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

Sir:

A new antibiotic, leucinostatin, was isolated from the culture filtrate of *Paecilomyces lilacinus* A-267. The peptide antibiotic has antitumor activity on Ehrlich solid carcinoma, antimicrobial activity against Gram-positive bacteria and a wide range of fungi.[1] Leucinostatin was found to be a mixture of several components which were separated by alumina column chromatography to give mainly leucinostatins A and B.

In this communication we describe the properties of leucinostatin A and the structure elucidation of one of its components, a novel amino acid.

Leucinostatin A (**1**), mp 98~101°C; $[\alpha]_D^{20}$ −11.0° (*c* 0.1, MeOH); λ_{max} (EtOH) 202 and 220 (sh) nm; ν_{max} ($CHCl_3$) 3280, 1705, and 1645 cm^{-1}, has the molecular formula $C_{62}H_{111}N_{11}O_{13}$ confirmed by field desorption mass spectrometry [*m/z* 1218 (MH^+)]. The ^1H and ^{13}C NMR spectra of **1** exhibited the presence of an *N,N*-dimethylamino moiety (δ_H 2.37, s), a γ-monosubstituted α,β-unsaturated amide (δ_H 6.19, d, J=16 Hz, and δ_C 120.9, d; δ_H 6.86, dd, J=16 and 8 Hz, and δ_C 150.6, d), a ketone (δ_C 211.0, s), and amides (δ_C 160~180, about 8×s). Leucinostatin A gives no reaction with ninhydrin, but a positive Dragendorff reaction. These data indicated that **1** is a basic peptide antibiotic.

Acid hydrolysis of leucinostatin A with 6 N HCl (110°C, 20 hours) followed by chromatography on a cellulose column (BuOH - AcOH - H_2O, 4: 1: 2) afforded an unidentified amino acid (**2**) as well as other amino acids; L-leucine, L-*threo*-β-hydroxyleucine,[2] *cis*-4-methyl-L-proline,[3] α-aminoisobutyric acid, β-alanine, and (*S*)-N^1,N^1-dimethylpropane-1,2-diamine.

The amino acid (**2**) gave a faint-yellow color with ninhydrin. Recrystallization from $CHCl_3$ - acetone gave the pure amino acid (**2**), colorless prisms, mp 197~199°C (decomp.); $[\alpha]_D^{22}$ +8.9° (*c* 0.09, MeOH); FDMS *m/z* 214 (MH^+). The IR spectrum shows absorptions at 3300~2400, 1718 (ketone), and 1630 cm^{-1} (carboxylate). The 400 MHz ^1H NMR spectrum (Fig. 1) consists of a series of well resolved peaks, which allow the assignment of all the hydrogens. Their coupling partners were assigned by proton spin-decoupling experiments and the results are summarized in the Table 1.

The unusually low chemical shift for C-6-H is probably due, in part, to the protonated nitrogen atom next to C-6. The equatorial protons at C-3 and C-5 were confirmed by the observation of W-letter long range coupling. Thus, irradiation of the signal at δ 1.72 leads the signal at δ 2.20 to a clean splitting (ddd, J=14.4, 3.7, and 3.4 Hz). On the basis of these results, the structure of the unidentified amino acid is that of 4-methyl-6-(2-oxobutyl)-2-piperidinecarboxylic acid (**2**). The substituents at C-2 and C-4 could be assigned to be as equatorial and that at C-6 as axial.

Fig. 1. 400 MHz ^1H NMR spectrum of the amino acid (**2**) in D_2O.
Chemical shifts are in parts per million (δ) from internal $(CH_3)_3Si(CH_2)_3SO_3Na$ (DSS).

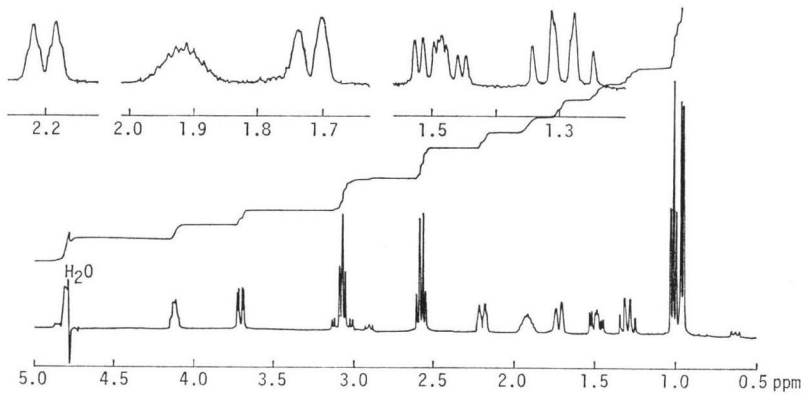
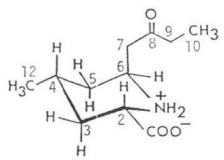


Table 1. Chemical shifts and proton coupling constants of the amino acid (**2**).

Proton (C-No)	Chemical shift (δ)	Coupling constant (Hz)
H(2)	3.70	$J_{2,3}$=12.2 and 3.7
H_{ax}(3)	1.29	J_{gem}=14.4, $J_{2,3}$=12.2, $J_{3,4}$=11.2
H_{eq}(3)	2.20	J_{gem}=14.4, $J_{2,3}$=3.7, $J_{3,4}$=3.4
H(4)	1.92	$J_{3,4}$=11.2 and 3.4, $J_{4,5}$=11.7 and 3.4, $J_{4,12}$=6.6
H_{ax}(5)	1.49	J_{gem}=14.4, $J_{4,5}$=11.7, $J_{5,6}$=4.9
H_{eq}(5)	1.72	J_{gem}=14.4, $J_{4,5}$=3.4, $J_{5,6}$=2.6
H(6)	4.12	$J_{5,6}$=4.9 and 2.6, $J_{6,7}$=6.8 and 6.6
H(7)	3.05	J_{gem}=18.3, $J_{6,7}$=6.8
	3.10	J_{gem}=18.3, $J_{6,7}$=6.6
H(9)	2.57	$J_{9,10}$=7.1
H(10)	1.20	$J_{9,10}$=7.1
H(12)	0.96	$J_{4,12}$=6.6



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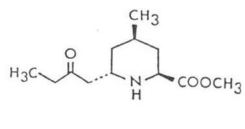

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The structure assignment is supported by the chemical ionization mass spectrometric analysis of the methyl ester (**3**). Only two fragment ions were observed at *m/z* 168 and 156 which correspond to the ions $MH^+ - CH_3OH - CO$ and $MH^+ - C_4H_8O$, respectively.

The structure of **2** reported herein was found to be identical with one of the amino acids of trichopolyns (trichoponamic acid),[4] but no precise ^1H NMR data have been reported,[4] whereas those of the C-6-epimer of **2** from antibiotic P 168 are available.[5] Thus, the ^1H NMR spectrum of **2** revealed complete agreement with the assigned structure.

The absolute configuration of **2** was established to be 2*S*, 4*R*, 6*S* by comparison with the optical rotations of **2** and trichoponamic acid.[4]

In a future communication[6] we will describe the full structure of leucinostatin A.

We thank for Prof. T. FUJITA for a generous gift of trichoponamic acid.

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(Received December 14, 1981)

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