A NEW ANTIBIOTIC SF-2370 PRODUCED BY *ACTINOMADURA*

Sir:

A new indolocarbazole antibiotic SF-2370 has been found in the culture broth of *Actinomadura* sp. SF-2370 which was isolated from a soil sample collected at Shimizu, Shizuoka Prefecture, Japan. The antibiotic is weakly active against some bacteria and fungi. In this communication, the isolation, characterization, and structural elucidation by the spectral studies are reported.

Actinomadura sp. SF-2370 was cultured at 28°C for 5 days in a medium (600 ml) containing glucose 1.5%, wheat germ 1.0%, corn steep liquor 1.0%, Pharmamedia (Traders Protein, Buckeye Cellulose Corp.) 0.5%, $CaCO_3$ (adjusted to pH 7.0) 0.3% in a 1-liter jar fermentor. Vegetative inoculum, 20 ml (3.3%) grown on a rotary shaker for 7 days at 28°C in a medium containing glucose 2.0%, wheat germ 1.0%, peptone 0.5%, yeast extract 0.5% and $CaCO_3$ (adjusted to pH 7.0) 0.1% was used. The antibiotic was assayed by the paper disc method against *Micrococcus luteus*.

The culture broth was filtered at pH 7.8 and the antibiotic in the mycelium was extracted with 70% aqueous acetone (500 ml). The extract was concentrated to remove acetone and the antibiotic in the concentrate was extracted twice with EtOAc (250 ml). The extract was dried on Na_2SO_4 and concentrated to give an oily residue. *n*-Hexane was added to the residue to give the precipitates (286 mg). The precipitates were chromatographed on a column of silica gel (60 ml) developed with chloroform - EtOAc (10: 1) to yield a pale yellow powder (216 mg). The powder was further purified by preparative TLC using EtOAc (Rf 0.31) as a developing solvent. Recrystallization from MeOH gave pale yellow crystals of antibiotic SF-2370 (79 mg) (**1**): mp 261~262°C (dec), $[\alpha]_D^{25}$ +57° (*c* 0.1, MeOH). *Anal* Calcd for $C_{27}H_{21}N_3O_5$: C 69.38, H 4.50, N 8.99. Found: C 69.50, H 4.74, N 8.88. MS *m/z* obsd: 467.1473 (M^+), calcd for $C_{27}H_{21}N_3O_5$: 467.1479. UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 228 (725), 250 (717), 265 (sh), 280 (sh), 290 (1,708), 320 (sh), 335 (432), 350 (325) and 367 (353). No characteristic shifts on UV absorption maxima were observed in acidic and alkaline solutions. IR ν_{max} (KBr) cm^{-1} 3400 (OH, NH), 1730 (ester), 1670 (amide), 1455, 1255 (ether) and 750 (aromatic). ^1H and ^{13}C

Table 1. ^1H NMR spectra of SF-2370 (**1**) and its derivatives.

Protons	Chemical shifts (ppm), *J*, Hz			
	1	**2**	**3**	**4**
5″-Me	2.16 3H, s	1.79 s	1.80 s	1.87 s
2″-CH_2	2.93 1H, dd, $J_{2''a,2''b}$ = 14.3	2.16 dd	2.17 dd	1.47 dd
	3.46 1H, dd	3.96 dd	3.95 dd	2.93 dd
OMe	4.05 3H, s	4.01 s	4.01 s	
7-CH_2	4.39 1H, d $J_{7a,7b}$ = 16.5	5.08 q	5.38 q	3.65 br s
	4.55 1H, d			
OH	5.41 1H, s			
NH	5.62 1H, br s	6.72 s		
1″-H	6.75 1H, dd $J_{1'',2''a}$ = 7.7	7.02 dd	7.01 dd	6.77 m
	$J_{1'',2''b}$ = 5.0			
2′-H	7.03 1H, t $J_{2',3'}$ = 7.0	7.39 m	7.37 t	6.77 m
4′-H	7.15 1H, d	7.39 m	7.43 d	6.87 brs
3′-H	7.23 1H, t $J_{3',4'}$ = 8.1	7.53 m	7.53 m	7.15 m
2-H	7.36 1H, t $J_{2,3}$ = 7.3	7.53 m	7.53 m	7.15 m
3-H	7.48 1H, t $J_{3,4}$ = 8.1	7.53 m	7.53 m	7.37 t
1-H	7.81 1H, d $J_{1,2}$ = 7.7	7.94 t	7.93 d	7.50 d
4-H	7.98 1H, d	7.94 t	8.06 d	7.56 d
1′-H	8.70 1H, d $J_{1',2'}$ = 7.7	9.35 d	9.21 d	8.56 d
OAc		2.28 s	2.26 s	
NAc			2.82 s	

400 MHz NMR: **1**, **2** and **3** in $CDCl_3$; **4** (Na salt) in D_2O.

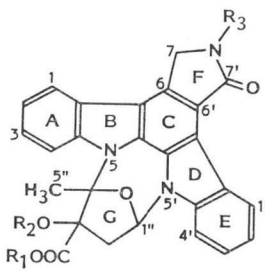
Table 2. ^{13}C NMR spectrum of SF-2370 (**1**).

Carbon	ppm	Carbon	ppm
5″	22.8 q	3′	120.8 d
2″	42.4 t	6	122.2 s
7	45.9 t	5c	123.9 s
OMe	53.4 q	5c′	124.9 s
1″	84.9 d	3	125.1 d
3″	85.7 s	2	125.5 d
4″	99.5 s	1′	125.9 d
4′	107.2 d	5a	129.0 s
5b	114.2 s	5a′	132.3 s
4	114.8 d	4a	136.9 s
5b′	116.5 s	4a′	140.3 s
6′	117.8 s	3″-CO	172.5 s
2′	119.3 d	7′	173.6 s
1	120.5 d		

100 MHz NMR in $CDCl_3$.

NMR data were shown in Tables 1 and 2. The antibiotic is soluble in chloroform, pyridine, DMF, DMSO and methylcellosolve; slightly soluble in MeOH and EtOAc; insoluble in water and *n*-hexane. The Rf values of TLC on Silica gel 60 F_{254} (E. Merck) developed with EtOAc, chloroform - MeOH (9: 1) and MeOH were 0.31, 0.57 and 0.89, respectively. It gave positive reactions with Greig-Leaback reagent[1] and negative with ninhydrin and $FeCl_3$ reagents on silica gel TLC.

The UV absorption spectrum suggested that SF-2370 (**1**) has an indolocarbazole moiety of staurosporine produced by *Streptomyces* sp.[2~4] Eight protons on the ring A and E were assigned by ^1H-^1H decoupling experiments and all carbons bearing protons of **1** were assigned by ^1H-^{13}C 2D NMR experiments as shown in Tables 1 and 2. Twelve quaternary carbons were also assigned by ^1H-^{13}C long range couplings which were confirmed by long range selective proton decoupling experiments, as shown in Table 2. It was suggested that hydroxyl and methyl groups in the sugar moiety (ring G) are located on C-3″ and C-4″, respectively by their chemical shifts (OH 5.41 s; C-3″ 85.7 s; 5″-Me 22.8 q, 2.16 s).



SF-2370 (**1**)	R_1=CH_3, R_2=R_3=H
Monoacetate (**2**)	R_1=CH_3, R_2=Ac, R_3=H
Diacetate (**3**)	R_1=CH_3, R_2=R_3=Ac
Acid (**4**)	R_1=R_2=R_3=H

The hydroxyl group on C-3″ was acetylated with acetic anhydride in pyridine at room temperature for 2 days to afford a mixture of the monoacetate (**2**): mp 212~234°C (dec), $C_{29}H_{23}N_3O_6$, MS *m/z* 509 (M^+) and diacetate (**3**): mp >270°C, $C_{31}H_{25}N_3O_7$, MS *m/z* 551 (M^+). The methoxycarbonyl group of **1** was hydrolyzed with an equimolar amount of NaOH in a mixture of pyridine - MeOH - water (10: 10: 1) at room temperature overnight to give an acid (**4**); Na salt: mp >270°C, $[\alpha]_D^{20}$ +136.4° (*c* 0.25, 50% MeOH), $C_{26}H_{18}N_3O_5Na$, SI-MS *m/z* 476 $(M+1)^+$. From the NMR of **1**, **2** and **4** (Tables 1 and 2), both methoxycarbonyl and hydroxyl groups were substituted to C-3″; the large shifts of 2″-methylene protons (1.47 dd, 2.93 dd) in **4** to the high field and the shifts (2.16 dd, 3.96 dd) in **2** were explained by deshielding or shielding effects based on elimination of the ester methyl group or introduction of the acetyl

Table 3. Antimicrobial activity of SF-2370.

Test organisms	MIC (μg/ml)
Staphylococcus aureus JC-1	>200
S. aureus Smith S-424	>200
S. epidermidis 109	>200
Bacillus anthracis No. 119	>200
B. subtilis ATCC 6633	>200
Escherichia coli JC-2	>200
Salmonella typhi O-901-W	>200
S. enteritidis No. 11	>200
Klebsiella pneumoniae PCI 602	>200
Proteus vulgaris OX-19	>200
Serratia marcescens MB-3848	>200
Pseudomonas aeruginosa MB-3829	>200
Micrococcus luteus	6.25
M. flavus FDA16	6.25
Corynebacterium bovis 1810	6.25
Candida albicans C-A-24	>100*
Cryptococcus neoformans Cr-1	25*
Trichophyton mentagrophytes	>100*
T. interdigitale	25*
Aspergillus fumigatus	>100*

Determined on Sensitivity Disk Agar medium (Nissui Seiyaku) and Sabouraud medium.*

group. Remarkable ^{1}H-^{1}H NOE were observed at 4-H by irradiation of 5″-Me and also 4′-H by 1″-H. The structure of the ring F was deduced to be a five-membered lactam from the chemical shifts of **1** (Tables 1 and 2).

From the above-mentioned NMR experiments, the gross structure of SF-2370 was deduced to be **1**. More details of the structural elucidation will be reported elsewhere.

The antibiotic showed weak antibacterial and antifungal activities as shown in Table 3. Protective effects against rice plant diseases in a green house test caused by *Rhizoctonia solani* (86% at 200 ppm), *Xanthomonas compestris* pv. *oryzae* (96% at 12.5 ppm) and *Pyricularia oryzae* (87% at 400 ppm) were observed. No acute toxicity of the antibiotic was observed at 300 mg/kg in mice intraperitoneally.

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