A NEW DESTOMYCIN-FAMILY ANTIBIOTIC PRODUCED BY *SACCHAROPOLYSPORA HIRSUTA*

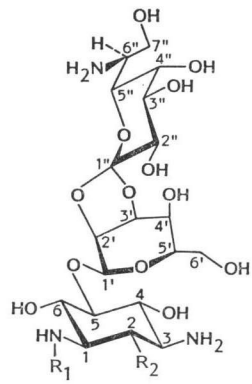
Sir:

As reported in our previous paper,[1] vanoxonin inhibiting thymidylate synthetase (EC 2.1.1.45) was found in the culture broth of *Saccharopolyspora hirsuta* KG245-CF2. Recently, we found that this strain produces two other antibiotics in a different medium. One of them is identical with nargenicin A_1 (CP-47,444) produced by *Nocardia argentinensis*,[2,3] and the other is a new antibiotic, 1-*N*-amidino-1-*N*-demethyl-2-hydroxydestomycin A. In this communication, the isolation and structural elucidation of the new antibiotic is reported.

The strain was cultured at 27°C for 5 days on a rotatory shaker (180 rpm) in a medium containing 3% soluble starch, 3% dry yeast, 0.3% K_2HPO_4, 0.1% KH_2PO_4, 0.2% NaCl, 0.1% $CaCO_3$ and 0.05% $MgSO_4 \cdot 7H_2O$ (adjusted to pH 7.2). Vegetative inoculum, 2% by volume grown for 4 days in a medium containing 1.0% glucose, 1.0% glycerol, 1.0% sucrose, 2.0% soybean meal, 1.0% press yeast, 0.5% oat meal, 0.5% Casamino Acids (Difco) and 0.1% $CaCO_3$ (adjusted to pH 7.0) was used. The culture broth in 45 flasks was collected and filtered (4.5 liters, 53 μg/ml assayed by the cylinder plate method using *Bacillus subtilis* PCI219 as the test organism).

Two antibiotics (**1** and **2**) in the filtrate were separated by adsorption on a column of Amberlite IRC-50 (Na^+ and H^+, 7:3, 250 ml). Antibiotic **1** in the effluent was purified by extraction with ethyl acetate followed by column chromatography of silica gel (eluted with chloroform - methanol) and Amberlite LH-20 (developed with methanol) to yield a colorless powder, EIMS *m/z* 515 (M^+), UV λ_{max} 267 nm. This antibiotic (**1**) inhibited the growth of *Staphylococcus aureus* but not *B. subtilis* and was found to be identical with nargenicin A_1 (CP-47,444)[2,3] in all respects.

Antibiotic **2** adsorbed on the resin column was eluted with 1 N HCl. The active eluate (500 ml) neutralized with Amberlite IR-45 (OH^-) was passed through a column of CM-Sephadex C-25 (80 ml) and after washing the column with 0.4 M NaCl, antibiotic **2** was eluted with a linear gradient of 0.4 M to 0.8 M NaCl (each 400 ml). The active eluate (195 ml) was concentrated to dryness and the residue was extracted with methanol (10 ml). The extract was passed through a column of Amberlite LH-20 (200 ml) and developed with methanol. The active effluent (28 ml) was concentrated to dryness yielding



	R_1	R_2
2	$-C(=NH)-NH_2$	OH
3	H	OH
Destomycin A	CH_3	H

Table 1. ^{13}C NMR chemical shifts (δ).

Carbon	**2** (pD 4.4)	**3** (pD 2.4)	Destomycin A (pD 4.4)
1	59.9 d	57.1	57.6
2	69.1* d	67.0*	26.4
3	56.9 d	57.1	50.8
4	68.8* d	68.7*	72.0
5	83.9 d	83.4	83.7
6	72.1* d	70.0*	71.6
7	159.1 s		30.9
1′	99.6 d	99.4	99.5
6′	62.1 t	62.1	62.1
1″	120.4 s	120.4	120.4
5″	71.0 d	71.0	71.0
6″	54.1 d	54.0	54.1
7″	59.2 t	59.1	59.2
2′~5′	76.1 d	76.1	76.1
	74.8 d	74.7	74.8
	72.6 d	72.5	72.6
	72.4 d	72.3	72.4
2″~4″	69.7 d	69.5	69.6
	69.5 d	69.5	69.5
	64.3 d	64.2	64.3

δ: ppm from TMS in D_2O using dioxane (δ 67.4 ppm) as the internal reference.

* Assignments within any vertical column may be reversed.

Table 2. The antibacterial spectrum on a nutrient agar.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA209P	>100
S. aureus Smith	50
Micrococcus flavus FDA16	50
M. luteus PCI1001	>100
Bacillus anthracis	50
B. subtilis NRRL B-558	50
B. subtilis PCI219	25
Corynebacterium bovis 1810	50
Mycobacterium smegmatis ATCC 607	6.25
Escherichia coli NIHJ	50
E. coli K-12	50
Klebsiella pneumoniae PCI602	25
Shigella dysenteriae JS11910	>100
S. flexneri 4b JS11811	>100
S. sonnei JS11746	>100
Salmonella typhi T-63	100
S. enteritidis 1891	100
Proteus vulgaris OX19	25
Serratia marcescens	>100
Pseudomonas aeruginosa A3	100

Table 3. The antifungal spectrum on a nutrient agar containing 1% glucose.

Test organism	MIC (μg/ml)
Candida tropicalis F-1	>100
C. pseudotropicalis F-2	25
C. albicans 3147	12.5
C. krusei F-5	>100
Saccharomyces cerevisiae F-7	50
Cryptococcus neoformans F-10	3.13
Aspergillus niger F-16	>100
Trichophyton asteroides 429	50
T. mentagrophytes 833	25
Helminthosporium oryzae	25
Pyricularia oryzae	>100
Pellicularia sasakii	100

antibiotic **2** hydrochloride (98 mg, 41.1% yield).

The hydrochloride was obtained as a colorless powder decomposing at 172~183°C. It shows $[\alpha]_D^{25}$ +7.7° (*c* 1.0, water), IR (KBr) 3370, 3050, 1640, 1500, 1400, 1250, 1160, 1085, 1040 and 900 cm^{-1}, and positive ninhydrin, RYDON-SMITH, SAKAGUCHI and pentacyanoaquoferriate reactions. *Anal* Calcd for $C_{20}H_{37}N_5O_{14}\cdot 3HCl\cdot H_2O$: C 34.37, H 6.06, N 10.02, Cl 15.22. Found: C 34.19, H 6.20, N 9.41, Cl 15.52. The ^{13}C NMR spectrum (Table 1) suggests that the structure is very similar to that of destomycin A[4] except for the inosadiamine moiety which is modified in this antibiotic.

Alkaline hydrolysis of **2** (82.4 mg) in 1 M NaOH (6 ml) at 100°C overnight followed by column chromatography of Amberlite CG-50 (NH_4^+, 100 ml) eluted with 0.5 M NH_4OH gave a colorless powder of deamidinyl antibiotic **3** (59.9 mg), SIMS *m/z* 530 (MH^+), $[\alpha]_D^{26}$ +11.0° (*c* 1.0, water). Antibiotic **3** was identical with antibiotic SS-56C[5] in all respects. Antibiotic **3** shows 45% and 54% activities of **2** against *B. subtilis* PCI219 and *Escherichia coli* K-12, respectively.

Acid hydrolysis of **2** (128 mg) in 0.5 M HCl (12 ml) at 105°C for 2.5 hours followed by chromatography on a column of Amberlite CG-50 (Na^+, 70 ml, eluted with 0.5 N HCl) and of Amberlite LH-20 (developed with methanol) gave a SAKAGUCHI-positive compound (42.3 mg) as the hydrochloride, SIMS *m/z* 221 (MH^+), $[\alpha]_D^{25}$ −10.2° (*c* 0.5, water). *Anal* Calcd for $C_7H_{16}N_4O_4\cdot 2HCl\cdot H_2O$: C 27.02, H 6.48, N 18.01. Found: C 26.77, H 6.49, N 16.69. The compound was identical with 1-*N*-amidinostreptamine (3-*N*-deamidinostreptidine) hydrochloride which was obtained by acid hydrolysis of 3-deamidinodihydrostreptomycin.* Furthermore, destomic acid was isolated from the effluent of Amberlite CG-50 column which was charged the acid hydrolysate of **2**. Talose was identified by gas chromatography of the trimethylsilylated derivative after methanolysis of **2**. From these results, the structure of the antibiotic (**2**) was determined to be 1-*N*-amidino-1-*N*-demethyl-2-hydroxydestomycin A as shown in the structure **2**.

Antibiotic **2** has weak antibacterial and antifungal activities (Tables 2 and 3) and an LD_{50} of 6.25~12.5 mg/kg in mice intravenously.

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