BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 1202—1207 (1970)

Studies of Peptide Antibiotics. XVII. Analogs of Gramicidin S Containing Glycine or Alanine in Place of Leucine

Okitoshi ABE[1] and Nobuo IZUMIYA[2]

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

(Received October 11, 1969)

In order to investigate the contribution to antibacterial activity of the leucine residues at 3- and 3′-position in gramicidin S, 3,3′-glycine- and 3,3′-L-alanine-gramicidin S (XII-G and XII-A) were prepared and tested for antibacterial properties. A crude product obtained after cyclization of a linear pentapeptide active ester consisted of a protected monomer and a dimer. The pure protected dimer was obtained by a Sephadex LH-20 column from the crude product. Hydrogenolysis of the pure protected dimer afforded the crystalline hydrochloride of XII-G or XII-A. It was found that XII-G showed no activity in any of the microorganisms tested, whereas XII-A exhibited substantial activity though weaker than that of gramicidin S.

Regarding the relationship between the chemical structure and the biological activity of gramicidin S (GS) (Fig. 1), it was reported in previous papers that 5,5′-glycine-GS[3] possessed higher activity than GS against several microorganisms, whereas 1,1′-

1) Present address: Institute for Muscle Disease, New York, N. Y., U.S.A.

2) To whom reprint requests should be addressed.

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

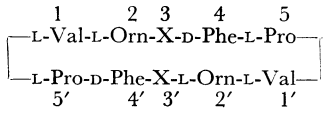

Fig. 1. Structure of gramicidin S (GS) and its analogs. X represents an amino acid residue such as L-Leu (GS), Gly (XII-G) or L-Ala (XII-A).

glycine-GS[4] and 4,4′-glycine-GS[5] exhibited no activity. Contrary to the inactivity of 1,1′-glycine-GS, it was found that 1,1′-L-alanine-GS was as active as GS.[4] These results indicate that the side chains, the isopropyls, of the valine residues in GS molecule can be replaced by smaller aliphatic side chains, the methyls, without influencing the activity, while glycine residues are too small to maintain it. In order to investigate to what extent the leucine residues in 3- and 3′-position of GS molecule contribute to the biological activity, we attempted to prepare several analogs of GS.

This paper deals with the syntheses and antibacterial properties of 3,3′-glycine- and 3,3′-L-alanine-GS besides the preparation of the cyclic pentapeptides, 3-glycine- and 3-L-alanine-cyclo-semiGS.

As shown in Fig. 2,[6] the acylpentapeptide ethyl ester (III-G or -A) was prepared by the coupling reaction of acyldipeptide azide derived from XIII with the tripeptide ester (II-G or -A). However, the ester (III-G or -A) showed considerable resistance to saponification. Hence, in the preparation of the acylpentapeptide acid (VI-G or -A), the azide from XIII was coupled with neutral free tripeptide (V-G or -A), as shown in Fig. 3.

Synthesis of the protected cyclic decapeptide (X-G or -A) was attempted by possible dimerization reaction of the pentapeptide active ester (VIII-G or -A) which was derived from the acylpentapeptide acid (VI-G or -A) (Fig. 3). Treatment of the active ester with a large amount of pyridine afforded a mixture of the protected monomer (IX-G or -A) and dimer (X-G or -A). Attempts to separate two components from the crude mixture by fractional recrystallization with methanol-ether failed because of no appreciable difference in solubility between the components. Separation of the components was achieved by the use of a Sephadex LH-20 column with methanol as an eluting solvent. The protected dimer was obtained from the faster eluting fraction, and the monomer from the slower fraction as shown in Fig. 4.

The weight of both components calculated are shown in Table 1 with the previous results. It is of interest to note that the ratio of formation of the protected monomer increased stepwise as 32→43→59 when the leucine residue in the active ester is replaced with alanine and glycine. The results indicate that a spacial conformation of a pentapep-

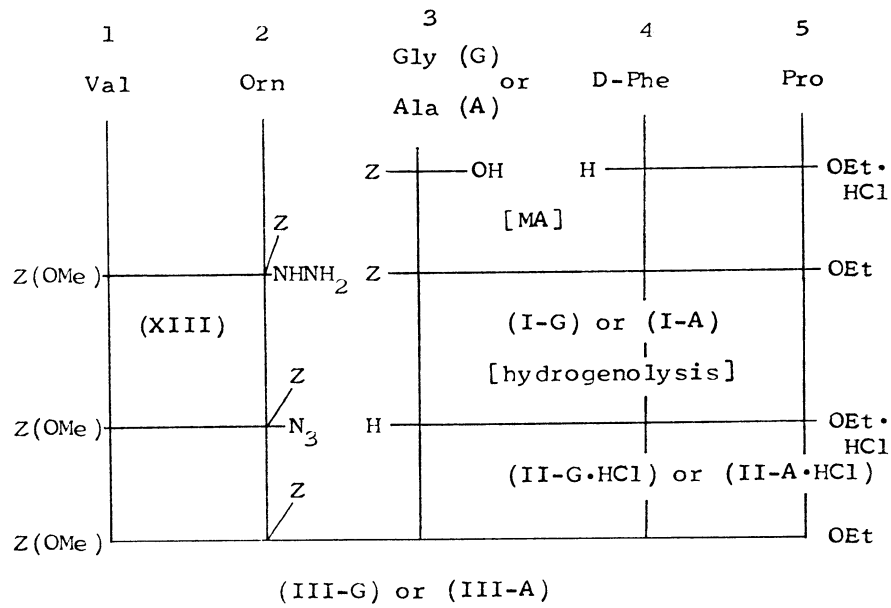

Fig. 2. Synthesis of acylpentapeptide ethyl ester.

4) M. Kondo and N. Izumiya, This Bulletin, **40**, 1975 (1967).

5) R. Nagata, M. Waki, M. Kondo, H. Aoyagi, T. Kato, S. Makisumi and N. Izumiya, *ibid.*, **40**, 963 (1967).

6) The following abbreviations are used; Z-, benzyloxycarbonyl; Z(OMe)-, *p*-methoxybenzyloxycarbonyl; -ONp, *p*-nitrophenoxy; -$NHNH_2$, hydrazide; -N_3, azide; MA, mixed anhydride method; DMF, dimethylformamide. Amino acid symbols except for D-Phe denote L-configuration.

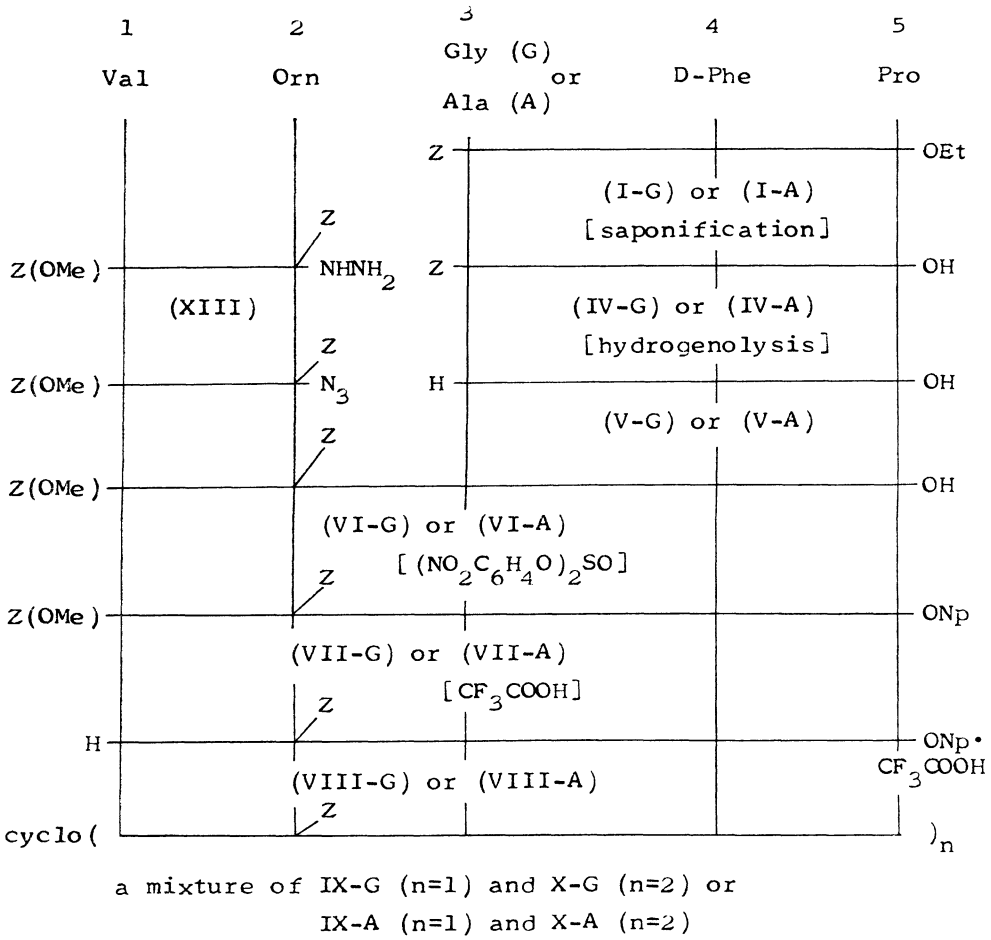

Fig. 3. Cyclization of linear pentapeptide active ester.

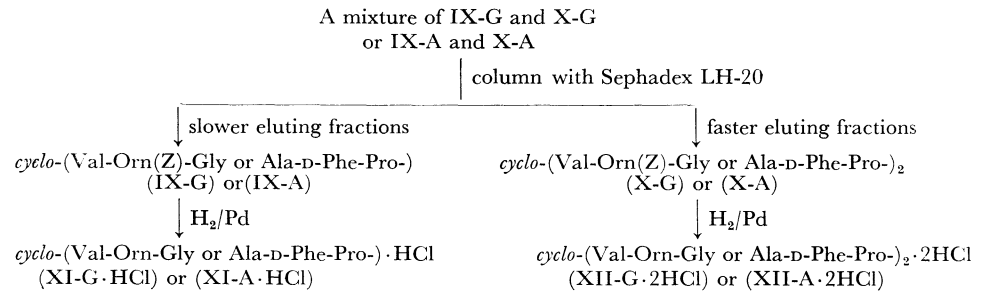

Fig. 4. Separation of the protected monomer and dimer, and preparation of the analogs of GS and cyclosemiGS.

tide active ester in pyridine is favorable for causing a monomerization reaction preferably when a small amino acid, glycine, occupies the third position in the pentapeptide active ester.

Hydrogenolysis of each of the protected cyclic peptides (IX and X) in the presence of hydrogen chloride yielded a crystalline hydrochloride of the required cyclic peptide (XI or XII). The antibacterial activities of these peptides toward several microorganisms were examined (Table 2). The two cyclosemiGS analogs (XI-G and -A) exhibited no activity as in the case of cyclosemiGS itself.[7] It was also found that 3,3′-glycine-GS (XII-G)

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

Table 1. Ratio of protected monomer and dimer after cyclization of linear pentapeptide active esters

p-Nitrophenyl ester of[a]	Ratio of compounds in product[b]	
	Z-cyclic monomer	diZ-cyclic dimer
H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH[7]	32	68
-Gly-[4]	100	0
-Ala-[4]	91	9
-Gly-[3]	59	41
-Ala-[3]	43	57
-Gly-[5]	0	100
-Gly-[3]	79	21

a) After H-Val-Orn(δ-Z)-Leu-D-Phe-Pro-OH listed, only variation of the residue is shown.
b) The concentrations of linear pentapeptide *p*-nitrophenyl esters in pyridine were of approximately 3×10^{-3}M.

exhibited no activity, whereas the alanine analog (XII-A) exhibited substantial activity toward two kinds of microorganisms though the degree of activity was weaker than that of GS (Table 2). These results indicate that the glycine residues which occupy the leucines are too small to maintain activity, while the alanines can replace the leucines without appreciable drop in activity.

Experimental

All melting points are uncorrected.

Z-Gly-D-Phe-Pro-OEt (I-G). To a solution of benzyloxycarbonyl-glycine (0.596 g, 2.85 mmol) and triethylamine (0.40 m*l*, 2.85 mmol) in tetrahydrofuran (5.8 m*l*), isobutyl chloroformate (0.37 m*l*, 2.85 mmol) was added at −5°C. After 15 min, a mixture of H-D-Phe-Pro-OEt·HCl[8] (0.932 g, 2.85 mmol) and triethylamine (0.40 m*l*, 2.85 mmol) in chloroform (5.8 m*l*) was added. The mixture was left to stand overnight at room temperature and then evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate. The solution was washed successively with 4% sodium bicarbonate, 2% hydrochloric acid and water, and dried over sodium sulfate. The filtrate was evaporated *in vacuo*; yield of oil, 1.01 g (74%); R_f, 0.97.[9]

Z-Ala-D-Phe-Pro-OEt (I-A). This was obtained from benzyloxycarbonyl-alanine (3.21 g, 14.4 mmol) and H-D-Phe-Pro-OEt·HCl (4.70 g, 14.4 mmol) as described above; yield of oil, 6.48 g (91%); R_f, 0.97.[9]

H-Gly-D-Phe-Pro-OEt·HCl (II-G·HCl). A solution of I-G (2.54 g, 7.34 mmol) dissolved in 0.52N ethanolic hydrogen chloride (15.5 m*l*) was subjected to hydrogenolysis in the presence of palladium black. The filtrate from the catalyst was evaporated to dryness *in vacuo*; yield of oil, 1.82 g (100%); R_f, 0.73.[9]

Table 2. Inhibitory activity of the compounds on microorganisms
Minimum inhibitory concentration, μg/m*l*

	A. Synthetic medium[a]				
	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*	*Mycobacterium avium*
GS	>100	>100	10	10	>100
$Gly^{3,3'}$-GS (XII-G)	>100	>100	>100	>100	>100
$Ala^{3,3'}$-GS (XII-A)	>100	>100	100	20	>100
Gly^{3}-semiGS (XI-G)	>100	>100	>100	>100	>100
Ala^{3}-semiGS (XI-A)	>100	>100	>100	>100	>100
	B. Bouillon agar medium[b]				
	Escherichia coli	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*	*Mycobacterium avium*
GS	>100	>100	5	2—5	>100
$Gly^{3,3'}$-GS (XII-G)	>100	>100	>100	>100	>100
$Ala^{3,3'}$-GS (XII-A)	>100	>100	50	20	>100
Gly^{3}-semiGS (XI-G)	>100	>100	>100	>100	>100
Ala^{3}-semiGS (XI-A)	>100	>100	>100	>100	>100

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

8) M. Ohno, T. Kato, S. Makisumi and N. Izumiya, This Bulletin, **39**, 1738 (1966).
9) The R_f values refer to the thin-layer chromatography with Merck silica gel G and 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 them with ninhydrin, and those with blocked amino groups, by spraying them with 48% hydrobromic acid, and then with ninhydrin.

H-Ala-D-Phe-Pro-OEt·HCl (II-A·HCl). This was obtained from I-A (6.36 g, 12.8 mmol) as described above; yield of oil, 5.22 g (103%); R_f, 0.74.[9]

Z(OMe)-Val-Orn(δ-Z)-Gly-D-Phe-Pro-OEt (III-G). To a solution of Z(OMe)-Val-Orn(δ-Z)-$NHNH_2$ (XIII)[10] (1.71 g, 3.15 mmol) dissolved in a mixture of acetic acid (50 ml), DMF (10 ml) and 1.92N hydrochloric acid (4.1 ml) was added sodium nitrite (261 mg). After 15 min, cold water (300 ml) was added to the solution. The azide which precipitated was collected by filtration, washed with 4% sodium bicarbonate and water, and then dried *in vacuo*. The azide was added to a solution of II-A·HCl (0.781 g, 3.15 mmol) and triethylamine (0.44 ml) in DMF (25 ml). The mixture was stirred for 2 days at 0°C and evaporated *in vacuo*. The precipitate which formed upon the addition of water was collected, washed successively with 4% sodium bicarbonate, 10% citric acid and water. It was recrystallized from dioxane-methanol-ether; yield, 1.92 g (70%); mp 151—154°C; $[\alpha]_D^{20}$ −18.0° (*c* 1, DMF); R_f, 0.95.[9]

Found: C, 62.65; H, 6.80; N, 9.80%. Calcd for $C_{45}H_{58}O_{11}N_6$: C, 62.91; H, 6.82; N, 9.78%.

Z(OMe)-Val-Orn(δ-Z)-Ala-D-Phe-Pro-OEt (III-A). The azide derived from XIII (2.18 g, 4.0 mmol) was condensed with II-A·HCl (1.59 g, 4.0 mmol) and triethylamine (0.56 ml) as described above. The crude product was recrystallized from ethanol-dioxane-ether; yield, 3.16 g (91%); mp 165—170°C; $[\alpha]_D^{20}$ −14.0° (*c* 1, DMF); R_f, 0.95.[9]

Found: C, 63.51; H, 6.70; N, 9.86%. Calcd for $C_{46}H_{60}O_{11}N_6$: C, 63.27; H, 6.94; N, 9.63%.

Z-Gly-D-Phe-Pro-OH (IV-G). To a solution of I-G (1.88 g, 3.9 mmol) in a mixture of dioxane (8 ml) and methanol (8 ml), N sodium hydroxide (8.15 ml) was added; the solution was then allowed to stand for 1.5 hr at room temperature. After the addition of water, the solution was evaporated *in vacuo*. The residue was acidified with N hydrochloric acid and extracted with ethyl acetate. The extract was dried over sodium sulfate and evaporated *in vacuo*. The residual oil was solidified by the addition of ether; yield of powder, 1.23 g (69%); R_f, 0.79.[9]

Z-Ala-D-Phe-Pro-OH (IV-A). This was obtained from I-A (1.94 g, 3.9 mmol) as described for the preparation of IV-G; yield of powder, 1.31 g (72%); R_f, 0.84.[9]

H-Gly-D-Phe-Pro-OH (V-G). A solution of IV-G (0.722 g, 1.6 mmol) in a mixture of acetic acid (10.8 ml), methanol (5.4 ml) and water (1.8 ml) was treated with hydrogen in the presence of palladium black. The filtrate from the catalyst was evaporated *in vacuo* to dryness. The hygroscopic crystals were collected with the aid of ether; yield, 0.52 g (102%); R_f, 0.46.[9]

H-Ala-D-Phe-Pro-OH (V-A). This was obtained from IV-A (1.31 g, 2.8 mmol) as described for the preparation of V-G; yield, 0.71 g (76%); $[\alpha]_D^{20}$ −36.0° (*c* 1, DMF); R_f, 0.46.[9]

Z(OMe)-Val-Orn(δ-Z)-Gly-D-Phe-Pro-OH (VI-G). The azide derived from XIII (1.31 g, 2.4 mmol) was added to a solution of V-G (0.765 g, 2.4 mmol) and triethylamine (0.34 ml) in DMF (25 ml). The mixture was stirred for 2 days at 0°C and evaporated *in vacuo*. The residue was triturated with 0.5M citric acid, and the precipitate was collected by filtration and washed with water. It was recrystallized from dioxane-ethanol-ether; yield, 1.54 g (77%); mp 110—112°C; $[\alpha]_D^{20}$ −30.0° (*c* 1, DMF); R_f, 0.83.[9]

Found: C, 61.05; H, 6.80; N, 10.19%. Calcd for $C_{43}H_{54}O_{11}N_6 \cdot H_2O$: C, 60.83; H, 6.60; N, 9.90%.

Z(OMe)-Val-Orn(δ-Z)-Ala-D-Phe-Pro-OH (VI-A). The azide derived from XIII (0.936 g, 1.72 mmol) was condensed with V-A (0.570 g, 1.72 mmol) and triethylamine (0.24 ml) as described for the preparation of VI-G. The crude product was recrystallized from dioxane-methanol-ether; yield, 1.22 g (84%); mp 150—153°C; $[\alpha]_D^{20}$ −20.0° (*c* 1, DMF); R_f, 0.77.[9]

Found: C, 61.35; H, 6.69; N, 9.76%. Calcd for $C_{44}H_{56}O_{11}N_6$: C, 61.23; H, 6.79; N, 9.74%.

Z(OMe)-Val-Orn(δ-Z)-Gly-D-Phe-Pro-ONp (VII-G). To a solution of VI-G (1.08 g, 1.3 mmol) in pyridine (25 ml), di-*p*-nitrophenyl sulfite (4.21 g, 1.3 mmol) was added. After the mixture had been allowed to stand overnight at room temperature, it was evaporated *in vacuo*. The residual solid was collected with the aid of a mixture of ether and petroleum ether; yield, 1.50 g. The *p*-nitrophenyl ester content was estimated to be 91% by measuring the optical density at 412 mμ.

Z(OMe)-Val-Orn(δ-Z)-Ala-D-Phe-Pro-ONp (VII-A). The compound VI-A (0.423 g, 0.5 mmol) was converted to VII-A (0.663 g) as described above in which *p*-nitrophenyl ester content was estimated to be 107%.

Mixture of *cyclo*-(Val-Orn(δ-Z)-Gly-D-Phe-Pro-) (IX-G) and *cyclo*-(Val-Orn(δ-Z)-D-Phe-Pro-)$_2$ (X-G). The compound VII-G (1.49 g) was treated with anisole (1 ml) and trifluoroacetic acid (6 ml) at 0°C. After 40 min, the solution was evaporated, and the solid was collected with the aid of a mixture of ether and petroleum ether. The compound VIII-G·CF_3COOH thus obtained was dissolved in DMF (13 ml) and acetic acid (0.5 ml). The solution was added dropwise into pyridine (650 ml) at 55—60°C during 5 hr and the stirring was continued for additional 2 hr. The solution was evaporated, and the residue was dissolved in a mixture of methanol (150 ml) and water (30 ml). The solution was treated with column (1.8×15 cm) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (450 ml), and the combined effluent was evaporated to dryness. The residual product was collected by filtration with the aid of water; yield, 0.51 g.[11] A part (0.1 g) of the product was dissolved in methanol (1.5 ml) and applied to a column (2.5×35 cm) with Sephadex LH-20, and the development continued with methanol. Elution was carried out at room temperature, at a flow rate of 30 ml per hour; a 2 ml fraction was collected. The peptide content in the fractions was determined as described previously.[7] The first peak appeared from tube number 31 to 51, and the second, from 56 to 70. The fractions 52 to 55 contained both components. The fractions were treated as follows.

***cyclo*-(Val-Orn(δ-Z)-Gly-D-Phe-Pro-) (IX-G).** The fractions 56—70 were combined and evaporated *in*

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

11) A few mg of this crude product was dissolved in methanol and subjected to hydrogenolysis. The paper electrophoresis of the hydrogenated material showed two spots.

vacuo, and the product was collected by filtration with the aid of water (yield, 39 mg). It was recrystallized from ethanol-ether; yield, 34 mg (20% from VI-G); mp 117—120°C; $[\alpha]_D^{20}$ −82.8° (*c* 0.5, MeOH); R_f, 0.98.[9]

Found: C, 62.03; H, 6.85; N, 12.90%; mol wt, 640.[12] Calcd for $C_{34}H_{41}O_7N_6 \cdot H_2O$: C, 62.07; H, 6.91; N, 12.78%; mol wt, 667.

***cyclo*-(Val-Orn(δ-Z)-Gly-D-Phe-Pro-)$_2$ (X-G).** The fractions 31—51 were treated as described above (yield, 27 mg). Recrystallization from ethanol-ether gave 25 mg (15% from VI-A); mp 208—210°C; $[\alpha]_D^{20}$ −232° (*c* 0.5, MeOH); R_f, 0.98.[9]

Found: C, 62.80; H, 6.83; N, 12.73%; mol wt, 1260. Calcd for $C_{68}H_{88}O_{14}N_{12}$: C, 62.93; H, 6.85; N, 12.96%; mol wt, 1298.

Mixture of *cyclo*-(Val-Orn(δ-Z)-Ala-D-Phe-Pro-) (IX-A) and *cyclo*-(Val-Orn(δ-Z)-Ala-D-Phe-Pro-)$_2$ (X-A). The compound VIII-A·CF_3COOH obtained from VII-A (0.662 g) was added to pyridine (250 ml) as described for the preparation of a mixture of IX-G and X-G. Yield of the crude product was 149 mg. A part (0.1 g) of the product was dissolved in methanol (2 ml) and applied to a column (2.5×35 cm) with Sephadex LH-20 as described. The first peak appeared from tube number 32 to 48, and the second 54 to 81. The fractions from 49 to 53 contained both components.

***cyclo*-(Val-Orn(δ-Z)-Ala-D-Phe-Pro-) (IX-A).** The fractions 54 to 81 were treated as described for the preparation of IX-G (yield, 26 mg). It was recrystallized from ethanol-ether; yield, 23 mg (10% from VI-B); mp 138—140°C; R_f, 0.98.[9]

Found: C, 61.62; H, 6.95; N, 12.30%; mol wt, 730.[12] Calcd for $C_{35}H_{46}O_7N_6 \cdot H_2O$: C, 61.74; H, 7.12; N, 12.35%; mol wt, 681.

***cyclo*-(Val-Orn(δ-Z)-Ala-D-Phe-Pro-)$_2$ (X-A).** The fractions 32 to 48 were treated as described above (yield, 35 mg). It was recrystallized from ethanol-ether; yield, 32 mg (15% from VI-B); mp 255—258°C; $[\alpha]_D^{20}$ −212° (*c* 0.5, MeOH); R_f, 0.98.[9]

Found: C, 61.38; H, 6.98; N, 11.97%; mol wt, 1320.[12] Calcd for $C_{70}H_{92}O_{14}N_{12} \cdot 2H_2O$: C, 61.74; H, 7.12; N, 12.35%; mol wt, 1362.

***cyclo*-(Val-Orn-Gly-D-Phe-Pro-)·HCl (XI-G·HCl).** A solution of IX-G (20 mg 0.03 mmol) dissolved in 0.01N methanolic hydrogen chloride (3.36 ml) was subjected to hydrogenolysis in the presence of palladium black. The filtrate was evaporated to dryness *in vacuo*. The crystals were collected with the aid of ether; yield of air-dried product, 16.0 mg (96%); mp 135—137°C (decomp); $[\alpha]_D^{20}$ −68.0° (*c* 0.5, MeOH); R_f, 0.84[9] and 0.76.[13]

Found: C, 51.30; H, 7.53; N, 13.86%. Calcd for $C_{26}H_{39}O_5N_6Cl \cdot 3H_2O$: C, 51.61; H, 7.50; N, 13.89%.

***cyclo*-(Val-Orn-Ala-D-Phe-Pro-)·HCl (XI-A·HCl).** The compound IX-A (20 mg 0.03 mmol) was converted to XI-A·HCl as described above; yield of air-dried product, 15.8 mg (95%); mp 140—142°C (decomp); $[\alpha]_D^{20}$ −55.3° (*c* 0.5, MeOH); R_f, 0.82[9] and 0.96.[13]

Found: C, 52.09; H, 7.68; N, 13.54%. Calcd for $C_{27}H_{41}O_5N_6Cl \cdot 3H_2O$; C, 52.37; H, 7.65; N, 13.57%.

***cyclo*-(Val-Orn-Gly-D-Phe-Pro-)$_2$·2HCl (XII-G·2HCl).** The compound X-G (20 mg; 0.015 mmol) was treated as described for the preparation of XI-G·HCl; yield of air-dried product, 15.4 mg (93%); mp 214—215°C (decomp); $[\alpha]_D^{20}$ −103° (*c* 0.5, MeOH); R_f, 0.86[9] and 0.97.[13]

Found: C, 51.44; H, 7.68; N, 13.76%. Calcd for $C_{52}H_{80}O_{10}N_{12}Cl_2 \cdot 6H_2O$: C, 51.51; H, 7.66; N, 13.87%.

***cyclo*-(Val-Orn-Ala-D-Phe-Pro-)$_2$·2HCl (XII-A·2HCl).** The compound X-A (20 mg, 0.015 mmol) was treated as described above; yield of air-dried product, 15.6 mg (92%); mp 260—263°C (decomp); $[\alpha]_D^{20}$ −101° (*c* 0.5, MeOH); R_f, 0.83[9] and 0.98.[13]

Found: C, 52.13; H, 8.00; N, 13.36%. Calcd for $C_{54}H_{84}O_{10}N_{12}Cl_2 \cdot 6H_2O$; C, 52.28; H, 7.82; N, 13.55%.

Electrophoresis. Electrophoresis on Toyo Roshi No. 52 paper was carried out with a solvent system, formic acid-acetic acid-methanol-water (1 : 3 : 6 : 10, v/v; pH 1.8) for 2 hr at 500 V/30 cm. Figure 5 shows

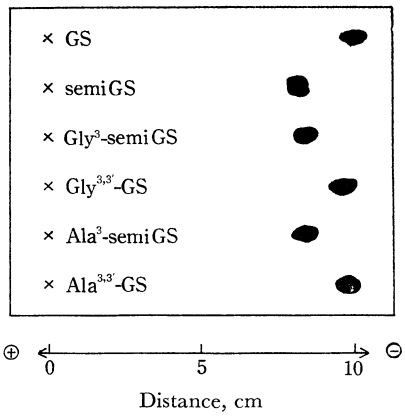

Fig. 5. Paper electrophoresis of the compounds.

that *cyclo*-decapeptides (XII-G and XII-A) migrate toward the cathode faster than *cyclo*-pentapeptides (XI-G and XI-A) and that mobilities of XII-G and XII-A are comparable with that of GS.

Microbiological Assays.[14] The minimum amount of the compounds necessary for the complete inhibition of growth was determined by a dilution method using a bouillon agar medium and a synthetic medium. As shown in Table 2, 3,3'-alanine-GS (XII-A) was found to exhibit weak antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, whereas 3,3'-glycine-GS (XII-G) and cyclosemiGS analogs (XI-G and XI-A) exhibited no activity against the microorganisms tested.

12) The molecular weight was determined on a Hitachi Osmometer, type 115, using methanol as the the solvent.

13) The R_f value 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.

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