NOTES

A NEW BROAD-SPECTRUM AMINOGLYCOSIDE ANTIBIOTIC COMPLEX, SPORARICIN V. SPORARICIN E

AKIO IWASAKI, TAKEO DEUSHI, ISAMU WATANABE, MASAO OKUCHI, HISAKATSU ITOH and TOSHIHITO MORI

Tokyo Research Laboratories, Kowa Co., Ltd., Higashimurayama, Tokyo 189, Japan

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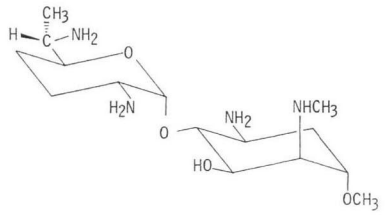
In the previous papers[1~4] of this series, we reported that *Saccharopolyspora hirsuta* subsp. *kobensis* KC-6606 produced sporaricins A, B, C and D. A new minor component, sporaricin E having weak antimicrobial activity, has been found in the culture broth of this strain. Furthermore, in the course of strain improvement, a high-yield mutant to produce sporaricin E has been isolated. In this paper, the fermentation, isolation, characterization and structural elucidation of sporaricin E are described. Sporaricin E is found to be identical with 2-deoxyfortimicin B.

This mutant, strain M-4-45 was cultured in 500-ml Erlenmeyer flasks which contained 50 ml of a medium composed of 2.5% corn starch, 1.5% soybean meal, 0.5% corn steep liquor, 0.1% Ebios (dried yeast, Ebios Yakuhin Kogyo Co., Ltd., Japan), 1.0% cotton seed oil, 0.3% NaCl, 0.3% $CaCO_3$ and 0.05% $MgSO_4 \cdot 7H_2O$ (pH was adjusted to 7.0 before sterilization) on a rotary shaker at 30°C for 7 days.

The fermentation broth (5 liters) was filtered at pH 2.0 by using Dicalite (Dicalite Orient Co., Ltd., Japan) as a filter aid. After neutralization, the antibiotics in the filtrate were adsorbed on a column of Amberlite IRC-50 (NH_4^+, 1 liter) and eluted with 1 N aqueous ammonia (5 liters). The active fractions were concentrated to 200 ml and the concentrate was diluted to 2 liters with water, adsorbed on a column of CM-Sephadex C-25 (NH_4^+, 300 ml) and developed by a gradient elution with 0.05 N (1.5 liters) to 0.4 N (1.5 liters) aqueous ammonia. The eluate was cut into 20-ml fractions. The fractions were monitored by bioactivity against *Bacillus subtilis* ATCC 6633 and thin-layer chromatography (silica gel, E. Merck DC-Alufolien 60 F_{254}; chloroform - methanol - 17% ammonia (1: 8: 3)). Sporaricin D (Rf 0.66) was eluted first, followed by sporaricins C (Rf 0.64), A (Rf 0.60), E (Rf 0.73) and B (Rf 0.52) in sequence. Fraction Nos. 93~103 were concentrated and lyophilized to give a colorless powder (184 mg) of sporaricin E. Further purification of sporaricin E was achieved by chromatography on a column of CM-Sephadex C-25 (NH_4^+, 100 ml) with a gradient elution between 0.1 N NH_4OH (500 ml) and 0.3 N NH_4OH (500 ml). Active fractions including sporaricin E were combined, concentrated and lyophilized to afford a colorless solid (103 mg) of pure sporaricin E.

Sporaricin E (**1**) shows $[\alpha]_D^{23}$ +54° (*c* 1, H_2O). The molecular formula is derived from elementary analysis, MS (*m/z* 332.2455, calcd. for $C_{15}H_{32}N_4O_4$, M^+, 332.2423) and ^{13}C NMR spectrum (Table 1). The ^1H NMR spectrum (D_2O, pD 12.1) of **1** indicates one anomeric proton (5.55 ppm, d, J=3.5 Hz) and three methyl groups assigned to C-CH_3 (1.53 ppm, d, J=6.5 Hz), N-CH_3 (2.84 ppm, s) and O-CH_3 (3.87 ppm, s).



Sporaricin E (**1**)

Fig. 1. IR spectrum of sporaricin E.

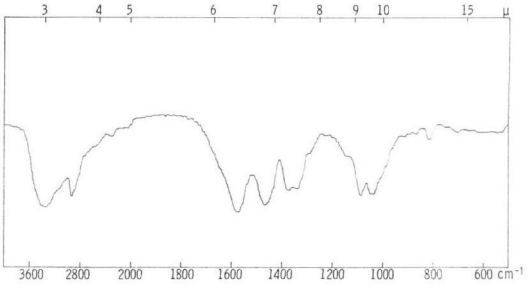


Table 1. Chemical shifts of ^{13}C NMR spectra of sporaricin E and 2-deoxyfortamine.

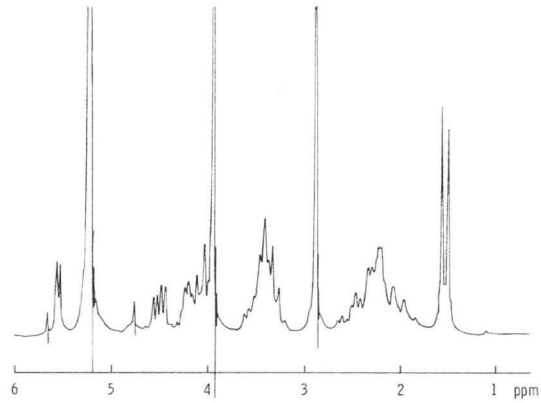
Carbons	Chemical shifts in D_2O (pD 12.1) (ppm)	
	Sporaricin E	2-Deoxy-fortamine
1′	102.3	
2′	51.0*	
3′	27.4	
4′	27.7	
5′	75.4	
6′	50.8*	
7′	18.9	
1	49.5*	50.4
2	31.5	31.3
3	76.7	77.0
4	63.7	63.8
5	71.6	72.2
6	85.0	76.4
N-CH_3	35.3	35.8
O-CH_3	57.3	57.3

* These chemical shifts may be interchangeable.

Anal. Calcd. for $C_{15}H_{32}N_4O_4 \cdot \frac{3}{2}H_2O$:
C 50.12, H 9.81, N 15.59.
Found: C 50.40, H 9.44, N 15.71.

Treatment of **1** (165 mg) with acetic anhydride in methanol at room temperature gave the tetra-*N*-acetate (**2**), quantitatively, $[\alpha]_D^{24}$ +112° (*c* 1, H_2O), MS *m/z* 500 (M^+), ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, J=6.5 Hz, CCH_3), 1.95, 1.97, 2.03, 2.10 (3H, s, $NCOCH_3$, respectively), 3.12 (3H, s, NCH_3) and 3.36 (3H, s, OCH_3). Methanolysis of tetra-*N*-acetylsporaricin E (**2**, 200 mg) with 5.5 N hydrogen chloride in methanol (8 ml) at 80°C for 8 hours in a sealed tube, followed by re-*N*-acetylation gave methyl di-*N*-acetyl-α- and β-aminoglycosides (**3**, 52 mg, $[\alpha]_D^{20}$ +61° (*c* 1, $CHCl_3$) and **4**, 15 mg, $[\alpha]_D^{20}$ −110° (*c* 1, $CHCl_3$)) and a di-*N*-acetylaminocyclitol (**5**, 79 mg, $[\alpha]_D^{20}$ +103° (*c* 0.8, H_2O)). These products were separated by silica gel column chromatography developed with chloroform - acetone (1: 2) and then ethyl acetate - methanol (7: 1). Compounds **3** and **4** were identified with methyl 2,6-di-*N*-acetyl-6-*epi*-α- and β-purpurosaminides B[3,5], respectively, obtained from sporaricin B in ^1H NMR and IR spectra, and optical rotations. The structure of the di-*N*-acetylaminocyclitol (**5**) was determined to be 1,4-di-*N*-acetyl-2-deoxyfortamine by comparing with authentic sample obtained from sannamycin B[6].

Fig. 2. ^1H NMR spectrum of sporaricin E (in D_2O).



The position of linkage of the aminosugar to the aminocyclitol was determined to be C-6 of 2-deoxyfortamine by ^{13}C NMR spectra of sporaricin E and 2-deoxyfortamine (Table 1). Finally, in the ^1H NMR spectrum of sporaricin E (Fig. 2) the coupling constant ($J_{1',2'}$=3.5 Hz) of C-1′ proton supported the same anomeric configuration as sporaricin B. Thus, the structure of sporaricin E was determined to be 2-deoxyfortimicin B.

Recently, MARTIN, *et al.* reported the syntheses of 2-deoxyfortimicins B and A from fortimicin B and have proved that 2-deoxyfortimicin A exhibited a broad antimicrobial activity[7].

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