Communications to the Editor

ISOLATION AND CHARACTERIZATION OF SPOREAMICIN C

Sir:

Sporeamicins A (Antibiotic L53-18 A)[1] and B[2] are new 14-membered macrolide antibiotics isolated from *Saccharopolyspora* sp. L53-18. Their taxonomy of the producing culture, fermentation, isolation, structure determination and biological properties have been described in the preceding communications.[2~5] During the search for other antibiotics in the culture filtrates of the sporeamicin-producing strain, we discovered a new minor component designated sporeamicin C (Fig. 1) which is described in this communication.

Fermentation was carried out at 28°C for 161 hours in 250-liter fermenter containing a medium consisting of glucose 3%, corn steep liquor 1%, dry yeast 0.6%, cobalt chloride 0.001% and FS-antifoam 028 (Dow Corning K. K., Japan) 0.04% (pH 7.0).

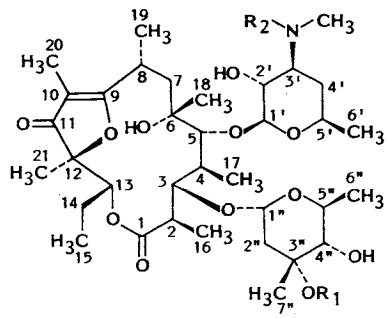
The culture broth (200 liters) was filtered and the filtrate was extracted with ethyl acetate at pH 9.0. The isolation of the sporeamicin C was accomplished using the general procedure for basic macrolide antibiotics and it was purified by precipitation, silica gel column chromatography and preparative reverse-phase HPLC (TSK gel 120T, Tosoh) as shown in Fig. 2. The fractionation of the antibiotics was monitored by bioautography using *Micrococcus luteus* ATCC 9341 and by HPLC analysis using a Hitachi gel No. 3056 column (416 mm × 15 cm) with UV detection at 275 nm. The mobile phase was CH_3CN - MeOH - 1/15 M $AcONH_4$ (50 : 25 : 35) with flow rate of 0.8 ml per minute. TLC systems as well as HPLC systems used clearly separated sporeamicins A, B and C.

Sporeamicin C is basic in nature and soluble in methanol, ethanol, acetone, ethyl acetate, benzene, chloroform and acidic water, but virtually insoluble in *n*-hexane and water. It gave positive color tests with potassium permanganate, iodine, Dragendorff and Molisch reagents, but was unreactive with ninhydrin or Sakaguchi reagent. The molecular formula of sporeamicin C was determined to be $C_{36}H_{61}NO_{12}$ based on FAB-MS (*m/z* 700, $(M+H)^+$) and elemental analysis. The UV spectrum suggested the presence of an enone function (276 nm). The IR spectrum also showed the presence of enone (1620, 1690 cm^{-1}), ester carbonyl (1740 cm^{-1}) and hydroxyl (3450 cm^{-1}) functions. Other physico-chemical properties of sporeamicin C are summarized in Table 1. These data are very similar to those of sporeamicins A and B.[1~3] However, among the known basic macrolide antibiotics, none shows UV spectra similar to that of sporeamicin A, B or C. The sporeamicin C is also distinguished from other macrolide antibiotics by their molecular weight and molecular formula as shown in Table 1. So we carried out the structure determination of sporeamicin C based on CI-MS and NMR data.

The molecular formula of sporeamicin C represents a compound possessing CH_2 atoms less that of sporeamicin A. In the CI-MS spectrum, the fragment ions *m/z* 542, 381 and 144 were observed. The physico-chemical properties, such as UV maximum at 276 nm and the fragment ion at *m/z* 381 in the CI-MS, suggested that the sporeamicins A and C have the same aglycon moiety in the structure. The fragment ions *m/z* 542 and 144 may be attributable to the aglycon-*O*-desosamine minus 14 and desosamine minus 14. It is indicated that the losing CH_2 atoms may be substituted on the desosamine moiety. The substituted position on the desosamine was determined by NMR data.

The ^1H and ^{13}C NMR spectra of sporeamicin C in $CDCl_3$ are shown in Tables 2 and 3. The assignments were made on the basis of ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C chemical shifts correlated with 2D NMR experiments. The ^1H NMR spectrum of sporeamicin C is very similar to that of sporeamicin A. However the

Fig. 1. The structures of sporeamicins A, B and C.



Sporeamicin A $R_1 = CH_3$ $R_2 = CH_3$
Sporeamicin B $R_1 = H$ $R_2 = CH_3$
Sporeamicin C $R_1 = CH_3$ $R_2 = H$

Fig. 2. Procedure for isolating sporeamicin C.

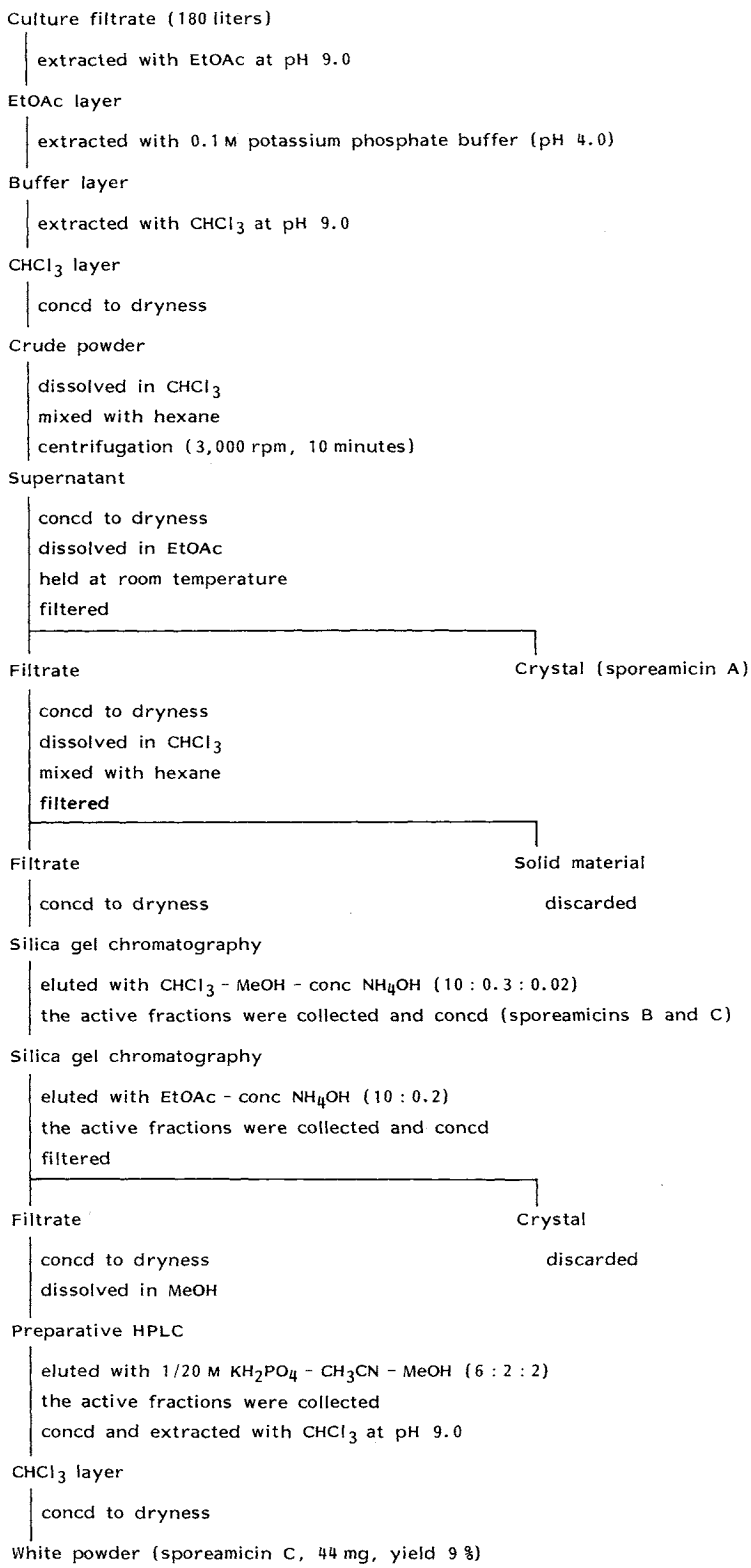


Table 1. Physico-chemical properties of sporeamicins A and C.

	Sporeamicin C	Sporeamicin A
Appearance	White powder	Colorless prism
FAB-MS (m/z)	700 $(M+H)^+$	714 $(M+H)^+$
Formula	$C_{36}H_{61}NO_{12}$	$C_{37}H_{63}NO_{12}$
Analysis Calcd for:	C 61.78, H 8.78, N 2.00	C 62.25, H 8.89, N 1.96
Found:	C 61.39, H 8.52, N 1.82	C 62.51, H 9.38, N 1.89
$[\alpha]_D^{22}$	$-41°$ (c 0.7, $CHCl_3$)	$-37°$ (c 0.8, $CHCl_3$)
MP	197~200°C	149~152°C
UV λ_{max}^{MeOH} nm (ε)	276 (10,600)	276 (10,550)
TLC (Rf)[a]	0.22	0.36
HPLC (Rt)[b]	6.21	6.70

[a] Absorbent; Silica gel f spot-film (Tokyo Kasei Co.). Solvent system; $CHCl_3$-MeOH-conc NH_4OH (10:0.5:0.05). Detection; UV lamp at 254 nm and bioautography using *Micrococcus luteus* ATCC 9341.

[b] Column; ODS, Hitachi gel No. 3056 (Hitachi, Ltd.). Equipment; Model 655 HPLC (Hitachi, Ltd.). Mobile phase; CH_3CN-MeOH-1/15 M $AcONH_4$ (50:25:35). Detection; UV absorption at 275 nm. Flow rate; 0.8 ml/minute, Rt value is expressed in minutes.

Table 2. ^1H NMR chemical shifts of sporeamicins A and C ($CDCl_3$, 400 MHz, 27°C).

Position	δ (ppm) J (Hz)		Position	δ (ppm) J (Hz)	
	Sporeamicin C	Sporeamicin A		Sporeamicin C	Sporeamicin A
15	0.89 (3H, t, J=7.33)	0.89 (3H, t, J=7.32)	NCH_3	2.41 (3H, s)	
17	1.00 (3H, d, J=7.33)	1.05 (3H, d, J=7.32)	$N(CH_3)_2$		2.33 (6H, s)
16	1.20 (3H, d, J=7.33)	1.19 (3H, d, J=6.84)	2	2.48 (1H, dq)	2.46 (1H, dq, J=5.86, 6.83)
7″	1.22 (3H, s)	1.23 (3H, s)	3′	2.59 (1H, ddd)	2.60 (1H, ddd, J=3.91, 10.25, 12.21)
4′a	1.23 (1H, m)	1.34 (1H, m)	8	2.98 (1H, m)	2.99 (1H, m)
6′	1.25 (3H, d, J=7.32)	1.27 (3H, d, J=6.34)	4″	3.02 (1H, d, J=9.28)	3.03 (1H, d, J=9.28)
18	1.28 (3H, s)	1.25 (3H, s)	OCH_3	3.25 (3H, s)	3.29 (3H, s)
6″	1.28 (3H, d, J=6.35)	1.30 (3H, d, J=6.34)	2′	3.29 (1H, dd, J=7.81, 9.28)	3.36 (1H, dd, J=7.33, 10.25)
19	1.31 (3H, d)	1.37 (3H, d, J=6.83)	5	3.67 (1H, dd, J=3.91)	3.67 (1H, dd, J=3.91)
21	1.35 (3H, s)	1.37 (3H, s)	5′	3.71 (1H, m)	3.64 (1H, m)
2″a	1.54 (1H, dd)	1.55 (1H, dd, J=4.88, 15.14)	3	3.97 (1H, dd, J=2.93, 5.37)	4.03 (1H, dd, J=2.93, 5.37)
4	1.57 (1H, m)	1.66 (1H, m)	5″	4.12 (1H, dq, J=6.35, 9.28)	4.09 (1H, dq, J=6.35, 9.28)
7a	1.70 (1H, dd)	1.86 (1H, dd, J=6.84, 15.14)	1′	4.41 (1H, d, J=7.81)	4.50 (1H, d, J=7.32)
20	1.73 (3H, s)	1.74 (3H, s)	1″	4.82 (1H, dd)	4.81 (1H, dd)
14a	1.73 (1H, m)	1.80 (1H, m)	13	5.07 (1H, dd, J=2.68, 11.48)	5.01 (1H, dd, J=3.42, 10.74)
4′b	1.98 (1H, m)	1.71 (1H, m)			
14b	2.13 (1H, m)	2.00 (1H, m)			
7b	2.25 (1H, dd)	2.12 (1H, dd, J=2.69, 14.90)			
2″b	2.33 (1H, dd)	2.31 (1H, m)			

$N(CH_3)_2$ signal (2.33 ppm, 6H) in sporeamicin A disappeared, and NCH_3 signal (2.41 ppm, 3H) appeared in sporeamicin C. In the ^{13}C NMR $N(CH_3)_2$ signal (40.42 ppm) in sporeamicin A shifted downfield and gave the NCH_3 signal (33.29 ppm) in sporeamicin C. Based on these data presented, we determined the structure shown in Fig. 1 for sporeamicin C.

Sporeamicin C exhibited antibacterial activity against Gram-positive bacteria (Table 4). The antibacterial activities of sporeamicin C were much less than those of sporeamicin A.

The difference of basic sugar moiety between sporeamicins A and C is the same as that between

Table 3. ^{13}C NMR chemical shifts of sporeamicins A and C ($CDCl_3$, 100 MHz, 27°C).

Position	δ (ppm)		Position	δ (ppm)	
	Sporeamicin C	Sporeamicin A		Sporeamicin C	Sporeamicin A
20	5.85 (q)	6.00 (q)	OCH_3	49.24 (q)	49.29 (q)
15	10.48 (q)	10.72 (q)	3′	60.16 (d)	64.65 (d)
17	11.25 (q)	10.93 (q)	5″	66.36 (d)	66.17 (d)
16	15.80 (q)	14.06 (q)	5′	69.78 (d)	69.69 (d)
6″	17.52 (q)	17.67 (q)	3″	72.77 (s)	72.80 (s)
21	20.14 (q)	20.62 (q)	2′	73.38 (d)	70.58 (d)
6′	20.75 (q)	21.01 (q)	6	74.15 (s)	74.76 (s)
14	20.95 (t)	21.34 (t)	4″	77.23 (d)	77.54 (d)
7″	21.40 (q)	21.53 (q)	13	77.61 (d)	77.93 (d)
19	22.47 (q)	21.10 (q)	3	79.72 (d)	78.55 (d)
18	26.98 (q)	26.37 (q)	12	87.63 (s)	87.14 (s)
8	31.23 (d)	31.79 (d)	5	89.33 (d)	86.28 (d)
NCH_3	33.29 (q)		1″	97.59 (d)	96.57 (d)
$N(CH_3)_2$		40.42 (q)	1′	105.88 (d)	104.80 (d)
2″	35.19 (t)	35.05 (t)	10	108.15 (s)	108.58 (s)
4′	36.87 (t)	29.13 (t)	1	174.88 (s)	175.90 (s)
7	41.36 (t)	41.82 (t)	9	192.76 (s)	193.02 (s)
4	43.31 (d)	43.05 (d)	11	205.13 (s)	204.96 (s)
2	48.26 (d)	46.31 (d)			

Table 4. Potency of sporeamicins A and C against a variety of bacteria.

Strain No.	MIC (μg/ml) (10^6 cells/ml)	
	Sporeamicin C	Sporeamicin A
Staphylococcus aureus FDA 209P JC-1	6.25	0.20
S. aureus Smith	6.25	0.39
S. epidermidis ATCC 27626	3.13	0.20
Streptococcus pyogenes N.Y. 5	0.39	≦0.05
S. pyogenes S-23	0.39	≦0.05
Micrococcus luteus ATCC 9341	0.78	≦0.05
Bacillus subtilis ATCC 6633	1.56	≦0.05
Escherichia coli NIHJ JC-2	>100	>100
Klebsiella pneumoniae NCTC 9632	>100	>100
Pseudomonas aeruginosa PA01	>100	>100

erythromycin A and de-*N*-methylerythromycin A.[6] The antibacterial spectra of the sporeamicins were also similar to those of erythromycin A and de-*N*-methylerythromycin A. Notably, sporeamicin C appeares to be biosynthetic precursor of sporeamicin A, whereas the de-*N*-methyl variant of erythromycin A was first noted as matabolite from animals fed the latter.

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