BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 2904—2909 (1967)

Studies of Peptide Antibiotics. XII. Syntheses of [2, 2′-α, γ-Diaminobutyric acid]- and [2, 2′-Lysine]-gramicidin S

Michinori Waki, Okitoshi Abe, Reiko Okawa, Tetsuo Kato, Satoru Makisumi and Nobuo Izumiya

Laboratory of Biochemistry, Faculty of Science, Kyushu University, Hakozaki, Fukuoka

(Received June 7, 1967)

To investigate the contribution for the antibacterial activity of the ornithine residues in gramicidin S, two analogs of gramicidin S, [2, 2′-L-α, γ-diaminobutyric acid]- and [2, 2′-lysine]-gramicidin S, were synthesized by the cyclization reaction of linear pentapeptide active esters with pyridine. It was indicated that the crude product of the cyclization of each of the linear active esters was composed of two components. The protected cyclic decapeptide, a less soluble material, was easily isolated by fractional crystallization, and the hydrogenolysis of this product in the presence of hydrogen chloride afforded the crystalline hydrochloride of [2, 2′-diaminobutyric acid]- or [2, 2′-lysine]-gramicidin S. These cyclic decapeptides were as active as natural gramicidin S; the results indicated that the side chains of the ornithines in gramicidin S could be replaced by other chains without a loss of activity.

It has been recognized that the basic character of ornithine residues in gramicidin S is necessary for antibacterial activity; Silaev and Stepanov have observed that acylation such as phthalylation of the δ-amino groups in gramicidin S brings about complete inactivation.[1] They have further reported that bis-δ-aminoacyl-gramicidin S, wherein the aminoacyls are glycyl, ε-amino caproyl, and ω-aminopelargonyl, shows lower activities than natural gramicidin S, decreasing with increasing chain length of the aminoacyl residues.[2] Schwyzer and Sieber have observed that a synthetic [Lys$^{2,2'}$]-gramicidin S[3] possesses antibacterial activity,[4] however, they did not describe the degree of activity in quantitative manner. Erlanger and Goode have reported that a linear decapeptide of gramicidin S, H-(Val-Orn-Leu-D-Phe-Pro)$_2$-OH,[5] was found to be approximately 1/10 as active as gramicidin S against *E. coli*, whereas a similar linear decapeptide, wherein L-lysine residues replace L-ornithines, to be approximately 1/40.[6] Therefore, it appeared of interest to reexamine quantitatively the antibacterial activity of [Lys$^{2,2'}$]-gramicidin S, and to synthesize [Dbu$^{2,2'}$]-gramicidin S, wherein L-α, γ-diaminobutyric acid residues replace the ornithines in gramicidin S, in order to determine what degree the side chain length of the ornithines contribute to the biological activity. It would be of interest to note that L-lysine residue is a constituent in natural antibiotic, bacitracins, and L- and D-α, γ-diaminobutyric acid in polymyxins.[7]

Synthesis of [Lys$^{2,2'}$]-gramicidn S was already reported by Schwyzer and Sieber; tosyl-substituted [Lys$^{2,2'}$]-gramicidin S, *cyclo*-(Val-Lys(ε-Tos)-Leu-D-Phe-Pro-)$_2$, was obtained by the cyclization reaction of a linear tosyl-substituted decapeptide active ester with pyridine.[4] They also reported that the cyclization of H-Val-Lys(ε-Tos or Mz)-Leu-D-Phe-Pro-ONp yielded exclusively Tos- or Mz-substituted [Lys$^{2,2'}$]-gramicidin S by the doubling reaction.[8,9] In a previous paper, we have described that the cyclization reaction of a linear pentapeptide active ester, H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-ONp, whose amino acid sequence is similar to that of the linear pentapeptide ester containing ε-Tos or Mz-lysine residue mentioned above, affords a mixture of the protected cyclic penta- and decapeptide, with a weight ratio of 32 : 68.[10] Therefore, it appeared also of interest to investigate the possibility of formation of the

1) A. B. Silaev and V. M. Stepanov, *Dokl. Akad. Nauk SSSR*, **112**, 297 (1957).
2) V. M. Stepanov, A. B. Silaev and A. N. Polin, *Antibiotiki*, **3**, 49 (1958).
3) We followed the rules naming synthetic modifications of natural peptides in *Biochemistry*, **6**, 362 (1967); *e. g.* the semitrivial name is [2, 2′-lysine]-gramicidin S and the abbreviated form is [Lys$^{2,2'}$]-gramicidin S.
4) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 1582 (1958).
5) The following abbreviations are from *Biochemistry*, **5**, 2485 (1966); Z-, benzyloxycarbonyl, Z(OMe)-, *p*-methoxybenzyloxycarbonyl; Mz-, *p*-methoxyphenylazobenzyloxycarbonyl; Tos-, tosyl; -ONp, *p*-nitrophenoxy; Dbu, α, γ-diaminobutyric acid. Amino acid symbols except D-Phe denote the L configuration.
6) B. F. Erlanger and L. Goode, *Nature*, **174**, 840 (1954); *Science*, **131**, 669 (1960).
7) M. W. Miller, "The Pfizer Handbook of Microbial Metabolites," McGraw-Hill Co., New York (1961).
8) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186 (1958).
9) R. Schwyzer and P. Sieber, *ibid.*, **43**, 1910 (1960).
10) M. Waki and N. Izumiya, *J. Am. Chem. Soc.*, **89**, 1278 (1967); This Bulletin, **40**, 1687 (1967).

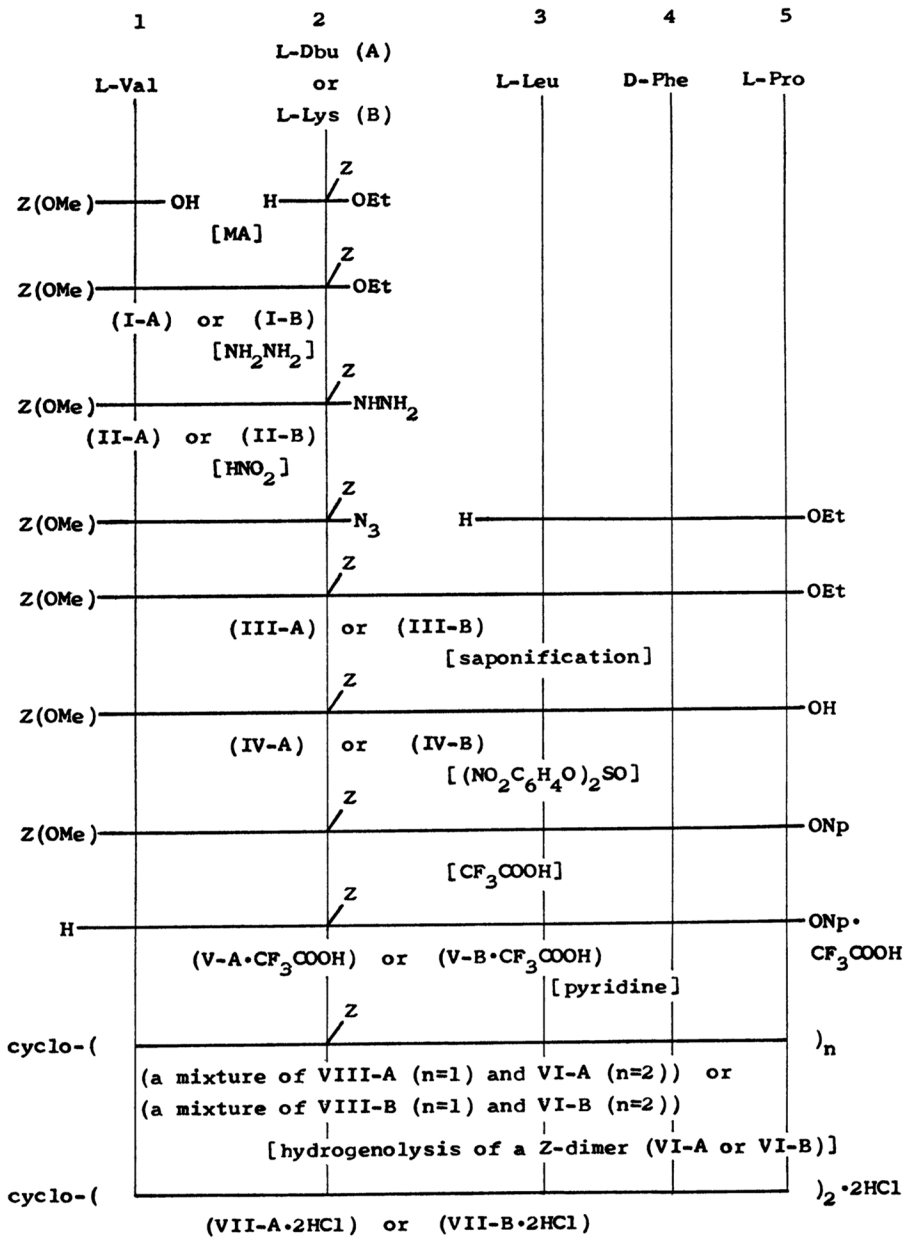

Fig. 1. Cyclization of linear pentapeptide active ester.

protected cyclic pentapeptide in the course of the cyclization reaction of a linear pentapeptide ester, H-Val-Lys(ε-Z)-Leu-D-Phe-Pro-ONp.

The sequence of reactions employed for the synthesis of VII-A or VII-B is shown in Fig. 1. The condensation of the azide derived from acyldipeptide hydrazide (II-A or II-B) with the tripeptide ester gave acylpentapeptide ester (III-A or III-B). The saponification of III-A or III-B afforded crystals of acylpentapeptide (IV-A or IV-B). The treatment of IV-A or IV-B with an excess of di-*p*-nitrophenyl sulfite gave an amorphous acylpentapeptide *p*-nitrophenyl ester, and its *p*-methoxybenzyloxycarbonyl group was removed by the action of trifluoroacetic acid. The pentapeptide active ester trifluoroacetate (V·CF_3COOH) obtained was treated with a large amount of pyridine (concentration of V in pyridine; 3×10^{-3} M) at 60°C for the cyclization reaction. After the evaporation of the reaction mixture, the residue dissolved in aqueous methanol was treated with columns of Dowex 1 and 50 to free from the undesired products; the subsequent evaporation of the effluent yielded a semi-solid residue. This

Table 1. Inhibitory activity of three compounds on microorganisms

Minimum inhibitory concentration, μg/ml

A. Bouillon agar medium[a)]

	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*
Natural GS	>100	>100	5	5
[Dbu$^{2,2'}$]-GS	>100	>100	10	10
[Lys$^{2,2'}$]-GS	>100	>100	10	10

B. Synthetic medium[a)]

	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*
Natural GS	>100	>100	5	5
[Dbu$^{2,2'}$]-GS	>100	>100	10	5
[Lys$^{2,2'}$]-GS	>100	>100	10	10

a) Usual bouillon agar medium, pH 7.0.
b) Stephenson-Whetham's medium (modified).

residue was found to be a mixture of two components by paper electrophoresis and column chromatography with a hydrogenated material of a small part of the residue. The less soluble component in the semi-solid residue was easily isolated, by the fractional recrystallization, as crystals in a yield of 36% from IV-A or 27% from IV-B; these crystals were identified as di-Z-substituted-[Dbu$^{2,2'}$]- or [Lys$^{2,2'}$]-gramicidin S (VI-A or VI-B). The hydrogenolysis of VI-A or VI-B in the presence of two equivalent of hydrogen chloride yielded a cyclic decapeptide dihydrochloride (VII-A·2HCl or VII-B·2HCl) as colorless crystals containing water of crystallization.

Another protected cyclic peptide in the semi-solid residue, which appears more soluble than VI-A or VI-B, was deduced as Z-substituted [Dbu^2]- or [Lys^2]-cyclosemigramicidin S (VIII-A or VIII-B) by the experiments of paper electrophoresis and column chromatography comparing with the cyclosemigramicidin S[10)] as is described in the experimental part in this paper. Furthermore, it was deduced that the weight ratios of VIII-A and VI-A in the crude product after the cyclization are 30 : 70, and the ratios of VIII-B and VI-B are 29 : 71. These results appeared to indicate that the replacement by a ω-benzyloxycarbonyl-α, γ-diaminobutyric acid or lysine residue of δ-benzyloxycarbonyl-ornithine residue in H-Val-Orn-(δ-Z)-Leu-D-Phe-Pro-ONp (XII) did not influence for the weight ratios of the monomer and the dimer after the cyclization since the ratios of Z-substituted monomer and di-Z-substituted dimer after the cyclization of XII were found to be 32 : 68.[10)]

The antibacterial activities of VII-A and VII-B toward several microorganisms were examined (Table 1). It was found that VII-A and VII-B were as active as gramicidin S against several microorganisms. These results indicated that the replacement of the ornithine residues by diamino acid residues with shorter or longer side chain than that of ornithine did not change the degree of antibacterial activity.

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 (VII-A·2HCl and VII-B·2HCl).

Z(OMe)-Val-Dbu(γ-Z)-OEt (I-A). To a chilled solution of *p*-methoxybenzyloxycarbonyl-L-valine[11)] (4.22 g, 15 mmol) and triethylamine (2.1 ml) in tetrahydrofuran (40 ml), isobutyl chloroformate (1.98 ml) was added at −5°C. After 15 min, a mixture of γ-benzyloxycarbonylamino-L-α-aminobutyric acid ethyl ester *p*-toluenesulfonate[12)] (6.79 g, 15 mmol) and triethylamine (2.1 ml) in chloroform (40 ml) was added. The mixture was left to stand overnight at room temperature and then evaporated *in vacuo*. The residual oil was solidified by adding water. The product was collected by filtration, washed successively with 10% citric acid, a 4% sodium bicarbonate solution, and water. It was recrystallized from methanol-ether-petroleum ether; yield, 6.07 g (74%); mp 156—158°C; $[\alpha]_D^{25}$ −14.7° (*c* 2, dimethylformamide).

Found: C, 61.87; H, 6.76; N, 7.82%. Calcd for $C_{28}H_{37}O_8N_3$: C, 61.86; H, 6.86; N, 7.73%.

Z(OMe)-Val-Lys(ε-Z)-OEt (I-B). This was prepared from *p*-methoxybenzyloxycarbonyl-L-valine (5.62 g, 20 mmol) and ε-benzyloxycarbonyl-L-lysine ethyl ester *p*-toluenesulfonate[12)] (9.61 g, 20 mmol) as has been described above. Yield, 8.92 g (78%); mp 123—126°C; $[\alpha]_D^{25}$ −4.5° (*c* 2, dimethylformamide).

Found: C, 62.82; H, 7.27; N, 7.50%. Calcd for $C_{30}H_{41}O_8N_3$: C, 63.03; H, 7.23; N, 7.35%.

11) T. Kato, M. Kondo, M. Ohno and N. Izumiya, This Bulletin, **38**, 1202 (1965); F. Weygand and E. Nintz, *Zeit. Naturforschung*, **20b**, 429 (1965).
12) T. Kato, S. Makisumi, M. Ohno and N. Izumiya, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **83**, 1151 (1962).

Z(OMe)-Val-Dbu(γ-Z)-NHNH$_2$ (II-A). A solution of I-A (2.72 g, 5 mmol) and hydrazine hydrate (5 ml, 100 mmol) in dimethylformamide (15 ml) was allowed to stand at room temperature for 48 hr. The excess hydrazine hydrate was evaporated *in vacuo*, and then water (60 ml) was added to the residue. The resulting crystals were collected by filtration; yield, 2.4 g (91%); mp 216—218°C; $[\alpha]_D^{20}$ −30.8° (*c* 1, acetic acid).

Found: C, 58.86; H, 6.58; N, 13.15%. Calcd for $C_{26}H_{35}O_7N_5$: C, 58.96; H, 6.66; N, 13.23%.

Z(OMe)-Val-Lys(ε-Z)-NHNH$_2$ (II-B). I-B (2.86 g, 5 mmol) was converted to the hydrazide (II-B) as has been described above. Yield, 2.48 g (89%); mp 193—195°C; $[\alpha]_D^{20}$ −18.8° (*c* 2, acetic acid).

Found: C, 60.35; H, 7.16; N, 12.58%. Calcd for $C_{28}H_{39}O_7N_5$: C, 60.31; H, 7.03; N, 12.56%.

Z(OMe)-Val-Dbu(γ-Z)-Leu-D-Phe-Pro-OEt (III-A). To a chilled solution of II-A (1.32 g, 2.5 mmol) in glacial acetic acid (30 ml) and dimethylformamide (15 ml), there were stirred N hydrochloric acid (5.5 ml), sodium nitrite (0.19 g) in water (2 ml). After 5 min, cold water (125 ml) was added. The azide which thereupon precipitated was collected by filtration, washed with water and a saturated sodium bicarbonate solution, and water, and then dried under a vacuum in a desiccator. The azide was added to a solution of L-leucyl-D-phenylalanyl-L-proline ethyl ester hydrochloride[10] (1.10 g, 2.5 mmol) and triethylamine (0.35 ml) in dimethylformamide (10 ml). The mixture was then stirred for 3 days at 0°C and evaporated *in vacuo*. The precipitate which formed upon the addition of water (100 ml) was collected, and then washed with a 4% sodium bicarbonate solution, 10% citric acid and water. Recrystallization from dioxane-ether gave 1.76 g (78%); mp 188—190°C; $[\alpha]_D^{25}$ −28.0° (*c* 2, dimethylformamide).

Found: C, 63.46; H, 7.09; N, 9.18%. Calcd for $C_{48}H_{64}O_{11}N_6 \cdot \frac{1}{2}H_2O$: C, 63.35; H, 7.20; N, 9.24%.

Z(OMe)-Val-Lys(ε-Z)-Leu-D-Phe-Pro-OEt (III-B). The azide prepared from II-B (1.39 g, 2.5 mmol) was coupled with H-Leu-D-Phe-Pro-OEt as has been described above. Yield, 1.88 g (82%); mp 145—146°C; $[\alpha]_D^{15}$ −18.0° (*c* 2, dimethylformamide).

Found: C, 64.04; H, 7.43; N, 9.09%. Calcd for $C_{50}H_{68}O_{11}N_6 \cdot \frac{1}{2}H_2O$: C, 64.01; H, 7.41; N, 8.96%.

Z(OMe)-Val-Dbu(γ-Z)-Leu-D-Phe-Pro-OH (IV-A). To a solution of III-A (901 mg, 1 mmol) in methanol (24 ml) and dioxane (12 ml), N sodium hydroxide (2 ml) was added; the solution then was allowed to stand for 5 hr at room temperature. After the addition of water (10 ml), the solution was acidified with 10% citric acid under cooling. The solution was concentrated *in vacuo* at a low temperature, and the residue was treated with water (100 ml). After the residue had been stored in a refrigerator for several hours, the precipitate was collected by filtration. The product was recrystallized from methanol-ether; yield, 776 mg (89%); mp 175—178°C; $[\alpha]_D^{15}$ −24.8° (*c* 1, dimethylformamide).

Found: C, 62.65; H, 6.73; N, 9.65%. Calcd for $C_{46}H_{60}O_{11}N_6 \cdot \frac{1}{2}H_2O$: C, 62.63; H, 6.97; N, 9.52%.

Z(OMe)-Val-Lys(ε-Z)-Leu-D-Phe-Pro-OH (IV-B). III-B (929 mg, 1 mmol) was saponified as has been described above. Yield, 838 mg (93%); mp 148—150°C; $[\alpha]_D^{15}$ −20.3° (*c* 1, dimethylformamide).

Found: C, 63.09; H, 7.27; N, 9.51%. Calcd for $C_{48}H_{64}O_{11}N_6 \cdot \frac{1}{2}H_2O$: C, 63.35; H, 7.20; N, 9.24%.

H-Val-Dbu(γ-Z)-Leu-D-Phe-Pro-ONp Trifluoroacetate (V-A·CF$_3$COOH). To a solution of IV-A (1.01 g, 1.16 mmol) in pyridine (5 ml), di-*p*-nitrophenyl sulfite[13] (1.49 g, 4.6 mmol) was added. After the mixture had been allowed to stand for 24 hr at room temperature, it was evaporated *in vacuo*. The residual solid was collected by filtration and washed with a mixture of ether and petroleum ether until no yellow color could be discerned on the addition of a sodium hydroxide solution to the filtrate. The yield was 1.16 g. The *p*-nitrophenyl ester content of this product was spectrophotometrically estimated to be 98% by measuring the optical density of the compound at 412 mμ.[14] To the acylpentapeptide *p*-nitrophenyl ester (1.155 g) thus obtained, anisole (0.8 ml) and trifluoroacetic acid (5.4 ml) were added at 0°C. The solution was then evaporated *in vacuo* at 0°C, and the residue was triturated with ether. The powder was collected by filtration and washed with ether. The yield was 1.09 g.

H-Val-Lys(ε-Z)-Leu-D-Phe-Pro-ONp Trifluoroacetate (V-B·CF$_3$COOH). A solution of IV-B (1.08 g, 1.2 mmol) and di-*p*-nitrophenyl sulfite (1.56 g, 2.4 mmol) in pyridine (3 ml) was allowed to stand for 24 hr, and then treated in the same manner as has been described above. The yield was 1.26 g, and the *p*-nitrophenyl ester content was estimated to be 96%. This product (1.255 g) was converted to V-B·CF$_3$COOH as has been described above; the yield was 1.18 g.

***cyclo*-(Val-Dbu(γ-Z)-Leu-D-Phe-Pro-)$_2$ (VI-A).** The trifluoroacetate (V-A·CF$_3$COOH) (545 mg) was dissolved in dimethylformamide (6 ml) containing glacial acetic acid (0.3 ml). The solution was then stirred, drop by drop and over a period of 4 hr, into pyridine (180 ml) which had been kept at 60°C; the stirring was then continued for an additional 2 hr at the same temperature. After the solvent had been removed, the residue was dissolved in a mixture of methanol (40 ml) and water (10 ml). The insoluble substance was removed by filtration, and the filtrate was passed successively through columns (1.6×10 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (300 ml), and the combined effluent was evaporated *in vacuo* to yield a semi-solid residue. The residual product was collected by filtration with the aid of water, and recrystallized from methanol - ether - petroleum ether; yield, 145 mg (36% from IV-A); mp 196—197°C (decomp.); $[\alpha]_D^{20}$ −202° (*c* 0.3, acetic acid); R_f 0.96.[15]

Found: C, 62.56; H, 7.34; N, 11.59%. Calcd for $C_{74}H_{100}O_{14}N_{12} \cdot 2H_2O$: C, 62.69; H, 7.39; N, 11.86%.

The molecular weight of VI-A was determined by a Hitachi Osmometer, type 115 (solvent; methanol).

Found: 1360. Calcd for $C_{74}H_{100}O_{14}N_{12} \cdot 2H_2O$: 1418.

***cyclo*-(Val-Lys(ε-Z)-Leu-D-Phe-Pro-)$_2$ (VI-B).** V-B·CF$_3$COOH (593 mg) was treated with pyridine (180 ml) as has been described above. The throughout

13) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1760 (1960).
14) R. Schwyzer and P. Sieber, *ibid.*, **40**, 624 (1957).
15) The R_f value of the 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).

effluent from Dowex 1 and 50 columns was evaporated, and the crystals were collected with the aid of water. This was recrystallized from methanol-ether-petroleum ether; yield, 121 mg (27% from IV-B); mp 213—214°C (decomp.); $[\alpha]_D^{20}$ −199° (*c* 0.1, acetic acid); R_f 0.96.[15]

Found: C, 63.60; H, 7.61; N, 11.48%; mol wt, 1490. Calcd for $C_{78}H_{108}O_{14}N_{12}\cdot 2H_2O$: C, 63.56; H, 7.66; N, 11.40%; mol wt, 1474.

***cyclo*-(Val-Dbu-Leu-D-Phe-Pro-)$_2$ Dihydrochloride (VII-A·2HCl).** VI-A (54 mg, 0.038 mmol), dissolved in 0.04 N methanolic hydrogen chloride (2.1 m*l*), was subjected to hydrogenolysis in the presence of palladium black. The solution, after being filtered from the catalyst, was evaporated *in vacuo*. The residual product was recrystallized from methanol-ether; yield of the air-dried product, 46 mg (90%); mp 280—283°C (decomp.); $[\alpha]_D^{20}$ −265° (*c* 0.1, ethanol); R_f, 0.78,[15] 0.95[16] and 0.93;[17] amino acid ratios in acid hydrolysate, $Val_{1.0}Dbu_{0.9}Leu_{1.0}Phe_{1.0}Pro_{1.0}$.

Found: C, 53.61; H, 7.89; N, 12.82%. Calcd for $C_{58}H_{88}O_{10}N_{12}\cdot 2HCl\cdot 6H_2O$: C, 53.81; H, 7.94; N, 12.99%.

***cyclo*-(Val-Lys-Leu-D-Phe-Pro-)$_2$ Dihydrochloride (VII-B·2HCl).** VI-B (45 mg, 0.03 mmol) in 0.04 N methanolic hydrogen chloride (1.7 m*l*) was hydrogenated as has been described above. The filtrate was evaporated, and the crystals which remained were collected with the aid of ether; yield of the air-dried product, 38 mg (88%); mp 268—269°C (decomp.); $[\alpha]_D^{20}$ −233° (*c* 0.1, ethanol); R_f, 0.74,[15] 0.95[16] and 0.93;[17] amino acid ratios in acid hydrolysate, $Val_{1.1}Lys_{0.9}Leu_{1.0}Phe_{1.0}Pro_{1.0}$. The reported value of $[\alpha]_D$ for VII-B·2HBr·H_2O was −260.5° (*c* 1, ethanol).[9]

Found: C, 52.37; H, 6.21; N, 10.81%. Calcd for $C_{62}H_{96}O_{10}N_{12}\cdot 2HCl\cdot 10H_2O$: C, 52.35; H, 6.10; N, 11.11%.

Electrophoresis and Carboxymethylcellulose (CMC) Chromatography. Paper electrophoresis and CMC column chromatography were carried out as has been described before,[10] and the results obtained are shown in Figs. 2 and 3. Figures show that the chromatographic patterns of [Dbu$^{2,2'}$]- and [Lys$^{2,2'}$]-gramicidin S are identical with natural gramicidin S within the limit of the experimental error.

Formation of Protected Monomer (VIII) and Protected Dimer (VI) after Cyclization of V. Each (110 mg) of the pentapeptide active ester trifluoroacetates (V-A and B·CF_3COOH) was treated with pyridine (36 m*l*) at 60°C as has been described for the preparation of VI-A (concentration of V-A or V-B in pyridine; about 3×10^{-3} M). The throughout effluent from columns of Dowex 1 and 50 was evaporated to afford the semi-solid residue (designated as IX-A or IX-B), and a few mg in acetic acid of IX-A or IX-B were subjected to hydrogenolysis; each of the hydrogenated materials was designated as X-A or X-B. The results of eleetrophoresis and CMC chromatography of X-A or X-B are shown in Figs. 2 and 3. It was confirmed by the comparison with the authentic samples that the faster moving spot (Fig. 2) and the slower eluting peak (Fig. 3) of X-A or X-B were originated from the cyclic decapeptide, VII-A or VII-B. Furthermore, it was observed that the position of the slower spot and the faster peak was identical with that of the cyclic pentapeptide, cyclosemigramicidin S, consequently, it was deduced, by analogy of the similar behaviors in electrophoresis and CMC chromatography between VII-A or VII-B and gramicidin S, that the slower spot and the faster peak were originated from

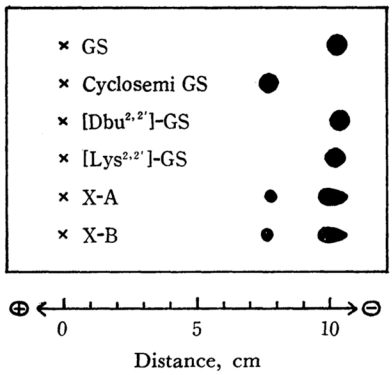

Fig. 2. Paper electrophoresis of the compounds. GS, gramicidin S; X-A or X-B, hydrogenated material after cyclization of pentapeptide ester containing Dbu or Lys.

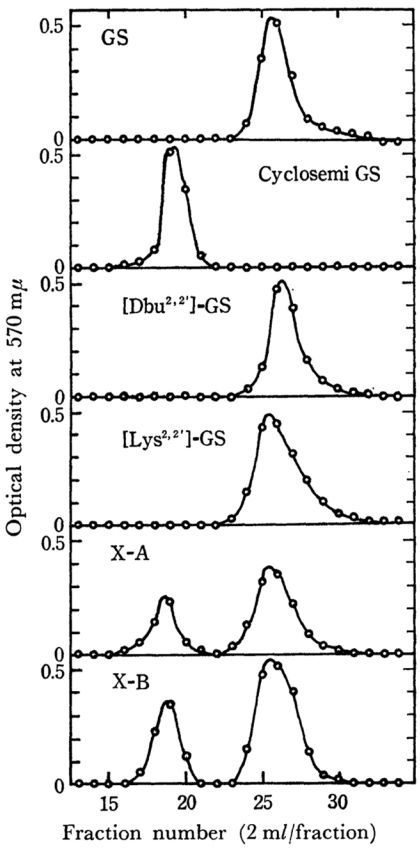

Fig. 3. Carboxymethylcellulose column chromatography of the compounds.

16) The R_f value of the paper chromatography refers to the same solvent system described.[15]

17) The R_f value of the paper chromatography refers to a solvent system of *t*-butanol - formic acid - water (75 : 15 : 10, v/v).

[Dbu^2]- (XI-A) or [Lys^2]-cyclosemigramicidin S (XI-B).[18] Therefore, it could be deduced that the cyclization product (IX-A or IX-B) contained two components, the Z-substituted monomer (VIII-A or VIII-B) and dimer (VI-A or VI-B). If the color intensities in CMC chromatography resulting from ninhydrin between XI-A or XI-B on the molar base and VII-A or VII-B on the base wherein a half mole used as an unit are assumed same,[19] it was calculated from Fig. 3 that the weight ratios of VIII-A and VI-A are 30 : 70, and of VIII-B and VI-B are 29 : 71. It appeared that the Z-substituted monomer (VIII-A or VIII-B) are more soluble in a solvent such as methanol than the Z-substituted dimer (VI-A or VI-B) because the recrystallization from methanol - ether of the crude product (IX-A or IX-B) yields easily the pure Z-substituted dimer.[20]

Microbiological Assays.[21] 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 using a bouillon agar medium and a synthetic medium. As is shown in Table 1, [Dbu$^{2,2'}$]-gramicidin S and [Lys$^{2,2'}$]-gramicidin S were found to be as active as natural gramicidin S against *Staph. aureus* and *B. subtilis*.

18) It should be noted that [Sar^5]-, [Gly^1]- or [Ala^1]-cyclosemigramicidin S is moved slower in paper electrophoresis, and each of them is eluted faster than that of the corresponding cyclic decapeptide; see, This Bulletin, **39**, 1747 (1966); **40**, 1975 (1967).

19) It was determined previously that the ratio of the color intensities between cyclosemigramicidin S (molar base) and gramicidin S (a half molar base) are 100 : 96.[10]

20) It was observed previously that the Z-substituted gramicidin S was very insoluble in many solvents compared with the Z-substituted cyclosemigramicidin S.[10]

21) We are indebted to Dr. M. Shibata of Takeda Chemical Industries, Ltd. for the biological assay.