SYNTHETIC STUDIES ON GRATISIN

Sir:

An antibiotic peptide, gratisin, showing activity toward *Bacillus subtilis* 720 was isolated from *Bacillus brevis* Y-33 by SILAEV *et al.*[1] It is a cyclododecapeptide composed of two each of Val, Orn, Leu, Phe, Pro and Tyr residues. The primary structure of gratisin was proposed as shown in Fig. 1.[2,3], but the configuration of the individual amino acid residues has not been established.

On the basis of the proposed structure, we synthesized a cyclic peptide containing only L-amino acid residues (all-L-gratisin) and another containing D-Phe residues ([D-Phe$^{4,4'}$]gratisin), and investigated the relationship between their structure and antibiotic activity.

The two compounds were synthesized by conventional methods. Boc-Tyr(Bzl)-Val-Orn(Z)-Leu-Phe-Pro-OBzl (**1**)* and its D-Phe containing isomer (**2**) were synthesized by the stepwise elongation form Pro-OBzl and coupling with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) and 1-hydroxy benzotriazole, and were converted to the hydrazides, respectively. In the synthesis of [D-Phe$^{4,4'}$]gratisin, coupling of the Boc-hexapeptide hydrazide and hexapeptide ester derived from **2** was carried out by an azide method and afforded the Boc-dodecapeptide ester. This ester was converted into the corresponding hydrazide, which was treated with HCl/dioxane and then cyclized by azide method in pyridine. The yield of cyclization was 43%. The protecting groups of cyclododecapeptide were removed by hydrogenolysis to afford [D-Phe$^{4,4'}$]gratisin. All-L-gratisin was prepared in the following way. Coupling of Boc-all-L-hexapeptide hydrazide and Tyr(Bzl) - Val-Orn(Z) - Leu - Phe - Pro - OH derived from **1** was carried out by an azide method and afforded Boc-dodecapeptide. This was converted into the corresponding active ester by WSCD and *N*-hydroxysuccinimide. The protected active ester was treated with trifluoroacetic acid and then cyclized in pyridine at 60°C for 2 hours. The yield of the cyclization was 29%. The protecting groups of cyclododecapeptide were removed by hydrogenolysis to afford all-L-gratisin. The homogeneity of [D-Phe$^{4,4'}$]- and all-L-gratisin were confirmed by thin-layer chromatography, amino acid analysis and elemental analysis.

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

The following are from J. Biol. Chem. 247: 977~983, 1972: Boc-, *t*-butoxycarbonyl; Z-, benzyloxycarbonyl; Bzl-, benzyl.

Fig. 1.

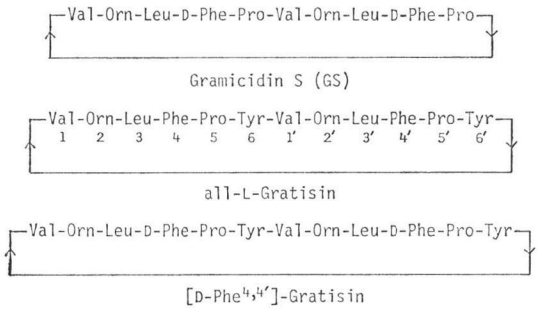


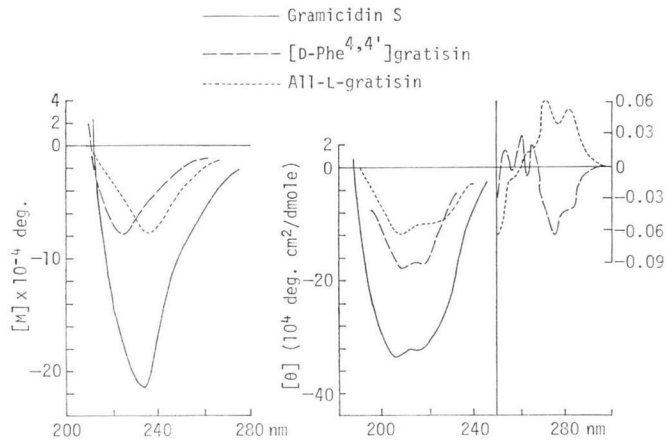
Table 1. Antibiotic activities of GS, [D-Phe$^{4,4'}$]- and all-L-gratisin
(Minimum inhibitory concentration, μg/ml).

Test organisms	GS	[D-Phe$^{4,4'}$]-	all-L-
Staphylococcus aureus ATCC 6538P	1.6	50	>50
Streptococcus pyogenes N.Y. 5	1.6	12.5	>50
Micrococcus flavus ATCC 10240	0.8	12.5	25
Corynebacterium diphtheriae P.W. 8	0.8	3.1	25
Bacillus subtilis ATCC 6633	3.1	50	>50
Escherichia coli NIHJ-JC2	>100	>50	>50
Proteus vulgaris OX 19	>100	>50	>50

The antibiotic activities of the two cyclic peptides and gramicidin S toward several organisms are summarized in Table 1. [D-Phe$^{4,4'}$]gratisin is active toward all Gram-positive microorganisms tested, but it is not as potent as gramicidin S. All-L-gratisin shows little activity.

The ORD and CD spectra of [D-Phe$^{4,4'}$]-gratisin, all-L-gratisin and gramicidin S in aqueous solution are shown in Fig. 2. The 200~250 nm region of these spectra was observed a curve similar to that of gramicidin S. These results indicate that both [D-Phe$^{4,4'}$]- and all-L-gratisin have a preferred conformation in water. In the 250~290 nm region, the CD spectrum of all-L-gratisin was closely similar to the inverted spectrum of [D-Phe$^{4,4'}$]gratisin. Based on the

Fig. 2. ORD and CD spectra of gramicidin S, [D-Phe$^{4,4'}$]gratisin and all-L-gratisin in water.



studies of SHIRAKI[4] and BEYCHOK *et al.*[5], the CD spectrum of [D-Phe$^{4,4'}$]gratisin at longer wavelengths showed result from the aromatic side chains of Phe and Tyr residues. The shape and reverse sign of all-L-gratisin suggests interaction between the two aromatic side chains.

These results are not sufficient for determination of the configuration of the constituent amino acids in natural gratisin. However, our results suggest that Phe residues in gratisin probably have D-configuration.

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