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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF AN IMMEDIATE PRECURSOR OF GRAMICIDIN S

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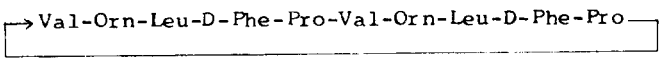
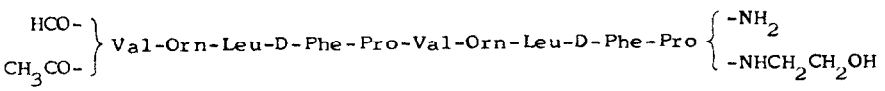
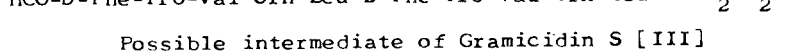
For clarifying the influence of the cyclic structure of gramicidin S [I] to its antibacterial activity, several linear decapeptide analogs [II] having an amino acid sequence of the gramicidin S in which the α-amino group is acylated with formyl or acetyl and C-terminal amino acid in the form of its amide or ethanolamide have been synthesized in this laboratory (1). Recently, Pollard et al. (2) observed the formation of an another linear decapeptide [III], with formate linked to the N-terminal amino acid and ethanolamine to the C-terminus of the chain, in the cell-free biosynthesis systems as a possible intermediate of gramicidin S, however, they did not describe any of the physical and biological proterties of the peptide. It can easily be seen that this natural analog [III] and the synthetic analogs [II] are very similar structurally, differing only by the sequence of amino acids. In connection with our original



Gramicidin S [I]



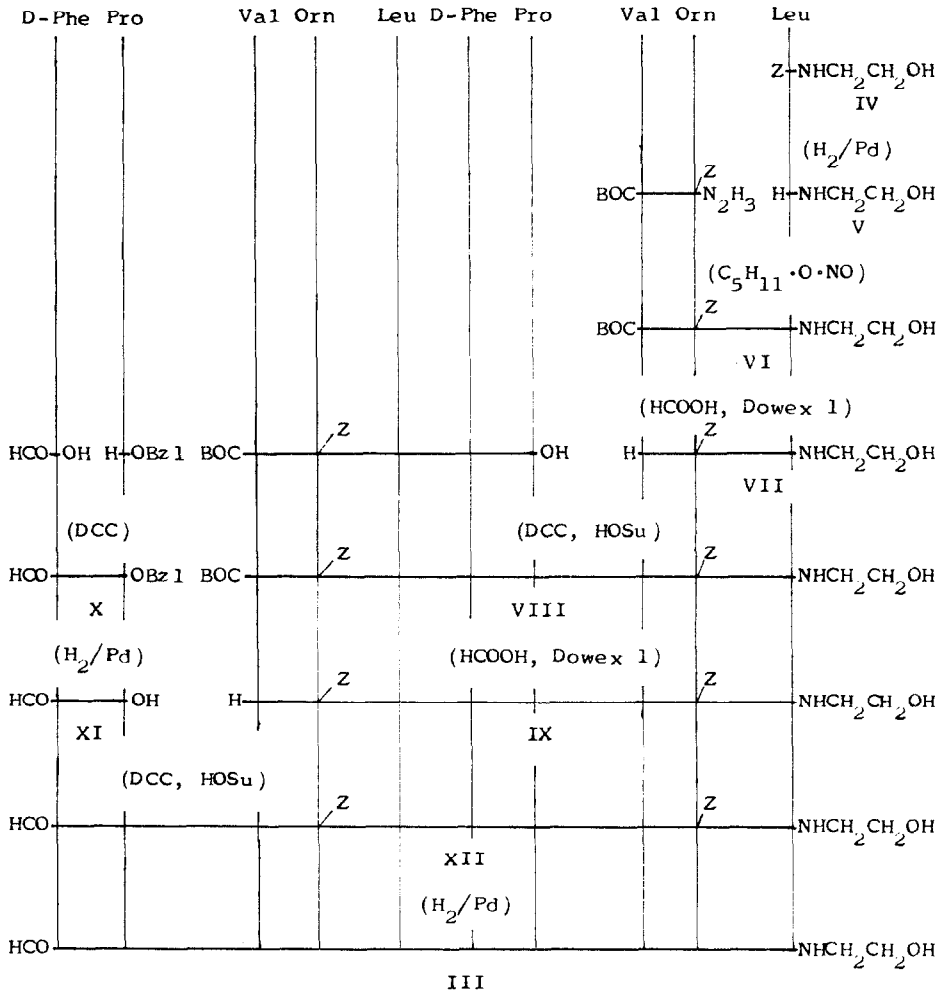
Model decapeptide analogs [II]



Possible intermediate of Gramicidin S [III]

program it is also interesting to synthesize this naturally occurring linear peptide and test its biological activity.

Details of the synthesis are summarized in the diagram and there only its more important features are outlined.



BOC-, t-Butyloxycarbonyl; Z-, Benzyloxycarbonyl; DCC, Dicyclohexylcarbodiimide; HOSu, N-Hydroxysuccinimide

Z-Leu-$NHCH_2CH_2OH$ [IV], mp 125-126°, $[\alpha]_D$ -179°, was prepared by coupling Z-Leu-OH with ethanolamine by the mixed anhydride method (3) in a yield of 71%. IV was converted to H-Leu-$NHCH_2CH_2OH \cdot HCl$ [V], mp 153-154°, $[\alpha]_D$ +22.7°, by catalytic hydrogenation in 93% yield. Condensation of the azide derived by treatment with isoamylnitrite from BOC-Val-Orn(δ-Z)-N_2H_3 (4) with V gave BOC-Val-Orn(δ-Z)-Leu-$NHCH_2CH_2OH$ [VI] in 76% yield, mp 197-198°, $[\alpha]_D$ -35.2°. Removal of the BOC-group from VI by an exposure to 98% formic acid (5) and subsequent neutralization with Dowex 1 in methanol yielded feee base of H-Val-Orn(δ-Z)-Leu-$NHCH_2CH_2OH$ [VII] in 94% yield, mp 180-182°, $[\alpha]_D$ -19.8°. Reaction of BOC-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (4) with VII by the dicyclohexylcarbodiimide(DCC)-N-hydroxycuccinimide(HOSu) procedure (6) with equivalent amount of DCC and HOSu gave BOC-Val-Orn(δ-Z)-Leu-D-Phe-Pro-Val-Orn(δ-Z)-Leu-$NHCH_2CH_2OH$ [VIII] in 94% yield, mp 156-158°, $[\alpha]_D$ -91.0°. The BOC-group was removed from VIII by the method described above giving H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-Val-Orn(δ-Z)-Leu-$NHCH_2CH_2OH$ [IX] in 91% yield, mp 113-117°, $[\alpha]_D$ -106.5°. Condensation of HCO-D-Phe-OH with H-Pro-OBzl by the DCC procedure (7) gave oily HCO-D-Phe-Pro-OBzl [X] in 87% yield. Hydrogenation of X afforded oily HCO-D-Phe-Pro-OH [XI] which was crystallized as dicyclohexylammonium(DCHA) salt in a yield of 72%, mp 177-178°, $[\alpha]_D$ -51.1°. The pure formyl dipeptide obtained from the DCHA salt of XI by treatment with Dowex 50 in methanol was coupled with IX by the DCC-HOSu procedure. This gave the protected decapeptide HCO-D-Phe-Pro-Val-Orn(δ-Z)-Leu-D-Phe-Pro-Val-Orn(δ-Z)-Leu-$NHCH_2CH_2OH$ [XII] in 86% yield, mp 132-135°, $[\alpha]_D$ -81.0°, (Anal. Calcd for $C_{79}H_{111}N_{13}O_{16} \cdot 2H_2O$: C, 61.82; H, 7.55; N, 11.86%. Found: C, 62.00; H, 7.50; N, 11.97%). The molecular weight of a dired sample of XII was determined with a Hitachi-Perkinelmer Type 115 apparatus using methanol as a solvent (Calcd: 1499, Found: 1447). Removal of the Z-groups from XII by catalytic hydrogenation in the presence of two equivalent amount of hydrogen chloride in methanol provided HCO-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-$NHCH_2CH_2OH$ [III] as dihydrochloride in 82% yield, mp 204-205°, $[\alpha]_D$ -112.7°, (Anal. Calcd for $C_{63}H_{99}N_{13}O_{12} \cdot 2HCl \cdot 4H_2O$: C, 55.01; H, 7.99; N, 13.24; H_2O, 5.2%. Found: C, 55.11; H, 8.06; N, 13.23; H_2O, 5.1%). Satisfactory elemental analyses and chromatographic data were obtained for all

crystalline compounds described above. $[\alpha]_D$ refers to 1% solution in methanol at 25-27°.

The final product III·HCl was practically homogeneous peptide with the following amino acid composition: phenylalanine 2.2, proline 1.9, valine 2.2, ornithine 1.8, leucine 2.3 and ethanolamine 1.0. However, this material was contaminated with a small quantity of the deformylated peptide which could be detected by electrophoresis on paper. It was found that the formyl group in this peptide is labile to be decomposed and the C-terminal ethanolamide group is apt to be rearranged by N-O acyl transfer under the condition of hydrogen chloride in methanol. Optical rotatory dispersion measurement at the region of 220-300 mμ revealed that this linear decapeptide exhibited apparent negative Cotton effect. As this feature of ORD measurement is quite similar to that of gramicidin S (8), it was expected that this peptide III would have antibacterial activity. However, it was found that the peptide possessed no activity for any of Gram-positive microorganisms tested, whereas gramicidin S showed substancial activity under the same conditions toward the microorganisms.

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