[4,4′-D-Diaminopropionic acid]gramicidin S: a synthetic gramicidin S analog with antimicrobial activity against Gram-negative bacteria

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Gramicidin S is especially active against Gram-positive bacteria; e.g., *Staphylococcus aureus*. An analog, [4,4′-D-diaminopropionic acid]gramicidin S, which contains D-diaminopropionic acid residues instead of D-phenylalanine residues, has been synthesized. This analog is active against some of the Gram-negative bacteria; e.g., *Escherichia coli* and *Salmonella typhosa*. Activities of several related analogs are discussed.

Gramicidin S *Gram-negative bacteria* *Peptide antibiotic* *Peptide synthesis* *Structure-activity relationship*

1. INTRODUCTION

Gramicidin S (fig.1) is a cyclic decapeptide antibiotic and exhibits high activity against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* [1]. Its decapeptide backbone holds a rigid β-sheet structure stabilized with 4 intramolecular hydrogen bonds as shown in fig.1. This conformation gives a cationic detergent-like character to the peptide [2]. In the course of studies on structure–activity relationships of gramicidin S, we synthesized analogs containing D-2,3-diaminopropionic acid (D-A_2pr) residues at 4,4′ positions in order to study the role of two additional cationic residues on antibacterial activity. We synthesized also an analog containing α,β-dehydroalanine (ΔAla) residues at 4,4′ positions from the analog containing D-A_2pr residues.

2. EXPERIMENTAL

Molecular mass was determined on a JEOL JMS-DX 300 mass spectrometer. CD spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter in methanol.

The route for the synthesis of [L-Orn(HCO)$^{2,2'}$, D-A_2pr(Z)$^{4,4'}$]gramicidin S is shown in fig.2. The peptide chain was elongated stepwise by use of Boc-amino acid active ester and successive TFA treatment until protected pentapeptide I was obtained. The C-terminus in intermediate linear pentapeptide was N^{ϵ}-formyl-L-ornithine. One half of compound I was converted to active ester II, and another half was treated with TFA to give pentapeptide trifluoroacetate III–TFA. Coupling of II with III afforded decapeptide (IV) (82%), which was again converted to active ester, treated with TFA, and then

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Abbreviations: see IUPAC-IUB Commissions (1974) Pure Appl. Chem. 40, 317–331; Others: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; HO-NSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid

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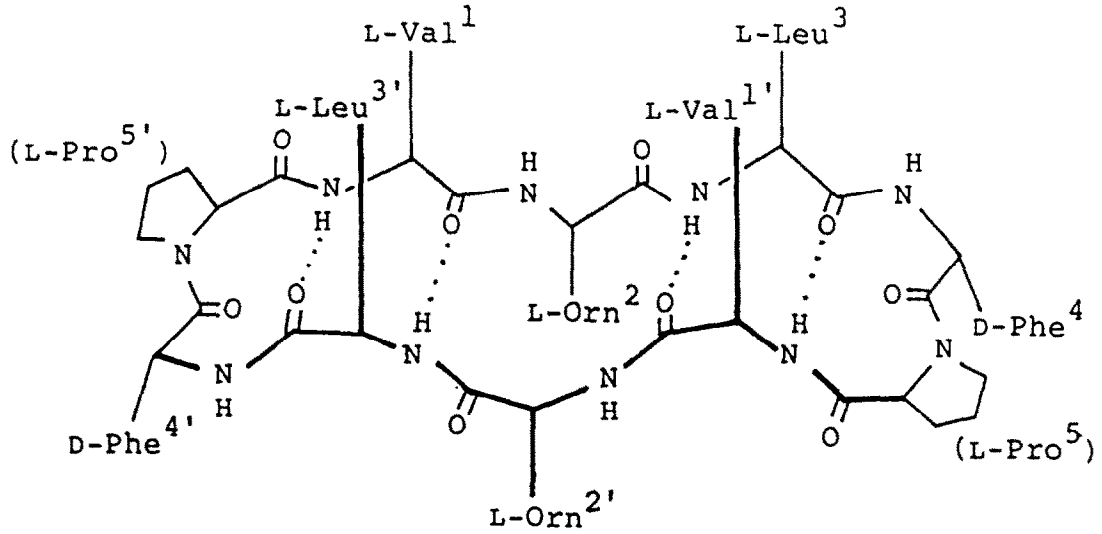

Fig.1. Structure of gramicidin S.

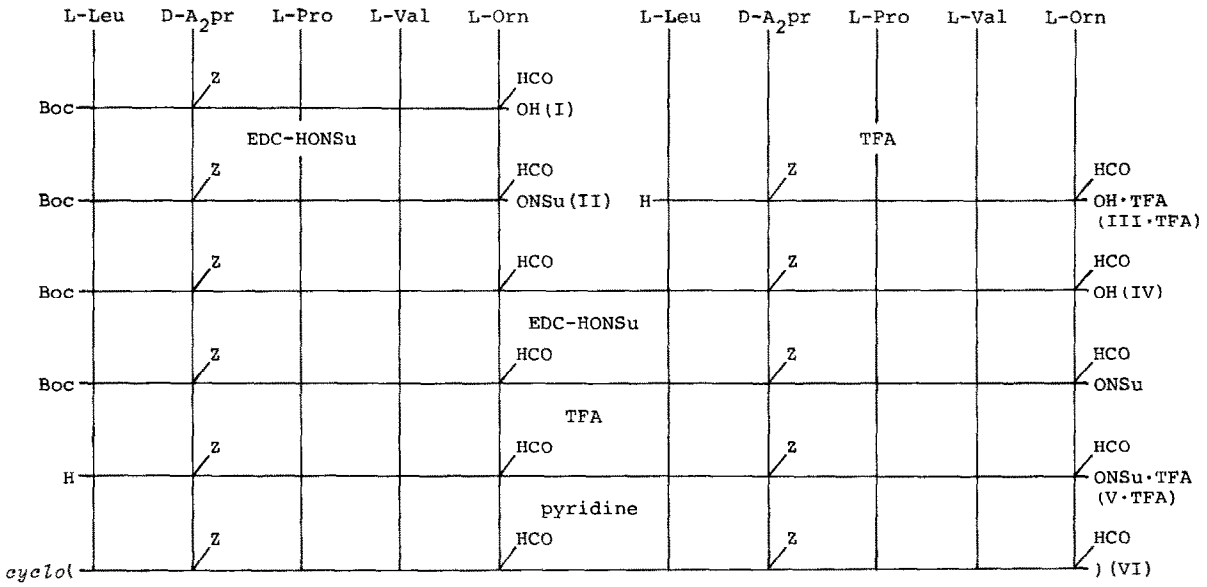

Fig.2. Synthesis of [L-Orn(HCO)$^{2,2'}$, D-A$_2$pr(Z)$^{4,4'}$]gramicidin S.

subjected to cyclization in pyridine at 3 mM of V. Purification with Amberlite CG-50, Dowex 1, and silica gel column chromatography yielded [L-Orn(HCO)$^{2,2'}$, D-A$_2$pr(Z)$^{4,4'}$]gramicidin S(VI) (39% from IV).

Treatment of VI with 0.5 M HCl in methanol for 3 days gave [D-A$_2$pr(Z)$^{4,4'}$]gramicidin S–2 HCl (VII–2 HCl) (75%, M_r $(m+1)^+$ 1288, calcd $(m+1)^+$1288). Hydrogenolysis of VII removed the Z groups to give [D-A$_2$pr$^{4,4'}$]gramicidin S–4 HCl (VIII–4 HCl) (76%). Treatment of VII with $(Boc)_2O$ [3] afforded [L-Orn(Boc)$^{2,2'}$, D-A$_2$pr(Z)$^{4,4'}$]gramicidin S (IX) (52%). Hydrogenolysis of IX gave [L-Orn(Boc)$^{2,2'}$, D-A$_2$pr$^{4,4'}$]-gramicidin S–2 HCl (X–2 HCl) (95%), which was treated with CH_3I and $KHCO_3$ as in [4,5] to give

Table 1

Antimicrobial activity of gramicidin S analogs

Organism	Minimum inhibitory concentration (μg/ml)				
	GS	VII	VIII	X	XII
Staphylococcus aureus FDA 209P	6.25	6.25	100	>100	12.5
Bacillus subtilis PCI 219	3.13	3.13	12.5	>100	6.25
Escherichia coli NIHJ JC-2	>100	>100	25	>100	>100
Salmonella typhosa Boxhill 58	>100	>100	25	>100	>100
Shigella flexneri EW-10	6.25	6.25	25	>100	50
Shigella sonnei EW-33	100	>100	50	>100	100
Klebsiella pneumoniae DT	12.5	50	50	>100	25
Proteus vulgaris IFO 3988	>100	>100	>100	>100	>100

[L-Orn(Boc)$^{2,2'}$, ΔAla$^{4,4'}$]gramicidin S (XI) (74%). This was treated with TFA to yield [ΔAla$^{4,4'}$]gramicidin S–2 TFA (XII–2 TFA) (88%). All crystalline compounds gave satisfactory elemental analyses.

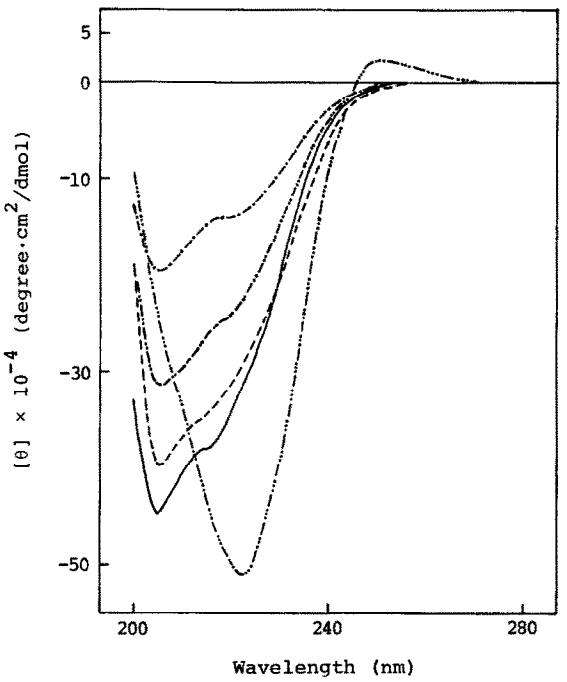

Fig.3. CD curves of gramicidin S analogs. Solvent: MeOH. Curve: (——) gramicidin S; (– – –) VII; (–·–·–) VIII; (–··–··–) X; (–···–···–) XII.

3. RESULTS

Results of the microbial assay are shown in table 1. Gramicidin S and compound VII show similar activities against Gram-positive bacteria and *Shigella flexneri*. On the other hand, compound VIII shows moderate activity against several Gram-negative bacteria including *Escherichia coli* but negligible activity against Gram-positive bacteria such as *Staphylococcus aureus*. Unexpectedly, compound XII shows high activity comparable to that of gramicidin S.

Fig.3 shows CD curves of gramicidin S and synthesized analogs. Characteristic features (troughs at about 205 and 215 nm) for gramicidin S are essentially preserved for VII, VIII and X. However, the intensity of the troughs was considerably decreased for VIII. Proximity of the 4 cationic charges may cause some disturbance in the backbone conformation. The CD curve of XII shows a trough at 222 nm, signifying change in conformation due to incorporation of ΔAla residues.

4. DISCUSSION

Dozens of amino acid-substituted analogs are synthesized for gramicidin S. All active analogs hold similar cationic detergent-like characters [2]. Analogs containing D-A$_2$pr residues also conserve the common character as suggested by similarity of CD curves. Complete inactivity of X suggests that the side-chain length of D-A$_2$pr is too short to interact with bacteria.

Compound VIII showed activity against some of Gram-negative bacteria. As the conformations of VIII and gramicidin S seem to be similar, the change in the activity should be due to the increase of cationic charges at the hydrophilic side of the molecule. It should be noted here that polymyxins, known by their high activity against Gram-negative bacteria, contain 6 basic amino acid residues out of 10 component amino acid residues [6].

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