

Title	Isolation and structures of new peptide antibiotics, cirratiomycin A and B
Sub Title	
Author	城座, 映明(Shiroza, Teruaki) 海老沢, 直之(Ebisawa, Naoyuki) コジマ, アツコ(Kojima, Atsuko) 降旗, 桂子(Furihata, Keiko) 島津, 昭(Shimazu, Akira) 遠藤, 豊成(Endo, Toyoshige) 瀬戸, 治男(Seto, Haruo) 大岳, 望(Otake, Noboru)
Publisher	共立薬科大学
Publication year	1982
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.27 (1982.) ,p.58- 60
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000027-0058

慶應義塾大学学術情報リポジトリ(KOARA)に掲載されているコンテンツの著作権は、それぞれの著作者、学会または出版社/発行者に帰属し、その権利は著作権法によって保護されています。引用にあたっては、著作権法を遵守してご利用ください。

The copyrights of content available on the Keio Associated Repository of Academic resources (KOARA) belong to the respective authors, academic societies, or publishers/issuers, and these rights are protected by the Japanese Copyright Act. When quoting the content, please follow the Japanese copyright act.

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

Teruaki SHIROZA*, Naoyuki EBISAWA,* Atsuko KOJIMA,
Keiko FURIHATA*, Akira SHIMAZU*, Toyoshige ENDŌ,
Haruo SETO* and Noboru ŌTAKE*

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 cirratimycin 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% CaCO₃ 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 (pK_a' 3.4, 6.7, 8.8), shows positive ninhydrin reaction (pale purple), $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 225 nm (ϵ 14400) and $\nu_{\text{max}}^{\text{KBr}}$ 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 ¹³C-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 C₃₁H₅₃N₉O₁₁·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

† Agr. Biol. Chem. **46** (3), 865~7 (1982) に発表.

* Institute of Applied Microbiology, The University of Tokyo.

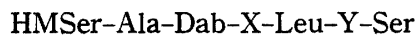
direct comparison (^1H -NMR, amino acid analysis) with an authentic sample.²⁾ The amino acid analysis and ^1H -NMR spectrum of **1** indicated the equimolar composition of these five amino acids, while its ^{13}C -NMR spectrum suggested further existence of two acid-labile amino acids, temporarily designated as X and Y. The 400 MHz ^1H -NMR spectrum of **1** ($\text{DMSO}-d_6$) 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 ^1H -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 ^1H -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 $\text{DMSO}-d_6$, 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.

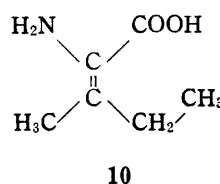
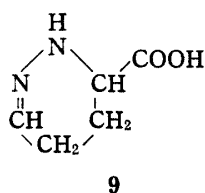
Mild acid hydrolysis of **1** (6 *N* HCl, 37°C , 7 days) and subsequent purification by column chromatography using Toyopearl HW 40 F and Dowex 50 W-X 2 (pyridine-AcOH buffer, pH 6.4) afforded four peptide fragments (**5**~**8**) containing X or Y in intact form. The N-terminal residues of these fragments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

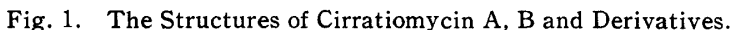
HMSer-Ala	5
Dab-X-Leu	6
Leu-Y	7
Leu-Y-Ser	8

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



In addition to these fragments, Dab was isolated without any racemization and identified to take an *L-erythro* configuration by ORD and ^1H -NMR spectra.¹⁾ The FD mass of **6** exhibited $(\text{M}+\text{Na})^+$ 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





The EI mass spectrum of *N*-acetyl derivative of **7** (*m/z* 284) suggested the molecular formula of C₆H₁₁NO₂ for **Y** and the ¹H-NMR spectral analysis of **7** revealed the structure of **Y** as 2,3-didehydroisoleucine (*Δ*Ile, **10**). Hydrogenation of **8** over PtO₂ 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-*Δ*Ile-Ser.

Cirratiomycin B (**2**) has closely related properties with **1** and the ^1H -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 confirmed its structure as shown in Fig. 1.

Reference

- 60 —