Studies of Peptide Antibiotics. III. Cyclo-(L-valyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl)$_2$[1]

By Haruhiko Aoyagi, Tetsuo Kato, Motonori Ohno, Michio Kondo
Michinori Waki, Satoru Makisumi and Nobuo Izumiya

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Certain peptide antibiotics, such as gramicidin S,

L-Val-L-Orn-L-Leu-D-Phe-L-Pro
↑ ↓
L-Pro-D-Phe-L-Leu-L-Orn-L-Val
Gramicidin S

possess several characteristic features in common. These features include a cyclic structure, D-amino acid residues, and a basic character due to the presence of diamino acid residues. The necessity of a cyclic structure for antibacterial activity is indicated by the finding that the activity of a synthetic linear decapeptide, with the same sequence of amino acid residues as that found in gramicidin S, is markedly less than that of gramicidin S.[2] In a previous paper from this laboratory,[3] a cyclic hexapeptide, cyclo-L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-glycyl, which contains a partial sequence found in gramicidin S, was prepared and its antibacterial activity was tested. The cyclic hexapeptide was, however, found to be devoid of antibacterial activity. Recently, another cyclic hexapeptide, cyclo-L-valyl-D-ornithyl-L-prolyl-D-phenylalanyl-D-leucyl-L-ornithyl, has been synthesized; it also was found to possess no antibacterial activity.[4] From these findings, it appears that, in addition to the characteristics mentioned above, other structural features, such as a ring size larger than that of the cyclic hexapeptide and/or specific amino acid sequence, are necessary in this type of molecule for an activity to be exhibited. Therefore, it became of interest to examine the effect on bacterial growth of a larger synthetic cyclic peptide than the cyclic hexapeptide.

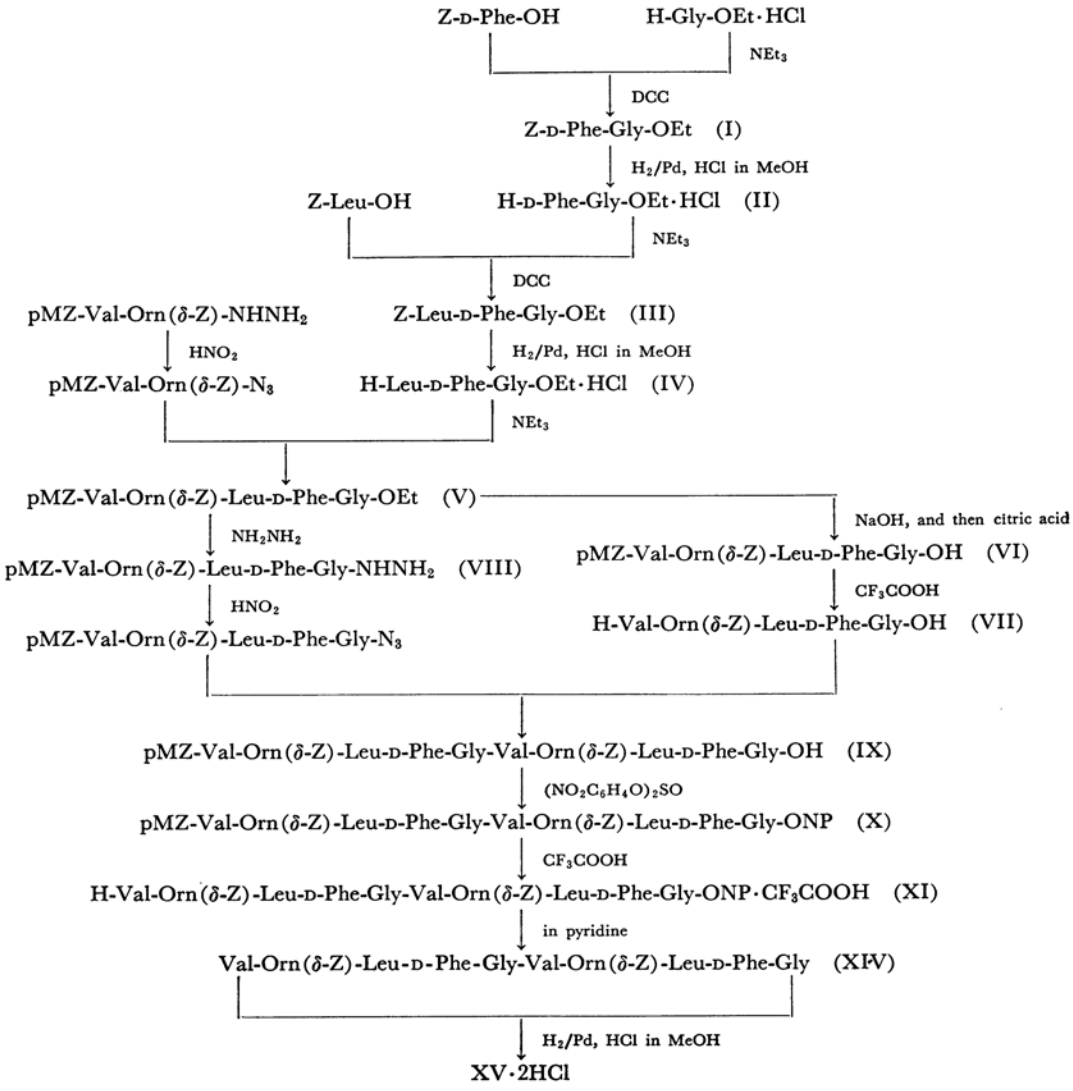
Syntheses of relatively large cyclic peptides have been reported from several laboratories in recent years. Cyclic hepta-[5] and octapeptide[6] related to polymyxin have been synthesized from the respective linear peptide via the carbodiimide. However, cyclic decapeptides have generally been obtained in better yields from the respective linear decapeptide active ester.[7,8] Furthermore, the cyclization reaction of linear pentapeptide active esters, wherein proline residues are C-terminuses, gave cyclic decapeptide in good yields by the dimerization reaction.[9,10] For these reasons, we have decided to attempt the synthesis of a cyclic decapeptide with the appropriate amino acid sequence.

It is well known that, in many polypeptides, a proline residue causes a kink in a peptide chain.[11] It has been reported that, in gramicidin S, the presence of proline might facilitate the formation of a stable conformation.[10] Therefore, it appeared of interest to synthesize an analog (XV) of the cyclic decapeptide gramicidin S, wherein proline residues are replaced by glycines, in order to determine to what degree the proline side chains contribute to the biological activity.

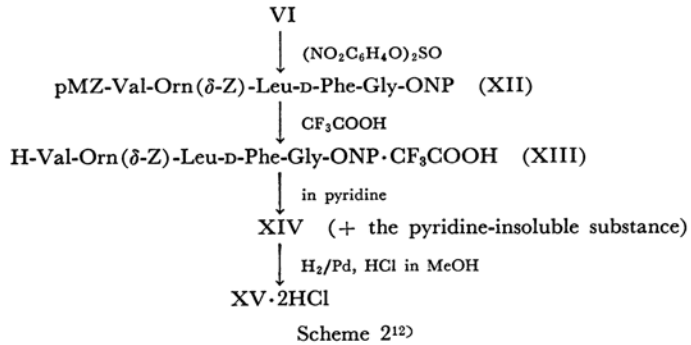
L-Val-L-Orn-L-Leu-D-Phe-Gly
↑ ↓
Gly-D-Phe-L-Leu-L-Orn-L-Val
XV

The reaction sequences employed for the synthesis of XV are shown in Schemes 1 and 2. The cyclic benzyloxycarbonyl-substituted decapeptide (XIV) was obtained both by the cyclization reaction of the decapeptide active ester (XI) (Scheme 1) and by the dimerization reaction of the pentapeptide active ester (XIII) (Scheme 2). It was found that the yield of XIV by the cyclization of XI was satisfactory, but that that by the dimerization reaction was only 10—15%, because a considerable amount of the unidentified pyridine insoluble product was formed. It should be noted that Schwyzer and Sieber, by the dimerization reaction, obtained tosyl-substituted gramicidin S in a satisfactory yield for the pertinent pentapeptide active ester, wherein a proline residue is the C-

1) A part of this work has already been briefly communicated: H. Aoyagi, T. Kato, M. Ohno, M. Kondo and N. Izumiya, *J. Am. Chem. Soc.*, **86**, 5700 (1964).
2) B. F. Erlanger and L. Goode, *Nature*, **174**, 80 (1954); *Science*, **131**, 669 (1960).
3) T. Kato, M. Kondo, M. Ohno and N. Izumiya, This Bulletin, **38**, 1202 (1965).
4) T. Kato and N. Izumiya, unpublished.
5) R. O. Studer, W. Lergier and K. Vogler, *Helv. Chim. Acta*, **46**, 612 (1963); K. Vogler, R. O. Studer, P. Lanz, W. Lergier, E. Bohni and B. Fust, ibid., **46**, 2823 (1963).
6) K. Vogler, R. O. Studer, W. Lergier and P. Lanz, ibid., **43**, 175 (1960); R. O. Studer, K. Vogler and W. Lergier, ibid., **44**, 131 (1961).
7) R. Schwyzer and P. Sieber, ibid., **40**, 624 (1957).
8) R. Schwyzer and P. Sieber, ibid., **41**, 1582 (1958).
9) German Patent, *Chem. Abstr.*, **57**, 949 (1962).
10) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186 (1958).
11) A. F. Cullis, H. Muirhead, M. F. Perutz, M. G. Rossmann and A. C. T. North, *Proc. Roy. Soc.*, **A265**, 161 (1962); M. F. Perutz, *Nature*, **194**, 914 (1962).



Scheme 1[12]



Scheme 2[12]

12) The abbreviations used are: pMZ, *p*-methoxybenzyloxycarbonyl; Z, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; ONP, *p*-nitrophenoxy ester. An amino acid residue except Gly and D-Phe is of L-configuration.

terminus.[10] The final product (XV) was obtained as colorless crystals with six moles of the water of crystallization, and its homogeneity was demonstrated by chromatography in many solvent systems.

The cyclic decapeptide (XV) was found to be much more active than gramicidin S against *B. subtilis* in a synthetic medium (Table I). This finding shows that the replacement of the proline residues by glycines in the sequence of gramicidin S does not reduce the activity, demonstrating that not all the original amino acid sequences in the natural peptide are necessary for the full activity; e.g., the side chain of proline is not required. It should be noted that homo-gramicidin S, wherein the ornithine residues are replaced by lysines, exhibits an activity nearly equal to that of the natural gramicidin S.[10] On the other hand, it appears that the valine residues in gramicidin S are of importance in the activity.[13]

In order to extend further the study of the relationship between the chemical structure and the biological activities of peptide antibiotics, experiments on the preparation of and studies in the properties of several cyclic and linear peptides are in progress in this laboratory.

Experimental

All melting points are uncorrected.

Benzyloxycarbonyl-D-phenylalanylglycine Ethyl Ester (I). — To a solution of benzyloxycarbonyl-D-phenylalanine (2.99 g.) and glycine ethyl ester hydrochloride (1.40 g.) in chloroform (40 ml.), triethylamine (1.4 ml.) and dicyclohexylcarbodiimide[14] (2.06 g.) were added at 0°C. After it had been permitted to stand overnight at 0°C, the mixture was evaporated in vacuo, the residue diluted with ethyl acetate (50 ml.), and the dicyclohexylurea formed was filtered off. The filtrate was washed successively with 4% sodium bicarbonate, 3% hydrochloric acid, and water and dried over anhydrous sodium sulfate. The filtered solution was evaporated in vacuo. The residual oil solidified after ether and petroleum ether had been added. Recrystallization from ethyl acetate - petroleum ether gave 3.18 g. (83%); m. p. 109—110°C; $[\alpha]_D^{18}$ +20.5° (*c* 2, methanol).

Found: C, 65.86; H, 6.56; N, 7.33. Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29%.

This compound had been previously obtained by the acid chloride method; m. p. 109—111°C.[15]

D-Phenylalanylglycine Ethyl Ester Hydrochloride (II).—I (2.69 g.) was subjected to hydrogenolysis in the presence of palladium black and 0.31 N methanolic hydrogen chloride (24.8 ml.). The filtrate from the catalyst was then evaporated to dryness in vacuo. The product was obtained as a hygroscopic powder; yield, 2.01 g. (100%); R_f 0.88[16] and 0.74[17].

Benzyloxycarbonyl-L-leucyl-D-phenylalanylglycine Ethyl Ester (III).—Benzyloxycarbonyl-L-leucine (1.86 g.) was coupled with II (2.01 g.) by dicyclohexylcarbodiimide (1.44 g), following the procedure described for the preparation of I. Recrystallization from ethyl acetate - ether gave 2.79 g. (80%); m. p. 122—124°C; $[\alpha]_D^{25}$ +13.0° (*c* 2, methanol).

Found: C, 64.75; H, 7.07; H, 7.07; N, 8.33. Calcd. for $C_{27}H_{35}O_6N_3$: C, 65.17; H, 7.09; N, 8.45%.

L-Leucyl-D-phenylalanylglycine Ethyl Ester Hydrochloride (IV).—III (1.99 g.) was subjected to hydrogenolysis in the presence of palladium black and 0.46 N methanolic hydrogen chloride (9.6 ml.), and then treated as described in the preparation of II. Yield of oily product: 1.61 g. (100%); R_f 0.90[16] and 0.77[17].

***p*-Methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine Ethyl Ester (V).**—The following operations were carried out in a cold room. Into a chilled solution of *p*-methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithine hydrazide[8] (0.98 g.) in glacial acetic acid (12 ml.), N hydrochloric acid (4 ml.) and sodium nitrite (136 mg.) in water (2 ml.) were stirred. After 6 min., cold water (100 ml.) was added to the solution. The azide which precipitated as a white mass was collected by filtration and washed with water, 4% sodium bicarbonate, and water, and then dried under a vacuum in a desiccator. The azide was added to a solution of IV (0.72 g.) and triethylamine (0.25 ml.) in dimethylformamide (20 ml.), and then the mixture was stirred for 3 days at 0°C. The precipitate which formed upon the addition of water (300 ml.) to the solution was collected, washed with 4% sodium bicarbonate, 0.5 M citric acd and water, and dried. The product was recrystallized from dioxane-ether; yield, 1.30 g. (83%); m. p. 206—208°C; $[\alpha]_D^{25}$ −23.5° (*c* 1, acetic acid); R_f 0.87.[17]

Found: C, 63.02; H, 7.15; N, 9.94. Calcd. for $C_{46}H_{62}O_{11}N_6$: C, 63.14; H, 7.14; N, 9.61%.

***p*-Methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine (VI).**—To a solution of V (872 mg.) in a mixture of methanol (50 ml.) and dioxane (20 ml.), N sodium hydroxide (1.5 ml.) was added; the solution was then allowed to stand overnight at room temperature. After the addition of 0.5 M citric acid (6 ml.) under cooling, the solution was concentrated in vacuo at low temperature, and the residue was treated with water (100 ml.). After they had been stored in a refrigerator, the crystals were collected by filtration, washed with water, and dried. Recrystallization from dioxane-ether gave 712 mg. (84%) of VI; m. p. 214—215°C; $[\alpha]_D^{25}$ −21° (*c* 1, acetic acid); R_f 0.82.[17]

Found: C, 61.95; H, 6.88; N, 10.00. Calcd. for $C_{44}H_{58}O_{11}N_6$: C, 62.39; H, 6.90; N, 9.92%.

13) T. Kato, M. Ohno, M. Kondo, Y. Fujita and N. Izumiya, 6th International Congress of Biochemistry, New York, N. Y. (1964), p. 159.

14) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

15) O. K. Behrens, D. G. Doherty and M. Bergmann, *J. Biol. Chem.*, **136**, 61 (1940).

16) The R_f of the paper chromatography with Toyo Roshi No. 52 refers to the *n*-butanol - acetic acid - pyridine - water (4 : 1 : 1 : 2, v./v.) system.

17) The R_f of the thin layer chromatography with Merck silica gel G refers to the *n*-butanol - acetic acid - pyridine - water (4 : 1 : 1 : 2, v./v.) system. Compounds possessing a free amino group were detected by spraying with ninhydrin, and those with blocked amino groups, by spraying with 47% hydrobromic acid, and then ninhydrin.

L-Valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine (VII).—To a mixture of VI (434 mg.) and anisole (0.16 ml.) trifluoroacetic acid (2 ml.) was added at 0°C.[18] After being permitted to stand for 20 min. at 0°C, the solution was evaporated in vacuo. The residue was triturated with ether (20 ml.) and washed repeatedly with ether by decantation. The residual crystals were dissolved in water. After triethylamine (1 ml.) had been added, the solution was evaporated to dryness in vacuo. The crystalline product was collected by filtration with the aid of water; yield, 260 mg. (76%); m. p. 191—192°C; $[\alpha]_D^{25}$ −4.2° (*c* 1, acetic acid); R_f 0.85.[17]

Found: C, 60.96; H, 7.37; N, 12.05. Calcd. for $C_{35}H_{50}O_8N_6 \cdot 1/2H_2O$; C, 60.76; H, 7.43; N, 12.15%.

***p*-Methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine Hydrazide (VIII).**—To a solution of V (440 mg.) in dimethylformamide (4 ml.) hydrazine hydrate (0.5 ml.) was added; the solution was allowed to stand for 2 days at room temperature. The reaction mixture was evaporated in vacuo. The hydrazide which precipitated upon the addition of water (20 ml.) was filtered, washed with water, and dried. It was recrystallized from methanol-ethyl acetate-ether; yield, 372 mg. (87%); m. p. 217—219°C; $[\alpha]_D^{25}$ −20° (*c* 1, acetic acid); R_f 0.91.[17]

Found: C, 60.84; H, 7.15; N, 12.89. Calcd. for $C_{44}H_{60}O_{10}N_8$: C, 61.38; H, 7.02; N, 13.02%.

***p*-Methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine (IX).**—Into a solution of VIII (345 mg.) in dimethylformamide (7 ml.), N hydrochloric acid (1.2 ml.) and sodium nitrite (30 mg.) in water (0.5 ml.) were stirred. After 5 min., cold water (100 ml.) was added to the solution. The azide which precipitated was filtered and treated as described in the preparation of V. The azide was added to a solution of VII (273 mg.) in a mixture of dimethylformamide (8 ml.) and triethylamine (0.055 ml.), and then the mixture was stirred for 3 days at 0°C. The insoluble material was removed by by filtration, and the filtrate was concentrated in vacuo. The precipitate which was formed upon the addition of ethyl acetate (10 ml.) and ether (4 ml.) to the concentrated filtrate was collected, washed with 0.5 M citric acid and water, and dried. Recrystallization from dimethylformamide-ethyl acetate-ether did not raise the melting point. The yield was 495 mg. (82%); m. p. 253—257°C; $[\alpha]_D^{25}$ −48° (*c* 0.5, acetic acid); R_f of hydrogenated product of IX, 0.60.[17]

Found; C, 61.77; H, 7.18; N, 10.94. Calcd. for $C_{79}H_{106}O_{18}N_{12} \cdot H_2O$; C, 62.02; H, 7.12; N, 10.99%.

Cyclo-(L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl)$_2$ (XIV).—*XIV (from deca).*—To a solution of IX (612 mg., 0.4 mmol.) in a mixture of dimethylformamide (3 ml.) and pyridine (0.95 ml.) di-*p*-nitrophenyl sulfite[19] (1.30 g., 4 mmol.) was added; the reaction mixture was then allowed to stand for 24 hr. at room temperature. After evaporation, the oily product was triturated with petroleum ether and washed repeatedly with a mixture of ether and petroleum ether (1:1) by decantation until no yellow color could be discerned on the addition of a sodium hydroxide solution to the washings. The product was filtered, washed with a mixture of ether and petroleum ether (1:1), and dried. It weighed 648 mg. This product was dissolved in dimethylformamide-N sodium hydroxide (1:1 v./v.), and the content of *p*-nitrophenyl ester was estimated by measuring the optical density of the solution at 412 mμ.[7] The purity of the compound was estimated to be 70%.

To the *p*-nitrophenyl ester X (638 mg.) anisole (0.5 ml.) and trifluoroacetic acid (3.5 ml.) were added at 0°C. The solution was evaporated in vacuo at 0°C, and the residue was triturated with ether. The decapeptide *p*-nitrophenyl ester trifluoroacetate (XI) was collected, washed with ether, and dissolved in dimethylformamide (12 ml.) containing glacial acetic acid (0.1 ml.). The solution was then stirred drop by drop into pyridine (120 ml.) which had been kept at 55—60°C over a period of 5 hr.; the stirring was then continued for an additional 2 hr. at the same temperature. After the solvent had been removed, the residual oil was dissolved in a mixture of methanol (150 ml.) and water (60 ml.). The insoluble substance was removed by filtration, and the filtrate was passed successively through columns of Dowex 1 (OH^- form, 2.7×11 cm.) and Dowex 50 (H^+ form, 2.5×10 cm.). The columns were then washed with the same solvent (200 ml.), the combined effluent was evaporated to dryness in vacuo, and the product was suspended in water (30 ml.), filtered, and dried (246 mg.). The product was recrystallized from methanol-ether; yield, 211 mg. (38%) (named as XIV (from deca)); m. p. 248—250°C; $[\alpha]_D^{17}$ −127° (*c* 0.5, acetic acid); R_f 0.93.[17]

Found: C, 60.62; H, 7.40; N, 12.25. Calcd. for $C_{70}H_{96}O_{14}N_{12} \cdot 3H_2O$: C, 60.76; H, 7.43; N, 12.15%.

The molecular weight of XIV (from deca) was determined by a Model 301A osmometer, Mechrolab Inc. solvent methanol).

Found: 1420. Calcd. for $C_{70}H_{96}O_{14}N_{12} \cdot 3H_2O$: 1384.

The air-dried compound lost 3.4% of its weight after it had been dried over phosphorus pentoxide for 2 hr. at 100°C; 2 mmHg. Calcd. for $3H_2O$: 3.9%.

XIV (from penta).—To a solution of VI (508 mg., 0.6 mmol.) in dimethylformamide (2 ml.) pyridine (0.2 ml.) and di-*p*-nitrophenyl sulfite (778 mg., 2.4 mmol.) were added. The reaction mixture was allowed to stand for 24 hr. at room temperature and treated as has been described above. The product (XII) obtained weighed 548 mg. The purity of the compound was estimated to be 78%.

To the *p*-nitrophenyl ester XII (538 mg.) anisole (0.5 ml.) and trifluoroacetic acid (3 ml.) were added. The solution was evaporated in vacuo, and the residue was triturated with ether. The pentapeptide *p*-nitrophenyl ester trifluoroacetate (XIII) was filtered, and then dissolved in dimethylformamide (10 ml.) containing glacial acetic acid (0.1 ml.). The solution was stirred drop by drop into pyridine (100 ml.) at 55—60°C as has been described above. During the stirring a crystalline precipitate appeared. After the completion of the reaction, the mixture was cooled to room temperature, and the crystals were filtered and washed with pyridine. It weighed 173 mg.[20] The filtrate was

18) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
19) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1760 (1960).
20) Experiments on the properties of this pyridine-insoluble substance are in progress in this laboratory.

evaporated in vacuo. The residue was dissolved in a mixture with methanol (120 ml.) and water (40 ml.), and the solution was treated with the columns of Dowex 1 and 50 as has been described above. Recrystallization from methanol-ether gave 40 mg. (10%); m. p. 247—251°C[21]; $[\alpha]_D^{17}$ −123° (*c* 0.5, acetic acid); R_f 0.93.[17]

Found: C, 61.31; H, 7.30; N, 12.20. Calcd. for $C_{70}H_{96}O_{14}N_{12}\cdot 3H_2O$: C, 60.76; H, 7.43; N, 12.15%.

When the procedure with the same scale was repeated, the yields of XIV (from penta) and the pyridine-insoluble substance were 62 mg. (15%) and 170 mg. respectively.

Cyclo-(L-valyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl)$_2$ Dihydrocloride (XV). — *XV (from deca).*— XIV (from deca) (82 mg., 0.06 mmol.), dissolved in methanol (1 ml.) and 0.065 N methanolic hydrogen chloride (2 m.), was subjected to hydrogenolysis in the presence of palladium black. After it had been filtered from the catalyst, the solution was evaporated to dryness in vacuo, The crystals which remained were then filtered with the aid of a mixture of acetone and ether (4: 1); yield of air-dried product, 66 mg. (88%); m. p. 227—230°C; $[\alpha]_D^{17}$ −190° (*c* 0.5, ethanol); R_f 0.90,[16] 0.77,[17] 0.91[22] and 0.78[23].

Found: C, 52.06; H, 7.98; N, 13.28. Calcd. for $C_{54}H_{86}O_{10}N_{12}Cl_2\cdot 6H_2O$: C, 52.20; H, 7.95; N, 13.53%.

The air-dried product lost 2.9% of its weight when it was left in a desiccator with calcium chloride at room temperature. Calcd. for $2H_2O$: 2.9%. The air-dried product lost 8.8% of its weight after it had been dried over phosphorus pentoxide for 2 hr. at 120°C; 2 mmHg. Calcd. for $6H_2O$: 8.7%. The product was treated with dinitrofluorobenzene in the usual manner.[24] After the hydrolysis of the dinitrophenylated compound (DNP-XV), only one DNP-amino acid identified as δ-DNP-ornithine was detected on the paper chromatogram; R_f 0.64.[16]

XV (from penta). — XIV (from penta) (6.9 mg., 5 μmol.) was dissolved in methanol (0.5 ml.) and 0.05 N methanolic hydrogen chloride (0.22 ml.) and treated as has been mentioned above. Crystals were obtained; yield, 4.5 mg. (80%). R_f values of the crystals (XV (from penta)) were identical with those of XV (from deca). 0.90,[16] 0.77,[17] 0.91[22] and 0.77[23]. Furthermore, the identity of XV (from deca) with XV (from penta) was confirmed by paper electrophoresis carried out with 500 V. for 2 hr., using a solvent of formic acid - acetic acid - methanol - water (1: 3: 6: 10, v./v.; pH 1.8) and Toyo Roshi No. 52 paper.

The Amino Acid Analysis of XV.—Quantitative amino acid analysis was performed as follows. XV (ca. 0.6 mg.) was hydrolyzed with 6 N hydrochloric acid at 110°C for 48 hr. Each of the hydrolysates of XV from deca and penta was dissolved in 0.1 ml. of

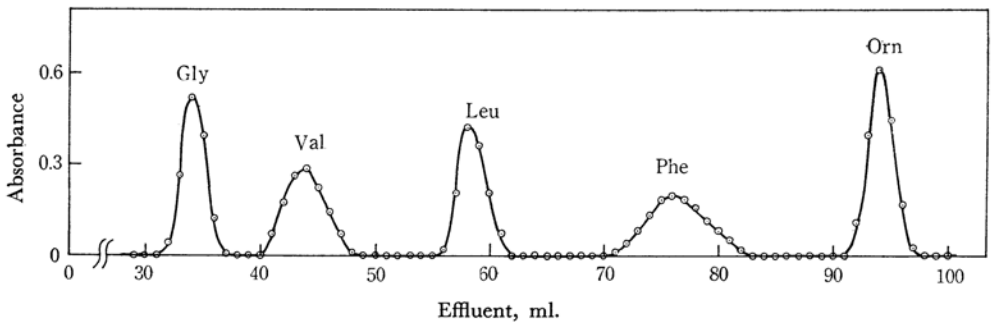


Fig. 1. Chromatogram of the hydrolysate of XV (from deca).

Table I. Amount of compound necessary for complete inhibition of growth (μg./ml.)

Compound	Synthetic medium[a]		Bouillon agar medium[b]	
	E. coli[c]	*B. subtilis*[d]	*E. coli*[c]	*B. subtilis*[d]
XV (from deca)	>100	1>	<100	20
XV (from penta)	>100	1>	<100	20
Gramicidin S sulfate[e]	>100	10	<100	10

a) Stephenson-Whetham's medium (modified); KH_2PO_4 1g., NaCl 1 g., $FeSO_4\cdot 7H_2O$ 0.7 g., Na-glutamate 4 g., glucose 5 g./1000 ml. and agar 20 g.
b) Usual bouillon agar medium, pH 7.0.
c) *Escherichia coli IRO 3044.*
d) *Bactllus subtilis PCI 219.*
e) A product of Astra Co., U. S. A. This contains eight moles of water of crystallization.

21) The melting point was not depressed by admixture with XIV (from deca).
22) The R_f of the paper chromatography with Toyo Roshi No. 52 refers to the *t*-butanol - formic acid - water (75 : 15 : 10, v./v.) system.
23) The R_f of the thin layer chromatography with Merck silica gel G refers to the *t*-butanol - formic acid - water (75 : 15 : 10, v./v.) system.
24) F. Sanger and E. O. P. Thompson, *Biochem. J.*, **53**, 353 (1953).

a 0.1 M citrate buffer (pH 3.1),[25] the solution was applied to a column (0.9×15 cm.) with Aminex Q-150 (31—45 microns, Na^+ form), and development was continued with the same solvent. Elution was carried out at about 20°C, the flow rate was 20—25 ml. per hr., and 1-ml. fractions were collected. The eluting solvent was replaced with a 0.1 M citrate buffer of pH 4.25[26] at fraction 35, and with a 0.2 N buffer of pH 9.2[26] at fraction 75. The amino acid contents in the fractions were determined by the modified ninhydrin reagent described by Yemm and Cocking.[27] A typical chromatogram is shown in Fig. 1. Quantitative amino acid determinations of XV from deca and penta gave the molar ratios of 1.0: 1.1: 1.0: 1.1: 1.1 and 1.0: 0.9: 1.0: 1.0: 1.0 for valine, ornithine, leucine, phenylalanine and glycine respectively.

Microbiological Assays.—The microrganisms employed are shown in Table I. A minimum amount of the compounds necessary for the complete inhibition of growth was determined by a dilution method with a bouillon agar medium and with a synthetic medium. As is shown in Table I, the cyclodecapeptide was found to be much more active than natural gramicidin S against *B. Subtilis* in a synthetic medium.

Summary

A cyclic decapeptide, cyclo-(L-valyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl)$_2$, has been synthesized in an attempt to obtain information about the relation of the structure to the antibacterial activity of gramicidin S.

The synthesis of the cyclic decapeptide was achieved in two ways. The condensation of *p*-methoxybenzyloxycarbonyl-L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine azide with L-valyl-δ-benzyloxycarbonyl-L-ornithyl L-leucyl-D-phenylalanylglycine afforded the corresponding N-blocked decapeptide, which was converted to the *p*-nitrpohenyl ester by the action of di-*p*-nitrophenyl sulfite. After the *p*-methoxybenzyloxycarbonyl group had been removed with trifluoroacetic acid, the decapeptide *p*-nitrophenyl ester was transformed to the cyclic benzyloxycarbonyl-substituted decapeptide by treatment with pyridine. On the other hand, this compound was also obtained by a dimerization reaction of L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanylglycine *p*-nitrophenyl ester. In the latter case, however, the yield was poor. The cyclic benzyloxycarbonyl-substituted decapeptide was hydrogenated in the presence of palladium black to give the desired cyclic decapeptide dihydrochloride.

The effects of the synthesized cyclic decapeptide on bacterial growth have also been examined. It was observed that the compound was about ten times as active as gramicidin S against *B. subtilis* in a synthetic medium; however, it showed silghtly less activity than gramicidin S in a bouillon agar medium.

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Laboratory of Biochemistry
Faculty of Science
Kyushu University
Fukuoka

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