Communications to the editor

A NOVEL β-LACTAMASE INHIBITOR, SF-2103 A PRODUCED BY A *STREPTOMYCES**

Sir:

A novel β-lactamase inhibitor, SF-2103 A, was discovered in culture filtrates of a new streptomycete strain SF-2103 for which was proposed the name *Streptomyces sulfonofaciens* sp. nov. In this communication, we report the isolation and structural elucidation of SF-2103 A.

The inhibitor was produced by submerged cultivation of strain SF-2103 in a 2,000-liter fermentor containing 1,000 liters of a medium consisting of 2% maltose syrup, 1.2% soybean meal, 1.2% wheat germ, 0.05% Na_2SO_4, 0.001% $CoCl_2 \cdot 6H_2O$ and 0.1% $CaCO_3$. Fermentation was carried out under agitation at 28°C for 86 hours, and the maximum titer was 12 μg of SF-2103 A per ml, based on inhibitory activity toward the β-lactamase of *Proteus vulgaris* GN-75[1].

The inhibitor in the culture filtrate (900 liters) was extracted with dichloromethane (300 liters) containing 0.2% benzyldimethyltetradecylammonium chloride at pH 6.1 and retransferred into 1% NaI aqueous solution (28 liters). The aqueous extract was passed through a column of active charcoal (1 liter, Wako Pure Chemical Industries), and the effluent was charged on a column of DEAE-Sephadex A-25 (3 liters). After washing with 0.02 M phosphate buffer containing 0.2 M NaCl (30 liters, pH 7.2), SF-2103 A was eluted with the same buffer containing 0.3 M NaCl. The active eluate was desalted by adsorption on a column of active charcoal (4 liters) and eluted with 50% aqueous acetone. The eluate was subjected to column chromatography with DEAE-Sephadex A-25 (500 ml) developed with 0.02 M phosphate buffer containing 0.2 M NaCl. The active eluate was desalted in the same manner as above, and applied to a column of Sephadex G-10 (800 ml) developed with water. Evaporation and lyophilization of the active effluent yielded the sodium salt of pure SF-2103 A (510 mg).

SF-2103 A sodium salt is a colorless powder which decomposes gradually over 168°C, and is soluble in water, dimethyl sulfoxide and *N,N*-dimethylformamide but insoluble in lower alcohols, acetone, ethyl acetate and benzene. It shows positive color reactions with GREIG-LEABACK's and EHRLICH's reagents, and negative with ninhydrin. SF-2103 A showed UV maxima (0.02 M phosphate buffer at pH 7.2) as follows: λ_{max} 230 nm (ε 5416), 266 nm (ε 5783) (extinguishable by addition of NH_2OH); IR (KBr) 1755 (β-lactam), 1610 (amide), 1220 cm^{-1} (S=O); $[\alpha]_D^{20}$ −16.3° (*c* 1, water); CD $[\theta]_{203}$ 0, $[\theta]_{232}$ −132,800, $[\theta]_{248}$ 0, $[\theta]_{264}$ +51,400, $[\theta]_{310}$ 0 (*c* 0.01, water).

Anal. Calcd. for $C_9H_8NO_{10}S_2Na_3 \cdot 2H_2O$:
C 23.53, H 2.61, N 3.05, S 13.94, Na 15.03.
Found: C 23.62, H 2.44, N 3.00, S 13.81, Na 14.97.

Table 1. ^1H NMR chemical shifts of SF-2103 A (**1**) and non β-lactam compounds **2a** and **2b**.

Proton	δ ppm (*J* Hz)		
	1	**2a**	**2b**
9-CH_3	1.60 (d, 3H, $J_{8,9}$=6.4)	1.52 (d, 3H, *J*=6)	1.53 (d, 3H, *J*=6)
8-CH	5.01 (m, 1H, $J_{8,6}$=8.8)	4.76 (m, 1H)	4.93 (m, 1H, *J*=6)
6-CH	3.99 (dd, 1H, $J_{6,5}$=5.9)	2.92 (t, 1H, *J*=8)	2.98 (dd, 1H, *J*=6)
5-CH	4.54 (m, 1H, $J_{5,4}$=10.3, $J_{5,4'}$=9.0)	4.61 (m, 1H)	4.33 (m, 1H)
4-CH_2	3.06 (dd, 1H, $J_{4,4'}$=16.9) 3.48 (dd, 1H)	2.54 (t, 2H, *J*=7)	2.05 (dd, 1H, *J*=7) 2.66 (dd, 1H, *J*=8)
3-CH		4.69 (m, 1H)	~4.6 (1H)

200 MHz, D_2O, DSS as external reference.

* This work was presented in the 225th Scientific Meeting of Japan Antibiotics Research Association, Tokyo, Sept. 25, 1981

Table 2. ^{13}C NMR chemical shifts of SF-2103 A (**1**) and non β-lactam compound (**2**).

Assignment	δ ppm				
	1		**2**		
9 $-CH_3$	19.5	q	18.9,	17.7	q
4 $-CH_2-$	33.0	t	30.7		t
6 >CH–	55.2	d	55.9,	60.2	d
5 >CH–	58.9	d	72.2,	71.5	d
8 >CH–	73.8	d	77.6,	77.4	d
3 >C=(>CH–)	129.2*	s	69.9,	69.7	d
2 >C=	139.4*	s	171.6*		s
7 >C=O	168.9**	s	170.9*,	170.5*	s
10 >C=O	178.2**	s	178.7*,	178.8*	s

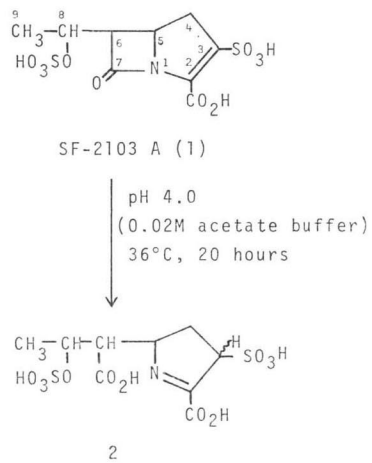
50 MHz, D_2O, DSS as external reference.
*,**: Assignments may be interconverted within each column.

The UV and IR absorption of SF-2103 A suggested the presence of a 1-carba-2-penem-3-carboxylate structure[2]. ^1H NMR and ^{13}C NMR spectra of SF-2103 A (Tables 1 and 2) showed close resemblance to those of MM 4550[3] and MC 696-SY2-A[*] except for their C-3 side chain. On high-voltage paper electrophoresis at 2,800V for 15 minutes in pyridine-acetate buffer (pH 6.4), SF-2103 A migrated toward the anode at the rate of Rm (relative mobility to MC696-SY2-A*) 1.6. Thus, the polyacidic nature of SF-2103 A coupled with elemental analysis suggested the presence of a sulfur-containing function, such as sulfate or sulfonate instead of the neutral C-3 side chain of MC696-SY2-A. Hydrolysis of SF-2103 A upon refluxing in 0.5 N HCl for 20 hours gave one mole of sulfate as analysed by gravimetric analysis.

From the physico-chemical properties and spectral data mentioned above, the structure of SF-2103 A was proposed to be **1** as shown in Scheme 1. The orientation of H-5 and H-6 in SF-2103 A was deduced to be *cis* from the vicinal coupling constants, $J_{5,6}=5.9$ Hz.

The presence of the sulfonate rather than the sulfate group at C-3 was confirmed by the following degradation. Mild hydrolysis in 0.02 M acetate buffer of pH 4.0 at 36°C for 20 hours yielded a non β-lactam compound (**2**), colorless powder, mp: gradually decomposed over 182°C, $[\alpha]_D^{20}$ −54° (*c* 1, water), no characteristic UV absorption, no IR band around 1755 cm^{-1}.

* MC696-SY2-A was kindly supplied by Dr. K. MAEDA, Institute of Microbial Chemistry, Japan.



Anal. Calcd. for $C_9H_9NO_{11}S_2Na_4 \cdot 2H_2O$:
C 21.64, H 2.61, N 2.81, S 12.82.
Found: C 21.93, H 2.94, N 2.82, S 12.87.

Most signals of the ^1H NMR and ^{13}C NMR spectra of **2** (Tables 1 and 2) were observed as pairs, suggesting that **2** was a mixture of diastereomers **2a** and **2b** (Table 1)[4]. Based on the ^1H NMR spectrum of **2**, the ratio of **2a** : **2b** was approximately 2 : 1. A pair of signals of C-3 (δ 69.7, 69.9 ppm) was observed at a higher field ($\Delta\delta$ 7 ~ 8 ppm) than that of C-8 (δ 77.4, 77.6 ppm) bearing a sulfate ester group. This result suggests that C-3 links to a sulfonate group.

SF-2103 A possessing a sulfonate group was obviously distinguishable from known carba-

Table 3. Antibacterial spectrum of SF-2103 A.

Organism	MIC (μg/ml)
Staphylococcus aureus 209P JC-1	25
Staphylococcus aureus Smith	25
Streptococcus faecalis ATCC 8043	50
Bacillus subtilis ATCC 6633	50
Escherichia coli NIHJ JC-2	12.5
Escherichia coli No. 29	6.25
Klebsiella pneumoniae PCI 602	50
Salmonella typhi O-901-W	12.5
Salmonella enteritidis No. 11	50
Shigella dysenteriae Shigae	3.13
Proteus vulgaris OX-19	25
Proteus morganii Kōno	50
Serratia marcescens No. 2	100
Pseudomonas aeruginosa E-2	>100
Pseudomonas cepacia M-0527	100

Table 4. β-Lactamase-inhibitory activities of SF-2103 A.

Source of β-lactamase[1)]	Substrate	I_{50} (μg/ml)*
Staphylococcus aureus MS258	PCG**	>50 (4.0)
Escherichia coli W3630 RGN823	PCG	1.6 (0.05)
Proteus vulgaris GN76/C-1	CET**	0.0036 (0.00015)
Citrobacter freundii GN346	CET	0.0086 (0.00068)

* Concentrations required to cause 50% inhibition were determined by the modified microiodometric method.[8)] The values in parentheses were obtained when inhibitor was preincubated with β-lactamase for 10 minutes at 30°C before substrate addition.

** PCG and CET are penicillin G and cephalothin, respectively.

penem antibiotics such as the olivanic acids[5)], epithienamycins[6)] and carpetimycins[7)].

SF-2103 A exhibits a broad antibacterial spectrum with moderate activity against Gram-positive and Gram-negative bacteria (Table 3). However, it shows powerful inhibition against both types of enzymes of penicillinases and cephalosporinases as shown in Table 4.

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