A NOVEL AMINOGLYCOSIDE ANTIBIOTIC, SUBSTANCE SF-2052

Sir:

Fortimicins[1~3] and sporaricins[4,5] represent a new class of aminoglycoside consisting of a pseudodisaccharide and an amino acid. We wish to report here a newcomer to this class, substance SF-2052 (**I**), which is the first aminoglycoside having a formimino moiety.

The antibiotic-producing organism, strain SF-2052, was isolated from soil collected at Matsuzaki-cho, Izu Peninsula, Japan. The isolate is a rare actinomycete, and was designated as *Dactylosporangium matsuzakiense* sp. nov. from a taxonomic study. It forms finger-shaped sporangia, each containing a single row of three spores. Spores are motile, and no aerial mycelium is formed.

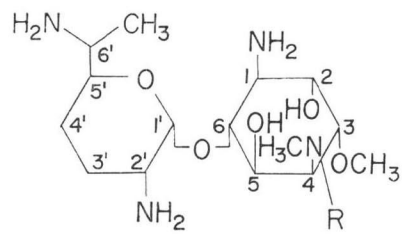
Substance SF-2052 was produced by submerged cultivation of *Dactylosporangium matsuzakiense* SF-2052 in a 300-liter jar fermentor. The medium consisted of 3.0% starch, 0.75% peptone, 0.9% wheat germ, 0.45% yeast extract, 0.0005% cobaltous chloride and 0.15% calcium carbonate (pH 7.0). Fermentation was carried out under agitation at 28°C for 4 days. The maximum titer, 80 μg/ml, was estimated by the paper disc method using *Bacillus subtilis* ATCC 6633 as a test organism.

The fermented broth was filtered at pH 2.0, and the filtrate (130 liters) was adjusted to pH 5 and passed through a column of Amberlite IRC-50 (Na^+, 5 liters). The antibiotic adsorbed was eluted with 0.4 N HCl (20 liters). The eluate was adjusted to pH 6, and passed through a column of active carbon (1.2 liters). The 50% aqueous acetone eluate was concentrated, and applied to a column of Amberlite IRC-50 (Na^+, 1 liter). Elution with 0.4 N HCl, followed by neutralization and desalting over active carbon gave a concentrated solution of the antibiotic. This was subjected to column chromatography over CM-Sephadex C-25 (Na^+, 600 ml), previously equilibrated with 0.1 M NaCl. After subsequent washing with 0.2 M, 0.3 M and 0.4 M NaCl, the antibiotic was eluted with 0.5 M NaCl. The eluate was desalted with active carbon, and concentrated to give a solution of SF-2052 hydrochloride. This was passed through a column of Amberlite IRA-400 (SO_4^{--}, 500 ml). Lyophilization of the aqueous effluent afforded SF-2052 sulfate (3.45 g). Alkaline instability of SF-2052 rendered the preparation of the free base difficult.

The antibiotic thus obtained is a white powder, melting at 176~178°C with decomposition. It is soluble in water, less soluble in methanol, but almost insoluble in acetone and ethyl acetate. It shows positive ninhydrin, GREIG-LEABACK and LEMIEUX reactions, but a negative SAKAGUCHI reaction. The antibiotic showed $[\alpha]_D^{25}$ +81° (*c* 1.0, water); UV (H_2O), end absorption; IR (KBr), 3400, 1720, 1643, 1520, 1110 and 1060 cm^{-1}; PMR (D_2O), 1.33 (3H d, C–CH_3), *ca.* 2.0 (4H m, CH_2), 3.16 (3H s, N–CH_3), 3.49 (3H s, OCH_3), 5.34 (1H d, anomeric H), 7.98 (1H s, CH=N), and CMR data listed in Table 1. The molecular formula, $C_{18}H_{36}N_6O_6 \cdot 2H_2SO_4 \cdot H_2O$, was determined on the basis of the number of carbon atoms in CMR and elemental analysis. Anal. Found: C 33.42, H 6.64, N 12.98. Calcd.: C 33.43, H 6.50, N 13.00.

SF-2052 showed Rf 0.46 on cellulose TLC developed with *n*-propanol - pyridine - acetic acid - water (15: 10: 3: 12, v/v), and Rf 0.09 on PPC developed ascendingly with *n*-butanol - acetic acid - water (2: 1: 1, v/v). Fortimicin A (**II**)[1~3], which is most closely related to SF-2052 (**I**), showed Rf values of 0.42 and 0.03 respectively. Definite differentiation of SF-2052 from fortimicin A could be made by the use of HPLC. Reverse-phase, ion-pair chromatography coupled with the fluorescent detection[6] gave a relative retention time of 1.3 against fortimicin A (1.0).

Refluxing SF-2052 with 3 N HCl for 3 hours yielded glycine and formiminoglycine, while alkaline hydrolysis by heating with 1 N NaOH at 95°C for 1 hour afforded fortimicin B (**III**). These



I R=-CO-CH_2-NH-CH=NH

II R=-CO-CH_2-NH_2

III R=-H

Table 1. 25 MHz CMR parameters of SF-2052 in D_2O solution.

	SF-2052 sulfate	Fortimicin A sulfate[3]		SF-2052 sulfate	Fortimicin A sulfate[3]
C-1′	95.4 ppm	95.4 ppm	C-3	72.5	72.4
C-2′	51.6	51.7	C-4	51.7	51.8
C-3′	21.6	21.6	C-5	71.5	71.6
C-4′	26.3	26.3	C-6	74.5	74.5
C-5′	70.8	70.9	O-CH_3	56.8	56.8
C-6′	49.4	49.4	N-CH_3	31.9	32.0
6′-CH_3	15.0	15.0	GlyCH_2	44.3	41.3
C-1	54.1	54.1	GlyC=O	169.0	168.8
C-2	66.2	66.3	CH=N	155.7	—

Table 2. *In vitro* antibacterial activity of SF-2052.

Test organism	MIC(μg/ml)*	Test organism	MIC(μg/ml)*
Staphylococcus aureus Rosenbach FDA 209-P JC-1	0.39	*Salmonella* sp. D-0006	3.13
Staphylococcus aureus Smith S-424	1.56	*Salmonella* sp. D-0029	6.25
Staphylococcus aureus No. 26	1.56	*Shigella dysenteriae* Shigae	3.13
Staphylococcus aureus ApO-1	1.56	*Klebsiella pneumoniae*	6.25
Staphylococcus aureus N-0089	1.56	*Klebsiella pneumoniae* 22 #3038	3.13
Staphylococcus epidermidis ATCC 14990	0.39	*Proteus morganii* Kōno	3.13
Staphylococcus epidermidis 109	0.78	*Proteus vulgaris* OX_{19}	6.25
Streptococcus faecalis ATCC 8043	50	*Proteus rettgeri* J-0026	12.5
Bacillus subtilis ATCC 6633	0.78	*Proteus mirabilis* J-0010	3.13
Bacillus anthracis No. 119	0.39	*Serratia marcescens* No. 1	1.56
Escherichia coli (M) Cast. & Chalm. NIHJ JC-2	3.13	*Serratia marcescens* No. 2	1.56
Escherichia coli No. 29	6.25	*Serratia marcescens* I-0043	3.13
Escherichia coli W 677 (A-20684)	0.78	*Serratia marcescens* MB-3848	1.56
Escherichia coli JR 66/W 677 (A-20683)	3.13	*Pseudomonas aeruginosa* NC-5	50
Escherichia coli A-0001	6.25	*Pseudomonas aeruginosa* E-2	50
Salmonella typhi 0-901-W	0.78	*Pseudomonas aeruginosa* IAM-1007	25
Salmonella typhimurium LT-2	3.13	*Pseudomonas aeruginosa* M-0085	0.78
Salmonella enteritidis No. 11 (Tōkai)	3.13	*Vibrio parahaemolyticus* K-5	6.25
		Mycobacterium smegmatis ATCC 607	1.56

* The MICs were determined on MUELLER-HINTON agar with inoculum size of 10^8 cell/ml, except for *Mycobacterium*. For the latter, glycerine bouillon agar was used.

results, together with the close similarity of CMR with fortimicin A (**II**) as shown in Table 1, supported the structure **I** for SF-2052.

The *in vitro* and *in vivo* antibacterial activities of SF-2052 are summarized in Tables 2 and 3. Substance SF-2052 showed activity against Gram-positive and Gram-negative bacteria, including aminoglycoside-resistant organisms. The acute LD_{50} value of SF-2052 sulfate in mice was 350 mg/kg by the intravenous route. The above results suggest that the novel aminoglycoside SF-2052 would be a useful antibiotic. Details of a taxonomic study of strain SF-2052, and of the structure determination and evaluation of SF-2052 will be described in separate papers.

Table 3. *In vivo* antibacterial activity of SF-2052.

Organism	Challenge dose (CFU/mouse)	ED_{50} (mg/mouse)*
Escherichia coli No. 29	7.9×10^4 ($31.9LD_{50}$)	0.094
Klebsiella pneumoniae 22 #3038	8.0×10^7 (1MLD)	0.066
Serratia marcescens MB-3848	3.6×10^6 ($3.5LD_{50}$)	0.11
Pseudomonas aeruginosa IAM-1007	3.3×10^7 ($24.5LD_{50}$)	1.1

* ED_{50} values were obtained by subcutaneous administration of SF-2052 immediately after intraperitoneal infection in mice.

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