# A NEW SYNTHETIC ANTIBIOTIC ANALOG OF GRAMICIDIN S, [2,2′-L-SERINE]-GRAMICIDIN S

Sir:

In studies of relationship between structure and antibacterial activity of gramicidin S, various analogs have been synthesized. From these studies, it has been recognized that the basic amino groups of ornithine residues in gramicidin S are necessary for the activity. For example, acylation of the amino groups in ornithine residues caused drastic decrease in the activity[1]. On the other hand, changing the length of the side chain in the ornithine residues[2,3] and conversion of the amino groups into strongly basic guanidino groups does not alter the activity[4]. Recently, [His$^{2,2'}$]-GS, in which the ornithine residues are replaced by histidine residues, was synthesized and shown to be 1/8 or 1/16 times as active as gramicidin S[5].

In order to investigate the biological role of $\delta$-amino groups in ornithine residues, we synthesized the gramicidin analog, [Ser$^{2,2'}$]-GS, in which these are replaced by serine residues (Fig. 1).

The compound was synthesized by conventional methods. Boc-Val-Ser hydrazide and Leu-D-Phe-Pro-OBzl, synthesized by DCC coupling using 1-hydroxybenztriazole, were joined to form Boc-Val-Ser-Leu-D-Phe-Pro-OBzl, which in part was converted to the Boc-pentapeptide hydrazide*,**. Coupling of Boc-pentapeptide hydrazide and peptapeptide ester derived from the protected ester afforded Boc-(Val-Ser-Leu-D-Phe-Pro)$_2$-OBzl. This ester was converted into the corresponding hydrazide, and then cyclized by azide method using pyridine. The cyclodecapeptide, [Ser$^{2,2'}$]-GS, was obtained in 60% yield. The homogeneity of [Ser$^{2,2'}$]-GS was confirmed by means of thin-layer chromatography, amino acid analysis, and elemental analysis.

The antibacterial activity toward several microorganisms was examined, and the results are shown in Table 1. This analog has antibacterial activity against some Gram-positive microorganisms, in spite of the absence of the ornithine amino groups. However, its activity is less by about one order than that of natural gramicidin S.

The ORD and CD spectra of this analog were measured in ethanol. Its ORD spectrum shape resembled that of gramicidin S, and a trough was observed at 234 nm like that of gramicidin S. In the CD spectrum was also observed a curve similar to that of gramicidin S, and the two troughs at 209 nm and 217 nm were slightly shallower than those of gramicidin S. The results of these spectra indicate that the conformation of the analog is similar to that of gramicidin S.

For the mode of action of gramicidin S, it has been reported by S. N. SEMENOV *et al.*[6] that the

Fig. 1

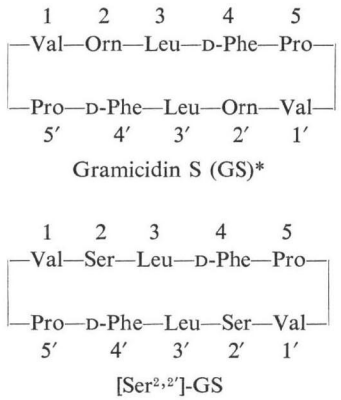


Table 1. Antibacterial activity of GS and its analog.

| Strain | Minimum inhibitory concentration, $\mu$g/ml | |
|---|---|---|
| | GS | [Ser$^{2,2'}$]-GS |
| *Staph. aureus* ATCC 6538P | 6.3 | >100 |
| *Strept. pyogenes* N.Y.5 | 1.6 | 25 |
| *Micrococcus flavus* ATCC 10240 | 1.6 | 12.5 |
| *Corynebact. diphtheriae* P.W.8 | 0.8 | 6.3 |
| *Bac. subtilis* ATCC 6633 | 1.6 | >100 |
| *E. coli* NIHJ-JC2 | >100 | >100 |

* Abbreviations with no prefix show L-amino acid residue.

** The following are from J. Biol. Chem. 247: 977~983, 1972: Boc-, t-butoxycarbonyl; DCC, dicyclohexylcarbodiimide.

most significant role for the gramicidin S membrane activity is played by the "pleated sheet" conformation, while free amino groups of ornithine residues are involved only in the formation of amino group-lipid complexes. In the present study, it is noteworthy that the activity of this synthetic analog might simulate not gramicidin S proper, but its active complex directly.

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## References

1) KAPREL'YANTS, A. S.; V. V. NIKIFOROV, A. I. MIROSHNIKOV, L. G. SNEZHKOVA, V. A. EREMIN & D. N. OSTROVSKI: Bacterial membranes and mechanism of action of gramicidin S. Biochemistry (translated from Russian) 42: 252~257, 1977
2) SCHWYZER, R. & P. SIEBER: A synthetic analog of gramicidin S, Cyclo (Val-Lys-Leu-D-Phe-Pro)$_2$. Helv. Chim. Acta 41: 1582~1587, 1958
3) WAKI, M.; O. ABE, R. OKAWA, T. KATO, S. MAKISUMI & N. IZUMIYA: Studies of peptide antibiotics. XII. Syntheses of [2,2′-α,γ-diaminobutyric acid]- and [2,2′-lysine]-gramicidin S. Bull. Chem. Soc. Jpn. 40: 2904~2909, 1967
4) SILAEV, A. B. & V. M. STEPANOV: Significance of free amino group of gramicidin S for its biological activity. Dokl. Akad. Nauk SSSR. 112: 297~299, 1957
5) NONAKA, K.; K. SATO, S. TERADA, T. KATO & N. IZUMIYA: Studies of peptide antibiotics. XXXIX. Syntheses of gramicidin S analogs containing L-histidine in place of L-ornithine. Bull. Chem. Soc. Jpn. 51: 2127~2130, 1978
6) SEMENOV, S. N.; E. I. MEL'NIK, L. G. SNEZHKOVA, A. I. MIROSHNIKOV & V. T. IVANOV: Study of the interaction of gramicidin S with model lipid systems. Bioorg. Khim. 3: 1055~1063, 1977
IVANOV, V. T.: Solution structures of peptides. *in* Peptides. GOODMAN, M. & J. MEIENHOFER, *Eds*, John Wiley and Sons, Inc., New York, pp. 307~321, 1977