Communications to the Editor

A NEW ANTIVIRAL ANTIBIOTIC SF-2140 PRODUCED BY *ACTINOMADURA*

Sir:

A new antiviral antibiotic SF-2140 has been found in the culture broth of *Actinomadura* sp. SF-2140 which was isolated from a soil sample collected in Hyogo Prefecture, Japan. In this communication, the isolation, properties and structural elucidation of antibiotic SF-2140 are reported.

Actinomadura sp. SF-2140 was cultured at 28°C for 96 hours in a medium (70 liters) containing maltose syrup 2%, soybean oil 0.15%, soybean meal 0.5%, distillers soluble 0.125%, Pharmamedia (Traders Protein, Traders Oil Mill Co.) 0.25%, peptone 0.4%, K_2HPO_4 0.1% and $FeSO_4 \cdot 7H_2O$ 0.0005% (pH 7.0) in two 50-liter jar fermentors. The antibiotic was assayed by the paper disc method against *Escherichia coli* NIHJ JC-2 on an agar plate.

The antibiotic in the culture filtrate (pH 7.0, 50 liters, 22 μg/ml) was adsorbed on a column of Diaion HP-20 (4 liters) and eluted with 50% aqueous acetone. The active eluate (10 liters) was concentrated to 4 liters and extracted with EtOAc (4 liters). The extract was concentrated to dryness and chromatographed on a column of silica gel (300 g) developed with $CHCl_3$ - MeOH (50: 1) to yield the crude powder (1.2 g). Further purification of the powder was carried out by column chromatography on Sephadex LH-20 (500 ml) using MeOH as a developing solvent. The active eluate was concentrated to dryness and the antibiotic in the residue was crystallized from $CHCl_3$ (440 mg). Recrystallization from a mixture of $CHCl_3$ and MeOH gave colorless crystals of antibiotic SF-2140 (**1**), mp 174~176°C. *Anal* Calcd for $C_{18}H_{20}N_2O_6$: C 60.00, H 5.56, N 7.78. Found: C 59.54, H 5.63, N 7.59. MS: *m/z* 360 (M^+); $[\alpha]_D^{20}$ +59° (*c* 1, MeOH); λ_{max}^{MeOH} nm (ε) 222 (34,560), 258 (sh 7,630), 265 (8,210), 284 (6,260) and 294 (6,910); ν_{max} (KBr) cm^{-1}: 3400 (OH), 2240 (CN) and 1735 (ester). ^1H NMR of the sugar moiety (400 MHz, acetone-d_6, *J* Hz): δ 6.33 (1H, d, $J_{1',2'}$=9.3, 1'-H), 4.16 (1H, ddd, $J_{2',3'}$=2.7, 2'-H), 4.36 (1H, d, $J_{2',OH}$=7.1, 2'-OH), 4.29 (1H, ddd, $J_{3',4'ax}$=2.2, $J_{3',4'eq}$=3.7, 3'-H), 4.21 (1H, dd, $J_{3',OH}$=2.7, $J_{4'ax,OH}$=1.2, 3'-OH), 2.28 (1H, dddd, $J_{4'ax,5'}$=6.8, $J_{4'ax,4'eq}$=14.4, 4'-Hax), 2.51 (1H, dddd, $J_{4'eq,5'}$=1.2, 4'-Heq), 4.45 (1H, br d, 5'-H) and 3.75 (3H, s, $COOCH_3$). The antibiotic is readly soluble in MeOH, EtOH, acetone, EtOAc and $CHCl_3$; insoluble in H_2O and *n*-hexane. The Rf values of TLC on Silica Gel G (E. Merck, F_{254}) developed with $CHCl_3$ - MeOH (5: 1) and EtOAc - C_6H_6 (2: 1) were 0.53 and 0.31, respectively. It gave positive color reactions with LEMIEUX's and sulfuric acid reagents and negative with ninhydrin.

Acetylation of **1** with acetic anhydride in pyridine gave the diacetate (**2**), colorless crystals, mp 114°C, MS: *m/z* 444 (M^+). *Anal* Calcd for $C_{22}H_{24}N_2O_8$: C 59.46, H 5.41, N 6.31. Found: C 59.35, H 5.39, N 6.32. λ_{max}^{MeOH} nm (ε) 222 (68,200), 260 (sh 14,030), 266 (15,150), 285 (11,600) and 294 (12,660); ν_{max} (KBr) cm^{-1} 2240 (CN) and 1735 (ester). ^1H NMR of the sugar moiety (200 MHz, $CDCl_3$, *J* Hz) δ 6.44 (1H, d, $J_{1',2'}$=9.6, 1'-H), 5.38 (1H, dd, $J_{2',3'}$=3.2, 2'-H), 5.57 (1H, ddd, $J_{3',4'ax}$=2.8, $J_{3',4'eq}$=3.8, 3'-H), 2.38 (1H, ddd, $J_{4'ax,5'}$=6.8, $J_{4'ax,4'eq}$=15.0, 4'-Hax), 2.62 (1H, ddd, $J_{4'eq,5'}$=2.2, 4'-Heq), 4.57 (1H, br d, 5'-H), 3.86 (3H, s, $COOCH_3$), 1.83, 2.12 (6H, s, $COCH_3$).

From the spectral data of **1** and **2**, it was suggested that **1** is an *N*-glycoside consisting of a deoxy sugar moiety and a chromophore of an indole derivative. Acid hydrolysis (1 N HCl, refluxed for 1 hour) of **1** gave a chromophore (**3**), colorless crystals, mp 136°C, $C_{11}H_{10}N_2O$, MS: *m/z* 186 (M^+); λ_{max}^{MeOH} nm (ε) 221 (88,380), 267 (15,770), 281 (10,840) and 291 (9,900); ν_{max} (KBr) cm^{-1} 3340 (NH) and 2250 (CN); ^1H NMR (400 MHz, $CDCl_3$, *J* Hz) δ 7.04 (1H, s, 2-H), 3.90 (3H, s, 4-OCH_3), 6.49 (1H, d, $J_{5,6}$=8.0, 5-H), 7.10 (1H, t, $J_{6,7}$=8.0, 6-H), 6.95 (1H, d, 7-H), 4.03 (2H, d, $J_{2,8-CH_2}$=1.0, 8-CH_2-) and 8.17 (1H, br s, NH). From these spectral data, the structure of **3** was suggested to be 4-methoxyindoleacetonitrile[1]. The chromophore (**3**) was identical with the synthetic one derived from 2-hydroxy-6-nitrotoluene by the method of GOVINDACHARI *et al.*[2], in all respects.

Fig. 1. Structures of SF-2140 and related compounds.

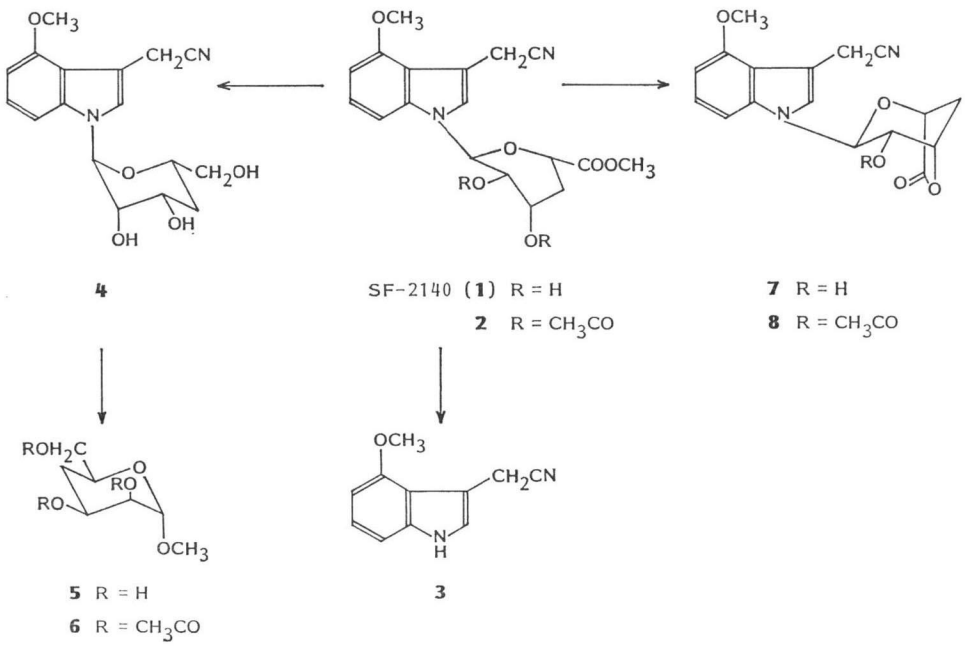

Table 1. *In vitro* antiviral activity and toxicity of SF-2140.

Virus strains	Concentration (μg/ml)		
	50% Toxicity against CAM	50% Virus proliferation inhibition	50% Virus inactivation
A_0/PR-8/34	>1,000	6.30 (>158.7)*	3.2 (>312.5)**
A_1/FM-1	>1,000	50.5 (>20.0)*	4.6 (>217.4)**
A_2/Adachi	>1,000	100 (>10.0)*	58.8 (>17.0)**
B/Lee	>1,000	200	46.4 (>21.6)**
Horse/Miami	>1,000	17.7 (>56.5)*	4.6 (>217.4)**

* Inhibition index $= \frac{50\%\text{ toxic concentration}}{50\%\text{ inhibitory concentration}}$

** Inactivation index $= \frac{50\%\text{ toxic concentration}}{50\%\text{ inactivation concentration}}$

Chorio-allantoic membrane (CAM) culture method was carried out as follows. One piece of chorio-allantoic membrane per tube was shaken for 18 hours at 36°C in a mixture of 0.9 ml of HANKS' BSS and 0.1 ml of SF-2140 solution. The membrane was washed 3 times with 5 ml of PBS and used to cultivate 10 MID_{100}/0.1 ml of the virus. The hemaglutination (HA) titer in the infected culture was determined at 48 hours after inoculation. The minimal concentration of SF-2140 which did not permit HA production was taken as the minimal toxic concentration.

Although the deoxy sugar moiety could not isolated from the acid hydrolysate of **1**, the structure of the sugar moiety of **1** was confirmed by isolation of a methyl glycoside (**5**) from the methanolysate of the reduction product (**4**). Compound **4** was obtained as a colorless powder by reduction of **1** with $LiAlH_4$ in tetrahydrofuran at room temperature for 2 hours, SI-MS: *m/z* 333 (MH^+); $[\alpha]_D^{20}$ +82° (*c* 1, MeOH). The methyl glycoside (**5**) was yielded as a colorless solid by reflux of **4** with 3% HCl in MeOH for 20 hours followed by purification of the main product on preparative silica gel TLC, FD-MS: *m/z* 179 (MH^+); $[\alpha]_D^{20}$ +86° (*c* 1, H_2O); $\Delta[M]_{436(CuAm)}$ +1,036° [3]. Acetylation of **5** with acetic anhydride in pyridine gave the triacetate (**6**), FD-MS: *m/z* 305 (MH^+); ^1H NMR (400 MHz, $CDCl_3$, *J* Hz) δ 4.67 (1H, d, $J_{1,2}$=1.7, 1-H), 5.01 (1H, t, $J_{2,3}$=3.2, 2-H), 5.19 (1H, ddd, $J_{3,4ax}$=11.5, $J_{3,4eq}$=5.6, 3-H), 1.72 (1H, m, $J_{4ax,5}$=10.3, $J_{4ax,4eq}$=12, 4-Hax), 1.77 (1H, m, $J_{4eq,5}$=3.4, 4-Heq), 4.00 (1H, m, $J_{5,6}$=6.6, 5-H), 4.08 (2H, ddd, $J_{6a,6b}$=11.6, 6-CH_2–), 1.95, 1.99, 2.00 (9H, s, $COCH_3$) and 3.31 (3H, s, 1-OCH_3). From ^1H NMR spectral data of **6** and the value of the difference in molecular rotation of **5**, the structure of **5** was determined to be methyl 4-deoxy-α-D-*lyxo*-hexopyranoside.

The antibiotic (**1**) was easily converted into a

Fig. 2. Antiviral activity of SF-2140 administered orally in mice infected with influenza virus A_0/PR-8/34 strain.

Three-week-old mice of ICR strain weighing 9~11 g were intranasally infected with LD_{80} of influenza virus A_0/PR-8/34 strain (inhalation: 1 kg/cm²/10 minutes). SF-2140 and amantadine were orally administered to mice (n=10) immediately after infection and thereafter once a day for five days.

● SF-2140 125 mg/kg, ○ SF-2140 62.5 mg/kg, ■ Amantadine·HCl 250 mg/kg, □ Without treatment.

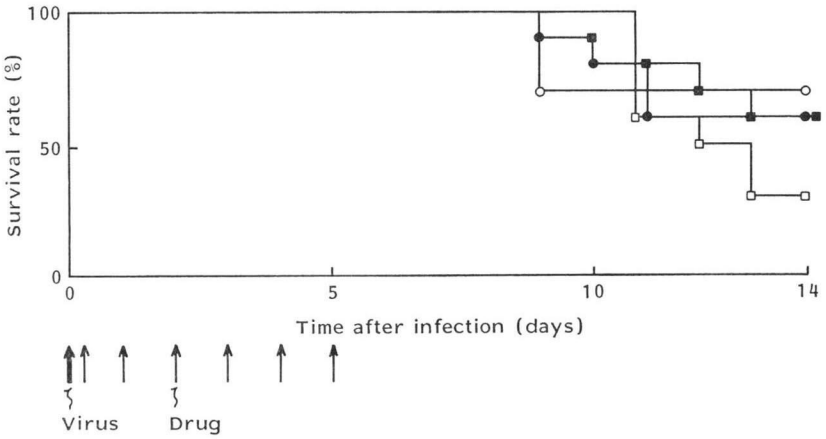

Table 2. Antibacterial spectrum of SF-2140.

Test organisms	MIC (μg/ml)	Test organisms	MIC (μg/ml)
Staphylococcus aureus 209P JC-1	25	*S. enteritidis* No. 11	12.5
S. aureus Smith (1)	100	*Micrococcus luteus*	50
S. aureus No. 26	100	*Shigella sonnei* EW33 Type I	>100
S. epidermidis ATCC 14990	100	*Klebsiella pneumoniae* PCI602	>100
S. epidermidis 109	100	*K. pneumoniae* 22#3038	>100
Streptococcus faecalis ATCC 8043	12.5	*Proteus vulgaris* OX-19	25
Bacillus anthracis No. 119	6.25	*P. rettgeri* J-0026	>100
Escherichia coli NIHJ JC-2	>100	*P. morganii* Kono	>100
E. coli No. 29	>100	*P. mirabilis* J-0013	>100
E. coli W3630 RGN 823	50	*Serratia marcescens* MB-3848	>100
E. coli JR66/W677	>100	*Pseudomonas aeruginosa* MB-3829	>100
Citrobacter freundii GN 346	>100	*P. cepacia* M-0527	100
Salmonella typhi O-901-W	100	*P. maltophilia* M-0627	>100

lactone (**7**) by saponification of **1** in a mild alkaline solution at room temperature overnight followed by treatment with dicyclohexylcarbodiimide in dichloromethane at room temperature for 3 hours, FD-MS: *m/z* 328 (M^+); ν_{max} (KBr) 1800 cm^{-1} (5-membered lactone). The lactone (**7**) gave the monoacetate (**8**) by acetylation with acetic anhydride in pyridine; MS: *m/z* 370 (M^+); ^1H NMR of the sugar moiety (400 MHz, $CDCl_3$, *J* Hz): δ 5.77 (1H, d, $J_{1',2'}$=8.4, 1′-H), 5.32 (1H, dd, $J_{2',3'}$=1.2, 2′-H), 5.13 (1H, dd, $J_{3',4'ax}$=1, $J_{3',4'eq}$=5.6, 3′-H), 2.47 (1H, d, $J_{4'ax,5'}$=1, $J_{4'ax,4'eq}$=13.4, 4′-Hax), 2.53 (1H, ddd, $J_{4'eq,5'}$=2.6, 4′-Heq), 4.51 (1H, s, 5′-H) and 1.94 (3H, s, $COCH_3$). ^1H NMR spectrum of **8** indicates that the sugar moiety has a $^1C_4$ conformation in a $CHCl_3$-*d* solution. However, ^1H NMR spectra of **1** and **2** show that the sugar moieties have twist-boat conformations in acetone-d_6 and $CHCl_3$-*d* solutions, respectively. From X-ray crystallographic analysis of **1**, the sugar moiety of the crystal has a $^1C_4$ conformation as like as those of **7** and **8**. Further detail will be reported elsewhere.

Based on the foregoing results, the structure of SF-2140 (**1**) was determined to be methyl (3-cyanomethyl-4-methoxyindol-1-yl 4-deoxy-α-D-*lyxo*-hexopyranosid)uronate. ^{1}H NMR spectra of **2** are very similar to those of the diacetate of neosidomycin[4] on chemical shifts and coupling constants. Although SF-2140 and neosidomycin have different chromophores, they show similar optical rotations (neosidomycin, +51°)[4]. These results suggests that the sugar moieties of SF-2140 and neosidomycin have the same stereostructure.

The antibiotic showed a proliferation-inhibiting activity and a marked inactivation activity against several influenza virus strains as shown in Table 1: 217 or higher inactivation index was seen against A_0/PR-8, A_1/FM-1 and Horse/Miami strains and 17 or higher against A_2/Adachi and B/Lee strains. The antibiotic was administered to mice orally immediately after infection with influenza virus A_0/PR-8 strain and thereafter once a day for five days. The survival rate of the mice treated with SF-2140 was superior to that of amantadine as shown in Fig. 2. SF-2140 showed a weak antibacterial activity against Gram-positive and -negative bacteria as shown in Table 2. No acute toxicity to mice was observed by intraperitoneal administration of SF-2140 at 2,000 mg/kg.

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