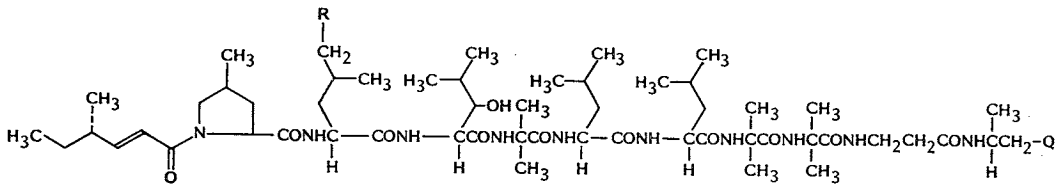
LEUCINOSTATINS H AND K, TWO NOVEL PEPTIDE ANTIBIOTICS WITH TERTIARY AMINE-OXIDE TERMINAL GROUP FROM *PAECILOMYCES MARQUANDII* ISOLATION, STRUCTURE AND BIOLOGICAL ACTIVITY

Sir:

In a recent communication[1] we reported the isolation and structure elucidation of leucinostatin D, a minor, biologically active, peptide component of the antibiotic mixture produced by submerged cultures of *Paecilomyces marquandii* (Massee) Hughes. In a search for further biologically active metabolites, the dark-brownish oily residue from the benzene extract of the culture broth was subjected to repeated flash chromatography on silica gel colums using $CHCl_3$ as the eluent to which MeOH and NH_3 were added in continuously increasing proportions up to final composition $CHCl_3$ - MeOH - NH_3, 80:18:2. At the end of this procedure, a fractions was obtained that consisted mainly of two new peptidic components we have labeled leucinostatins H and K. These were separated and purified through repeated flash chromatography under N_2 atmosphere using final solvent mixture $CHCl_3$ - MeOH - NH_3 as the eluent. Evaporation of the solvents gave leucinostatin H (**1**, Rf 0.4) as a white, partly crystalline material (mp 166~168°C) and leucinostatin K (**2**, Rf 0.45) as white amorphous solid (mp 138~141°C). (Rf values refer to the final solvent mixture.)

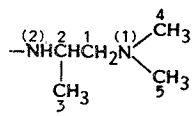
While the fourier transform (FT) IR and UV/vis spectra of the new metabolites provided values very similar to those obtained for other leucinostatin components described earlier[1~3], fast atom bombardment (FAB) mass spectra, remarkably displaying the M+H ions only, suggested that peptides **1** [*m/z* (nominal mass) 1,134] and **2** (*m/z* 1,234) might correspond to leucinostatins D (**3**, *m/z* 1,118)[1] and A (**4**, *m/z* 1,218)[2,3] with an additional oxygen atom in the molecules.

Supporting evidence for this structural relationship and information as to the site of oxidation were conveniently inferred from the high-field NMR spectra. Detailed analysis of the conventional (1D) and two-dimensional (2D) ^{13}C (100 MHz) and ^{1}H (400 MHz) spectra disclosed that **1** and **3** and, in a similar manner, **2** and **4** consist, pairwise, of the same amino acid residues arranged in identical sequences. Multiplicity-selected carbon-13 spectra, on the other hand, revealed that incorporation of the extra oxygen atom into the metabolites entails no changes in the total number of carbon-bonded hydrogen atoms, a finding that suggests *N*-oxidation. From the comparison of the fully assigned ^{13}C and ^{1}H chemical shift data of **1** and **2** with those of their assumed non-oxygenated counterparts, **3** and **4**, it became evident that the oxidation-induced changes in the chemical shifts are restricted to nuclei in the 1-(dimethylamino)-2-aminopropane unit. More specifically, as attested by the chemical shift data in Table 1, we found that, *e.g.* from **3** to **1**, the chemical shift values of carbon atoms located in this part of the molecule undergo variations typical of oxygen substitution at the quaternary nitrogen atom. (Note the characteristic β, γ and δ substituent effects at the pertinent carbon sites.) Less distinct, but equally characteristic, are the changes observed for the chemical shifts of protons in this moiety. Here, we found ~0.3 ppm downfield shift of resonances due to protons in β-position relative to the site of oxidation, again an effect best accounted for by considering oxygen substitution of the (protonated) quaternary nitrogen atom. A practically identical



1	R=H	Q=$NO(CH_3)_2$
2	R=$CH(OH)CH_2COCH_2CH_3$	Q=$NO(CH_3)_2$
3	R=H	Q=$N^+H(CH_3)_2$
4	R=$CH(OH)CH_2COCH_2CH_3$	Q=$N^+H(CH_3)_2$

Table 1. ^{13}C and ^{1}H Chemical shifts of the 1-(dimethylamino)-2-amino-propane unit in leucinostatins D (**3**) and H (**1**)*.



	3 (free base)	**3** (HCl salt)	**1**
Carbon			
C1	64.69	62.65	75.19
C2	43.44	40.12	39.93
C3	19.07	18.45	21.50
C4	45.76	46.58	59.68
C5	45.76	42.50	54.69
Proton			
1-H_A	2.22	3.01	3.21
1-H_B	2.35	3.81	4.13
2-H	4.02	4.63	4.80
3-H_3	1.18	1.28	1.31
4-H_3	2.22	3.04	3.34
5-H_3	2.22	3.13	3.38
N(1)$^+$-H	—	8.13	—
N(2)-H	6.83	7.26	7.61

* Chemical shifts ($CDCl_3$) are in ppm relative to internal TMS.

Table 2. Antibacterial and antimycotic activity of leucinostatins H (**1**) and K (**2**)* (MIC, μg/ml).

Microorganism	**1**	**2**
Bacillus subtilis ICI	100	100
B. subtilis var. *niger*	25	25
B. cereus B43 1335	100	50
Micrococcus luteus ISS	25	25
Staphylococcus aureus	10	25
Pseudomonas aeruginosa 6750	>100	>100
Salmonella typhimurium	>100	>100
Proteus vulgaris	>100	>100
Escherichia coli 982	>100	>100
Citrobacter freundii	>100	>100
Pseudomonas fluorescens	>100	>100
Candida albicans CBS 562	10	25
C. tropicalis 5711 IMAT	25	50
C. guilliermondii 5319	25	>10
C. krusei CBS 1910	10	<2
Cryptococcus laurentii 4685	10	10
C. neoformans 4711 IMAT	2	2

* MIC values were determined after 48 hours of incubation. Media for bacteria consisted of nutritive agar broth, for fungi SABOURAUD's dextrose broth.

set of chemical shift data was found for the pair **2** and **4**. Corroborative chemical evidence for the correctness of structures **1** and **2** was readily available by *N*-oxidation of **3** *via m*-Cl-perbenzoic acid treatment (in dry CH_2Cl_2 at room temperature). The resulting product proved, in every respect, identical with **1**.

It may be noted that structures **1** and **2** also provide an immediate rationale for the aforementioned lack of fragmentation ions in the FAB mass spectra of the new metabolites. In contrast to the non-oxygenated leucinostatins, where formation of the M+H ions is greatly facilitated by the availability of quaternary N-atoms, *N*-oxidation, as in **1** and **2**, clearly renders this process highly unfavored. This results in a major drop in the ion currents making the fragmentation pattern undetectable under normal experimental conditions.

Leucinostatins H and K show biological activity against Gram-positive bacteria and fungi as do the respective leucinostatins D and A[1,2]. However, as demonstrated by the MIC values in Table 2, both the antibacterial and the antimycotic activities become significantly reduced upon *N*-oxidation. In a similar manner, *N*-oxidation causes a nearly 2-fold decrease in the phytotoxic activity[4]; irreversible withering of tomato cuttings became visible after 72 hours at 10 and 5 μg/ml concentrations of **1** and **2**, respectively.

N-Oxides are known to occur frequently in nature[5] and it is generally believed that their presence is connected with processes leading to *N*-dealkylation of natural products[6~9]. While the exact biogenetic role of **1** and **2** may require further clarification, we mention here that sizable amounts of nor-[3] and bisnor-leucinostatin A[10] have, in fact, been detected by us in the culture filtrates of *P. marquandii*.

In a recent communication describing the identification of leucinostatin components from *Paecilomyces lilacinus* A-257 by means of directly coupled liquid chromatography/FAB mass spectrometry, STROH *et al.*[11] have reported the detection of two minor components in such a low abundance that only molecular weight information could be obtained. The reported mass values, however, suggest that the two new components may prove to be identical with leucinostatins H and K.

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