STRUCTURE OF LEUCINOSTATIN B, AN UNCOUPLER ON MITOCHONDRIA

Sir:

Peptide antibiotic leucinostatin, isolated from the culture filtrate of *Paecilomyces lilacinus* A-267, was originally reported as a single compound.[1] However, in the course of structural study, it was found to be a complex of two components, leucinostatins A and B which are active against yeasts, both pathogenic and non-pathogenic strains, and filamentous fungi as well as Gram-positive bacteria. Both antibiotics act as uncouplers of oxidative phosphorylation.

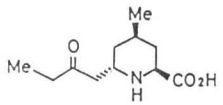
We have recently reported the structure of leucinostatin A[2,3] that contains unusual amino acids: *cis*-4-methyl-L-proline (MePro), L-*threo*-β-hydroxyleucine (HyLeu), α-aminoisobutyric acid (Aib), and 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD). KENNER *et al.*[4] and ISOGAI *et al.*[5,6] isolated the antibiotics No. 13959 and P168, respectively, which had the same amino acids as those of leucinostatin A. The latter was identified with leucinostatin A. Another isolation of peptide antibiotic No. 1907-II[7] having the same components as with leucinostatin B, whose sequence except for stereochemistry was determined only by in-beam EI mass spectrometry, prompted us to report the structure of leucinostatin B including the absolute stereochemistry and the uncoupling effect on mitochondria.

Leucinostatin B (**1**) was obtained as amorphous powder, mp 132~140°C; $[\alpha]_D^{20}$ −30.8° (*c* 0.09, MeOH); λ_{max} (EtOH) 204 (log ε 4.38) and 213 nm (log ε 4.27, sh); ν_{max} ($CHCl_3$) 3310 (NH and OH), 1705 (CO), and 1655 cm^{-1} (amide CO); and showed positive reactions for ninhydrin and Dragendorff reagents, suggesting it a basic peptide antibiotic. The composition $C_{81}H_{109}N_{11}O_{13}\cdot HCl$ was established by elemental analysis and fast atom bombardment (FAB) and field desorption (FD) mass spectometric analyses [*m/z* 1,204 (MH^+)].

The ^1H and ^{13}C NMR spectra of **1** exhibited signals assignable to an *N*-methyl group (δ_H 2.46, s), a γ-monosubstituted α,β-unsaturated amide (δ_H 6.18, d, J=16 Hz, and 6.86, dd, J=16 and 8 Hz; δ_C 120.1, d, 151.9, d, and 166.9, s), and a ketone (δ_C 211.7) as well as amides (δ_C 176.8, 176.4, 175.7, 175.2, 174.3, 173.9, and 172.6). Considering the intense absorptions of the signals at δ_C 175.2 and 173.9, the antibiotic contains ten amide carbonyls.

Hydrolysis of leucinostatin B (**1**) in refluxing 6 N HCl (110°C, 20 hours) followed by cellulose column chromatography yielded six amino acids: MePro[8,9], HyLeu[10], Aib, L-leucine (Leu), β-alanine (β-Ala), and (2*S*,4*R*,6*S*)-4-methyl-6-(2-oxobutyl)-2-piperidinecarboxylic acid (**2**)[2,3,5,6,11] and its C-6 epimer[5,6], and an amine: (2*S*)-N^1-methyl-propane-1,2-diamine (**3**). The *S*-configuration of **3** was established by comparison of the N^1,N^2-diacetyl derivative (**4**) with an authentic sample prepared from BOC-alanine by successive treatments with (i) $ClCO_2Et$, (ii) $MeNH_2$, (iii) CF_3CO_2H, (iv) $LiAlH_4$, (v) $(CH_3CO)_2O$/pyridine. From the diethyl ether-soluble fraction of the hydrolysate were isolated (4*S*)-(2*E*)-4-methylhex-2-enoic acid (**5**)[2,3] and its acid-catalyzed lactonization product, 4-hydroxy-4-methylhexanoic-1,4-lactone (**6**).

Partial hydrolysis [conc. HCl - HCO_2H (1: 1), 37°C, 30 hours] of **1** followed by partition afforded mainly two fragments (**7**) and (**8**) from the ethyl acetate-soluble fraction and three fragments (**9**), (**10**), and (**11**) from the water-soluble fraction. The fragment **7** consisted of MePro and the acid



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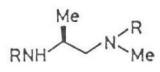
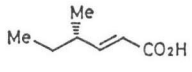


3 R=H
4 R=COCH3



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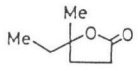

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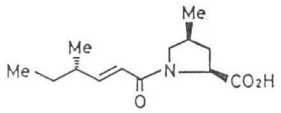

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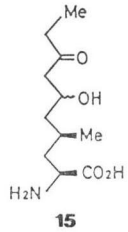

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Fig. 1. CI Mass spectrum of the methyl ester of the fragment **8**.

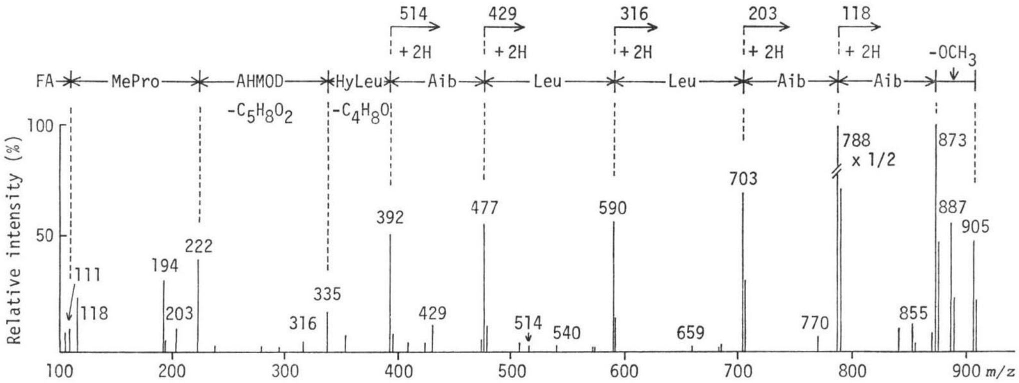


Fig. 2. Sequences of the fragments (**7**)~(**13**).

FA=(4*S*)-(2*E*)-4-methylhex-2-enoic acid. X=(2*S*)-N^1-methylpropane-1,2-diamine. Singly underlined: mass spectrometric analysis. Doubly underlined: dansylation. Arrow: dansyl-Edman analysis. * Detected after acid hydrolysis.

7 FA-MePro

8 FA-MePro-[AHMOD+HyLeu]-Aib-Leu-Leu-Aib-Aib

9 Leu-Aib-Aib-[β-Ala+X]*

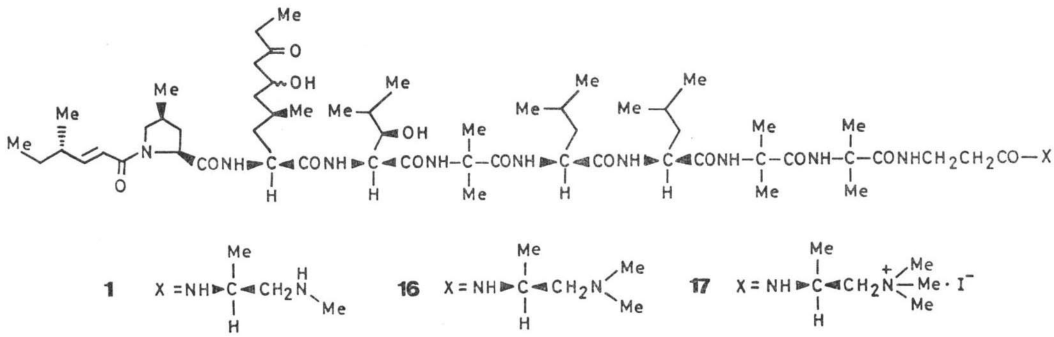
10 Aib-Aib-[β-Ala+X]*

11 β-Ala-X

12 HyLeu-Aib-Leu-Leu-Aib-Aib-β-Ala-X-Ac

13 Leu-Aib-Aib-β-Ala-X-Ac

14 FA-MePro-[Y]-HyLeu-Aib-Leu-Leu-Aib-Aib-β-Ala-X



(**5**), and found to be the *N*-terminal moiety of leucinostatin B. Fig. 1 shows the chemical ionization mass spectrum of the methyl ester of **8**, which enabled to established the amino acid sequence of **8** except for that of [AHMOD+HyLeu]. Sequences of **9**, **10**, and **11** were determined by dansyl-Edman degradation and dansylation. Another partial hydrolysis (6 N HCl, 25°C, 20 hours) of the acetylated compound of **1** gave the oligopeptides (**12**) and (**13**). The sequences determined are summarized in Fig. 2.

Replacement of Y in the sequence (**14**) by the amino acid (**2**) constitutes a peptide, $C_{61}H_{107}N_{11}O_{12}$, which corresponds to the dehydration product of **1** and suggests that **2** must present as the amino acid (AHMOD; **15**) as in the case of leucinostatin A (**16**).[2,3] Moreover, the *C*-terminal amine (**3**) has the possibility of linking to β-Ala in two ways. In oder to establish the structure unambiguously, leucinostatin B (**1**) was selectively

N-methylated. Treatment of **1** with methyl iodide in ethanol gave the trimethylammonium compound (**17**) (δ_H 3.27, s; δ_C 53.8, s; $-N(CH_3)_3$). The secondary ion mass spectrum (SIMS) of **17** showed the ion peak at *m/z* 1,232, corresponding to the mass of the cationic part of **17**. The ammonium compound was identified with the *N*-methylated compound of leucinostatin A (**16**), obtained by treatment with methyl iodide in ethanol, by the spectral (IR, ^1H, and ^{13}C NMR, and SIMS) comparisons including the specific rotation. These results revealed the structure of leucinostatin B which was depicted as **1**.

In the study on biological activity, leucinostatins A and B showed the uncoupling effect on mitochondria. Both of antibiotics stimulated the respiration of the state four on rat liver mitochondria using succinate as a substrate. The minimum concentration to obtain the effect was 0.3 μg/ml with leucinostatin A, and 0.9 μg/ml with leucinostatin B. Thus, it was concluded that leucinostatins A and B act as uncouplers on mitochondria. We will report on the outcome of these investigations in the near future.

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References

1) ARAI, T.; Y. MIKAMI, K. FUKUSHIMA, T. UTSUMI & K. YAZAWA: A new antibiotic, leucinostatin, derived from *Penicillium lilacinum*. J. Antibiotics 26: 157~161, 1973
2) MORI, Y.; M. TSUBOI, M. SUZUKI, K. FUKUSHIMA & T. ARAI: Isolation of leucinostatin A and one of its constituents, the new amino acid, 4-methyl-6-(2-oxobutyl)-2-piperidinecarboxylic acid, from *Paecilomyces lilacinus* A-267. J. Antibiotics 35: 543~544, 1982
3) MORI, Y.; M. TSUBOI, M. SUZUKI, K. FUKUSHIMA & T. ARAI: Structure of leucinostatin A, new peptide antibiotic from *Paecilomyces lilacinus* A-267. J. Chem. Soc., Chem. Commun. 1982: 94~96, 1982
4) KENNER, G. W. & R. C. SHEPPARD: α-Aminoisobutyric acid, β-hydroxyleucine, and γ-methylproline from the hydrolysis of a natural product. Nature 181: 48, 1958
5) ISOGAI, A.; A. SUZUKI, S. HIGASHIKAWA, S. KUYAMA & S. TAMURA: Constituents of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (THOM) Samson. Agric. Biol. Chem. 44: 3029~3031, 1980
6) ISOGAI, A.; A. SUZUKI, S. HIGASHIKAWA, S. KUYAMA & S. TAMURA: Structure of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (THOM) Samson. Agric. Biol. Chem. 44: 3033~3035, 1980
7) SATO, M.; T. BEPPU & K. ARIMA: Properties and structure of a novel peptide antibiotic No. 1907. Agric. Biol. Chem. 44: 3037~3040, 1980
8) ABRAHAM, R. J.; K. A. MCLAUCHLAN, S. DALBY, G. W. KENNER & R. C. SHEPPARD: Structures and conformations of some naturally occurring derivatives of proline. Nature 192: 1150~1153, 1961
9) DALBY, J. S.; G. W. KENNER & R. C. SHEPPARD: Peptide. XII. The stereoisomers of 4-methylproline. J. Chem. Soc. 1962: 4387~4396, 1962
10) DALBY, S.; G. W. KENNER & R. C. SHEPPARD: Peptide. X. β-Hydroxyleucine. J. Chem. Soc. 1960: 968~973, 1960
11) FUJITA, T.; Y. TAKAISHI, A. OKAMURA, E. FUJITA, K. FUJI, N. HIRATSUKA, M. KOMATSU & I. ARITA: New peptide antibiotics, trichopolyns I and II, from *Trichoderma polysporum*. J. Chem. Soc., Chem. Commun. 1981: 585~587, 1981