Communications to the Editor

ISOLATION AND CHARACTERIZATION OF SPOREAMICIN B

Sir:

Sporeamicin A is a 14-membered macrolide antibiotic produced by *Saccharopolyspora* sp. L53-18, which has strong antibacterial activity against Gram-positive bacteria.[1~4] During the search for new antibiotic from the culture filtrates of the sporeamicin A-producing strain, we have discovered a new minor component designated sporeamicin B (Fig. 1). In this communication, we wish to describe the isolation and characterization of sporeamicin B.

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 sporeamicin B 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 yield of the antibiotic 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.

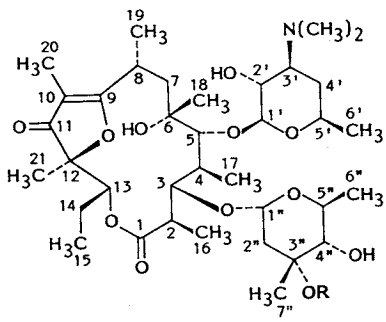
Sporeamicin B is basic in nature and soluble in methanol, ethanol, acetone, ethyl acetate, benzene, chloroform and acidic water, but barely soluble or insoluble in *n*-hexane and water. It gave positive color reactions with potassium permanganate, iodine, Dragendorff and Molisch reagents, but was negative with ninhydrin and Sakaguchi reagent. The molecular formula of sporeamicin B was determined to be $C_{36}H_{61}NO_{12}$ based on FAB-MS ($(M+H)^+$, *m/z* 700) 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. The other physico-chemical properties of sporeamicin B are summarized in Table 1. These data are very similar to those of sporeamicin A except for the molecular formula.[1,2] Of the known basic macrolide antibiotics, none have this molecular formula along with a UV spectrum similar to that of sporeamicin B. So we carried out the structure determination of sporeamicin B based on CI-MS and NMR data.

The molecular formula of sporeamicin B represents a compound differing from sporeamicin A by CH_2. The presence of a desosamine and aglycon moieties was shown by the fragment ions at *m/z* 158, 381, 556 in the CI-MS. This suggests that the missing CH_2 atoms are associated with the neutral sugar moiety. The ^1H and ^{13}C NMR spectra of sporeamicin B 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 B is very similar to that of sporeamicin A, however, the OCH_3 signal observed at δ_H 3.29 ppm in sporeamicin A, is missing. The structure was confirmed by ^{13}C NMR spectral data. Based on the data presented, sporeamicin B has the structure shown in Fig. 1. This structure indicates that the neutral sugar, cladinose, of sporeamicin A is replaced by mycarose in sporeamicin B.

Sporeamicin B exhibited antibacterial activity against Gram-positive bacteria (Table 4). The antibacterial activities of sporeamicin B were about two fold less than those of sporeamicin A.

The difference of neutral sugar moiety between sporeamicins A and B is the same as that between

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



Sporeamicin A R = CH_3
Sporeamicin B R = H

Fig. 2. Isolation procedure of sporeamicin B.

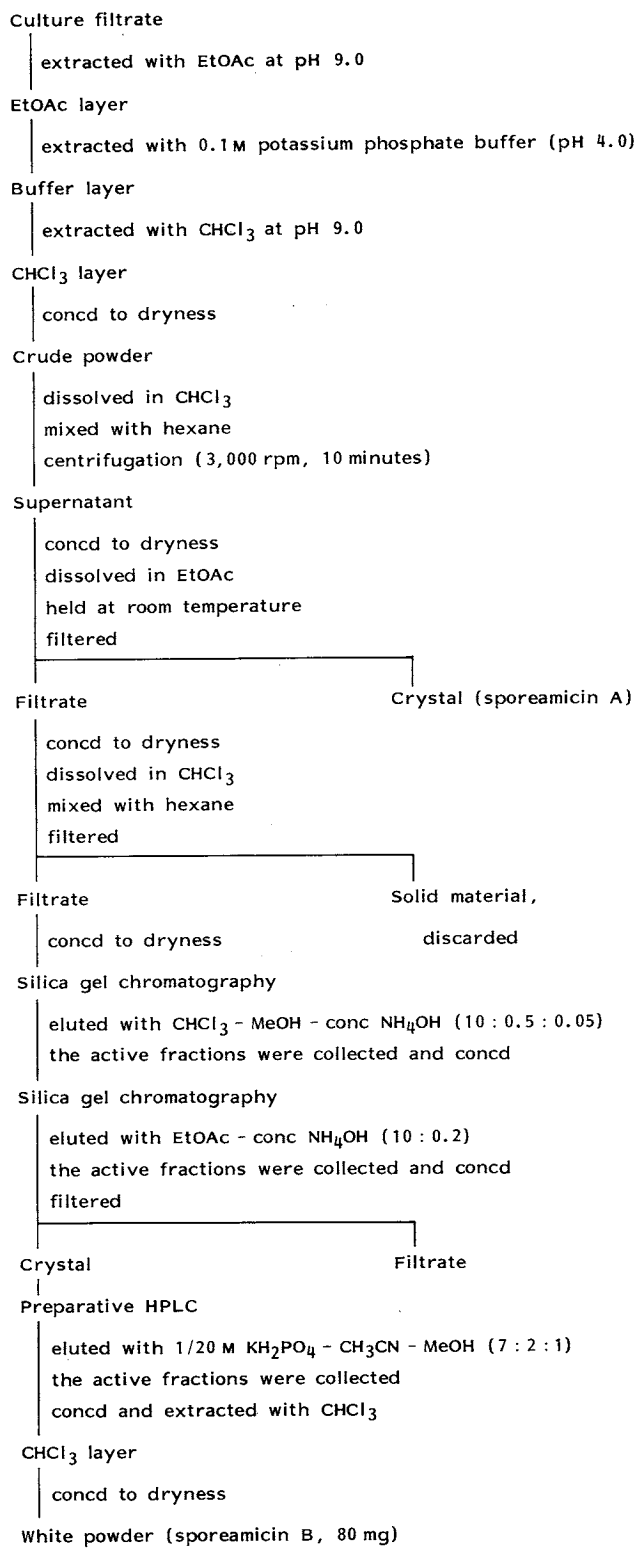


Table 1. Physico-chemical properties of sporeamicins A and B.

	Sporeamicin B	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:	C 61.78, H 8.78, N 2.00	C 62.25, H 8.89, N 1.96
Found:	C 61.68, H 8.57, N 2.04	C 62.51, H 9.38, N 1.89
$[\alpha]_D^{22}$	$-29°$ (*c* 0.7, $CHCl_3$)	$-37°$ (*c* 0.8, $CHCl_3$)
MP (°C)	108~111	149~152
UV λ_{max}^{MeOH} nm (ε)	276 (9,800)	276 (10,550)
TLC (Rf)[a]	0.18	0.36
HPLC (Rt)[b]	5.03	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 B ($CDCl_3$, 400 MHz, 27°C).

Position	δ (ppm) *J* (Hz)	
	Sporeamicin B	Sporeamicin A
15	0.88 (3H, t, J=7.57)	0.89 (3H, t, J=7.32)
17	1.06 (3H, d, J=7.32)	1.05 (3H, d, J=7.32)
16	1.20 (3H, d, J=6.84)	1.19 (3H, d, J=6.84)
7″	1.24 (3H, s)	1.23 (3H, s)
18	1.28 (3H, s)	1.25 (3H, s)
6′	1.28 (3H, d)	1.27 (3H, d, J=6.34)
6″	1.36 (3H, d, J=6.35)	1.30 (3H, d, J=6.34)
21	1.37 (3H, s)	1.37 (3H, s)
19	1.37 (3H, d)	1.37 (3H, d, J=6.83)
4	1.71 (1H, m)	1.66 (1H, m)
4′b	1.73 (1H, m)	1.71 (1H, m)
20	1.74 (3H, s)	1.74 (3H, s)
2″a	1.79 (1H, dd)	1.55 (1H, dd, J=4.88, 15.14)
14a	1.79 (1H, m)	1.80 (1H, m)
7a	1.83 (1H, dd, J=6.35, 15.13)	1.86 (1H, dd, J=6.84, 15.14)
14b	2.03 (1H, m)	2.00 (1H, m)
7b	2.09 (1H, dd)	2.12 (1H, dd, J=2.69, 14.90)
2″b	2.17 (1H, dd)	2.31 (1H, m)
$N(CH_3)_2$	2.30 (6H, s)	2.33 (6H, s)
2	2.49 (1H, dq)	2.46 (1H, dq, J=5.86, 6.83)
3′	2.56 (1H, ddd)	2.60 (1H, ddd, J=3.91, 10.25, 12.21)
8	2.87 (1H, m)	2.99 (1H, m)
4″	2.98 (1H, d, J=9.28)	3.03 (1H, d, J=9.28)
OCH_3		3.29 (3H, s)
2′	3.33 (1H, dd, J=7.33, 10.26)	3.36 (1H, dd, J=7.33, 10.25)
5	3.60 (1H, d, J=5.37)	3.71 (1H, d, J=4.88)
5′	3.63 (1H, m)	3.64 (1H, m)
3	4.13 (1H, dd, J=2.44, 5.86)	4.03 (1H, dd, J=2.93, 5.37)
5″	3.92 (1H, dq)	4.09 (1H, dq, J=6.35, 9.28)
1′	4.37 (1H, d, J=7.32)	4.50 (1H, d, J=7.32)
1″	4.99 (1H, dd)	4.81 (1H, dd)
13	5.05 (1H, dd, J=3.42, 10.74)	5.01 (1H, dd, J=3.42, 10.74)

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

Position	δ (ppm)		Position	δ (ppm)	
	Sporeamicin B	Sporeamicin A		Sporeamicin B	Sporeamicin A
20	5.92 (q)	6.00 (q)	OCH_3		49.29 (q)
15	10.61 (q)	10.72 (q)	3′	65.17 (d)	64.65 (d)
17	10.87 (q)	10.93 (q)	5″	66.36 (d)	66.17 (d)
16	14.07 (q)	14.06 (q)	3″	69.54 (s)	72.80 (s)
6″	18.08 (q)	17.67 (q)	5′	70.10 (d)	69.69 (d)
21	20.55 (q)	20.62 (q)	2′	70.48 (d)	70.58 (d)
19	20.77 (q)	21.10 (q)	6	74.77 (s)	74.76 (s)
6′	21.04 (q)	21.01 (q)	4″	76.44 (d)	77.54 (d)
14	21.30 (t)	21.34 (t)	13	77.99 (d)	77.93 (d)
7″	25.68 (q)	21.53 (q)	3	80.74 (d)	78.55 (d)
18	26.49 (q)	26.37 (q)	5	87.13 (d)	86.28 (d)
4′	28.81 (t)	29.13 (t)	12	87.22 (s)	87.14 (s)
8	31.92 (d)	31.79 (d)	1″	98.33 (d)	96.57 (d)
$N(CH_3)_2$	40.38 (q)	40.42 (q)	1′	106.01 (d)	104.80 (d)
2″	40.64 (t)	35.05 (t)	10	108.67 (s)	108.58 (s)
7	41.61 (t)	41.82 (t)	1	175.61 (s)	175.90 (s)
4	42.50 (d)	43.05 (d)	9	192.53 (s)	193.02 (s)
2	46.30 (d)	46.31 (d)	11	204.78 (s)	204.96 (s)

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

Strain No.	MIC (μg/ml) (10^6 cells/ml)	
	Sporeamicin B	Sporeamicin A
Staphylococcus aureus FDA 209P JC-1	0.39	0.20
S. aureus Smith	0.78	0.39
Staphylococcus epidermidis ATCC 27626	0.39	0.20
Streptococcus pyogenes N.Y. 5	≦0.05	≦0.05
S. pyogenes S-23	0.10	≦0.05
Micrococcus luteus ATCC 9341	0.10	≦0.05
Bacillus subtilis ATCC 6633	0.20	≦0.05
Escherichia coli NIHJ JC-2	>100	>100
Klebsiella pneumoniae NCTC 9632	>100	>100
Pseudomonas aeruginosa PA01	>100	>100

erythromycins A and C.[5] The antibacterial spectra of the sporeamicins are also similar to those of the erythromycins.

ATSUKI MORISHITA
SAKAE MUROFUSHI
KENYA ISHIZAWA
NAOKI MUTOH
SATOSHI YAGINUMA

Research Laboratories, Toyo Jozo Co., Ohito, Shizuoka 410-23, Japan

(Received December 16, 1991)

References

1) YAGINUMA, S.; A. MORISHITA, N. MUTOH, K. ISHIZAWA, M. HAYASHI & T. SAITOH (Toyo Jozo): Antibiotic L53-18 A. Jpn. Kokai 190189 ('90), July 26, 1990 [EP 379 395 July 25, 1990]
2) YAGINUMA, S.; A. MORISHITA, K. ISHIZAWA, S. MUROFUSHI, M. HAYASHI & N. MUTOH: Sporeamicin A, a new macrolide antibiotic. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 45: 599~606, 1992
3) MORISHITA, A.; K. ISHIZAWA, N. MUTOH, T. YAMAMOTO, M. HAYASHI & S. YAGINUMA: Sporeamicin A, a new macrolide antibiotic. II. Structure determination. J. Antibiotics 45: 607~612, 1992
4) MORISHITA, A.; N. MUTOH, K. ISHIZAWA, T. SUZUKI, S. YOKOIYAMA & S. YAGINUMA: Sporeamicin A, a new macrolide antibiotic. III. Biological properties. J. Antibiotics 45: 613~617, 1992
5) WILEY, P. F.; R. GALE, C. W. PETTINGA & K. GERZON: Erythromycin. XII. The isolation, properties and partial structure of erythromycin C. J. Am. Chem. Soc. 79: 6074~6077, 1957