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Studies of Peptide Antibiotics. XVIII. The Synthesis of Tyrocidine E[1,2]

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The synthesis of cyclic decapeptide, *cyclo*-L-phenylalanyl-D-phenylalanyl-L-asparaginyl-L-glutaminyl-L-phenylalanyl-L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl, which has the amino acid sequence designated as tyrocidine E by Kurahashi, and the chemical and biological properties of the synthetic product, have been described. The degree of the activity of the synthetic product toward the Gram positive microorganisms is nearly the same as that of tyrocidine A. The results indicate that the L-tyrosine residue in the molecule of tyrocidine A can be replaced by L-phenylalanine without influencing activity.

Tyrocidine E is a new basic polypeptide isolated by Kurahashi and his coworkers[4] from an incubation mixture in a cell-free enzyme system of *Bacillus brevis* ATCC 8185 when both tryptophan and tyrosine were subtracted from the standard culture mixture for the biosynthesis of tyrocidine A, B and C. They have suggested the structure of tyrocidine E to be a cyclic decapeptide shown as XVI[4] in Fig. 1 by comparison of the amino acid composition of a sample designated as tyrocidine E with that of tyrocidine A, since the amino acid sequence of tyrocidine A has been already established.[5,6] However, they did not mention the property of an antibacterial activity of tyrocidine E and data such as melting point, specific rotation and elemental analysis.

We reported previously the synthesis of tyrocidine A[6] and B,[7] and have been attempting to synthesize other tyrocidines. This paper describes the synthesis of cyclic decapeptide hydrochloride (XVI·HCl) having the amino acid sequence of tyrocidine E (Fig. 1), and the chemical and biological properties of the synthetic product.

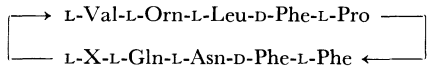

Fig. 1. Structure of tyrocidine E (XVI) and A (XVII).
X represents an amino acid residue such as Phe (XVI) and Tyr (XVII).

The synthesis of the pentapeptide derivative, Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-$NHNH_2$ (VI), was achieved as follows (Fig. 2). The tripeptide derivative (III) of the C-terminal moiety was built up by stepwise elongation from the carboxyl toward the amino end to minimize racemization. The condensation of benzyloxycarbonyl-glutamine *p*-nitrophenyl ester with phenylalanine ethyl ester gave dipeptide derivative (I). This was hydrogenated in the presence of palladium black in a mixture of methanol and dimethylformamide containing an equivalent of hydrogen chloride to give dipeptide ester hydrochloride (II). The condensation of benzyloxycarbonyl-asparagine *p*-nitrophenyl ester with II gave acyl tripeptide ester (III) which was converted to tripeptide ester hydrochloride (IV) by the catalytic hydrogenation as described for II. In a previous study[6] the decarbobenzoxylation of the protected di- and tripeptide stages was performed by treatment with hydrogen bromide in acetic acid and the oily products obtained were contaminated by a small amount of impurities. However, the decarbobenzoxylation step in the

1) A part of this work was presented at the 21st Annual Meeting of Chemical Society of Japan, Osaka, April, 1968 (Preprints, Vol. 3, p. 2242), and communicated briefly; N. Mitsuyasu and N. Izumiya, *Experientia*, **26**, in press (1970).

2) The following abbreviations are used; Z-, benzyloxycarbonyl; Z(OMe)-, *p*-methoxybenzyloxycarbonyl; -ONp, *p*-nitrophenyl ester; -OEt, ethyl ester; $-NHNH_2$, hydrazide; $-N_3$, azide; TEA, triethylamine; DMF, dimethylformamide. Amino acid symbols except for D-Phe denote L-configuration.

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4) K. Fujikawa, Y. Sakamoto, T. Suzuki and K. Kurahashi, *Biochim. Biophys. Acta*, **169**, 520 (1968).

5) A. Paladini and L. C. Craig, *J. Amer. Chem. Soc.*, **76**, 688 (1954).

6) M. Ohno and N. Izumiya, *ibid.*, **88**, 376 (1966); M. Ohno, T. Kato, S. Makisumi and N. Izumiya, This Bulletin, **39**, 1738 (1966).

7) K. Kuromizu and N. Izumiya, presented at the 7th Symposium on Peptide Chemistry at Tokyo University, Tokyo, Nov. 21, 1969; submitted for publication in *Experientia*.

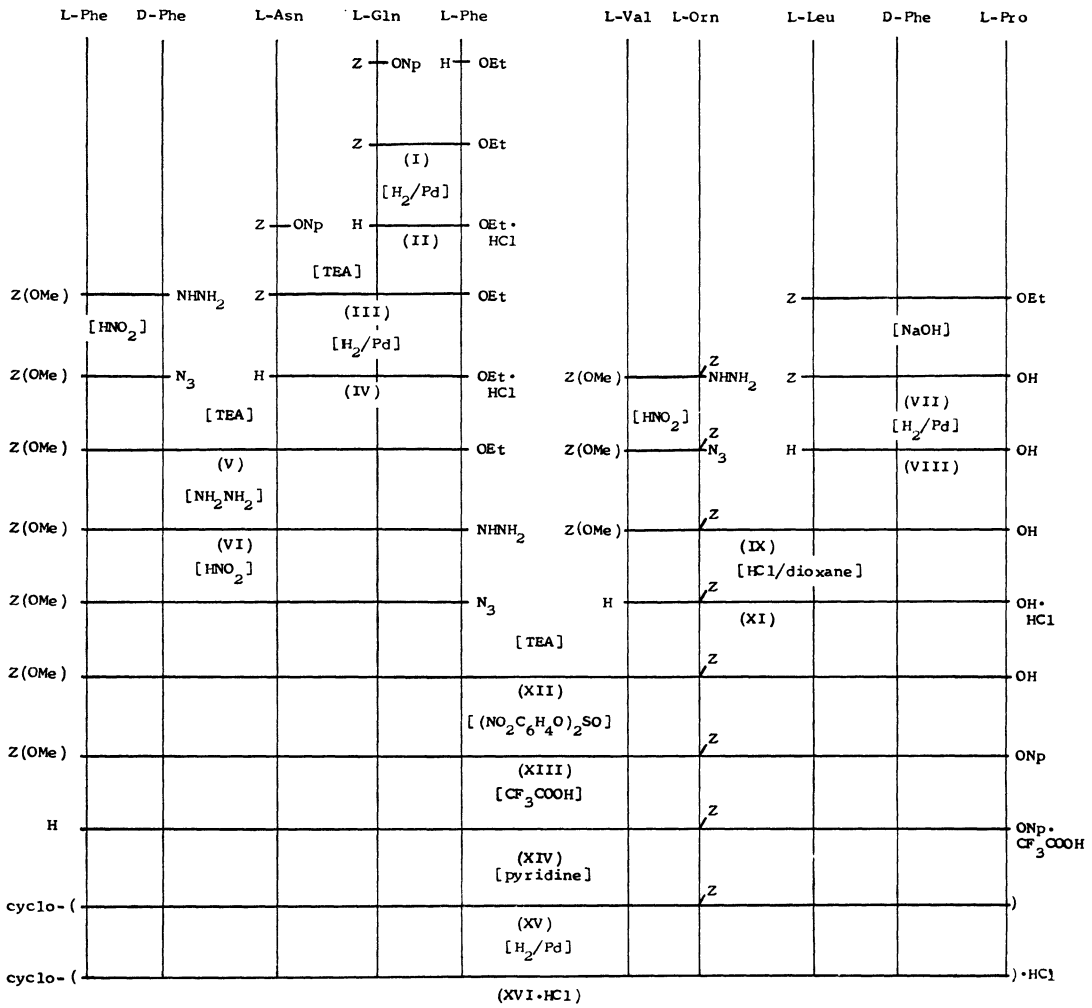

Fig. 2. Synthesis of tyrocidine E.

present study was much improved by this catalytic hydrogenation procedure which afforded pure crystalline products. The azide derived from Z(OMe)-Phe-D-Phe-$NHNH_2$[6] was allowed to react with tripeptide ester (IV) to afford acylpentapeptide ester (V) in a 73% yield. Compound V was converted to the corresponding hydrazide (VI) in a 97% yield by treatment with hydrazine.

Another pentapeptide derivative, H-Val-Orn-(δ-Z)-Leu-D-Phe-Pro-OH·HCl (XI), was prepared as follows (Fig. 2). The free tripeptide (VIII) was prepared from Z-Leu-D-Phe-Pro-OEt by saponification and successive hydrogenation in a mixture of methanol, acetic acid and water. The azide derived from Z(OMe)-Val-Orn(δ-Z)-$NHNH_2$[8] was allowed to react with VIII in the presence of an equivalent amount of triethylamine to yield crystalline protected pentapeptide (IX)[9] in a 73% yield. Treatment of IX with twenty equivalents of hydrogen chloride in dioxane afforded pure crystalline pentapeptide hydrochloride (XI)[10] in a yield of 90%.

Subsequent synthesis of the cyclic decapeptide hydrochloride (XVI·HCl) was achieved as follows (Fig. 2). Condensation of the azide derived from VI with XI in the presence of two equivalents of triethylamine gave amorphous protected decapeptide (XII) in a yield of 80%. XII was converted

8) T. Kato, M. Kondo, M. Ohno and N. Izumiya, This Bulletin, **38**, 1202 (1965).

9) Acylpentapeptide acid (IX) was prepared previously by saponification of Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OEt; M. Waki and N. Izumiya, *J. Amer. Chem. Soc.*, **89**, 1278 (1967); This Bulletin, **40**, 1687 (1967).

10) Hydrochloride (XI) of the neutral pentapeptide was prepared by the action of hydrogen chloride on BOC-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH: see Ref. 6.

to the corresponding decapeptide *p*-nitrophenyl ester (XIII) with the action of twenty equivalents of di-*p*-nitrophenyl sulfite in the presence of pyridine. Removal of the *p*-methoxybenzyloxycarbonyl group from XIII by the action of trifluoroacetic acid yielded amorphous decapeptide *p*-nitrophenyl ester trifluoroacetate (XIV). Cyclization reaction of XIV in a large amount of hot pyridine gave crude benzyloxycarbonyl-substituted cyclic decapeptide (XV), which was then purified by passing its aqueous dioxane - methanol solution through columns of cation and anion exchangers. The yield of XV from XII was 40% after recrystallization. The homogeneity of XV was established by the criteria of thin-layer chromatography and by elemental analysis. Molecular weight determination using a vapor pressure osmometer demonstrated that the molecular weight of XV corresponded to that of cyclic decapeptide. The hydrogenation of XV in the presence of an equivalent amount of hydrogen chloride in methanol afforded cyclic decapeptide hydrochloride (XVI·HCl) in an 84% yield. The homogeneity of hydrochloride (XVI·HCl) was confirmed by thin-layer and paper chromatography, paper electrophoresis, elemental analysis and amino acid analysis.

Antibacterial activity toward several microorganisms was examined[11] (Table 1). In addition to synthetic XVI, synthetic tyrocidine A[6] and natural gramicidin S[12] were examined as reference compounds. It was found that the degree of the activities of synthetic XVI toward the Gram positive microorganisms was nearly the same as that of tyrocidine A, indicating that the L-tyrosine residue in the molecule of tyrocidine A can be replaced by the L-phenylalanine without influencing activity.

Syntheses of the cyclic decapeptides corresponding to the proposed structures for tyrocidine C[13] and D[14] is in progress in this laboratory.

Experimental

Melting points were uncorrected. The optical rotations were determined with a Yanagimoto Photometric Polarimeter, OR-20 type. Paper chromatography was performed on Toyo Roshi No. 52 with the following solvent systems: R_f^{I}, *n*-butanol - acetic acid - pyridine - water, 4 : 1 : 1 : 2 v/v; R_f^{II}, *n*-butanol - acetic acid - water, 4 : 1 : 2 v/v. Thin layer chromatography was performed on Merck silica gel G with the following solvent systems: R_f^{1}, *n*-butanol - acetic acid - pyridine - water 4 : 1 : 1 : 2 v/v; R_f^{2}, chloroform-methanol, 5 : 1 v/v; R_f^{3}, *n*-butanol - acetic acid - water, 4 : 1 : 2 v/v; R_f^{4}, *s*-butanol - pyridine - water, 75 : 15 : 10 v/v; R_f^{5}, chloroform - benzene - methanol, 6 : 3 : 1 v/v. Spots of materials possessing a free amino group on a thin layer plate were detected by spraying ninhydrin, and those of the amino group-blocked materials, by spraying 47% hydrobromic acid and then ninhydrin. Prior to analysis, the compounds were dried over phosphorus pentoxide at 60°C and 2 mmHg to a constant weight unless otherwise described.

Z-Gln-Phe-OEt (I). To a solution of phenylalanine ethyl ester hydrochloride (1.37 g, 6 mmol) dissolved in a mixture of TEA (0.84 m*l*, 6 mmol) and DMF (10 m*l*), a solution of benzyloxycarbonyl-glutamine *p*-nitrophenyl ester (2.0 g, 5 mmol) in DMF (5 m*l*) was added. After the solution had been allowed to stand for 6 hr at room temperature, it was diluted with water (250 m*l*). The crystalline precipitate was collected by filtration and washed successively with N hydrochloric acid, a 3% sodium bicarbonate solution and water. It was recrystallized from methanol-ether. Yield, 2.12 g (93%); mp 167—169°C; R_f^{1} 0.86; R_f^{2} 0.61; $[\alpha]_D^{15}$ −4.0° (*c* 1.0, DMF).

Found: C, 63.28; H, 6.40; N, 8.83%. Calcd for $C_{24}H_{29}O_6N_3$: C, 63.28; H, 6.42; N, 9.23%.

H-Gln-Phe-OEt·HCl (II). A solution of I (1.28 g, 2.8 mmol) dissolved in a mixture of 2.53N hydrogen chloride in methanol (1.23 m*l*) and DMF (10 m*l*) was hydrogenated in the presence of palladium black. After 6 hr the filtrate from the catalyst was concentrated to a small volume *in vacuo* and then ether was added. The resulting crystals were collected by filtration. Yield, 0.99 g (99%); mp 178—182°C; R_f^{1} 0.79; R_f^{2} 0.16; $[\alpha]_D^{15}$ +17.0° (*c* 1.0, DMF).

Found: C, 52.55; H, 6.69; N, 11.58%. Calcd for $C_{16}H_{23}O_4N_3\cdot HCl\cdot \frac{1}{2}H_2O$: C, 52.38; H, 6.87; N, 11.46%.

Z-Asn-Gln-Phe-OEt (III). To a solution of II (0.54 g, 1.5 mmol) dissolved in a mixture of TEA (0.224 m*l*, 1.6 mmol) and DMF (10 m*l*), a solution of benzyloxycarbonyl-asparagine *p*-nitrophenyl ester (0.58 g, 1.5 mmol) in DMF (5 m*l*) was added. After the solution had been stood overnight at room temperature, it was diluted with water (150 m*l*). The crystalline product deposited was collected by filtration and washed successively with N hydrochloric acid, a 3% sodium bicarbonate solution and water (0.69 g). It was recrystallized from DMF-ethanol-ethyl acetate. Yield, 0.66 g (77%); mp 225—227°C; R_f^{1} 0.82; R_f^{2} 0.28; $[\alpha]_D^{15}$ −16.0° (*c* 0.5, DMF).

Found: C, 58.76; H, 6.25; N, 12.10%. Calcd for $C_{28}H_{35}O_8N_5$: C, 59.04; H, 6.19; N, 12.30%.

H-Asn-Gln-Phe-OEt·HCl (IV). This compound was prepared by the same procedure as for II. Hydrogenolysis of III (0.62 g, 1.09 mmol) in the presence of a 1.1 equivalent of hydrogen chloride afforded IV as amorphous solid. Yield, 0.50 g (97%); mp 199—202°C; R_f^{1} 0.73; R_f^{2} 0.05; $[\alpha]_D^{15}$ −8.0° (*c* 0.3, DMF).

Found: C, 49.08; H, 6.88; N, 14.10%. Calcd for $C_{20}H_{29}O_6N_5\cdot HCl\cdot H_2O$: C, 49.03; H, 6.58; N, 14.30%.

Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-OEt (V). A solution of Z(OMe)-Phe-D-Phe-$NHNH_2$[6] (0.44 g, 0.9 mmol) dissolved in a mixture of DMF (9 m*l*) and acetic acid (6 m*l*) was cooled to −5°C. To this solution, N hydrochloric acid (2.0 m*l*) was added, followed by N sodium nitrite (1.0 m*l*). After it had been stood for 5 min at

11) We are indebted to Dr. M. Shibata of Takeda Chemical Industries for the bioassay.

12) We wish to express our thanks to Mr. I. Kitazato of Meiji Seika Co. for the generous gift of crystalline gramicidin S dihydrochloride.

13) M. A. Ruttenberg, J. P. King and L. C. Craig, *Biochemistry*, **4**, 11 (1965).

14) M. A. Ruttenberg and B. Mach, *ibid.*, **5**, 2864 (1966).

this temperature, the solution was diluted with cold water (50 ml). The azide deposited was collected by filtration, washed with a 3% sodium bicarbonate solution and water, and then dried *in vacuo* at 0°C. To a chilled (−5°C) solution of IV (0.45 g, 0.9 mmol) dissolved in a mixture of TEA (0.14 ml, 1.0 mmol) and DMF (15 ml), the azide was added. The solution was stirred for 2 days at 0°C and then diluted with water (200 ml). The precipitate formed was collected by filtration and washed successively with 0.5M citric acid, a 3% sodium bicarbonate solution and water. Yield, 0.59 g (73%); R_f^1 0.88; R_f^2 0.57; R_f^3 0.76.

For analysis 80 mg of the product was recrystallized from DMF-ethanol-ether (60 mg); mp 212—215°C; $[\alpha]_D^{15}$ −16.4° (*c* 0.5, DMF).

Found: C, 62.78; H, 6.19; N, 10.86%. Calcd for $C_{47}H_{55}O_{11}N_7$: C, 63.14; H, 6.20; N, 10.97%.

Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-NHNH$_2$ (VI). A solution of V (0.49 g, 0.55 mmol) and hydrazine hydrate (0.53 ml, 11 mmol) in DMF (15 ml) was allowed to stand at room temperature. After 24 hr hydrazine hydrate (0.27 ml, 5.5 mmol) was added to allow the reaction to proceed completely and the reaction mixture was stood at room temperature for another 24 hr. The solution was then evaporated *in vacuo* to remove the excess hydrazine, after which water (200 ml) was added to the residual solution; the gelatinous product deposited was collected by filtration. Yield, 0.47 g (97%); R_f^1 0.78; R_f^2 0.10; R_f^3 0.66.

For analysis 48 mg of the product was recrystallized from DMF-ether (36 mg); mp 218—221°C; $[\alpha]_D^{15}$ −23.5° (*c* 0.5, dimethylsulfoxide).

Found: C, 60.59; H, 6.27; N, 14.12%. Calcd for $C_{45}H_{53}O_{10}N_9\cdot\frac{1}{2}H_2O$: C, 60.80; H, 6.12; N, 14.18%.

Z-Leu-D-Phe-Pro-OH (VII). To a solution of Z-Leu-D-Phe-Pro-OEt[6] (1.07 g, 2 mmol) dissolved in a mixture of methanol (15 ml) and dioxane (10 ml), N sodium hydroxide (4 ml) was added. Saponification was carried out at 0°C for 2 hr and then at room temperature for 2 hr. The progress of the reaction was followed by thin-layer chromatography. The solution was evaporated *in vacuo*, after which the residual solution was diluted with water (20 ml) and then acidified to pH 3 with N hydrochloric acid. The resulting oil was taken up twice with each 20 ml of ethyl acetate. The combined ethyl acetate solutions were washed with water, dried over sodium sulfate, and then evaporated *in vacuo*. Yield of an oily product, 0.91 g (89%); R_f^1 0.84; R_f^2 0.72.

H-Leu-D-Phe-Pro-OH (VIII). A solution of VII (0.52 g) dissolved in a mixture of methanol (3 ml), water (1 ml) and acetic acid (6 ml) was hydrogenated in the presence of palladium black. After 3 hr the filtrate from the catalyst was concentrated *in vacuo* and then a mixture of ether and petroleum ether (1 : 1) was added. The resulting amorphous powder was collected by filtration. Yield, 0.36 g (73%); R_f^1 0.75; R_f^2 0.00; R_f^1 0.68.

Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (IX). A solution of Z(OMe)-Val-Orn(δ-Z)-NHNH$_2$[8] (0.50 g, 0.95 mmol) in acetic acid (15 ml) was cooled to −5°C. To this, N hydrochloric acid (2.1 ml) was added, followed by N sodium nitrite (1.0 ml). After it had been stirred for 5 min at this temperature, the solution was diluted with cold water (150 ml). The azide precipitated was treated as for the preparation of V. The dried azide was added to a chilled solution of VIII (0.36 g, 0.95 mmol) dissolved in a mixture of TEA (0.133 ml, 0.95 mmol) and DMF (10 ml). After the solution had been stirred for 2 days at 0°C, it was concentrated to a small volume and then acidified with cold 0.5M citric acid. The resulting crystals were collected by filtration, washed with 0.5M citric acid and water. It was recrystallized from methanol-ether-petroleum ether. Yield, 0.62 g (73%); mp 138—140°C; R_f^1 0.83; R_f^2 0.54; $[\alpha]_D^{17}$ −20.1° (*c* 1.0, DMF). In order to confirm the homogeneity of the product a small portion was hydrogenated; a single ninhydrin positive spot was observed on a thin-layer chromatogram, R_f^1 0.65.

We prepared compound (IX) by saponification of the corresponding acyl-pentapeptide ethyl ester; $[\alpha]_D^{20}$ −18.2° (*c* 1.0, DMF).[9]

H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH·HCl(XI). To a solution of IX (0.53 g, 0.6 mmol) in dioxane (10 ml), 2.57N dry hydrogen chloride in dioxane (4.67 ml, 12 mmol) and anisole (0.1 ml) were added. The solution was then allowed to stand at room temperature and the reaction was followed by thin layer chromatography. After 9 hr the solution was concentrated to a small volume *in vacuo* and ether was added. The crystals precipitated were collected by filtration and washed thoroughly with ether. Yield, 0.41 g (90%); mp 139—142°C (decomp.); R_f^1 0.73; R_f^2 0.11; R_f^3 0.56; $[\alpha]_D^{15}$ −19.8° (*c* 0.5, DMF).

Found: C, 58.29; H, 7.41; N, 10.54%. Calcd for $C_{38}H_{54}O_8N_6\cdot HCl\cdot\frac{3}{2}H_2O$: C, 58.04; H, 7.43; N, 10.69%.

We prepared compound (X) by the treatment with hydrogen chloride of the corresponding BOC-pentapeptide acid; $[\alpha]_D^{20}$ −22.5° (*c* 2.12, DMF).[10]

Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH (XII). A solution of VI (0.42 g, 0.475 mmol) dissolved in DMF (6 ml) was treated with N hydrochloric acid (0.6 ml) and N sodium nitrite (0.52 ml). After it had been stood for 5 min, the solution was diluted with cold water (50 ml). The azide deposited was treated as described previously. The dried azide was added to a chilled solution of XI (0.38 g, 0.5 mmol) dissolved in a mixture of TEA (0.14 ml, 1.0 mmol) and DMF (8 ml). The solution was stirred for 2 days at 0°C, and then diluted with 0.25M citric acid (30 ml). The resulting product was collected by filtration and washed with water. Yield, 0.65 g (80%); R_f^1 0.82; R_f^2 0.00; R_f^3 0.90; R_f^4 0.81; R_f^5 0.77. In order to establish further the homogeneity of the product, a small portion was hydrogenated; R_f^1 0.26; R_f^5 0.07; a single ninhydrin-positive spot was observed on paper electrophoresis with pH 1.8 buffer:[6] relative mobility to gramicidin S[12] 0.89.

For analysis 100 mg of the product was recrystallized from DMF-ether (61 mg); mp 236—239°C; $[\alpha]_D^{15}$ −31.8° (*c* 0.5, dimethyl sulfoxide).

Found: C, 61.98; H, 6.73; N, 11.30%. Calcd for $C_{83}H_{103}N_{13}O_{18}\cdot 2H_2O$: C, 62.04; H, 6.71; N, 11.33%.

Z(OMe)-Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro-ONp(XIII). To a suspension of XII (196 mg, 0.125 mol) in anhydrous pyridine (5 ml) was added di-*p*-nitrophenyl sulfite (810 mg, 25 mmol) The mixture was stirred for 2 days at room temperature and then evaporated *in vacuo*. The oily residue was triturated with a mixture of ether and petroleum ether (1 : 1). The solid mass was collected by filtration and

washed with a mixture of ether and petroleum ether (1 : 1) until yellow color could not be discerned upon the addition of N sodium hydroxide to the filtrate. A faintly yellow product was thus obtained (179 mg); R_f^1 0.98; R_f^2 0.74. The *p*-nitrophenyl ester content of this product was estimated to be 104% by means of the method described by Schwyzer and Sieber,[15] except that a mixture of DMF and N sodium hydroxide (1 : 1) was used as the solvent and the absorption measurements were performed at the wavelength of 412 mμ. This product was used for the next reaction without further purification.

H-Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro-ONp·CF_3COOH (XIV). To a mixture of XIII (179 mg) and anisole (0.2 m*l*), anhydrous trifluoroacetic acid (2.3 m*l*) was added at −5°C. When swirled, the reaction mixture formed a solution after 10 min. The solution was then evaporated *in vacuo* at 0°C, and the resulting oily residue was triturated with ether. The product was collected by filtration and washed with ether: yield, 199 mg; R_f^1 0.87.

***cyclo*-(Phe-D-Phe-Asn-Gln-Phe-Val-Orn(δ-Z)-Leu-D-Phe-Pro) (XV).** A solution of XIV (199 mg) dissolved in a mixture of acetic acid (0.3 m*l*) and DMF (10 m*l*) was stirred, drop by drop, into anhydrous pyridine (60 m*l*) kept at 65—70°C over a 3.5 hr period. The solution was further stirred for 1.5 hr at this temperature, and then evaporated to dryness *in vacuo*. The resulting residue was collected by filtration with the aid of water, dried *in vacuo* (131 mg), and then dissolved in 15 m*l* of a mixture of dioxane, methanol and water (1 : 3 : 1). This solution was passed successively through columns (2×6.5 cm) of Dowex −50 (H^+ form) and Dowex-1 (OH^- form) which had been washed with a mixture of dioxane, methanol and water (1 : 3 : 1). The columns were then washed with the same solvent. The filtrate and washings were combined (total 150 m*l*) and evaporated *in vacuo*. The oily residue crystallized upon the addition of water (76 mg). It was recrystallized from dioxane-water. Yield, 68 mg (40% from XII); mp 252—255°C (decomp.); R_f^1 0.92; R_f^2 0.57; $[\alpha]_D^{23}$ −129° (*c* 0.5, methanol).

Found: C, 62.66; H, 6.80; N, 12.32%. Calcd for $C_{74}H_{93}O_{14}N_{13}\cdot 2H_2O$: C, 62.39; H, 6.86; N, 12.78%.

The molecular weight was determined on a Hitachi Osmometer, type 115, using methanol as the solvent. Found: 1435. Calcd for the benzyloxycarbonyl-substituted cyclic decapeptide dihydrate: 1425.

***cyclo*-(Phe-D-Phe-Asn-Gln-Phe-Val-Orn-Leu-D-Phe-Pro)·HCl (XVI·HCl).** A solution of XV(51 mg, 0.036 mmol) dissolved in a mixture of 0.046 N dry methanolic hydrogen chloride (0.87 m*l*, 0.041 mmol) and methanol (3.5 m*l*) was hydrogenated in the presence of palladium black. The progress of the reaction was followed by thin-layer chromatography. After 3 hr the filtrate from the catalyst was concentrated to a small volume (0.1 m*l*), and 2 m*l* of ether was added to give crystals. The crystals were collected by filtration and washed with ether. Yield, 39 mg (84%); mp 265—267°C (decomp.); $[\alpha]_D^{23}$ −126° (*c* 0.2, methanol). Homogeneity of the compound was ascertained as follows.

a) Paper chromatography: R_f^I 0.96; R_f^{II} 0.95.

b) Thin-layer chromatography: R_f^1 0.78; R_f^2 0.40.

c) Paper Electrophoresis: Paper, Toyo Roshi No. 52; solvent, formic acid-acetic acid-methanol-water (1 : 3 : 6 : 10 v/v) (pH 1.8); voltage gradient, 17 V/cm; charged period, 2.5 hr. Gramicidin S dihydrochloride[12] was used as the reference compound. Figure 3 shows

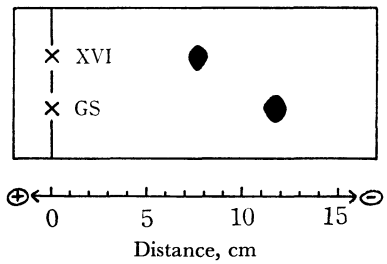

Fig. 3. Paper electrophoresis of synthetic cyclic decapeptide hydrochloride (XVI·HCl) and natural gramicidin S (GS) dihydrochloride.[12]

Table 1. Inhibitory activity of the three compounds on microorganisms

Minimum inhibitory concentration, μg/m*l*

A. Bouillon agar medium[a]

	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*	*Mycobacterium avium*
Synthetic XVI	>100	>100	10	5	>100
Tyrocidine A	>100	>100	8	8	>100
Gramicidin S	>100	>100	2	2	>100

B. Synthetic medium

	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*	*Mycobacterium avium*
Synthetic XVI	>100	>100	10	10	>100
Tyrocidine A	>100	>100	10	8	>100
Gramicidin S	>100	>100	2	2	>100

a) Usual bouillon agar medium, pH 7.0.

b) Stephenson-Whetham's medium (modified).

15) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).

that XVI migrates more slowly toward the cathode than gramicidin S as observed for tyrocidine A.[6]

d) Elemental Analysis: The elemental analysis was carried out using a sample which had been dried over anhydrous phosphorus pentoxide at room temperature and 2 mmHg.

Found: C, 58.81; H, 6.96; N, 13.32%. Calcd for $C_{66}H_{87}O_{12}N_{13}\cdot HCl\cdot 3H_2O$: C, 58.94; H, 7.04; N, 13.54%.

e) Amino Acid ratios in the acid hydrolysate: Phe 3.90, Asp 1.00, Glu 1.03, Val 0.92, Orn 1.03, Leu 1.03, Pro 1.00, NH_3 1.89.[16]

Microbiological Assays.[11] The microorganisms employed are listed in Table 1. The minimum amount of the compound necessary for the complete inhibition of growth was determined by a dilution method with a bouillon agar medium and with a synthetic medium. In addition to the synthetic XVI·HCl, synthetic tyrocidine A hydrochloride[6] and natural gramicidin S dihydrochloride[12] were examined as reference compounds. Table 1 shows that the degree of the activities of synthetic XVI toward the Gram positive microorganisms was nearly the same as that of tyrocidine A.

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