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Isolation and Structures of New Peptide Antibiotics, Cirratiomycin A and B†

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In the course of screening for antifolic metabolites using *Lactobacillus casei* as a test organism growing on a synthetic medium with a limiting amount of folic acid, two peptide antibiotic cirratiomycin A (**1**) and B (**2**) have been isolated from the culture filtrate of *Streptomyces cirratus* 248.-Sq 2 In this paper, we describe the isolation and structural elucidation of these antibiotics.

Streptomyces cirratus 248-Sq 2 was cultivated in a medium consisting of 2.0% soybean meal, 2.5% dextrin, 0.5% NaCl and 0.4% $CaCO_3$ in jar fermenters at 27°C for 72 hr. The filtered broth (50 liters) was treated with activated carbon (1%) and the carbon cake was eluted with 70% aqueous acetone (pH 3.0). After concentration, the eluate was passed through a column of Dowex 50 W-X 2 (H^+ form) and the column was developed with 5% pyridine. The active principles were separated by a series of column chromatographies using Dowex 50 W-X 2 (0.5 M pyridine-AcOH buffer, pH 6.4). Sephadex G-25 (partition, *n*-BuOH-AcOH-water 4 : 1 : 2)and Toyopearl HW 40 F to afford 50 mg of **1** and 30 mg of **2** in pure form.

1, an amorphous white powder (p*K*a′ 3.4, 6.7, 8.8), shows positive ninhydrin reaction (pale purple), $\lambda_{max}^{H_2O}$ 225 nm (ε 14400) and υ_{max}^{KBr} 1650 cm^{-1} suggesting the presence of peptide bonds. In the FD mass spectrum, two ion peaks were found at *m/z* 728 $(M+H)^+$ and 750 $(M+Na)^+$ indicating the molecular weight of 727 for **1**. Based on the elemental analysis and ^{13}C-NMR spectrum which revealed seven CO, one C=C and one C=N bonds, the molecular formula of monohydrochloride of **1** was deduced to be $C_{31}H_{53}N_9O_{11}\cdot HCl$ (calcd. C 48.72, H 7.07, N 16.50, O 23.05, Cl 4.65, obs. C 48.39, H 7.20, N 16.98, O 24.11, Cl 4.16%. 10-degree of unsaturation), indicating the presence of a ring structure in **1**.

1 (10 mg) was hydrolyzed with 6 N HCl at 120°C for 16 hr in a sealed tube. After removal of HCl *in vacuo*, the residue was charged on a column of Dowex 50 W-X 2 (pyridine-AcOH buffer, pH 6.4) to separate into a neutral amino acid mixture and one basic amino acid which was identified as a diastereomeric mixture of α,β-diaminobutyric acid (abbreviated as Dab) by ^1H-NMR.[1] The neutral amino acid fraction was successfully separated into four amino acids by a Sephadex G-25 partition column chromatography (*n*-BuOH-AcOH-water 4 : 1 : 2). The first three were identified as L-Leu, L-Ala, L-Ser and the last unknown amino acid was determined as hydroxymethylserine (HMSer) by

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direct comparison (^{1}H-NMR, amino acid analysis) with an authentic sample.[2] The amino acid analysis and ^{1}H-NMR spectrum of **1** indicated the equimolar composition of these five amino acids, while its ^{13}C-NMR spectrum suggested further existence of two acid-labile amino acids, temporarily designated as X and Y. The 400 MHz ^{1}H-NMR spectrum of **1** (DMSO-d_6) afforded four doublet α-amide protons, due to Leu, Ala, Ser and Dab as well as a singlet.

Treatment of **1** with acetic anhydride in MeOH (0°C for 30 min) gave *N*-monoacetyl cirratiomycin A (**3**) and *N*-diacetyl cirratiomycin A (**4**), and re-acetylation of **3** under same condition gave **4**. In the ^{1}H-NMR spectrum of **3** (D_2O) the β-methine proton of Dab was observed at 4.4 ppm which was shifted down-field from 4.0 ppm of the parent antibiotic, indicating the β-amino group of Dab to be free. On the other hand, the ^{1}H-NMR spectrum of **4** resembled very closely to that of **3** suggesting that the second acylation should occur at a free amino group attached to a quaternary carbon. In agreement with this, a new singlet amide proton appeared in the spectrum of **4** in DMSO-d_6, together with the doublet of β-amide proton of Dab. Treatment of **1** with dansyl chloride (DNS-Cl) at pH 9.5 only dansylated the β-amino group of Dab but not the other one.

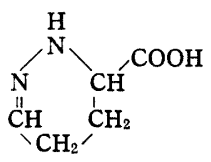
Mild acid hydrolysis of **1** (6 N HCl, 37°C, 7 days) and subsequent purification by column chromatography using Toyopearl HW 40 F and Dowex 50 W-X 2 (pyridine-AcOH buffer, pH 6.4) afforded four peptide fragments (**5**~**8**) containing X or Y in intact form. The N-terminal residues of these fragments were determined by DNS method.

HMSer-Ala	**5**
Dab-X-Leu	**6**
Leu-Y	**7**
Leu-Y-Ser	**8**

Dansylated **5** could not be obtained due probably to steric hindrance around the amino group of HMSer. This result indicated that the α-amino group of Ala was acylated in this dipeptide. Isolation of these peptide fragments together with the fact that the α-amino group of Dab was protected in the parent antibiotic, established the amino acid sequence of **1** as

HMSer-Ala-Dab-X-Leu-Y-Ser

In addition to these fragments, Dab was isolated without any racemization and identified to take an L-*erythro* configuration by ORD and ^{1}H-NMR specta.[1] The FD mass of **6** exhibited $(M+Na)^+$ ion peaks at *M/z* 342 and 364, respectively, indicating the molecular weight of 341, and further spectral data allowed to assign the molecular formula of X as



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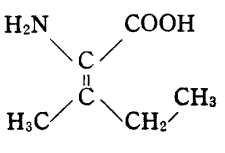

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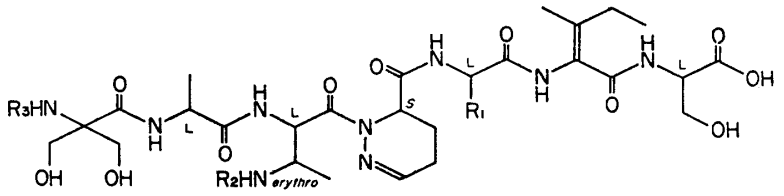

	R_1	R_2	R_3
1	$CH_2CH(CH_3)_2$	H	H
2	CH_3	H	H
3	$CH_2CH(CH_3)_2$	Ac	H
4	$CH_2CH(CH_3)_2$	Ac	Ac

Fig. 1. The Structures of Cirratiomycin A, B and Derivatives.

$C_5H_8N_2O_2$. Catalytic hydrogenation of **6** over PtO_2 in 1 N AcOH (room temperature, 5 days) followed by hydrolysis gave Leu, Dab and L-ornithine which was identified by TLC, amino acid analysis and ORD. This result and the ^{1}H-NMR spectrum of **6** showing the partial structure -CH-CH_2-CH_2-CH= for X, proved the unknown amino acid to be 2,3,4,5-tetrahydropyridazine-3-carboxylic acid (THPC) **9**. Thus, the structure of **6** was elucidated as L-*erythro* Dab-(S)-THPC-L-Leu.

The EI mass spectrum of *N*-acetyl derivative of **7** (*m/z* 284) suggested the molecular formula of $C_6H_{11}NO_2$ for Y and the ^{1}H-NMR spectral analysis of **7** revealed the structure of Y as 2,3-didehydroisoleucine (⊿Ile, **10**). Hydrogenation of 8 over PtO_2 in 1 N AcOH (room temperature, 5 days) followed by acid hydrolysis gave Leu, Ser and Ile, confirming the above conclusion and the sequence of 8 as Leu-⊿Ile-Ser.

Upon irradiation of an amide proton (9.31 ppm singlet, assignable to the amide proton of ⊿Ile) in the ^{1}H-NMR spectrum (DMSO-d_6), NOE effect was observed in singlet methyl protons of ⊿Ile indicating its configuration to be *E*. These experimental results confirmed the linear structure for **1** having the N-terminal of HMSer which could not be dancylated because of its presumable steric hindrance, and the total structure of cirratiomycin A has been determined as described in **1**.

Cirratiomycin B (**2**) has closely related properties with **1** and the ^{1}H-NMR and ^{13}C-NMR spectra were essentially superimposable to **1**, except the presence of Ala moiety instead of Leu. Mild acid hydrolysis of **2** gave amino acid segments HMSer-Ala, Dab-X-Ala and Ala-Y-Ser, and comfirmed its steucture as shown in Fig. 1.

CirratiomycinA and B are active against a narrow range of *Lactobacillus* including *L. casei* and some strains of *Streptococci* and *Mycobacterium* but substantially inactive against filamentous fungi and yeasts. Detailed data will be published successively.

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