromatography on silica gel developed with a solvent mixture of benzene and ethyl acetate (1 : 1) to yield 180 mg of colorless crystals of **2**. **2** (160 mg) dissolved in a mixture of 4 ml of ethyl alcohol, 4 ml of dioxane and 2 ml of 0.1 M

sodium phosphate buffer pH 7.5 was reduced by catalytic hydrogenation with 160 mg of Pd-C for 2 hours. After filtration, the reaction mixture was passed through Diaion HP-20AG column. The eluate with deionized water was concentrated to a small volume under reduced pressure and lyophilized to obtain colorless powder of **1** (68 mg).

The physico-chemical properties of **1** and **2** are summarized in Table 1. **1** is a water soluble, colorless powder and has no characteristic ultraviolet absorption maximum. The molecular weight of **1** was calculated to be 338 from the results of FD-MS of **2**, 452 $(M+H)^+$.

The molecular formula for **1**, arrived at by elemental analysis and this molecular weight, is $C_{13}H_{19}N_2O_5SNa$. ^1H NMR spectrum of **1** is shown in Fig. 1. The absorption at 1760 cm^{-1} in the IR spectrum of **2** strongly indicated the presence of a β-lactam ring (Fig. 2).

^1H NMR of **2** as shown in Fig. 3 indicating a singlet methyl (1.97 ppm), a doublet methyl (1.35 ppm, $J=6.5$ Hz) and other signals, together with other physico-chemical properties suggested that the structure was related to that of the epithienamycins.

A multiplet (3.42 ppm) assigned to C-6 methine was coupled to C-5 multiplet methine (4.10 ppm, $J=5.5$ Hz) and C-8 multiplet methine (4.10~4.12 ppm, $J=4.5$ Hz). The relative configuration of the C-5 and C-6 protons on β-lactam ring was, therefore, established as *cis* from their coupling constant. A doublet methine (4.77 ppm) assigned to the C-2 proton collapsed to a singlet upon irradiation of the C-3 multiplet methine at 3.75 ppm with coupling constant $J=7.5$ Hz. The relative configuration at C-2 and C-3 protons was assumed to be β-*cis* from the coupling constant $J_{2,3}=7.5$ Hz and the chemical shift of the C-2 proton (4.77 ppm).

These values were identical with the data on synthesized carbapenams described by BATESON *et al.*[4]. Two methylenes attributed to a cysteaminyl side chain at 2.70 and 3.38 ppm, and C-4 methylenes at 2.0~2.6 ppm were also observed. These findings described above have unequivocally elucidated the structure of No. 17927 D as **1**.

Further confirmation of the relative stereochemistry of **1** was achieved by X-ray analysis of **2** crystallized from a mixture of acetonitrile and benzene. The single crystals of **2** are monoclinic, space group $P2_1$, with $a=18.826(4)$, $b=8.668(2)$, $c=6.715(2)$Å, $\beta=92.64(8)°$, $Z=2$. Intensity data were measured on a Rigaku four-circle diffractometer (graphite-monochromated CuKα radiation).

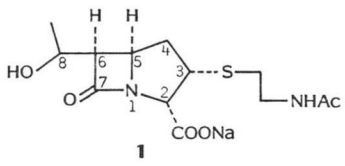


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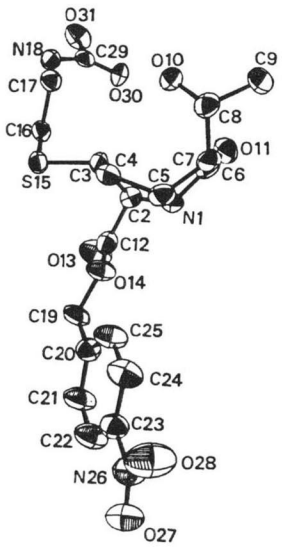
Fig. 4. Stereoscopic view of the molecule **2** with the labeling of the non-hydrogen atoms.



The structure was solved by direct methods using the MULTAN program[5] and refined by block diagonal least-squares methods.

The positions of all hydrogen atoms were obtained from a difference Fourier synthesis. The final discrepancy index R is 0.065 for 1723 observed reflections ($Fo \geq 3\sigma Fo$). The final atomic parameters have been deposited with the Crystallographic Data Center. Fig. 4 shows a stereoscopic drawing of molecule **2**. From this figure the relative configurations of the chiral centers can be seen as 2*S*, 3*S*, 5*R*, 6*R* and 8*R* or those of enantiomer. The torsion angles H(2)–C(2)–C(3)–H(3) and H(5)–C(5)–C(6)–H(6) are $-22.4°$ and 26.5°, corresponding to a *cis* conformation about both the C(2)–C(3) and C(5)–C(6) bonds. The sum of the three bond angles about the nitrogen atom of the β-lactam ring is 322.6° and the deviation of the nitrogen atom from the plane defined by the three adjacent atoms is 0.500

Å. These values agree with those for thienamycin[6]. The hydrogen of the hydroxyl group is separated by 2.00 Å from the carboxyl oxygen atom of the *N*-acetyl group of the neighboring molecule (x, y, z+1), suggesting that there is hydrogen bonding in the crystal between those two oxygen atoms (O ... O=2.786 Å). Furthermore, the molecules are linked by an intermolecular N(H) ... O hydrogen bond (3.017 Å) between the N-acetyl groups of symmetry-related molecules (−x, 1/2+y, −z).

No antimicrobial activity of **1** was detected against several strains of Gram-positive and Gram-negative bacteria tested by paper disc-agar diffusion assay even at a concentration of 1,000 μg/ml and there was no inhibitory activity of β-lactamase.

The possibility of participation of **1** in the biosynthetic pathway of carbapenem antibiotics was tested in a preliminary fashion as follows: After incubation of **1** with the resting cells of *S. fulvoviridis* SANK 61278 in 0.1 M phosphate buffer, pH 7.5, at 24°C for 4 hours on a rotary shaker, antimicrobial activity of the reaction mixture increased in comparison with that of the incubation mixture of the resting cells alone but the properties of the active principle(s) remained to be determined.

Further efforts are also under way to determine the absolute configuration of **1**.

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