ANTIBIOTICALLY ACTIVE TRIPEPTIDES WITH THE SEQUENCE L-ARGININE–*N*-α-METHYL-L-X–L-PHENYLALANINE

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1. Introduction

To investigate the structure–activity relationship of an antibiotically active tripeptide (the sequence of which is L-Arg–D-aThr–L-Phe) isolated by König et al. [1], a sequence variation approach was used. Earlier experiments showed, that D-allo-threonine could be substituted in the above sequence by a number of other D-amino acids without total loss of antibiotic activity [2]. It was hence of interest to find out if the replacement of D-allo-threonine by *N*-α-methyl-L-amino acids (which are found in some peptide antibiotics) would also lead to antibiotically active tripeptides. It was also of interest whether the action of these peptides could also be neutralized by L-amino acids as was shown [2] for the peptides with D-amino acids . These experiments should also clarify whether the conformation of these tripeptides (which is quite different from those with a middle D-amino acid) must be specific or not.

2. Materials and methods

The peptides were synthesised in the solid phase and purified by column chromatography on silica gel at 4°C, using *n*-butanol: H_2O: glacial acetic acid = 4 : 2 : 1.

The *N*-α-methyl-L-amino acids were prepared according to [3], the Boc-amino acids* to [4] and Z_3-L-arginine to [5]. Test-organisms used were those described by König et al. [1] (*Paecilomyces varioti, Mucor miehei, Bacillus subtilis* and *E. coli*). The plate diffusion test followed the method in [6], complex medium was prepared according to [7] and the cross-stripe test followed the method of [8]. Pathogenic moulds used were *Trichophyton mentagrophytes* and *Epidermophyton floccosum.*

* *Abbreviations:* Boc = tert. butyloxycarbonyl, DMF = dimethylformamide, EtOH = ethanol, Me = methyl, Z = benzyloxycarbonyl, Z_3L-Arg = tris(benzyloxycarbonyl)-L-arginine.

3. Results and discussion

The tripeptides synthesised, together with their analytical data, are shown in table 1.

Six of the peptides synthesised showed antibiotic activity on fungi and on the pathogenic moulds used (table 2) but none towards Gram positive and Gram negative bacteria. These facts agree with the results described by König et al. [1] for the isolated peptide (L-Arg–D-aThr–L-Phe) and with those of the synthetic peptides, where D-allo-threonine is replaced by other D-amino acids [2].

The results of the biological tests agree with those of earlier works [1,2] in the following points:

1) The antibiotic activity increases with the lipophilicity of the amino acid used for the replacement of D-allo-threonine.
2) Trifunctional, aliphatic amino acids used for the replacement of D-allo-threonine yield inactive tripeptides.
3) The results of the cross-stripe test show that the middle amino acid in the tripeptides most probably determines the activity.

As the tripeptides with an *N*-α-Me-L-amino acid in their middle positions yield the same results in the

Table 1
Synthetic tripeptides

Peptide	Yield, %*	N,%	α^{20}_{546} α^{20}_{578} c = 1 in water	Amino acid analyses**		Formulae
L-Arg–L-MeAla–L-Phe	50	calc. 17.24 found 17.13	−33.0	Arg MeAla Phe	0.96 – 1.00	$C_{19}H_{30}N_6O_4 \cdot HBr$ MW: 487.4
L-Arg–L-MeGlu–L-Phe	56	calc. 15.41 found 15.33	−18.0 −16.5	Arg MeGlu Phe	0.98 – 1.00	$C_{21}H_{32}N_6O_4 \cdot HBr$ MW: 545.5
L-Arg–L-MeIle–L-Phe	52.8	calc. 15.87 found 15.75	−41.0	Arg MeIle Phe	1.02 – 1.00	$C_{22}H_{36}N_6O_4 \cdot HBr$ MW: 529.5
L-Arg–L-MeLeu–L-Phe	61.3	calc. 15.87 found 15.77	+15.0 +13.0	Arg MeLeu Phe	0.98 – 1.00	$C_{22}H_{36}N_6O_4 \cdot HBr$ MW: 529.5
L-Arg–L-MePhe–L-Phe	58.7	calc. 14.92 found 14.83	−15.0 −17.0	Arg MePhe Phe	1.02 – 1.00	$C_{25}H_{36}N_6O_4 \cdot HBr$ MW: 563.5
L-Arg–Sar–L-Phe	77.2	calc. 17.75 found 17.62	+20.5 +25.5	Arg Sar Phe	0.96 – 1.00	$C_{18}H_{28}N_6O_4 \cdot HBr$ MW: 473.4
L-Arg–L-MeSer–L-Phe	56.9	calc. 16.70 found 16.59	+12.0 +10.5	Arg MeSer Phe	0.95 – 1.00	$C_{19}H_{30}N_6O_5 \cdot HBr$ MW: 503.4
L-Arg–L-MeThr–L-Phe	67	calc. 16.24 found 16.09	−27.0 −25.0	Arg MeThr Phe	1.01 – 1.00	$C_{20}H_{32}N_6O_5 \cdot HBr$ MW: 517.4
L-Arg–L-MeVal–L-Phe	73.1	calc. 16.30 found 16.12	−48.0 −42.0	Arg MeVal Phe	0.93 – 1.00	$C_{21}H_{34}N_6O_4 \cdot HBr$ MW: 515.5

* The yield is related to the first amino acid on the resin support, and was precipitated from EtOH/ether in each case.
** L-Methyl amino acids not determined.

Table 2
Antibiotic activity of synthetic tripeptides

Peptide	Minimal* inhibitory concn., mg/ml	Cross-stripe test	
		Neutralizing amino acid	Neutralizing ratio
L-Arg–L-MeAla–L-Phe	2.1	L-Ala	1:1
L-Arg–Sar–L-Phe	1.5	none found	–
L-Arg–L-MeVal–L-Phe	0.48	L-Val	1:3
L-Arg–L-MeLeu–L-Phe	0.45	L-Leu	1:4
L-Arg–L-MePhe–L-Phe	0.22	L-Phe	1:5
L-Arg–L-MeIle–L-Phe	0.20	L-Ile	1:6

L-Arg–L-MeGlu–L-Phe, L-Arg–L-MeSer–L-Phe and L-Arg–L-MeThr–L-Phe were not inhibitory.

* Inhibition tested on *Paecilomyces varioti.*

biological test as those with a D-amino acid in this position, the conclusion can be drawn, that a special conformation is not essential for antibiotic activity of tripeptides of general sequence L-Arg–X–L-Phe.

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