Structure Activity Studies on Chemically Modified Homologues of the Antibiotic Phytotoxic Leucinostatin A

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The synthesis and a conformational study of a number of homologues of the well known antibiotic, phytotoxic leucinostatin A are reported. The circular dichroism of all the compounds are discussed. Some conclusions on the SAR of these compounds are drawn. The influence of the α-helical conformation and/or the increased lipophile character on their interesting biological activities is emphasized.

The nonapeptide leucinostatin A is the major antibiotic, cytotoxic, phytotoxic component isolated, as hydrochloride salt, from cultures filtrates of *Paecilomyces marquandii* (Massee) Hughes. Additional minor metabolites leucinostatin B, C, D, F, H and K have been isolated from the same submerged cultures. The chemical structure of leucinostatin A has been worked out by several groups[1~3]. It turned out to be composed by the unsaturated fatty acid (4*S*,2*E*)-4-methylhex-2-enoic acid at the N-terminal; the (2*S*)-N^1,N^1-dimethylpropane-1,2-diamine at the C-terminal; and of the following nine amino acids: *cis*-4-methyl-L-proline, (2*S*,4*S*,6*S*)-2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid, *threo*-β-hydroxy-L-leucine, three α-aminoisobutyric acids, two L-leucines and β-alanine. The composition of other leucinostatins is closely related[4~7] to that of leucinostatin A. All these co-metabolites of the phytopathogenic microorganism are endowed with a similar wide spectrum of antibacterial, antimycotic, cytotoxic, and phytotoxic activity. However, these biological activities are exhibited to a different extent by different leucinostatins[2~7]. In particular, leucinostatin D is active against some strains of staphilococcus that are known to be resistant against more common antibiotics, and shows promising cytotoxic and phytotoxic activities[5]. CD and IR spectroscopy studies on leucinostatin A hydrochloride have investigated the preferred conformations of the molecule in solvents of different polarity and at different concentrations[8]. The results provide evidence that the peptide antibiotic, in apolar solvents and in the range of concentration used in the spectroscopic measurements, folds its backbone in a helical structure. Similar behaviour has been observed[9] in some synthetic fragments of leucinostatin A in lipophilic solvents, indicating a strong tendency to fold in an ordered structure. The crystal and molecular structure of the nonapeptide antibiotic has been also determined[10]. It was found that the peptide backbone folds in a regular right-handed α-helix conformation with six intramolecular hydrogen bonds. In the crystal the helices are linked head to tail by hydrogen bonds and electrostatic interactions, forming continous helical rods.

Since the molecular structure of leucinostatin A is important in providing a basis for structure-activity studies and in obtaining an insight into the possible role of specific side chains and backbone folding in determining the biological activity, in this paper we report the results of a structure-activity study in solution carried out using CD and biological activity measurements of the nonapeptide homologues which have been obtained by chemical modification of native leucinostatin A.

Materials and Methods

Isolation of Leucinostatin A

Leucinostatin A hydrochloride was obtained from the benzene extract of the cultural broth by extensive flash chromatography fractionations on silica gel column in a nitrogen atmosphere. As eluent $CHCl_3$ containing increasing amounts of CH_3OH, (up to 9%) and of conc. NH_4OH (up to 1%) was used. After solvent evaporation the white material obtained was crystallized several times

using dry acetone and ethyl acetate in order to obtain the separation between salt and free forms of leucinostatin A. Further details of isolation and purification of leucinostatin A are reported elsewhere[11].

Spectroscopic Measurements

The CD spectra were recorded at room temperature (25°C) on a Jasco model J-500A automatic recording circular dichrograph interfaced (IF-500 II) with a IBM AT computer. Cylindrical fused quartz cells of 0.2, 0.5 mm pathlengths were used for CD measurements. The usual instrumental precautions were taken to avoid artefacts. The values are expressed in terms of $[\theta]_M$ molar ellipticity (deg cm^2 dmol^{-1}), using the molecular weight of the compounds. 2,2,2-Trifluoroethanol (TFE) was purchased from Fluka (Switzerland); dioxane, methanol and *n*-hexane (spectrophotometric grade) were acquired from Merck (FRG). The peptide concentrations ranged from 2.09×10^{-4} to 2.5×10^{-4} M.

Bioassays

Leucinostatin A derivatives showed interesting antimicrobial and antimycotic activities against microorganisms (Table I). Cultures were grown for 48 hours at 30°C on nutrient agar broth (one liter contains Constantino ACAS (10 g), NaCl (5 g), Constantino peptone (10 g)) and then centrifuged, washed with physiological solution and adjusted to a final concentration by means of a colorimeter. Cultures were seeded by means multipoint inoculator on 10 mm Petri dishes containing 19 ml of jelled broth, 1 ml of DMSO and scalar quantities (from 100 to 3 μg) of derivatives per ml of broth. Control dishes containing only broth-DMSO and broth containing cephaloridine or nistatine were prepared as well and were incubated for 48 hours at 30°C.

Chemical Modification of Leucinostatin A

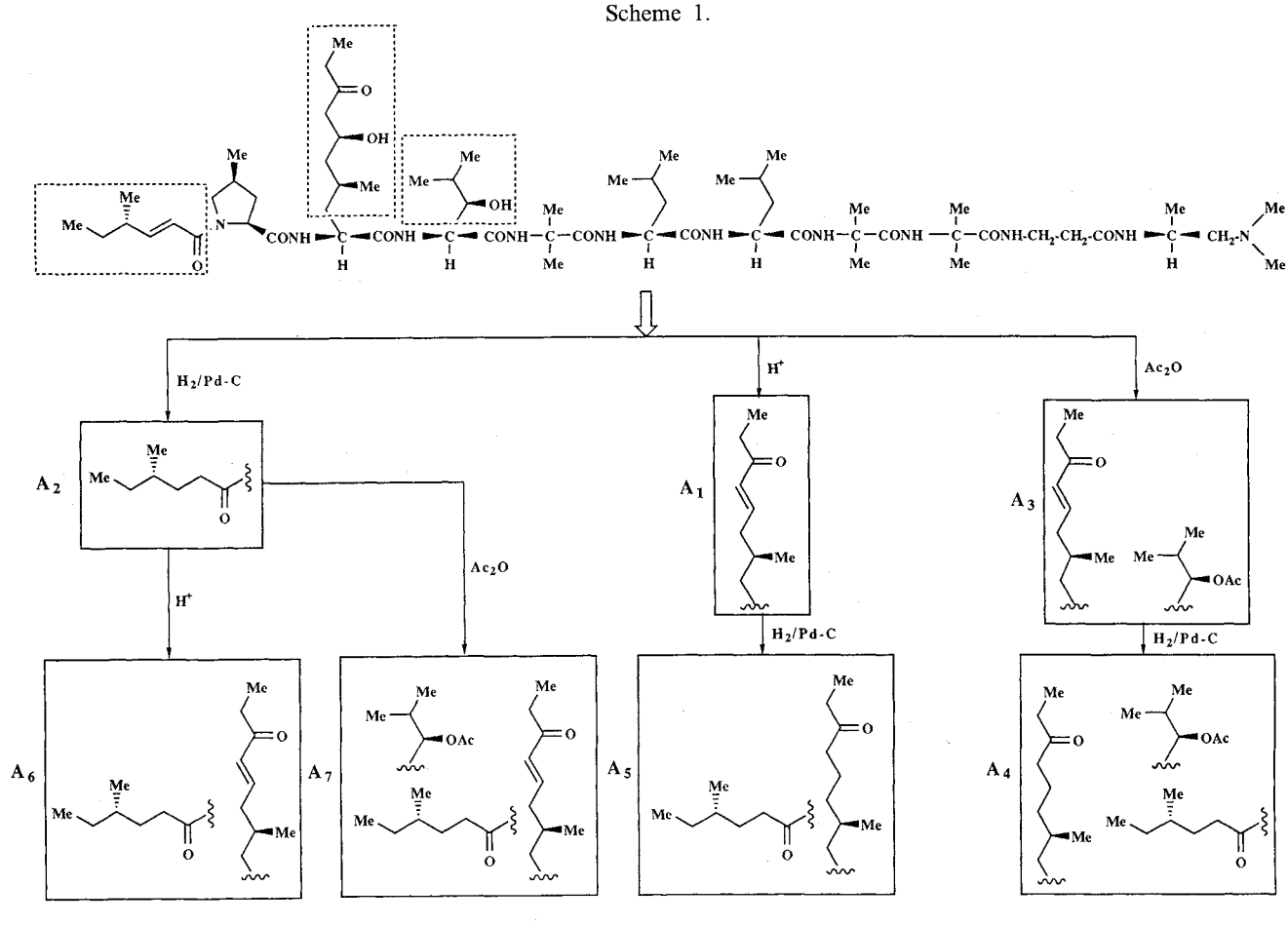
Scheme 1 reports the leucinostatin A homologues obtained by reduction, dehydration and acetylation of the (4*S*,2*E*)-4-methylhex-2-enoic acid, (2*S*,4*S*,6*S*)-2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid and *threo*-β-hydroxy-L-leucine residues.

Leucinostatins A2, A5, A4

Leucinostatin A hydrochloride, A1 and A3 (70 mg each) were reduced by adding them to a suspension of 5% Pd on charcoal in 20 ml of MeOH - EtOH (1 : 1) previously saturated with H_2 at 4 atm at room temperature for 12 hours. Following usual work up, an oily residue was obtained which, after purification by Silica gel column chromatography (eluent $CHCl_3$ - MeOH - conc. NH_4OH, 93.1 : 6 : 0.9) and several crystallizations from CH_3CN, carefully acidified by gaseous HCl, afforded compounds A2, A5 and A4 respectively, (80% yield) as hydrochlorides.

Compound A2: white prisms; mp 173 ~ 175°C; Rf 0.78 ($CHCl_3$ - MeOH - conc. NH_4OH, 80 : 18 : 2); UV λ_{max} (EtOH): 213 (ε 9,325); IR ν_{max} ($CDCl_3$): 3307, 1705 and

Scheme 1.



1654 cm^{-1}; ^1H NMR δ ($CDCl_3$): 9.05 (1H, bs), 8.32 (1H, d, J=4 Hz), 8.22 (1H, dd, J=8 and 2 Hz), 8.1 (1H, s), 7.9 (1H, s), 7.8 (1H, d, J=4 Hz), 7.68 (1H, s), 7.40 (1H, d, J=6 Hz), 7.31 (1H, d, J=4 Hz), 3.08 (3H, d, J=2.5 Hz), 3.18 (3H, d, J=2.5 Hz), 1.50 (3H, s), 1.48 (3H, s), 1.43 (3H, s), 1.40 (3H, s), 1.38 (3H, s), 1.37 (3H, s), 1.28 (3H, d, J=6.50 Hz), 1.12 (3H, d, J=6.54 Hz), 1.05 (3H, d, J=6.50 Hz), 1.03 (3H, d, J=7 Hz), 1.02 (3H, t, J=7 Hz), 0.96 (3H, d, J=7 Hz), 0.94 (3H, d, J=6 Hz), 0.93 (3H, d, J=6 Hz), 0.91 (3H, d, J=6 Hz), 0.90 (3H, t, J=6.5 Hz), 0.88 (3H, d, J=6 Hz), 0.86 (3H, d, J=6 Hz); ^{13}C NMR δ ($CDCl_3$): 211.53 (s), 177.69 (s), 176.68 (s), 176.48 (s), 176.06 (s), 175.83 (s), 174.76 (s), 174.62 (s), 174.53 (s), 174.34 (s), 172.93 (s), 74.92 (d), 63.96 (d), 63.60 (d), 50.80 (t), 45.49 (t), 45.20 (2 × q), 31.25 (t), 29.55 (t).

Compound A5: small white cubes; mp 123~125°C; Rf 0.72 ($CHCl_3$ - MeOH - conc. NH_4OH, 80:18:2); UV λ_{max} (EtOH): 213 nm (ε 11,300); IR ν_{max} ($CHCl_3$): 3307, 1705, 1654 cm^{-1}; ^1H NMR δ ($CDCl_3$): 9.00 (1H, bs), 8.68 (1H, d, J=4 Hz), 8.48 (1H, s), 8.45 (1H, dd, partially obscured), 8.28 (1H, s), 8.09 (1H, d, J=4 Hz), 7.73 (1H, s), 7.58 (1H, d, J=6 Hz), 7.20 (1H, d, J=4 Hz), 3.15 (3H, d, J=2.5 Hz), 3.05 (3H, d, J=2.5 Hz), 1.63 (3H, s), 1.62 (3H, s), 1.58 (3H, s), 1.50 (3H, s), 1.49 (3H, s), 1.48 (3H, s), 1.30 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=6.50 Hz), 1.05 (3H, d, J=6.5 Hz), 1.02 (3H, t, J=7.00 Hz), 0.98 (3H, d, J=7.0 Hz), 0.97 (3H, d, J=6.0 Hz), 0.90~0.80 (18H, m); ^{13}C NMR δ ($CDCl_3$): 212.64 (s), 176.62 (s), 176.11 (s), 175.14 (s), 174.98 (s), 174.69 (s), 174.46 (s), 173.54 (s), 172.74 (s), 172.31 (s), 75.60 (d), 63.60 (d), 45.41 (2 × q), 42.46 (t), 36.88 (2 × t), 31.38 (t), 29.64 (t).

Compound A4: white amorphous powder; mp 100~102°C; Rf 0.72 ($CHCl_3$ - MeOH - conc. NH_4OH, 80:18:2); UV λ_{max} (EtOH): 213 nm (ε 10,628); IR ν_{max} ($CHCl_3$): 3317, 1705 and 1645 cm^{-1}; ^1H NMR δ ($CDCl_3$): 8.85 (1H, m), 8.30 (1H, s), 8.22 (1H, dd, J=8 and 2 Hz), 7.90 (1H, s), 7.85 (1H, d, J=4 Hz), 7.70 (1H, s), 7.65 (1H, d, J=4 Hz), 7.58 (1H, d, J=4 Hz), 7.39 (1H, d, J=6 Hz), 5.10 (1H, dd, J=4.5 and 7.5 Hz), 3.15 (2 × 3H, s), 2.08 (3H, s), 1.5 (3H, s), 1.49 (3H, s), 1.47 (3H, s), 1.43 (6H, s), 1.40 (3H, s), 1.23 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=6.55 Hz), 1.05 (3H, d, J=6.5 Hz), 1.01 (3H, t, J=7 Hz), 0.98~0.8 (24H, m); ^{13}C NMR δ ($CDCl_3$): 212.02 (s), 177.65 (s), 176.31 (s), 176.17 (s), 176.09 (s), 175.75 (s), 174.50 (s), 174.45 (s), 174.28 (s), 174.17 (s), 171.60 (s), 170.78 (s), 75.61 (d), 63.30 (d), 44.41 (2 × q), 42.58 (t), 37.40 (t), 36.36 (t), 31.84 (t), 29.55 (t), 19.02 (q).

Leucinostatins A1, A6

Leucinostatin A hydrochloride and A2 (100 mg each) in $CHCl_3$ (30 ml) were treated with gaseous HCl for 10 minutes and then deluted with $CHCl_3$ (60 ml). The solutions brought to dryness at reduced pressure left oily yellow residues which were purified by column chromatography (eluent $CHCl_3$ - MeOH - conc. NH_4OH, 93.1:6:0.9) and eventually crystallized by CH_3CN, carefully acidified by gaseous HCl, to afford compounds A1 and A6 respectively (85% yield) as hydrochloride salts. Compound A6 was further purified by flash column chromatography using the usual ternary eluent.

Compound A1: white small cubes; mp 145~147°C; Rf 0.77 ($CHCl_3$ - MeOH - conc. NH_4OH, 80:18:2); UV λ_{max} (EtOH): 219.5 nm (ε 29,629) and 211 nm (ε 15,946); IR ν_{max} ($CHCl_3$): 3307 and 1654 cm^{-1}; ^1H NMR δ ($CDCl_3$): 9.12 (1H, bs), 8.68 (1H, d, J=4 Hz), 8.29 (1H, dd, partially obscured), 8.28 (1H, s), 8.02 (1H, s), 7.95 (1H, d, J=4 Hz), 7.68 (1H, s), 7.42 (1H, d, J=6 Hz), 7.22 (1H, d, J=4 Hz), 6.88 (1H, m), 6.73 (1H, dd, J=15.4 and 7.4 Hz), 6.20 (1H, d, J=15.4 Hz), 6.05 (1H, d, J=15.4 Hz), 3.15 (3H, d, J=2.5 Hz), 3.07 (3H, d, J=2.5 Hz), 1.58 (3H, s), 1.57 (3H, s), 1.49 (3H, s), 1.47 (3H, s), 1.44 (3H, s), 1.43 (3H, s), 1.23 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=6.55 Hz), 1.07 (3H, d, J=6.50 Hz), 1.05 (3H, d, J=7 Hz), 1.03 (3H, t, J=7 Hz), 0.98 (3H, d, J=7 Hz), 0.96 (3H, d, J=6 Hz), 0.94 (3H, d, J=6 Hz), 0.92 (3H, d, J=6 Hz), 0.91 (3H, t, J=6.5 Hz), 0.90 (3H, d, J=6 Hz), 0.88 (3H, d, J=6 Hz); ^{13}C NMR δ ($CDCl_3$): 201.02 (s), 177.71 (s), 176.54 (s), 175.95 (s), 175.81 (s), 175.75 (s), 174.45 (s), 174.34 (s), 174.23 (s), 172.64 (s), 167.03 (s), 151.23 (d), 145.18 (d), 131.61 (d), 120.31 (d), 74.34 (d), 63.60 (d), 46.1 (2 × q), 36.80 (t).

Compound A6: amorphous white powder; mp 110~112°C; Rf 0.77 ($CHCl_3$ - MeOH - conc. NH_4OH, 80:18:2); UV λ_{max} (EtOH): 214.7 nm (ε 14,700) and 210.5 nm (ε 4,732); IR ν_{max} ($CHCl_3$): 3309 and 1654 cm^{-1}; ^1H NMR δ ($CDCl_3$): 8.77 (1H, bs), 8.58 (1H, d, J=4 Hz), 8.12 (1H, s), 8.10 (1H, dd, partially obscured), 7.95 (1H, s), 7.90 (1H, d, J=4 Hz), 7.50 (1H, s), 7.30 (1H, d, J=6 Hz), 7.22 (1H, d, J=4 Hz), 6.85 (1H, m), 6.07 (1H, d, J=15.4 Hz), 2.9 (2 × 3H, s), 1.54 (3H, s), 1.53 (3H, s), 1.50 (3H, s), 1.48 (3H, s), 1.47 (3H, s), 1.43 (3H, s), 1.23 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=7.0 Hz), 1.08 (3H, d, J=6.50 Hz), 1.06 (3H, d, J=6 Hz), 1.05 (3H, d, J=6 Hz), 0.96 (3H, d, J=6 Hz), 0.95 (3H, d, J=6 Hz), 0.93 (3H, t, J=6.5 Hz), 0.92 (3H, d, J=6 Hz), 0.90 (3H, d, J=6 Hz), 0.88 (3H, d, J=6 Hz); ^{13}C NMR δ ($CDCl_3$): 201.2 (s), 177.36 (s), 176.62 (s), 175.86 (s), 175.78 (s), 175.72 (s), 174.65 (s), 174.59 (s), 174.40 (s), 173.89 (s), 172.76 (s), 145.31 (d), 131.69 (d), 74.47 (d), 63.60 (d), 44.85 (2 × q), 36.9 (t), 31.36 (t), 29.44 (t).

Leucinostatins A3, A7

Leucinostatin A hydrochloride and A2 (100 ml each), dissolved with dry pyridine (2 ml) and acetic anhydride (1 ml) were stirred for 24 hours at room temperature and then poured in iced water. The cloudy solutions were repeatedly extracted with $CHCl_3$ (5 × 30 ml). The combined organic layers, dried over anhydrous Na_2SO_4, were brought to dryness at reduced pressure and the oily yellow residues were purified by flash column chromato-

graphy (eluent $CHCl_3$-MeOH-conc. NH_4OH, 93.1:6:0.9) in a nitrogen atmosphere to afford compounds A3 and A7 respectively (90% yield) as free base forms.

Compound A3: white amorphous powder; mp 98~100°C, Rf 0.74 ($CHCl_3$-MeOH-conc. NH_4OH, 80:18:2), UV λ_{max} (EtOH): 219 nm (ε 26,420) and 211 nm (ε 16,060); IR ν_{max} ($CHCl_3$): 3321, 1735 and 1634 cm^{-1}; ^1H NMR δ ($CDCl_3$): 8.48 (1H, bs), 8.10 (1H, s), 7.82 (2H, m), 7.68 (1H, s), 7.49 (2H, m), 7.32 (1H, s), 7.10 (1H, d, J=6 Hz), 6.88 (1H, m), 6.73 (1H, dd, J=15.4 and 7.4 Hz), 6.15 (1H, d, J=15.4 Hz), 6.05 (1H, d, J=15.4 Hz), 5.10 (1H, dd, J=4.5 and 7.5 Hz), 2.65 (6H, s), 2.06 (3H, s), 1.49 (3H, s), 1.48 (3H, s), 1.46 (6H, s), 1.44 (3H, s), 1.42 (3H, s), 1.23 (3H, d, J=6.5 Hz), 1.13 (3H, d, J=6.55 Hz), 1.05 (3H, d, J=6.5 Hz), 1.02 (3H, t, J=7 Hz), 1.00 (3H, d, J=6.0 Hz), 0.92~0.81 (21H, m); ^{13}C NMR δ ($CDCl_3$): 200.95 (s), 176.56 (s), 176.06 (s), 175.10 (s), 175.02 (2s), 174.39 (s), 174.00 (s), 172.90 (s), 171.06 (s), 170.68 (s), 166.75 (s), 152.72 (d), 144.76 (d), 131.55 (d), 119.71 (d), 75.98 (d), 63.15 (d), 44.99 (2×q), 39.8 (t), 20.7 (q).

Compound A7: white amorphous powder; mp 106~110°C; Rf 0.75 ($CHCl_3$-MeOH-conc. NH_4OH, 80:18:2); UV λ_{max} (EtOH): 214.6 nm (ε 15,810); IR ν_{max} ($CHCl_3$): 3318, 1735 and 1654 cm^{-1}; ^1H NMR ($CDCl_3$): 9.55 (1H, bs), 8.35 (1H, dd, partially obscured), 8.33 (1H, s), 8.01 (1H, d, J=4 Hz), 7.80 (1H, s), 7.77 (1H, s), 7.70 (1H, d, J=4 Hz), 7.64 (1H, d, J=4 Hz), 7.49 (1H, d, J=6 Hz), 6.90 (1H, m), 6.05 (1H, d, J=15.4 Hz), 5.12 (1H, dd, J=4.5 and 7.5 Hz), 2.33 (6H, s), 2.18 (3H, s), 1.52 (3H, s), 1.50 (6H, s), 1.47 (6H, s), 1.40 (3H, s), 1.28 (3H, d, J=6.5 Hz), 1.18 (3H, d, J=6.55 Hz), 1.08 (3H, d, J=6.5 Hz), 1.02 (3H, t, J=7.0 Hz), 1.00~0.87 (24H, m); ^{13}C NMR δ ($CDCl_3$): 200.80 (s), 176.089 (s), 176.0 (s), 174.76 (2×s), 174.39 (s), 174.14 (s), 173.94 (s), 172.33 (s), 170.95 (s), 170.86 (2×s), 144.47 (d), 131.60 (d), 76.06 (d), 45.05 (2×q), 36.90 (t), 31.39 (t), 29.55 (t), 20.76 (q).

Results and Discussion

Fig. 1 represents the CD spectra of leucinostatin A hydrochloride homologues A1, A2 and A5 in solvents of different polarity. They show the same spectral behaviour observed for leucinostatin A hydrochloride[8]. In TFE the CD profile (negative maximum at 203 nm and a large shoulder at 210~220 nm) indicates the existence of a conformational equilibrium between aperiodic structure and folded conformations (3_{10} or α-helical structures). This equilibrium shifts towards populations in ordered conformation in the less polar solvent MeOH and, chiefly, in the more apolar dioxane-*n*-hexane (1:1, v/v) mixture. In this solvent the CD band of the amide π-π* transition moved to 208 nm and the negative maximum of the amide *n*-π* transition is well resolved at 220~222 nm (α-helix structure). This spectral behaviour means that chemical modifications do not influence macroscopically the ordered conformation

Fig. 1. CD spectra of homologues A1, A2, A5 in TFE (curve 1), MeOH (curve 2) and dioxane-*n*-hexane (1:1, v/v) (curve 3).

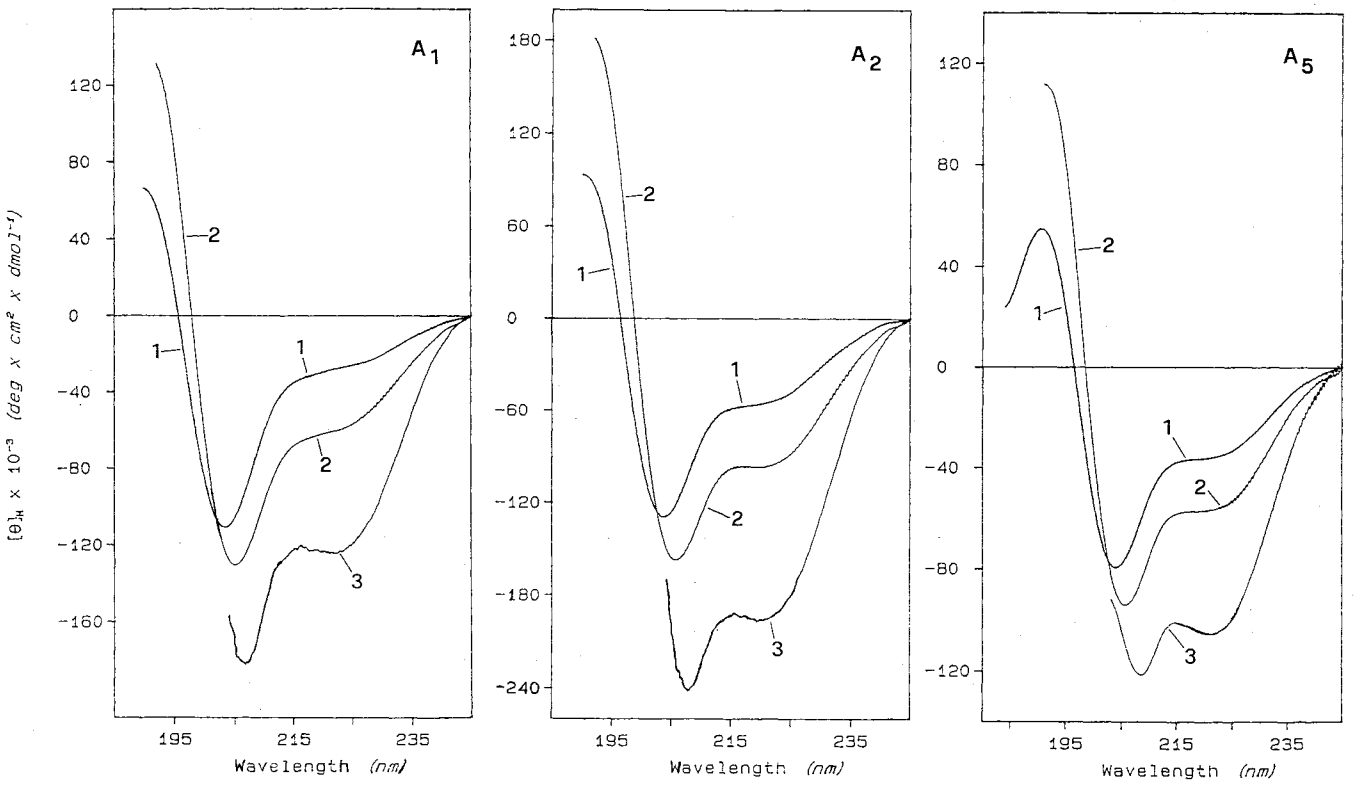


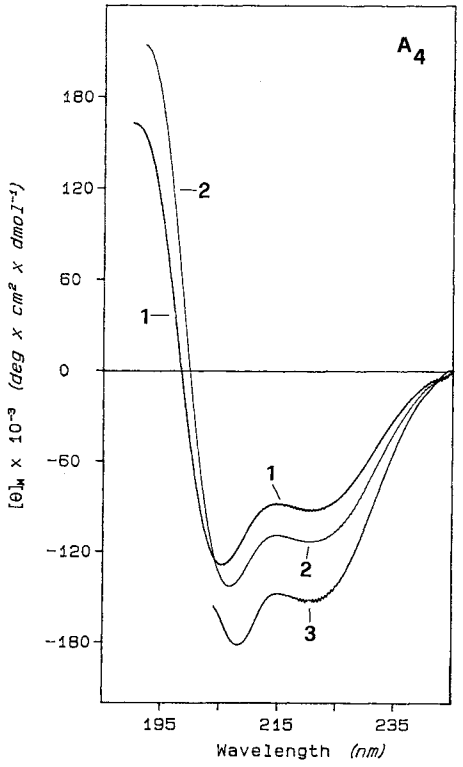
Table 1. Antibacterial and antimycotic activity of leucinostatin A·HCl homologues. (MIC, μg/ml)

Microorganism	Compounds							
	A*	A1	A2	A3	A4	A5	A6	A7
Bacillus subtilis ICI	3	<3	<3	<3	3	3	12	6
Micrococcus luteus 9341	<3	3	<3	<3	<3	<3	<3	<3
Bacillus subtilis var. niger	3	3	12	<3	<3	3	12	<3
Bacillus cereus B43 1335	12	12	12	<3	6	6	12	6
Staphilococcus aureus	12	12	12	6	6	3	12	6
Pseudomonas aeruginosa 6750	>100	>100	>100	>100	>100	>100	>100	>100
Salmonella typhimurium	>100	>100	>100	>100	>100	>100	>100	>100
Proteus vulgaris	>100	>100	>100	>100	>100	>100	>100	>100
Escherichia coli 982	>100	>100	>100	>100	>100	>100	>100	>100
Citrobacter freundii	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas fluorescens C3	>100	>100	>100	>100	>100	>100	>100	>100
Candida albicans CBS 562	<3	<3	<3	50	3	<3	12	12
Candida tropicalis 5711 IMAT	25	25	25	100	25	12	25	25
Candida krusei CBS 1910	25	50	25	>100	100	25	50	>100
Cryptococcus laurentii 4685	50	100	25	100	100	100	50	>100
Cryptococcus neoformans 4711 IMAT	<3	<3	<3	100	3	3	12	25

* Leucinostatin A·HCl.

of the parent natural compound. The spectroscopic results parallel the biological activities (Table 1) which do not show any significative variations if compared to those of leucinostatin A hydrochloride, with the exception of A5 which resulted more active against *Staphilococcus aureus*. Interestingly CD spectra of A4 (Fig. 2) revealed the presence of two sharp negative bands centered at 208 and 222 nm in TFE and MeOH too, whose intensities approach those observed in dioxane-*n*-hexane solution. This indicates a remarkable increase of an ordered molecular population in these solvents probably due to the acetylation of the *threo*-β-hydroxy-L-leucine residue. In fact the lack of a free hydroxy group prevents the formation of an intermolecular hydrogen bond with the solvent and, at the same time, increases the lipophile character of the molecule cooperating thus to the formation of an ordered structure. The CD spectra of compounds A3 and A7 (Fig. 3), insoluble in buffer solution, show in TFE and MeOH a similar behaviour of A4 while in a very apolar medium, such as dioxane-*n*-hexane the equilibrium ordered conformation versus disordered conformation is clearly displaced towards the latter. Since NMR spectra undoubtedly indicate that these compounds are in the free base and not in the hydrochloride form, it was inferred that molecules with positively charged C-terminal moiety assume an ordered conformation in an apolar medium and vice versa. Such a hypothesis was confirmed by performing CD spectra of leucinostatin A free base (Fig. 4); in this case a similar behaviour was observed, in fact by dissolving this compound in dioxane-*n*-hexane, a

Fig. 2. CD spectra of homologues A4 in TFE (curve 1), MeOH (curve 2) and dioxane-*n*-hexane (1 : 1, v/v) (curve 3).



complete inversion of the behaviour, as compared to what showed by leucinostatin A hydrochloride, was noticed. In acqueous buffer medium, the spectrum profile of leucinostatin A free base, as in the case of hydrochloride form[8], indicates the presence of a limited molecular population in ordered structure (low intensities of the negative bands and blue shift of π-π* transition). By comparing these data with the biological activities

Fig. 3. CD spectra of homologues A3, A7 in TFE (curve 1), MeOH (curve 2) and dioxane-*n*-hexane (1 : 1, v/v) (curve 3).

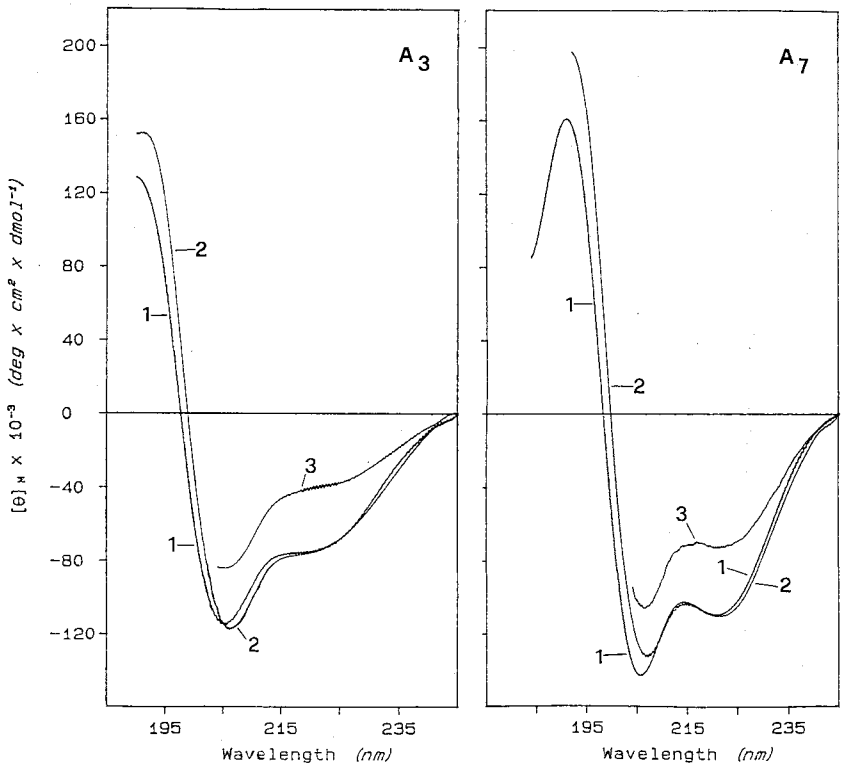


Fig. 4. CD spectra of leucinostatin A free base in TFE (curve 1), MeOH (curve 2), dioxane-*n*-hexane (1 : 1, v/v) (curve 3) and in TFE 2 mM phosphate buffer, pH 7.4 (1 : 19, v/v) (curve 4).

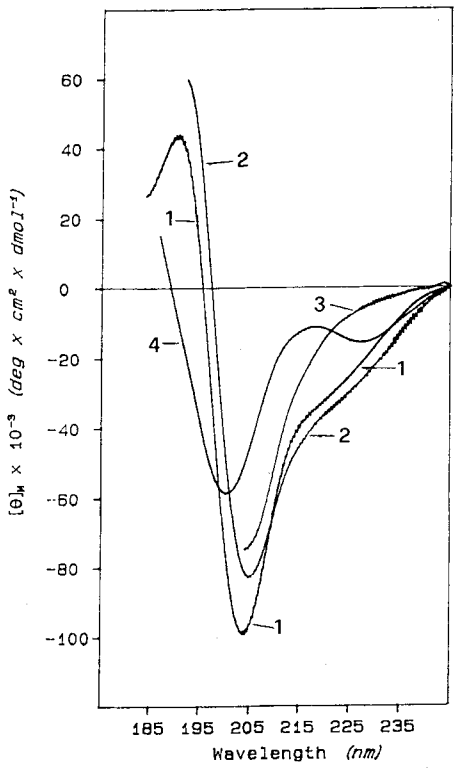
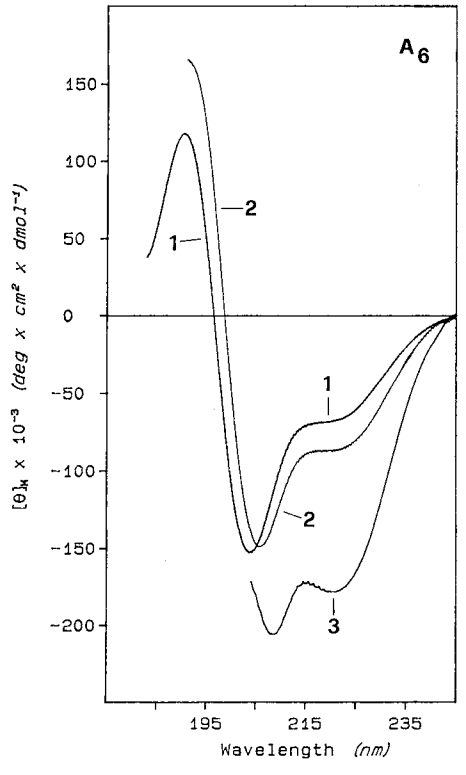


Fig. 5. CD spectra of homologues A6 in TFE (curve 1), MeOH (curve 2) and dioxane-*n*-hexane (1 : 1, v/v) (curve 3).



(Table 1) it is possible to argue that in addition to the presence of a charged C-terminal moiety, which appeared at the first glance indispensable for the activity, is also important the higher lipophyle character of these compounds. In fact A3, A7 and A4 show a higher antimicrobial and a lower antifungal activity if compared to what displayed by the parent leucinostatin A hydrochloride. The apparent anomalous behaviour noticed in the CD spectra of compound A6 (Fig. 5) is due to the fact that A6 exists in the hydrochloride-free base form mixture (75.25) as inferred from leucinostatin A NMR shift titration experiments[12]. In fact the CD curves in MeOH and TFE approach those observed for compounds A3 and A7 while the CD curve in dioxane-*n*-hexane (1 : 1, v/v) is very similar to that one shown by compounds A1, A2, A4 and A5. Accordingly with these findings, A6 shows a mild activity against Gram-positive bacteria and no significant differences in activity against fungi if compared to that observed for the hydrochloride homologues.

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References

1) FUKUSHIMA, K.; T. ARAI, Y. MORI, M. TSUBOI & M. SUZUKI: Studies on peptide antibiotics, Leucinostatins II. The structures of Leucinostatins A and B. J. Antibiotics 36: 1613～1630, 1983
2) CASINOVI, C. G.; L. TUTTOBELLO, C. ROSSI & Z. BENCIARI: Structural elucidation of the phytotoxic antibitic peptides produced by submerged cultures of *Paecilomyces marquandii* (Massee) Hughes. Phytopathol. Mediterr. 22: 103～106, 1983
3) ISOGAI, A.; A. SUZUKI, S. TAMURA, S. HIGASHIKAWA & S. KUYAMA: Structure of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (Thom) Samson. J. Chem. Soc. Perkin Trans. I: 1405～1411, 1984
4) CASINOVI, C. G.; C. ROSSI, L. TUTTOBELLO & M. RICCI: The structure of Leucinostatin C, a minor peptide from *Peacilomyces marquandii*. Eur. J. Med. Chem. Chim. Ther. 21: 527～528, 1986
5) ROSSI, C.; L. TUTTOBELLO, M. RICCI, C. G. CASINOVI & L. RADICS: Leucinostatin D, a novel peptide antibiotic from *Paecilomyces marquandii*. J. Antibiotics 40: 130～133, 1987
6) RADICS, L.; M. KAJTAR-PEREDY, C. G. CASINOVI, C. ROSSI, M. RICCI & L. TUTTOBELLO: Leucinostatins H and K, two novel peptide antibiotics with tertiary amine-oxide terminal group from *Paecilomyces marquandii*. Isolation, structure and biologycal activity. J. Antibiotics 40: 714～716, 1987
7) ROSSI, C.; M. RICCI, L. TUTTOBELLO, S. CERRINI, A. SCATTURIN, G. VERTUANI, V. AMBROGI & L. PERIOLI: Leucinostatin F. A minor co-metabolite from *Paecilomyces marquandii*. Acta Technologiae et Legis Medicamenti 1: N.2, 109～112, 1990
8) VERTUANI, G.; C. FALCOMER, M. BOGGIAN, G. POCHETTI, S. CEERINI, M. RICCI, C. ROSSI & A. SCATTURIN: Structural studies of Leucinostatin A and its Boc-Aib-Leu-Leu-Aib-OMe tetrapeptide fragment. Int. J. Peptide Protein Res. 33: 162～170, 1989
9) FALCOMER, C.; G. VERTUANI, M. BOGGIAN, G. POCHETTI, S. CERRINI & A. SCATTURIN: Conformational studies of synthetic Leucinostatin A fragments. In Second Forum on Peptides. *Ed.* A. AUBRY *et al.* Vol. 174 pp. 383～386, INSERM/J. Libbey Eurotext Ltd., London-Paris, 1989
10) CERRINI, S.; D. LAMBA, A. SCATTURIN, C. ROSSI & C. UGHETTO: The crystal and molecular structure of the α-helical nonapeptide antibiotic Leucinostatin A. Biopolymers 28: 409～420, 1989
11) CASINOVI, C. G.; C. ROSSI & L. TUTTOBELLO (Istitute Superiore di Sanità): A now peptide: Microbiologic procedures for its production and its uses as an antibiotic, cytotoxic and phytotoxic agent. Italian Pat. 47889A85, Mar. 28, 1985
12) RADICS, L.; M. KAJTAR-PEREDY, M. RICCI & C. ROSSI: Leucinostatin A: High Field NMR Spectroscopy and Solution Conformation. To be published