Notes

2″-*N*-FORMIMIDOYLSPORARICIN A PRODUCED BY *SACCHAROPOLYSPORA HIRSUTA* SUBSP. *KOBENSIS*

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Sporaricin A, an aminoglycoside antibiotic containing a 1,4-diaminocyclitol was found by DEUSHI *et al.*[1] in the culture filtrate of *Saccharopolyspora hirsuta* subsp. *kobensis*. Related *N*-formimidoyl antibiotics, 2″-*N*-formimidoylfortimicin A (dactimicin)[2] and 2″-*N*-formimidoylistamycins A and B[3] were found by INOUYE *et al.* and by KONDO *et al.* in culture filtrates of *Dactylosporangium matsuzakiense* SF-2052 and *Streptomyces tenjimariensis* SS-939, respectively. In this paper, we will report the isolation and characterization of another related antibiotic, 2″-*N*-formimidoylsporaricin A produced by the strain MG937-14F1. This strain was isolated from a soil sample collected at a garden of our Institute and classified into *Saccharopolyspora hirsuta* subsp. *kobensis*.[4] The production of 2″-*N*-formimidoylsporaricin A by the sporaricin-producing strain has never been reported before.

Fermentation and Isolation

Strain MG937-14F1 was cultured at 30°C for 3 days on a rotatory shaker (180 rpm) in a 500-ml baffled Erlenmeyer flask containing 110 ml of a seed medium [dextrin 2%, galactose 2%, peptone (Bacto-Soytone) 1%, corn steep liquor (Nisshoku) 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2%, pH 7.4]. The seed culture (2.2 ml) thus prepared was inoculated into 110 ml of a production medium consisting of starch 3%, soybean meal (Nisshin) 1.5%, corn steep liquor 0.5%, yeast extract (Oriental) 0.2%, NaCl 0.3%, $CaCO_3$ 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CoCl_2 \cdot 6H_2O$ 0.001% (pH 7.0) in a flask and cultured at 27°C for 4 days on a rotatory shaker.

The culture broth was harvested from 45 flasks and combined. It was suggested to contain 5.7 μg/ml of 2″-*N*-formimidoylsporaricin A by the cylinder plate test using *Bacillus subtilis* PCI219 as the test organism and pure 2″-*N*-formimidoylsporaricin A sulfate (610 μg/mg) as the standard. After filtration at pH 2.0, the antibiotic in the filtrate (adjusted to pH 7.0, 4.03 liters) was adsorbed on a column of Amberlite IRC-50 (70% Na^+, 200 ml) and eluted with 1 N HCl. The antibiotic in the active eluate (pH 7.2, 230 ml, 12.4 μg/ml) was re-adsorbed on a column of charcoal (2 g) and eluted with 0.05 N HCl in 80% aq methanol. The active eluate (65 ml) was concd to dryness to give the crude powder (152 mg, 21.3 μg/mg) in 14.1% yield from the filtrate. By HPLC analysis[5] of components, the crude powder was shown to contain 2″-*N*-formimidoylsporaricin A (32.84 minutes) at 1.6% and sporaricin A (21.49 minutes) at 0.3%.

The crude powder was combined with other similar samples (91 mg of 63.9 μg/mg and 72 mg of 21.4 μg/mg) and dissolved in water (10 ml, 1,059.3 μg/ml). To the solution was added sodium *p*-toluenesulfonate (200 mg) as the organic counter ion and the solution was adsorbed on a column of Diaion HP-20 (10 ml). The column was developed successively with 50 ml of water, each 50 ml, of 2.5%, 5.0%, 7.5%, 10.0% and 12.5% aq methanol and 100 ml of 15% aq methanol. The eluates were cut into 5-ml fractions. Fractions (Nos. 41~82, 260 ml, 24 μg/ml) containing the antibiotic were combined and concd to 10 ml. Purification

Table 1. ^{1}H NMR chemical shifts of 2″-*N*-formimidoylsporaricin A (**1**) and sporaricin A (**2**) in D_2O at pD 5.0.

Proton	1		2
	δ (ppm)	Multiplicity	δ (ppm)
1-H	3.92	ddd	3.90
2-H_{ax}	1.86	ddd	1.84
2-H_{eq}	2.66	dddd	2.63
3-H	4.09	ddd	4.06
4-H	4.38	dd	4.36
5-H	4.47	dd	4.43
6-H	4.23	br	4.21
3-OCH_3	3.48	s	3.45
4-NCH_3	3.14	s	3.09
1′-H	5.51	d	5.49
2′-H	3.64	ddd	3.63
3′-H_2	2.03~2.14	m	2.02~2.12
4′-H_{ax}	1.60	m	1.58
4′-H_{eq}	2.03~2.14	m	2.02~2.12
5′-H	3.86	ddd	3.84
6′-H	3.39	dq	3.38
7′-H_3	1.35	d	1.33
2″-H_a	4.37	d	4.03
2″-H_b	4.47	d	4.12

δ (ppm) were measured from sodium 3-trimethylsilyl-1-propanesulfonate (0 ppm) as the internal reference.

Table 2. ^{13}C NMR chemical shifts of 2″-*N*-formimidoylsporaricin A (**1**) and sporaricin A (**2**) in D_2O at pD 5.0.

Carbon	1		2
	δ (ppm)	Multiplicity	δ (ppm)
1	47.5	d	47.3
2	29.5	t	29.5
3	72.2	d	72.0
4	56.7	d	56.7
5	68.1	d	68.1
6	73.1	d	73.2
3-OCH_3	56.7	q	56.6
4-NCH_3	32.1	q	32.1
1′	92.6	d	92.6
2′	49.8	d	49.7
3′	21.5	t	21.4
4′	26.4	t	26.5
5′	71.2	d	71.0
6′	52.0	d	51.9
7′	15.2	q	15.3
1″	169.2	s	168.4
2″	44.3	t	41.4
3″	156.1	d	

δ (ppm) were measured from dioxane (67.4 ppm) as the internal reference.

Table 3. Minimum inhibitory concentrations of 2″-*N*-formimidoylsporaricin A.

Test organism	μg/ml	Test organism	μg/ml
Staphylococcus aureus FDA 209P	0.78	*Klebsiella pneumoniae* PCI602	1.56
S. aureus Smith	0.39	*K. pneumoniae* 22#3038	3.13
S. aureus Ap01	6.25	*Shigella dysenteriae* JS11910	3.13
S. epidermidis 109	0.78	*S. flexneri* 4b JS11811	3.13
Micrococcus flavus FDA16	1.56	*S. sonnei* JS11746	3.13
M. luteus PCI1001	0.78	*Salmonella typhi* T-63	0.78
Bacillus anthracis	0.78	*S. enteritidis* 1891	3.13
B. subtilis PCI219	0.78	*Proteus vulgaris* OX19	1.56
B. subtilis NRRL B-558	0.39	*P. rettgeri* GN311	0.78
B. cereus ATCC10702	6.25	*P. rettgeri* GN466	0.78
Corynebacterium bovis 1810	0.39	*Serratia marcescens*	3.13
Mycobacterium smegmatis ATCC607	0.78	*Serratia* sp. SOU	50
Escherichia coli NIHJ	0.78	*Serratia* sp. 4	25
E. coli K-12	0.78	*Providencia* sp. Pv16	1.56
E. coli K-12 R5	3.13	*Providencia* sp. 2991	1.56
E. coli K-12 R388	0.78	*Pseudomonas aeruginosa* A3	1.56
E. coli K-12 J5R11-2	0.78	*P. aeruginosa* No. 12	25
E. coli K-12 ML1629	1.56	*P. aeruginosa* H9	25
E. coli K-12 ML1630	1.56	*P. aeruginosa* H11	50
E. coli K-12 ML1410	1.56	*P. aeruginosa* TI-13	25
E. coli K-12 ML1410 R81	1.56	*P. aeruginosa* GN315	25
E. coli K-12 LA290 R55	1.56	*P. aeruginosa* 99	>100
E. coli K-12 LA290 R56	1.56	*P. aeruginosa* B-13	>100
E. coli K-12 LA290 R64	1.56	*P. aeruginosa* 21-75	50
E. coli W677	0.78	*P. aeruginosa* PSTI	50
E. coli JR66/W677	0.78	*P. aeruginosa* ROS134/PU21	>100
E. coli K-12 C600 R135	25	*P. aeruginosa* K-Ps102	50
E. coli JR225	0.78	*P. maltophilia* GN907	>100

of the antibiotic in the concentrate was achieved by Amberlite CG-50 (70% Na^+, 6 ml) column chromatography eluted with 0.5 N H_2SO_4 followed by charcoal (500 mg) column chromatography eluted with 0.01 N H_2SO_4. After neutralizing the eluate with Amberlite IR-45 (OH^-) and concentrating to 4 ml, the concentrate was passed through a column of Amberlite IRA-400 (SO_4^{2-}, 4 ml) and developed with water. The active effluent (4 ml) was lyophilized to give 7.6 mg of pure 2″-*N*-formimidoylsporaricin A disulfate tetrahydrate (610 μg/mg) as a colorless hygroscopic powder in 43.8% yield from the crude powder.

Properties and Structure

2″-*N*-Formimidoylsporaricin A disulfate tetrahydrate: No definite mp $[\alpha]_D^{27}$ +65.8° (*c* 0.5, H_2O), secondary ion mass spectrum (SI-MS) *m/z* 417 (MH^+), IR (KBr) cm^{-1} 3400, 2950, 1710 ($C{=}N^+$), 1640, 1490, 1120, 1050, 990. *Anal* Calcd for $C_{18}H_{36}N_6O_5 \cdot 2H_2SO_4 \cdot 4H_2O$: C 31.57, H 7.07, N 12.27, S 9.36. Found: C 31.37, H 6.48, N 11.47, S 9.20. By high-voltage paper electrophoresis with 3,500 V for 15 minutes in HCOOH - CH_3COOH - H_2O (1: 3: 36), the antibiotic moved toward the cathode with Rm (relative mobility to alanine) 2.01. The ^1H and ^{13}C NMR chemical shifts in D_2O (pD 5.0) were compared with those of sporaricin A[6] as shown in Tables 1 and 2, respectively. The ^{13}C NMR data of the antibiotic showed good agreements with those of sporaricin A except the 2″-C and 3″-C signals as shown in Table 2. These two signals were easily assigned from those (44.1 and 155.8 ppm) of 2″-*N*-formimidoylistamycin B.[5] Therefore, the structure of the antibiotic was determined to be 2″-*N*-formimidoylsporaricin A.

As shown in Table 3, the antibiotic had strong antibacterial activity against Gram-positive and Gram-negative bacteria including aminoglycoside-resistant bacteria.

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