NATURAL HOMOLOGS OF GRAMICIDIN S

Sir:

As natural gramicidin S only a single compound, cyclo(Val-Orn-Leu-D-Phe-Pro)$_2$, is known, although peptide antibiotics produced by microorganisms usually consist of compounds with closely related structures.

We wish to report here that gramicidin S consists of two minor components, named gramicidins S-2 and S-3, and of a major one named gramicidin S-1, with the already known structure (Fig. 1).*

Analytical HPLC (LiChrosorb RP-18, methanol - 5% $NaClO_4$) of gramicidin S (produced by *Bacillus brevis* Nagano) showed the presence of four components (Fig. 2), three of which were isolated as the perchlorates by semipreparative scale HPLC. The molar ratio of the isolated compounds was 1 (gramicidin S-1) : 0.034 (S-2) : 0.020 (S-3) (Total recovery of the peptides was 80%). Their antibiotic activities toward several test microorganisms resembled each other (Table 1).

The amino acid compositions found in the acid-hydrolysates (6 M HCl, 110°C, 20 hours) were as follows.

Gramicidin S-1 : Pro 2.12, Val 1.75, Leu 2.12, Phe 1.94, Orn 2.07. Gramicidin S-2 : Pro 2.01, Aba 1.04, Val 0.99, Leu 2.02, Phe 1.87, Orn 2.07. Gramicidin S-3 : Pro 1.97, Aba 1.91, Leu 2.04, Phe 2.01, Orn 2.07.

The data show that gramicidin S-1 is composed of the five amino acid constituents of the known structure. In gramicidins S-2 and S-3, one or both valyl residues in the known structure are replaced by one or two 2-aminobutyric acid residues respectively. The presence of 2-aminobutyric acid was confirmed by amino acid analyses under several conditions.

The secondary ion mass spectra of the acetyl derivatives showed the following peaks (m/z).

Acetyl gramicidin S-1 : 1,247 (M, $C_{64}H_{96}O_{12}N_{12}$, $+Na)^+$, 1,225 $(M+H)^+$, 417 (Ac-Orn-Leu-Phe$+H)^+$, 353 (Pro-Val-Ac-Orn$+H)^+$.

Acetyl gramicidin S-2 : 1,233 (M, $C_{63}H_{94}O_{12}N_{12}$, $+Na)^+$, 1,211 $(M+H)^+$, 417 (Ac-Orn-Leu-Phe$+H)^+$, 353 (Pro-Val-Ac-Orn$+H)^+$, 339 (Pro-Aba-Ac-Orn$+H)^+$.

Acetyl gramicidin S-3 : 1,219 (M, $C_{62}H_{92}O_{12}N_{12}$, $+Na)^+$, 1,197 $(M+H)^+$, 417 (Ac-Orn-Leu-Phe$+H)^+$, 339 (Pro-Aba-Ac-Orn$+H)^+$.

The differences of 14 mass numbers found between the molecular ions of gramicidins S-1 and S-2, and between those of S-2 and S-3 were interpreted by the successive replacement of valyl

Fig. 1. Structures of gramicidins S-1, S-2 and S-3.

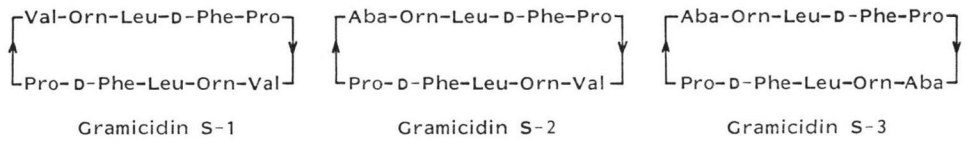


Table 1. Antibiotic activities of gramicidins S-1, S-2 and S-3.

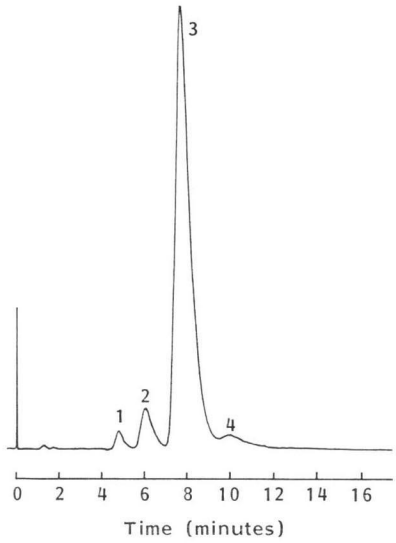
Test organisms	Minimum inhibitory concentration (μg/ml)		
	Gramicidin S-1	Gramicidin S-2	Gramicidin S-3
Staphylococcus aureus JC-1	3.13	3.13	3.13
Streptococcus pyogenes Cook	6.25	6.25	6.25
Corynebacterium diphtheriae Type Gravis	3.13	3.13	3.13
Bacillus subtilis ATCC 6633	3.13	1.56	3.13
Escherichia coli NIHJ JC-2	>50	>50	>50

* Amino acids with no prefix are of L-configuration.
Aba=2-Aminobutyric acid.

Fig. 2. HPLC profile of gramicidins S-1, S-2 and S-3.

Column: LiChrosorb RP-18, 4×250 mm. Mobile phase: CH_3OH - 5% $NaClO_4$, 5: 1. Flow rate: 1.83 ml/minute. Monitored at 220 nm.

1: Gramicidin S-3, 2: gramicidin S-2, 3: gramicidin S-1, 4: unidentified.



residues by 2-aminobutyric acid residues. The data on the fragment ions also agreed with the structures shown in Fig. 1.

The CD spectra of gramicidins S-1, S-2 and S-3 in ethanol are closely similar to each other with troughs at 207 nm and shoulders near 219 nm, indicating that the three compounds have similar ring conformations. Considering configurational homology usually found in peptide antibiotics produced by a single species, it is reasonable to conclude that the constituent amino acids in gramicidins S-2 and S-3 have L-configurations with the exception of phenylalanine, which has D-configuration.

Isolation of the homologous peptides showed that the well-known antibiotic, gramicidin S, is a member of a "family" as other antibiotics[1] yielded by *B. brevis*. The HPLC profile indicates the presence of an additional minor homolog (Fig. 2, peak 4), which we have not identified so far.

Acknowledgment

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SUKEKATSU NOZAKI†
ICHIRO MURAMATSU

†Department of Microbiology,
Faculty of Pharmaceutical Sciences,
Josai University,
Sakado, Saitama, 350-02, Japan

Department of Chemistry,
College of Science,
Rikkyo University,
Nishi-Ikebukuro, Tokyo, 171, Japan

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