Communications to the Editor

1-EPIDACTIMICIN, A NEW AMINOGLYCOSIDE ANTIBIOTIC CONVERTED FROM FORTIMICIN B BY A BLOCKED MUTANT OF ISTAMYCIN-PRODUCING *STREPTOMYCES TENJIMARIENSIS*

Sir:

In our previous paper[1], we showed similarities in antibiotic biosynthesis and antibiotic resistance between *Streptomyces tenjimariensis* and *Micromonospora olivasterospora* that produce istamycins (IS's) and fortimicins (FT's), respectively. It was demonstrated that these organisms were capable of converting biosynthetic intermediates derived from each other to antibiotic substances including a new one. In this paper, we report the structure determination and some physicochemical properties of the new antibiotic (named 1-epidactimicin; EDC) isolated from the FT-B supplemented cultured broth of *S. tenjimariensis*. Antimicrobial activity of the novel antibiotic is also provided.

Table 1. ^{13}C NMR chemical shifts of dactimicins.

Carbon	DC sulfate[a]	EDC sulfate[a]
C-1	52.3	49.1
C-2	72.1	65.4
C-3	76.7	74.0
C-4	54.9	51.5
C-5	69.6	67.4
C-6	72.8	73.7
3-OCH_3	56.3	56.4
4-NCH_3	32.0	31.7
C-1′	95.1	92.0
C-2′	49.2	49.2
C-3′	21.8	21.1
C-4′	26.2	26.0
C-5′	70.4	70.7
C-6′	51.4	51.3
6′-CH_3	15.0	15.0
C-1″	166.8	169.0
C-2″	44.6	44.0
CH=NH	155.6	155.6

Spectra were taken in D_2O.

[a] Dactimicin (DC) sulfate and 1-epidactimicin (EDC) sulfate correspond to substances **III** and **IV**, respectively. ^{13}C assignment was based on ^1H-^{13}C correlation spectroscopy.

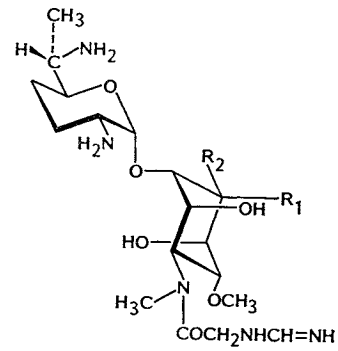
Antibiotics accumulated by *S. tenjimariensis* U41, a blocked mutant of IS-biosynthesis incubated in a fermentation medium (100 ml) supplemented with 200 μg/ml of FT-B[1] were isolated as follows. The filtrate of the cultured broth (adjusted to pH 2 with H_2SO_4) was neutralized (up to pH 6) with NaOH and loaded on a column (95 ml) of Amberlite IRC-50 (Na^+ - H^+, 7:3). Antibiotics adsorbed on the column were eluted with 0.5 N H_2SO_4. The active fractions thus eluted were added with sodium *p*-toluenesulfonate (3 g) as an organic counter ion, adjusted to pH 5.0 with NaOH and then passed through a Diaion CHP-20P column (75 ml). The adsorbed antibiotics were eluted with a linear gradient (0~8%) of MeOH containing 0.01 mM HCl. Four peak fractions (**I**, **II**, **III** and **IV**) were visualized by HPLC monitoring[1] of the eluates. Subsequently, the two peak fractions (**III** and **IV**) with antibiotic activity were purified. The fractions were concentrated to remove MeOH, neutralized with Amberlite IRA-45 and then chromatographed on an Amberlite IRC-50 (Na^+ - H^+, 7:3) column (3 ml). The

Table 2. 400 MHz ^1H NMR parameters for 1-epidactimicin sulfate (**IV**).

Proton	Chemical shifts (ppm)	Coupling constants (Hz)
1′-H	5.46 (d, 1H)	$J_{1',2'}$=3.3
2′-H	3.60 (m, 1H)	
3′-H_2, 4′-H	2.03~2.15 (m, 3H)	
4′-H	1.60 (m, 1H)	
5′-H	3.88 (m, 1H)	
6′-H	3.41 (m, 3H)	J_{6',CH_3}=6.7, $J_{5',6'}$=6.7
6′-CH_3	1.35 (d, 1H)	$J_{CH_3,6'}$=6.7
2″-H_2	4.38 (d, 1H), 4.48 (d, 1H)	$J_{2'',2''}$=18.0
CH=NH	7.98 (s, 1H)	
1-H	3.93 (t, 1H)	$J_{1,2}$=3.6, $J_{1,6}$=3.6
2-H	4.70 (t, 1H)	
3-H	4.06 (dd, 1H)	$J_{2,3}$=3.3, $J_{3,4}$=12.0
4-H	4.66 (dd, 1H)	
5-H	4.42 (t, 1H)	$J_{4,5}$=$J_{5,6}$=3.0
6-H	4.23 (t, 1H)	
OCH_3	3.51 (s, 3H)	
NCH_3	3.16 (s, 3H)	

antibiotics were eluted with 0.5 N H_2SO_4, neutralized (up to pH 6.0) with NaOH and loaded on a carbon column (25 ml) using charcoal (Wako Pure Chemical Industries, Ltd., Japan). The antibiotics adsorbed were then eluted with 80% MeOH containing 0.05 N H_2SO_4. The resultant active fractions were evaporated to remove MeOH, adjusted to pH 5.0 with Amberlite IRA-45 (OH^-), concentrated to 1 ml *in vacuo* and passed through a column (25 ml) of Amberlite IRA-400 (SO^{2-}). The antibiotics were eluted with water, concentrated and lyophilized. These procedures gave pure antibiotic substances, **III** and **IV**, as their sulfates.

Fig. 1. Structure of 1-epidactimicin.



Dactimicin	R_1=NH_2	R_2=H
1-Epidactimicin	R_1=H	R_2=NH_2

For structure determination, the ^{13}C and ^1H NMR spectra were recorded on a Jeol JNM-GX400 spectrometer. Table 1 shows the ^{13}C chemical shifts of **III** and **IV**. Both substances exhibited the identical chemical shifts to each other except for chemical shits of C-1 and C-2. Differences in chemical shifts of C-1 and C-2 could be regarded as reflecting the conforma-

Table 3. Antimicrobial spectra of dactimicin (DC) and 1-epidactimicin (EDC).

Organism	MIC (μg/ml)		Organism	MIC (μg/ml)	
	DC	EDC		DC	EDC
Staphylococcus aureus FDA 209P	3.13	3.13	*Klebsiella pneumoniae* PCI 602	3.12	3.12
S. aureus Smith	0.78	0.78	*K. pneumoniae* 22 No. 3038	6.25	6.25
S. aureus Ap01	3.12	3.12	*Shigella dysenteriae* JS11910	6.25	6.25
S. epidermidis 109	1.56	1.56	*S. flexneri* 4b JS11811	6.25	6.25
Micrococcus flavus FDA 16	1.56	3.12	*S. sonnei* JS11746	3.12	3.12
M. luteus PCI 1001	0.78	0.78	*Salmonella enteritidis* 1891	6.25	6.25
Bacillus subtilis PCI 219	0.78	0.78	*S. typhi* T-63	0.78	1.56
B. subtilis NRRL B-558	1.56	1.56	*Proteus vulgaris* OX19	0.78	1.56
B. cereus ATCC 10702	12.5	6.25	*P. rettgeri* GN311	1.56	1.56
B. anthracis	1.56	1.56	*P. rettgeri* GN466	1.56	1.56
Corynebacterium bovis 1810	1.56	3.12	*Serratia marcescens*	3.12	6.25
Mycobacterium smegmatis ATCC 607	0.39	0.39	*Serratia* sp. SOU	100	100
			Serratia sp. 4	50	50
Escherichia coli NIHJ	0.78	1.56	*Providencia* sp. Pv16	1.56	3.12
E. coli K-12	1.56	1.56	*Providencia* sp. 2991	3.12	3.12
E. coli K-12 R5	3.12	3.12	*Pseudomonas aeruginosa* A3	1.56	3.12
E. coli K-12 R388	1.56	1.56	*P. aeruginosa* No. 12	50	50
E. coli K-12 J5R11-2	0.78	1.56	*P. aeruginosa* H9	25	50
E. coli K-12 ML 1629	1.56	3.12	*P. aeruginosa* H11	100	100
E. coli K-12 ML 1630	3.12	3.12	*P. aeruginosa* TI-13	25	25
E. coli K-12 ML 1410	3.12	3.12	*P. aeruginosa* GN315	50	50
E. coli K-12 ML 1410 R81	3.12	3.12	*P. aeruginosa* 99	>100	>100
E. coli K-12 LA290 R55	1.56	3.12	*P. aeruginosa* B-13	>100	>100
E. coli K-12 LA290 R56	1.56	1.56	*P. aeruginosa* 21-75	100	100
E. coli K-12 LA290 R64	1.56	3.12	*P. aeruginosa* PST1	50	100
E. coli W677	1.56	3.12	*P. aeruginosa* ROS134/PU21	>100	>100
E. coli JR66/W677	3.12	3.12	*P. aeruginosa* K-Ps102	50	50
E. coli C600 R135	50	50	*P. maltophilia* GN907	>100	>100
E. coli JR225	1.56	3.12			

tional difference of amino group at C-1 position. Since chemical shifts of **III** were totally identical with those of dactimicin (DC; 2″-*N*-formimidoyl-FT-A) reported[2], it was assigned as DC. Substance **IV** was thereby determined as EDC (a novel antibiotic; Fig. 1). The ^1H chemical shifts of EDC (Table 2) were homologous to those of DC. Thus, our postulation that EDC would be expected by incubating *S. tenjimariensis* with FT-B was demonstrated. Conversion rate of FT-B to 1-*epi*-FT-B, DC and EDC was estimated as low as 1%.

EDC sulfate is a white powder melting at 205°C or higher with decomposition. Its optical rotation, $[\alpha]_D^{21}$, was +92° (*c* 0.15, H_2O), while that of DC, $[\alpha]_D^{21}$, was +114° (*c* 0.2, H_2O). Elemental analysis gave the following: Calcd for $C_{18}H_{36}N_6O_6 \cdot 2H_2SO_4 \cdot H_2O$: C 30.90, H 6.90, N 11.99. Found: C 30.78, H 7.01, N 12.00. Its molecular weight was determined as 432 from the result of secondary ion mass spectrum. Thus, the distinct difference in physico-chemical properties between DC and EDC were recognized in the ^{13}C NMR spectrum (see Table 1) and the optical rotation. In a comparative study both EDC and DC exhibited an identical broad antimicrobial spectrum (Table 3).

Substances **I** and **II** were also purified and analyzed for physico-chemical properties. Their physico-chemical properties were totally consistent with those of FT-B and its epimer, 1-*epi*-FT-B (data not shown).

Antibiotics accumulated by *M. olivasterospora* ATCC 21819 in a fermentation medium supplemented with IS-A_0 or -B_0[1] were also isolated. Antibiotics purified as converted products from IS-A_0 and -B_0 exhibited totally the same physico-chemical properties as those of IS-A_3 and -B_3[3,4], respectively (data not shown). These results were also predictable on the basis of the biosynthetic pathway of FT's by *M. olivasterospora*[1,5,6].

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