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Studies of Peptide Antibiotics. XVI. Analogs of Gramicidin S Containing β-Alanine in Place of L-Proline[1]

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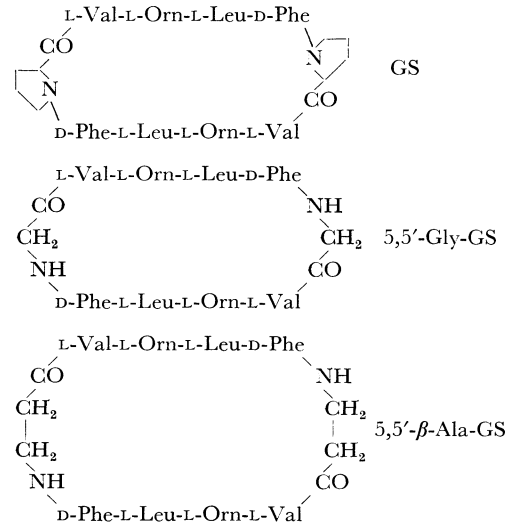
5,5′-β-Alanine-gramicidin S having a larger ring size by two methylene groups than that of gramicidin S was synthesized to investigate the contribution of the ring size of gramicidin S to antibacterial activity. Cyclization of a protected linear decapeptide active ester in pyridine gave the protected 5,5′-β-alanine-gramicidin S in a good yield. The product obtained after the cyclization of a protected linear pentapeptide active ester consisted of the protected 5-β-alanine-cyclosemi- and 5,5′-β-alanine-gramicidin S. Pure compounds were obtained by column chromatographic separation of Sephadex LH-20. Hydrogenolysis of these protected cyclic peptides in the presence of hydrogen chloride afforded crystalline hydrochlorides of cyclic deca- and pentapeptide. These hydrochlorides exhibited no antibacterial activity against any of the microorganisms tested. Optical rotatory dispersion measurements of these peptides were also carried out.

For a study of the relationship between chemical structure and biological activity of gramicidin S, various analogs have been synthesized in this laboratory. Particularly in regard to ring size, a number of synthetic cyclic peptides, dipeptide anhydrides,[2] cyclo-L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-glycyl,[3] cyclo-L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-glycyl-glycyl[4] and cyclo-D-phenylalanyl-D-leucyl-L-ornithyl-L-valyl D-ornithyl-L-prolyl,[5] were found to be devoid of antibacterial activity. These results seemed to show that a certain minimum ring size and/or a specific amino acid sequence are necessary for the exhibition of antibacterial activity. On the other hand, 5,5′-glycine-gramicidin S[6] possessed activity five times higher than that of natural gramicidin S. Thus it is of interest to investigate the influence of a change in the ring size of gramicidin S or 5,5′-glycine-gramicidin S molecule on biological activity. Replacement of proline or glycine residues in gramicidin S or 5,5′-glycine-gramicidin S molecule by β-alanine residues, which results in the insertion of two methylene groups into the thirty-members ring structure of gramicidin S, may cause considerable change in the conformation and biological activity. The present paper will describe the synthesis, antibacterial properties and optical rotatory dispersion data of 5,5′-β-alanine-gramicidin S and 5-β-alanine-cyclosemigramicidin S.



The sequence of reaction employed for the synthesis of XIII·2HCl is shown in Fig. 1. Acylpentapeptide acid (VIII) was prepared efficiently as follows. Acyltripeptide ester (IV) was prepared by stepwise elongation by means of the dicyclohexylcarbodiimide method. Saponification of IV gave acyltripeptide acid (V), which was then transformed into free tripeptide (VI). Condensation of azide (VII) with VI afforded the acylpentapeptide acid (VIII) in a good yield. Condensation of active ester (IX) derived from VIII with penta-

1) Presented at the 22nd Meeting of the Chemical Society of Japan, Tokyo, April, 1969.

2) N. Izumiya, T. Kato and Y. Fujita, This Bulletin, **37**, 1810 (1964).

3) T. Kato, M. Kondo, M. Ohno and N. Izumiya, *ibid.*, **38**, 1202 (1965).

4) O. Abe, K. Kuromizu, M. Kondo and N. Izumiya, *ibid.*, **43**, 1202 (1970).

5) T. Kato and N. Izumiya, *ibid.*, **39**, 2242 (1966).

6) H. Aoyagi, T. Kato, M. Ohno, M. Kondo and N. Izumiya, *J. Amer. Chem. Soc.*, **86**, 5700 (1964); This Bulletin, **38**, 2139 (1965).

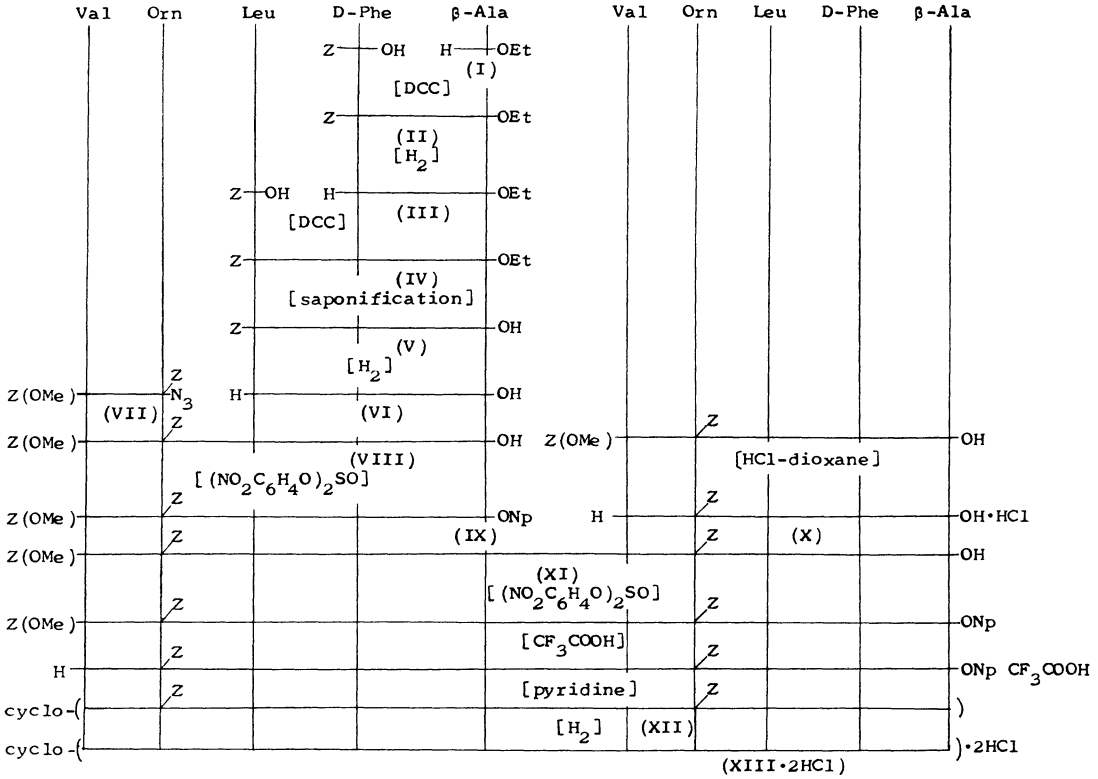


Fig. 1. Synthesis of 5,5′-β-alanine-gramicidin S.

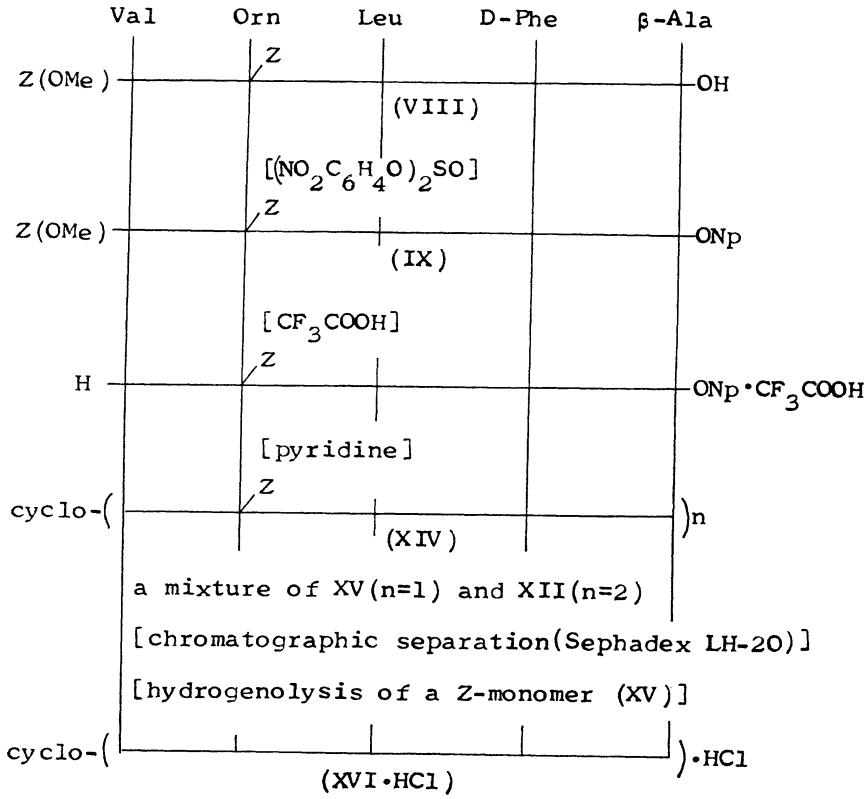


Fig. 2. Synthesis of 5-β-alanine-cyclosemigramicidin S.

peptide hydrochloride (X), which had been obtained from VIII by treatment with hydrogen chloride in dioxane, gave acyldecapeptide acid (XI). Successive treatment of XI with di-*p*-nitrophenyl sulfite, trifluoroacetic acid and excess pyridine afforded the benzyloxycarbonyl-substituted cyclic decapeptide (XII) in a good yield (42% from XI). Hydrogenation of XII in the presence of two equivalents of hydrogen chloride yielded the desired 5,5′-β-alanine-gramicidin S dihydrochloride. Synthesis of the protected cyclic decapeptide (XII) was also attempted by the possible dimerization reaction of acylpentapeptide active ester (IX) (Fig. 2). Active ester (IX) was treated successively with trifluoroacetic acid and excess pyridine. After evaporation, the reaction mixture was passed through Dowex 1 and 50 columns; the subsequent evaporation of the effluent yielded a semi solid residue. This was found to be a mixture of two components by analyzing the hydrogenated product of a small part of the residue (see, Figs. 3 and 4). The remains were subjected to a Sephadex LH-20 column to be separated into components; dimer (XII) was obtained in a yield of 3.7% and monomer (XV) obtained in 29.3%. Thus, the weight ratio of XII and XV is calculated as 11 : 89; the results indicate that the cyclization from acylpentapeptide active ester is favored for the formation of the cyclic monomer.

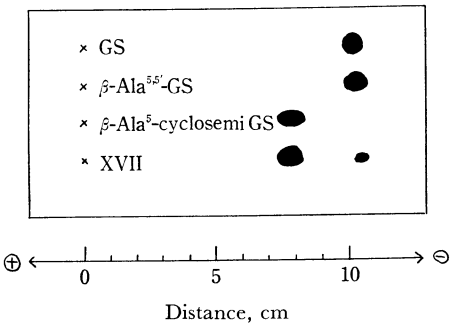


Fig. 3. Paper electrophoresis of the compounds. GS, gramicidin S; XVII; hydrogenated material after cyclization of pentapeptide ester.

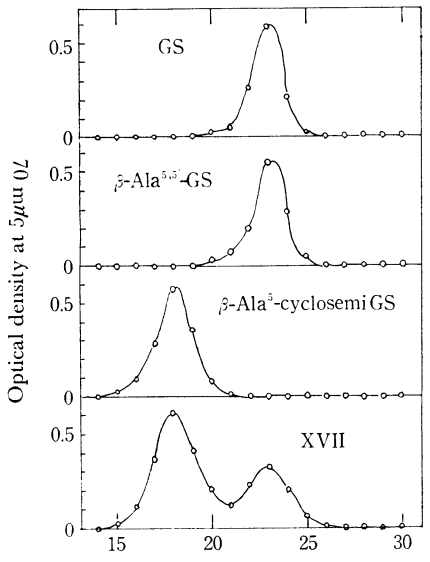


Fig. 4. Carboxymethylcellulose column chromatography of the compounds.

The antibacterial activity of gramicidin S, 5,5′-β-alanine-gramicidin S and 5-β-alanine-cyclosemi-gramicidin S toward several microorganisms was examined. Both monomer (XVI·HCl) and dimer (XIII·2HCl) showed no activity for any of the microorganisms even at 100 γ/ml of the assay medium, whereas minimum concentration of growth inhibition for *B. subtilis* was found to be 3 γ/ml with gramicidin S.

These results suggest that the replacement of proline or glycine residues in gramicidin S or 5,5′-glycine-gramicidin S molecule by β-alanine residues causes change in the conformation of the molecule. We measured the optical rotatory dispersion (ORD) of XIII·2HCl and XVI·HCl in ethanol for comparison with that of gramicidin S[7] (see Fig. 5). The result indicates that the conformation of XIII differs apparently from that of gramicidin S in ethanol. The relationship between ORD patterns and biological activity of gramicidin S analogs is now under investigation.

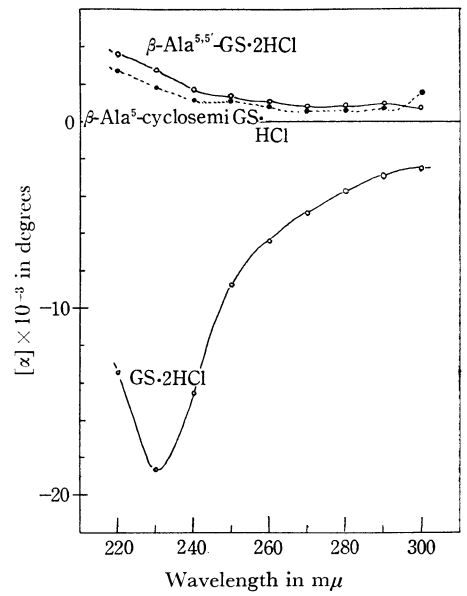


Fig. 5. Optical rotatory dispersion curves of the three compounds.

7) D. Balasubramanian, *J. Amer. Chem. Soc.*, **89**, 5445 (1967).

Experimental

All the melting points are uncorrected. Prior to analysis, the compounds were dried over phosphorus pentoxide to a constant weight at 80°C and 2 mmHg, except for the cyclic peptide hydrochlorides.

H-β-Ala-OEt·p-TsOH (I·p-TsOH).[8] This compound was prepared according to the general procedure of Kato *et al.*[9] A solution of β-alanine (4.45 g, 50 mmol) and *p*-toluenesulfonic acid monohydrate (9.51 g, 55 mmol) in a mixture of ethanol (40 ml) and carbon tetrachloride (200 ml) was refluxed azeotropically for 64 hr in an oil bath. The reaction mixture was concentrated *in vacuo*, and the residual oil was solidified by treatment with ether and petroleum ether. The hygroscopic product was collected by filtration in a cold room and dried. Recrystallization from hot acetone-ether gave 12.1 g (83.5%); mp 49—51°C; R_f 0.75.[10]

Found: C, 49.23; H, 6.61; N, 5.05%. Calcd for $C_{12}H_{19}O_5NS \cdot 1/4H_2O$; C, 49.06; H, 6.69; N, 4.77%.

Z-D-Phe-β-Ala-OEt (II). Dicyclohexylcarbodiimide[11] (4.26 g, 20.6 mmol) was added to a solution of benzyloxycarbonyl-D-phenylalanine (6.18 g, 20.6 mmol) in anhydrous tetrahydrofuran (40 ml) with stirring at 0°C. After 30 min, to this mixture was added a solution of β-alanine ethyl ester *p*-toluenesulfonate (6.56 g, 22.7 mmol) and triethylamine (3.49 ml, 22.7 mmol) in chloroform (40 ml). The reaction mixture was stirred for 3 hr at 0°C and was then allowed to stand in a refrigerator overnight. The mixture was evaporated *in vacuo*, and ethyl acetate (60 ml) was added to the residue. The insoluble dicyclohexylurea was removed by filtration and the filtrate was washed successively with water, a 4% sodium bicarbonate solution, 2% hydrochloric acid and water. The organic layer was dried over anhydrous sodium sulfate and was then evaporated *in vacuo*. The residual oil was solidified by addition of ether and petroleum ether. The product was recrystallized from ethyl acetate-ether-petroleum ether; yield, 6.83 g (87%); mp 102—104°C; $[\alpha]_D^{20}$ +12.5° (*c* 1.04, DMF); R_f 0.98.[10]

Found: C, 66.40; H, 6.77; N, 7.35%. Calcd for $C_{22}H_{26}O_5N_2$: C, 66.31; H, 6.58; N, 7.03%.

HCl·H-D-Phe-β-Ala-OEt (III). A solution of II (3.38 g, 8.49 mmol) in methanol (30 ml) was subjected to hydrogenolysis in the presence of palladium black and 2.48N methanolic hydrogen chloride (4.13 ml) for 8 hr. The filtrate from the catalyst was evaporated to dryness *in vacuo*. The product was obtained as an oil; yield, 2.25 g (100%). R_f 0.82.[10]

Z-Leu-D-Phe-β-Ala-OEt (IV). Benzyloxycarbonyl-leucine dicyclohexylammonium salt (2.25 g, 5.04 mmol) was coupled with III (1.52 g, 5.04 mmol) by dicyclohexylcarbodiimide (1.04 g, 5.04 mmol) following the procedure employed for the preparation of II. Recrystallization from ethyl acetate-ether gave 1.32 g (81%); mp 130—132°C; $[\alpha]_D^{20}$ +15.8° (*c* 1.04, DMF); R_f 0.98.[10]

Found: C, 65.50; H, 7.47; N, 8.51%. Calcd for $C_{28}H_{37}O_6N_3$: C, 65.73; H, 7.29; N, 8.21%.

Z-Leu-D-Phe-β-Ala-OH (V). To a solution of IV (1.65 g, 3.21 mmol) in a mixture of methanol (10 ml) and dioxane (5 ml) was added 1.0N sodium hydroxide (4.05 ml), and the solution was allowed to stand for 4 hr at room temperature. After the addition of water (30 ml) and 1.0N hydrochloric acid (4.68 ml), the solution was concentrated *in vacuo* at a low temperature, and the residual oily product was extracted with ethyl acetate (50 ml). The organic layer was dried over anhydrous sodium sulfate and was then evaporated *in vacuo*. The residual foam weighed 1.7 g (100%). R_f 0.90.[10]

H-Leu-D-Phe-β-Ala-OH (VI). A solution of V (1.5 g, 3.22 mmol) in a mixture of methanol-water-acetic acid (3 : 1 : 6, v/v, 20 ml) was subjected to hydrogenolysis in the presence of palladium black for 6 hr. The filtrate from the catalyst was evaporated to dryness *in vacuo* and the oily residue was triturated with ether-petroleum ether. The crystals were collected by filtration in a cold room and dried *in vacuo*; yield, 1.17 g (95%); mp 189—191°C; $[\alpha]_D^{25}$ +57.7° (*c* 0.44, methanol); R_f 0.85.[10]

Found: C, 60.91; H, 7.84; N, 11.49%. Calcd for $C_{18}H_{27}O_4N_3 \cdot 1/3H_2O$: C, 60.82; H, 7.84; N, 11.82%.

Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-OH (VIII). To a chilled solution of Z(OMe)-Val-Orn(δ-Z)-$NHNH_2$ (VII)[12] (1.68 g, 3.21 mmol) in glacial acetic acid (40 ml) were added successively with stirring 1.0N hydrochloric acid (6.42 ml) and sodium nitrite (265.8 mg) in 9 ml of water. After 6 min, cold water (520 ml) was added to the solution. The azide which precipitated as a white mass was collected by filtration and washed with cold water (200 ml), a 9% sodium bicarbonate solution (200 ml) and cold water (200 ml), and then dried in a vacuum in a desiccator. The azide was added to a solution of VI (1.35 g, 3.12 mmol) and triethylamine (0.87 ml) in dimethylformamide (25 ml), and the reaction mixture was stirred for 3 days at 0°C. The precipitate formed by the addition of 10% citric acid (15 ml) was collected, washed with 10% citric acid and water and then dried. Recrystallization from methanol-ether-petroleum ether gave 2.32 g (86%); mp 181—183°C; $[\alpha]_D^{20}$ +11.6° (*c* 0.53, DMF); R_f 0.87.[10]

Found: C, 62.52; H, 7.20; N, 9.68%. Calcd for $C_{45}H_{60}O_{11}N_6$: C, 62.77; H, 7.02; N, 9.76%.

Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-ONp (IX). To a solution of VIII (600 mg, 0.69 mmol) in pyridine (10 ml) was added di-*p*-nitrophenyl sulfite[13] (2.25 g, 6.96 mmol), and the reaction mixture was allowed to

8) The following abbreviations are from *Biochemistry*, **5**, 2485 (1966); Z-, benzyloxycarbonyl; Z(OMe)-, *p*-methoxybenzyloxycarbonyl; -ONp, *p*-nitrophenoxy; DCHA, dicyclohexylammonium salt; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide. Amino acid symbols except D-Phe and β-Ala denote the L-configuration.

9) T. Kato, S. Makisumi, M. Ohno and N. Izumiya, *Nippon Kagaku Zasshi*, **83**, 1151 (1962).

10) The R_f value of thin-layer chromatography with Merck silica gel refers to a solvent system of *n*-butanol-acetic acid-pyridine-water (4 : 1 : 1 : 2, 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.

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

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

13) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1760 (1960).

stand for 24 hr at room temperature. After evaporation of the mixture to dryness, the oily product was triturated with petroleum ether and washed repeatedly with a mixture of ether-petroleum ether (1 : 1) by decantation until no yellow color could be discerned upon the addition of a sodium hydroxide solution to the washings. The resulting product was collected by filtration, washed with a mixture of ether-petroleum ether (1 : 1) and dried. The yield was 694 mg (108%); the *p*-nitrophenyl ester content in the product was estimated to be 95% spectrophotometrically by measuring the optical density of the solution of the compound in dimethylformamide - N sodium hydroxide (1 : 1) at 404 mμ.[14]

HCl·H-Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-OH (X). To a solution of VIII (700 mg, 0.814 mmol) in dioxane (6 ml) were added anisole (0.6 ml) and 2.0N hydrogen chloride in dioxane (16.28 ml). The reaction mixture was allowed to stand for 7.5 hr at room temperature. After removal of the solvent *in vacuo*, the residue was triturated with ether. The product solidified was collected by filtration, washed with ether and dried in a vacuum in a desiccator; yield, 580 mg (97%); mp 144—146°C; $[\alpha]_D^{25}$ +22.4° (*c* 0.5, DMF); R_f 0.80.[10]

Found: C, 56.45; H, 7.50; N, 11.00%. Calcd for $C_{36}H_{53}O_8N_6Cl \cdot 2H_2O$: C, 56.20; H, 7.46; N, 10.93%.

Z(OMe)-Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-Val-Orn-(δ-Z)-Leu-D-Phe-β-Ala-OH (XI). To a solution of X (273 mg, 0.37 mmol) and triethylamine (0.05 ml) in a mixture of pyridine (5 ml) and dimethylformamide (5 ml) was added IX (367 mg, 0.37 mmol), and the reaction mixture was stirred for 8 days at room temperature. The precipitate formed by the addition of 10% citric acid (30 ml) was collected, washed with 10% citric acid and water and then dried. Recrystallization from dimethylformamide-ether gave 464 mg (81%); mp 237—240°C; $[\alpha]_D^{20}$ +9.2° (*c* 0.5, DMF); R_f 0.99.[10]

Found: C, 62.53; H, 7.22; N, 10.41%. Calcd for $C_{81}H_{110}O_{27}N_{12} \cdot H_2O$: C, 62.45; H, 7.24; N, 10.79%.

***cyclo*-(Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-)$_2$ (XII).** *From XI.* To a solution of XI (358 mg, 0.23 mmol) in pyridine (8 ml), di-*p*-nitrophenyl sulfite (3.01 g, 9.30 mmol) was added. After the mixture had been allowed to stand for 5 days at room temperature, it was evaporated *in vacuo*. The residual solid was collected by filtration with the aid of a mixture of ether and petroleum ether (1 : 1); yield, 476 mg. The *p*-nitrophenyl ester content for this product was estimated to be 100% by measuring the optical density of the compound at 404 mμ. To the active ester thus obtained, anisole (0.3 ml) and trifluoroacetic acid (4.5 ml) were added at 0°C. The solution was evaporated *in vacuo*, and the residual powder was collected by filtration with the aid of ether. This decapeptide *p*-nitrophenyl ester trifluoroacetate was dissolved in dimethylformamide (8 ml) and acetic acid (0.1 ml). The solution was added drop by drop over a period of 6 hr to pyridine (100 ml) which had been kept at 60°C; stirring was then continued for an additional 2 hr at the same temperature. After removal of the solvent by evaporation *in vacuo*, the residue was dissolved in a mixture of methanol (50 ml), dioxane (20 ml) and water (15 ml). The insoluble substance was removed by filtration, and the filtrate was successively passed through columns (1.8×15 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (400 ml), and the combined effluent was evaporated *in vacuo* to yield a crystalline product. The product was collected by filtration with the aid of water, and recrystallized from methanol-ether-petroleum ether; yield, 132 mg (42% from XI); mp 231—234°C (decomp); $[\alpha]_D^{25}$ −5.3° (*c* 0.6, acetic acid).

Found: C, 62.43; H, 7.44; N, 12.07%; mol wt, 1407.[15] Calcd for $C_{72}H_{100}O_{14}N_{12} \cdot 2H_2O$: C, 62.04; H, 7.53; N, 12.06%; mol wt, 1394.

From VIII. The acylpentapeptide VIII (1.82 g, 2.1 mmol) was converted to the pentapeptide *p*-nitrophenyl ester trifluoroacetate following the same procedure as described above, and then added to pyridine (300 ml). After the filtrate was passed through columns of Dowex 1 and Dowex 50 in the same way as described above, the effluent was evaporated to dryness, and the residual powder was collected by filtration with the aid of water; yield of the crude product (XIV), 482 mg. The solution of XIV (107 mg) in methanol (2 ml) was applied to a column (2.7×110 cm) of Sephadex LH-20, and development was continued with methanol. Elution was carried out at room temperature, and 3 ml fraction were collected under a flow rate 30 ml per hr. When the peptide content in the fractions was determined on a thin-layer plate, the peak of XII appeared from the test tube numbers 41 to 80 and the peak of XV from 90 to 130. The same chromatography was repeated further three times; thus, 430 mg of XIV was separated into two components. The four groups of the fractions of 41—80 were combined, evaporated *in vacuo* and the crystals (44 mg, 3.7% from VIII) of XII were obtained; yield after recrystallization, 40 mg; mp 230—233°C (decomp); $[\alpha]_D^{25}$ −5.0° (*c* 0.58, acetic acid).

***cyclo*-(Val-Orn(δ-Z)-Leu-D-Phe-β-Ala-) (XV).** The four groups of the fractions of 90—130 obtained above were combined and evaporated *in vacuo* to afford the crystals (363 mg, 29.3% from VIII). It was recrystallized from methanol-ether-petroleum ether, yield, 358 mg; mp 213—215°C (decomp); $[\alpha]_D^{25}$ −12.5° (*c* 0.68, acetic acid).

Found: C, 61.00; H, 7.52; N, 11.34%; mol wt, 636.[15] Calcd for $C_{36}H_{50}O_7N_6 \cdot 3/2H_2O$: C, 61.16; H, 7.56; N, 11.80%; mol wt, 678.

5,5′-β-Alanine-gramicidin S, *cyclo*-(Val-Orn-Leu-D-Phe-β-Ala-)$_2$ (XIII). A solution of XII (51.6 mg, 0.038 mmol) in ethanol (6 ml) and 0.25N methanolic hydrogen chloride (0.37 ml), was subjected to hydrogenolysis in the presence of palladium black for 6 hr. The solution, after being filtered from the catalyst, was evaporated *in vacuo*. The residual product was recrystallized from methanol-ether-petroleum ether; yield of the air-dried product, 38 mg (86%); mp 280—282°C (decomp); $[\alpha]_D^{25}$ +43.2° (*c* 0.32, ethanol); R_f 0.77.[10]

Found: C, 52.46; H, 7.77; N, 12.66%. Calcd for $C_{56}H_{88}O_{10}N_{12} \cdot 2HCl \cdot 7H_2O$: C, 52.19; H, 8.12; N, 13.04%.

β-Alanine-cyclosemigramicidin S, *cylco*-(Val-Orn-Leu-D-Phe-β-Ala-) (XVI). XV (50.4 mg, 0.074 mmol) was treated as described for the preparation of XIII·2HCl; yield, 41 mg (95%); mp 250—252°C (dec.); $[\alpha]_D^{25}$ −13.4° (*c* 0.55, ethanol); R_f 0.70.[10]

Found: C, 51.61; H, 7.89; N, 12.60%. Calcd for

14) R. Schwyzer and P. Sieber, *ibid.*, **40**, 624 (1957).

15) Molecular weight was determined by a Hitachi Osmometer, type 115 (Solvent, methanol).

Table 1. Inhibitory activity of three compounds on microorganisms
Minimum inhibitory concentration, μg/ml

	Staphylococcus aureus 209P	*Bacillus subtilis ATCC 6633*	*Escherichia coli IAM 1253*	*Candida albicans IAM 4888*	*Proteus vulgaris IAM 1025*	*Shigella sonnei*	*Salmonella Paratyphi A*
Natural GS	6	3	50	13	100	50	100
5,5′-β-Alanine-GS	100	100	>100	>100	>100	>100	>100
5-β-Alanine-cyclosemi GS	>100	>100	>100	>100	>100	>100	>100

$C_{28}H_{44}O_5N_6 \cdot HCl \cdot 4H_2O$: C, 51.48; H, 8.17; N, 12.86%.

Electrophoresis and Carboxymethylcellulose (CMC) Chromatography. These experiments were carried out as previously described.[16] A part of the crude product (XIV), which was obtained after the cyclization reaction of the acylpentapeptide *p*-nitrophenyl ester (IX), was hydrogenated, and the product obtained was designated as XVII. As shown in Figs. 3 and 4, 5,5′-β-alanine-gramicidin S and 5-β-alanine-cyclosemigramicidin S were clearly separated in paper electrophoresis and in CMC chromatography.

Optical Rotatory Dispersion (ORD).[7,17] ORD measurements were performed with a Jasco Model ORD/UV5 spectropolarimeter. Cell of path length 1.0 cm was used and the runs were made at ambient temperature. As shown in Fig. 5, the ORD curve of gramicidin S is similar to that obtained by Balasubramanian,[7] and a trough is encountered at 230 mμ with a trough specific rotation of $-18560°$. 5,5′-β-Alanine-gramicidin S and 5-β-alanine-cyclosemigramicidin S showed the monotonic change toward positive at 230 mμ.

Microbiological Assays.[18] The microorganisms employed are listed in Table 1. Minimum amount of the compound necessary for the complete inhibition of growth was determined by the dilution method. As shown in Table 1, 5,5′-β-alanine-gramicidin S and 5-β-alanine-cyclosemigramicidin S were found to exhibit no antibacterial activity against the microorganisms tested, whereas gramicidin S exhibited activity against *Bacillus subtilis* and *Staphylococcus aureus*.

16) M. Waki and N. Izumiya, This Bulletin, **40**, 1687 (1967); *J. Amer. Chem. Soc.*, **89**, 1278 (1967).

17) We are indebted to Dr. K. Hayashi and Mr. T. Shimoda of Department of Biochemistry, Faculty of Agricalture, Kyushu University, for the ORD measurement.

18) We are indebted to Meiji Seika Co., Ltd., for the microbiological assays.