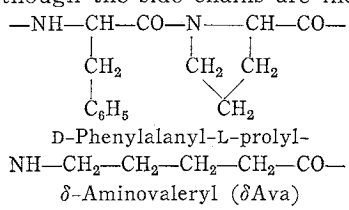
A NEW SYNTHETIC AND ANTIBIOTIC ANALOG OF GRAMICIDIN S, [4-5-δ-AMINOVALERIC ACID]-GRAMICIDIN S

Sir:

A number of gramicidin S analogs have been synthesized to elucidate the relationship between structure and antimicrobial activity[1]. In most of the cases, amino acid residues of the natural peptide were replaced with ones having different side chains. Some of them had antibiotic activity. Some cyclic peptides containing a smaller ring than that of gramicidin S were also synthesized but had no activity. KATO *et al.*[2] and BALASUBRAMANIAN[3] reported the correlation between activity and structure of these antibiotic peptides on the basis of their optical rotatory dispersion.

In order to investigate the contribution of the amide bond in the peptide to the activity and conformation, we attempted to synthesize an analog of a new type, [δ-Ava$^{4-5}$]-gramicidin S, in which one of D-phenylalanyl-L-prolyl residues of the natural peptide was replaced with δ-aminovaleryl residue. This analog and the natural peptide have the same number of members in the ring although the side chains are inevitably changed, and thus it was expected that the analog peptide might have a similar ring structure to the natural peptide and also might have antimicrobial activity.



The analog peptide[4], cyclo-[L-valyl-L-ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl], was synthesized by a conventional method. *p*-Methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-δ-aminovaleryl azide and L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline were synthesized by the stepwise elongation method and were joined to form the linear nonapeptide. This nonapeptide was converted to its *p*-nitrophenyl ester, and the protection on the terminal valine was removed with trifluoroacetic acid. Cyclization was carried out in an excess of pyridine in a good yield (70%) and the benzyloxycarbonyl group of ornithine was removed by hydrogenolysis.

The elementary analyses of the final product were in good agreement with the value calculated for $C_{51}H_{87}N_{11}O_9 \cdot 2HCl \cdot 7H_2O$. $[\alpha]_D^{28}$ −204.0° (*c* 0.1, EtOH). An amino acid analysis gave the following ratio: valine 2.09, ornithine 1.91, leucine 2.09, phenylalanine 1.00, proline 1.05, δ-aminovaleric acid 0.83. In paper electrophoresis (HCOOH-AcOH-H_2O, 4:15:180, v/v, pH 1.9, 15 cm ×40 cm, 600 V, 2 hours), one ninhydrin-positive spot was found (11.8 cm toward the cathode) and it moved a little faster than gramicidin S. This analog peptide has antimicrobial activity for some microorganisms as shown in Table 1. The acute toxicity (LD_{50}) of the peptide dihydrochloride in mice

Table 1. Antimicrobial activity of gramicidin S and [δAva$^{4-5}$]-gramicidin S

	Test organisms	M. I. C. (mcg/ml)	
		Gramicidin S	[δAva$^{4-5}$]-gramicidin S
Agar dilution method	*Shigella dysenteriae* E-1	3.2	6.3
	Bacillus subtilis PCI 219	6.3	6.3
	Staphylococcus aureus FDA 209P	6.3	12.5
	〃 *albus*	6.3	6.3
	〃 *citreus*	12.5	12.5
	Micrococcus flavus	12.5	12.5
	Sarcina lutea	12.5	25
	〃 〃 ATCC 1001	12.5	25
	Proteus vulgaris OX 19	100	50
Broth dilution method	*Candida albicans* Yu-1200	6.3	25

was 40~62.5 mg/kg when given intravenously.

KATO[2] described a correlation between antibiotic activity and the appearance of COTTON effect in the ORD of gramicidin S and related peptides. Our analog peptide gave a smilar type of ORD to gramicidin S. A negative COTTON effect was observed at 232 mμ with about two third of the depth of gramicidin S. Several authors[5~8] have discussed possible conformations of gramicidin S, and STERN *et al.*[7] proposed a conformation in solution deduced from nmr spectra. According to STERN's results, two amide bonds joining D-phenylalanine with L-proline do not paricipate in intramolecular hydrogen bonds. The fact that this analog peptide, lacking in one of these amide bonds, has biological activity and, by ORD, appears to have a gramicidin S-like conformation, suggests that not every peptide bond is indispensable to the function and backbone structure of gramicidin S.

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