Structure of Leucinostatin A, New Peptide Antibiotic from *Paecilomyces lilacinus* A-267

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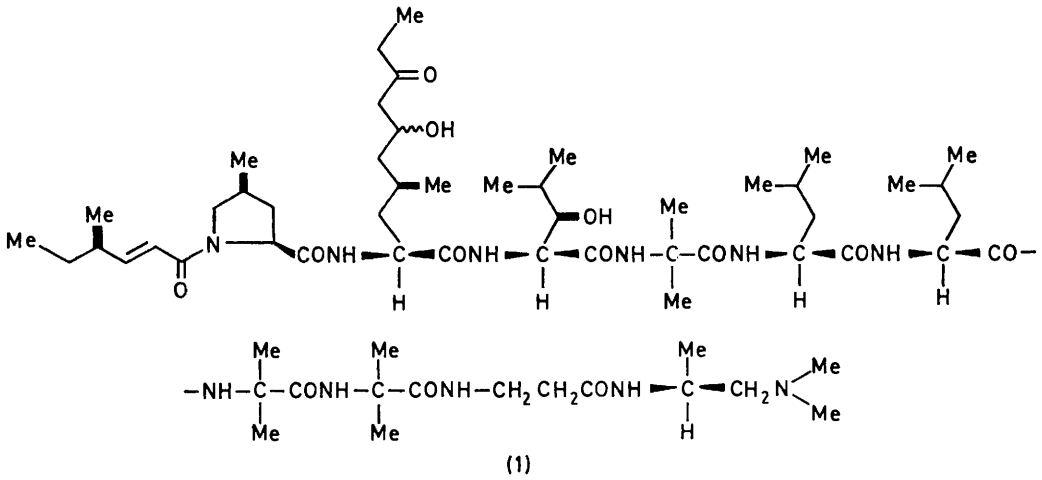
A new antibiotic leucinostatin A was isolated from the culture filtrate of *Paecilomyces lilacinus* A-267 and its structure was elucidated by mass spectrometric and degradative methods.

The peptide antibiotic leucinostatin isolated from *Paecilomyces lilacinus* A-267 has aroused considerable interest owing to its antitumour activity on Ehrlich solid carcinoma and antibacterial activity against Gram-positive bacteria and a wide range of fungi.[1] A structural study revealed leucinostatin to be a new basic peptide composed of unusual amino-acids: *cis*-4-methyl-L-proline (MePro),[2] L-*threo*-β-hydroxyleucine (HyLeu),[3] and α-aminoisobutyric acid (Aib). In an independent study, Kenner *et al.*[4] reported the isolation of antibiotic I.C.I. No. 13959 which contains the same amino-acids as leucinostatin but which has not yet been characterized.

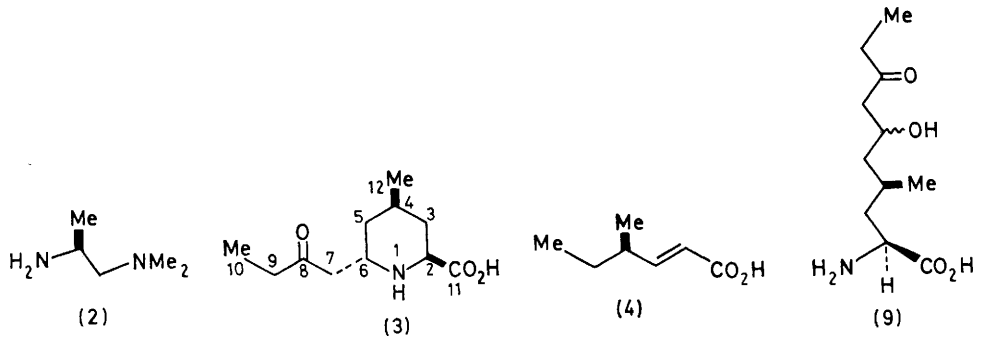
Leucinostatin is a mixture of several components which were separated by alumina column chromatography to give mainly leucinostatin A and B. We report here the structure of leucinostatin A.

Leucinostatin A (**1**), $C_{62}H_{111}N_{11}O_{13}$; m.p. 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 (NH), 1705 (CO), and 1645 (amide CO) cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 3·10 (*N*,*N*-dimethyl); ^{13}C n.m.r. ($CDCl_3$) δ 211·0 (s, CO), 180—160 (*ca.* 8 × s, amide CO), and 150·6 and 120·9 p.p.m. (each d, C=C), has a molecular weight of 1217 from its field desorption mass spectrum [f.d.m.s. *m/z* 1218 (MH^+)] and showed a negative reaction for ninhydrin, but a positive Dragendorff reaction. These data indicated that (**1**) is a basic peptide antibiotic with one ketone carbonyl, one conjugated double bond, and dimethylamino-groups.

Acid hydrolysis (6N HCl, 110 °C, 20 h) of (**1**) followed by amino-acid analysis gave the following results: $(HyLeu)_1$ $(Aib)_{2-3}$ $(Leu)_{2-3}$ $(\beta\text{-Ala})_1$ $(MePro)_1$. Cellulose column chromatography of the hydrolysate gave (*S*)-N^1,N^1-dimethylpropane-1,2-diamine (**2**)·2HCl, m.p. 115—117 °C: $[\alpha]_D^{20}$ +9·8° (*c* 0·12, MeOH); chemical ionization (c.i.) m.s. *m/z* 103 (MH^+); ^1H n.m.r. ($CDCl_3$) δ 1·50 (3H, d, *J* 6 Hz), 3·00 (6H, s), 3·25—3·65 (2H, m), and 3·95 (1H, m), and an unidentified amino-acid (**3**). The *S*-configuration of (**2**) was established by comparison with an authentic sample prepared from Boc-Ala by successive treatment with i, $ClCO_2Et$, ii, $HNMe_2$, iii, CF_3CO_2H, and iv, $LiAlH_4$. The ^1H n.m.r., i.r., and mass



(1)



(2) (3) (4) (9)

spectra of the amino-acid (3)† revealed that (3) is 4-methyl-6-(2-oxobutyl)-2-piperidinecarboxylic acid whose stereochemistry was established by proton spin-decoupling experiments. The structure of (3) corresponds to trichoponamic acid obtained by the hydrolysis of trichopolyns.[5]

From the diethyl ether extract of the hydrolysate was isolated (*S*)-(*E*)-4-methylhex-2-enoic acid (**4**), $[\alpha]_D^{20}$ + 49·7° (*c* 0·25, $CHCl_3$); *m/z* 128 ($M^{+\cdot}$); λ_{max} (EtOH) 207 nm; ν_{max} ($CHCl_3$) 3600—2400 (OH), 1685 (CO), and 1640 (C=C) cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 0·89 (3H, t, *J* 7 Hz), 1·05 (3H, t, *J* 7 Hz), 1·43 (2H, q, *J* 7 Hz), 2·26 (1H, m), 5·77 (1H, d, *J* 16 Hz), and 6·98 (1H, dd, *J* 16, 8 Hz). Catalytic hydrogenation of (**4**) afforded a saturated acid, $[\alpha]_D^{20}$ + 7·6° (*c* 0·15, $CHCl_3$), which is identical to (*S*)-4-methylhexanoic acid (lit.,[6] $[\alpha]_D^{20}$ +7·4°). Since leucinostatin A (**1**) is negative for ninhydrin and methylation with CH_2N_2 recovered the starting material the *C*- and *N*-termini of (**1**) could be protected by the diamine (**2**) and the fatty acid (**3**), respectively. The u.v. absorptions and ^{13}C n.m.r. chemical shifts of (**1**) at 150·6 and 120·9 p.p.m. are, therefore, ascribed to the *N*-terminal α,β-unsaturated amide structure.

Partial hydrolysis (6N HCl, room temp., 40 h) of (**1**) gave mainly two peptides (**5**) and (**6**), and the diamine (**2**). Hydrolysis with 2N HCl (reflux, 2 h) afforded fragments (**7**) and (**8**), the one containing the *N*-terminal fatty acid and the other the *C*-terminal diamine. Sequences of the fragments (**5**)—(**8**) were determined by dansylation, dansyl–Edman degradation, c.i.m.s., and ^1H n.m.r. spectroscopy. The results are summarized in Figure 1. As the *C*- and *N*-termini of leucinostatin A were blocked with X[=(**2**)] and FA[=(**4**)], respectively, the amino-acid (**3**) should be placed between the fragments (**5**) and (**7**). The above-mentioned components constitute a peptide, $C_{62}H_{109}N_{11}O_{12}$, which corresponds to the dehydration product of leucinostatin A (**1**).

(**5**) HyLeu → Aib → Leu → Leu-Aib-Aib-β-Ala-X

(**6**) Leu → Aib → Aib-β-Ala-X

(**7**) FA-MePro (**8**) β-Ala-X

Figure 1. Sequences of the fragments (**5**)—(**8**) obtained by partial hydrolyses of (**1**). The methods of determination are indicated as follows; singly underlined: mass spectrometry, doubly underlined: dansylation, arrow: dansyl–Edman analysis. FA = (*S*)-(*E*)-4-methylhex-2-enoic acid (**4**): X = (*S*)-N^1,N^1-dimethylpropane-1,2-diamine (**2**).

Alumina treatment of the diacetyl compound obtained by acetylation of (**1**) gave the *O*-monoacetyl derivative, f.d.m.s. *m/z* 1264 (*M* + Na^+) and 1242 (MH^+); ν_{max} ($CHCl_3$) 1745, 1680, and 1660 cm^{-1}; ^{13}C n.m.r. ($CDCl_3$) δ 119·3 (d), 131·6 (d), 144·2 (d), 153·9 (d), and 200·8 (s) p.p.m. The chemical shifts at 131·6, 144·2, and 200·8 p.p.m. can be ascribed to the newly formed α,β-unsaturated ketone system.

These data suggested that the amino-acid (**3**) is present in (**1**) as 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (**9**), which, upon hydrolysis, is converted into an α,β-conjugated ketone by elimination of water and then cyclized to (**3**) by Michael addition.[5] On the basis of these results, the structure of leucinostatin A can be represented as (**1**).‡

† (**3**): m.p. 197—199 °C (decomp.); $[\alpha]_D^{22}$ + 8·9° (*c*. 0·09, MeOH); c.i.m.s. *m/z* 214 (MH^+); ν_{max} ($CHCl_3$) 3300—2400, 1718, and 1630 cm^{-1}; 400 MHz ^1H n.m.r. (D_2O) δ 0·96 (d, *J* 6·6 Hz), C-12-Me), 1·02 (t, *J* 7·1 Hz, C-10-Me), 1·29 (q, *J* 14·4 Hz, C-3-H_{ax}), 1·49 (ddd, *J* 14·4, 11·7, and 4·9 Hz, C-5-H_{ax}), 1·72 (d, *J* 14·4 Hz, C-5-H_{eq}), 1·92 (m, C-4-H_{ax}), 2·20 (d, *J* 14·4 Hz, C-3-H_{eq}), 2·57 (q, *J* 7·1 Hz, C-9-H), 3·05 (dd, *J* 18·3 and 6·8 Hz, C-7-H), 3·10 (dd, *J* 18·3 and 6·6 Hz, C-7-H), 3·70 (dd, J 12·2 and 3·7 Hz, C-2-H_{ax}), and 4·12 (m, C-6-H_{eq}).

‡ Recently the isolation of peptide antibiotics from the *Paecilomyces lilacinus* strain have been reported; see A. Isogai, A. Suzuki, S. Higashikawa, S. Kuyama, and S. Tamura, *Agric. Biol. Chem.*, 1980, **44**, 3029 and 3033; M. Sato, T. Beppu, and K. Arima, *Agric. Biol. Chem.*, *ibid.*, p. 3037.

It is interesting that the amino-acids contained in leucinostatin A are unusual and that the *C*-terminal linkage of the propanediamine (**2**) of the antibiotic has not been found previously.

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