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THE STRUCTURE OF LIPOPEPTIN A

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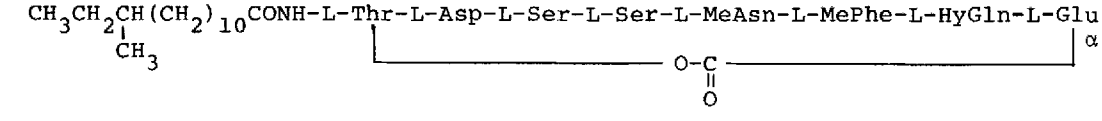
Summary: The structure of lipopeptin A, an antifungal peptolide antibiotic was determined as 1 *on the basis of chemical and spectroscopic evidence.*

Recently, we reported isolation of lipopeptin A, an antifungal antibiotic from a streptomycete, which resembles *Streptomyces violaceochromogenes*.[1] In this communication, we wish to propose the structure 1 for this antibiotic.

Lipopeptin A (1), $C_{54}H_{84}N_{10}O_{19}$ [m.p. 206-208°C (dec), $[\alpha]_D^{20}$ -45.4° (*c* 1.06, methanol), $(M + Na)^+$ 1199 (FD-MS)] is a dibasic acid with pKa' of 4.6. On acid hydrolysis, it gave eight amino acids and a fatty acid. All the amino acids were isolated and identified using authentic samples. The results are summarized in Table 1.



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MeAsn, N-methylasparagine; MePhe, N-methylphenylalanine; HyGln, threo-β-hydroxyglutamine.

The fatty acid was esterified with diazomethane and analyzed by GC/MS [M^+ 256, $(M-C_4H_9)^+$ 199, $(M-C_2H_5)^+$ 227, $(M-OCH_3)^+$ 225]. Enhanced abundance of ion 199 suggested branching at C-12. The anteiso structure is further reflected by the unusual ion abundance ratio, $(M-C_2H_5)^+ > (M-OCH_3)^+$ in contrast to normal chain esters.[2] The methyl ester of authentic 12-methyltetradecanoic acid[3] gave an essentially identical mass spectrum in particular with respect to the distinguishing features which indicate the anteiso terminus. Identity was further

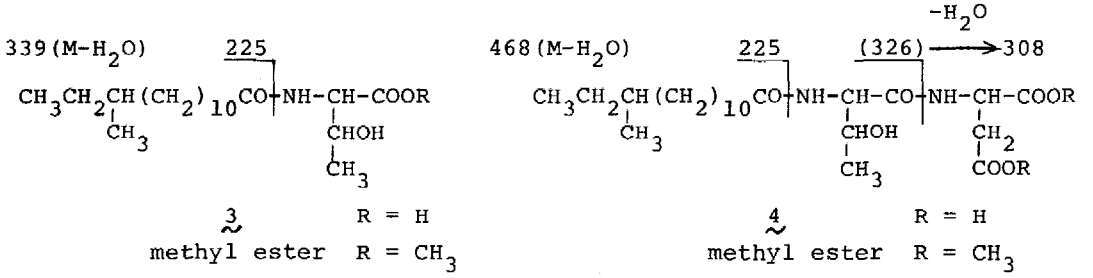
Table 1.

Amino acid	$[\alpha]_D^{20}$	Molar ratio
L-Serine	-6.5° (*c* 0.52. H_2O)	1.84
L-Threonine	-25.4° (*c* 0.50, H_2O)	0.96
L-Aspartic acid	+22.0° (*c* 0.33, 6 N HCl)	1.04
L-Glutamic acid	+9.2° (*c* 0.74, H_2O)	1.00
L-N-Methylphenylalanine[4]	+9.2° (*c* 0.50, H_2O)	1.00
L-threo-β-hydroxyglutamic acid[5]	+16.1° (*c* 0.18, 1 N HCl)	0.78
L-N-Methylaspartic acid[6]	+24.6° (*c* 0.12, 1 N HCl)	(*ca.*1)

confirmed by identical GC retention time [3% OV-17]: 14.68 relative to methyl pentadecanoate (15.00).

The molecular weight of 1 can reasonably be explained by the sum of eight amino acids and one fatty acid linked by eight amide bonds (λ_{max}^{KBr} 1650, 1525 cm^{-1}) and one lactone bond (λ_{max}^{KBr} 1735 cm^{-1}) plus the presence of two primary amides (δ_H 6.66, 6.78, 7.08, 7.35 ppm in DMSO-d_6).

The presence of a lactone group was proved by obtaining an open chain acid (2), $[\alpha]_D^{20}$ -72.4° (*c* 0.50, MeOH), $(M + Na)^+$ 1217 (FD-MS), pKa' 4.8 (dibasic),[7] by treatment of 1 with 0.03 N NaOH at room temperature. The C-terminal amino acid of 2 was identified as glutamic acid by carboxypeptidase[7] treatment. Selective acid hydrolysis (0.25 N AcOH, reflux 8 hrs) of 2 gave, after separation, aspartic acid, lipoamino acid (3), lipopeptide (4), and hexapeptide (5). The structure of 3 and 4 were deduced from EI-MS analysis of the corresponding methyl esters as illustrated below.



3 R = H
methyl ester R = CH_3

4 R = H
methyl ester R = CH_3

Ser-Ser-MeAsn-MePhe-HyGln-Glu

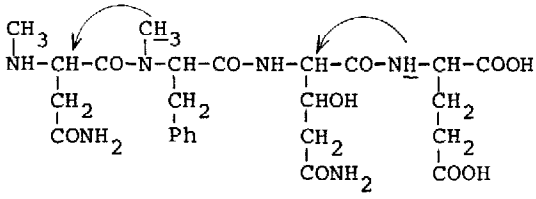
5

Amino acid analysis of 5 showed serine, threo-β-hydroxyglutamic acid, and glutamic acid in 1.88 : 0.91 : 1.00 molar ratios as well as the presence of N-methylphenylalanine and N-methylaspartic acid. The C-terminus was determined as glutamic acid by the tritium-labeling method,[9] and three successive Edman-Dansyl degradations[10] revealed the sequence, Ser-Ser-MeAsn-MePhe-. From the molecular weight [FD-MS $(M + H)^+$ 755, $(M + Na)^+$ 777], 5 should have two primary amides and

the only possible positions are the γ-carboxyl group of threo-β-hydroxyglutamic acid and β-carboxyl group of N-methylaspartic acid. The glutamic acid γ-carboxyl possibility can be excluded because carboxypeptidase treatment of 2 gave glutamic acid but not glutamine.

The position of the lactone was determined as follows. Chromic acid oxidation of 1 in acetic acid-pyridine followed by acid hydrolysis resulted in recovery of threonine but none of threo-β-hydroxyglutamic acid and serine. Participation of the β-hydroxy group of threonine in the lactone formation was further supported by ^{1}H-NMR. The β-methine proton of threonine residue (δ_H 5.04 ppm in DMSO-d_6) in 1 appeared about 1 ppm down-field compared with that of 2 (δ_H 4.05 ppm in DMSO-d_6). Borohydride reduction of 1 in water followed by acid hydrolysis resulted in disappearance of glutamic acid, proving that either α- or γ-carboxyl of glutamic acid participates in the lactone formation in 1. Because 1 is a dibasic acid with pKa' of 4.6, the γ-carboxyl must be free. Thus the lactone position was concluded as between the β-hydroxyl group of threonine and the α-carboxyl group of glutamic acid.

Two successive Edman degradations of 5 afforded tetrapeptide (6) after purification. To determine the position of two primary amides, the NOE of 6 in DMSO-d_6 solution at 63°C was measured from the difference spectrum on a 270 MHz spectrometer.[11] Observed NOE enhancements of each proton signal upon irradiation of specific protons are listed in Table 2. On irradiation of N-methyl protons of N-methylphenylalanine, the α-methine proton (δ_H 3.70 ppm) of N-methylasparagine was enhanced (3.75 %) but β-methylene protons (δ_H 2.29 ppm) were not. Similarly, on irradiation of the NH proton of glutamic acid, the α-methine proton (δ_H 4.36 ppm) of threo-β-hydroxyglutamine was enhanced (4.5 %) but γ-methylene protons (δ_H 2.16 ppm) were not. The results indicate the presence of β-carboxamide of N-methylasparagine and γ-carboxamide of threo-β-hydroxyglutamine as well as the presence of α-peptide linkages.



6

Table 2. NOE Enhancement of 6.

Proton irradiated	δ_H (ppm)	Observed NOE (%)			
		α-CH(MeAsn)	β-CH_2(MeAsn)	α-CH(HyGln)	γ-CH_2(HyGln)
N-CH_3	2.97	3.75	0	-	-
NH	7.76	-	-	4.46	0

From the data described above, we propose structure 1 for lipopeptin A.[12] As will be reported in a separate paper,[13] lipopeptin A inhibits in vitro peptidoglycan synthesis of *Escherichia coli* and in vitro proteoheteroglycan synthesis of *Piricularia oryzae*.

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References and Notes

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2) R. Ryhage and E. Stenhagen, Arkiv. Kemi., 15, 291 (1960).
3) Authentic sample was purchased as a component of standard mixtures from Applied Science, State College, Pennsylvania.
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7) A newly generated α-carboxyl group was not titrable because of the low pKa' value (∿2).
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9) H. Matsuo and K. Narita, "Protein Sequence Determination", ed. S. B. Needleman, Springer-Verlag, Berlin, P. 104 (1975); K. Narita, H. Matsuo, and T. Nakajima, ibid., P. 30.
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11) The sample was degassed 3 times by the freeze-thaw method and sealed in a NMR tube under N_2 gas. NOE's were determined by applying a 5-s low power saturating pulse at the appropreate peak positin, followed by a high power 90° observing pulse after 0.01-s waiting time. Off-resonance control spectra were measured in the same way, except that the presaturating pulse was offset 1000 Hz higher field from tetramethylsilane so that no solute resonances were perturbed. On-resonance and off-resonance spectra were the sum of 512 scans.
12) A minor component was isolated and designated as lipopeptin B. The methyl ester of the isolated fatty acid (M^+ 242) was identical to authentic methyl 12-methyltridecanoate, as judged from its mass spectrum and GC retention behavior.
13) M. Nishii, K. Isono, and K. Izaki, Agric. Biol. Chem., submitted.

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