Studies of Peptide Antibiotics. XXXI.[1] Syntheses of *iso*-Gramicidin S and *iso*-[2,2′-Lysine]-Gramicidin S[2]

Hideo TAKIGUCHI and Nobuo IZUMIYA*

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma, Fukuoka 814
**Laboratory of Biochemistry, Faculty of Science, Kyushu University, Hakozaki, Fukuoka 812*

(Received September 17, 1973)

Structural isomers of gramicidin S and [2,2′-lysine]-gramicidin S, namely *iso*-gramicidin S and *iso*-[2,2′-lysine]-gramicidin S, were prepared to investigate the influence of the ring size for antibacterial activity and conformation of natural gramicidin S. Each isomer involves two peptide bonds between carboxyl group of valine residue and ω-amino group of ornithine or lysine residue. Two isomers showed no antibacterial activity toward several microorganisms. In the experiment of optical rotatory dispersion, two isomers showed similar shaped curves as gramicidin S in a solvent of ethanol. In 6 M urea, the trough of two isomers was moved significantly, whereas that of gramicidin S remained constant.

In studies of relationship between ring size and antibacterial activity of gramicidin S (GS), several analogs have been synthesized.[3] As one analog with a larger ring size than GS, [β-Ala$^{5,5'}$]-GS showed entirely different ORD[4] pattern compared with that of GS and possessed no activity.[5] Recently, two macro-ring analogs of GS, namely sesquiGS and diGS, were synthesized, and the analogs showed similar ORD patterns with GS in a solvent of ethanol and possessed weak activities.[6]

In order to determine further the influence of larger ring size on the activity and the conformation, we have designed the syntheses of *iso*-gramicidin S (*iso*-GS)[7] and *iso*-[2,2′-lysine]-gramicidin S (*iso*-[Lys$^{2,2'}$]-GS) which are the structural isomers of GS and [Lys$^{2,2'}$]-GS, respectively, as shown in Fig. 1. A related analog of GS, [Lys$^{2,2'}$]-GS, was already synthesized, and the analog showed the same ORD curve and antibacterial activity as GS.[8,9]

Among many natural antibiotics with a character of cyclic peptide, it has been reported that several antibiotics involve a peptide bond participating ω-amino group of ornithine or lysine. Tamura and his collaborators reported the structure of aspochracin as *cyclo*-[δ-(-*N*-Me-Val-*N*-Me-Ala)-Orn(α-octatrienoyl)-].[10]

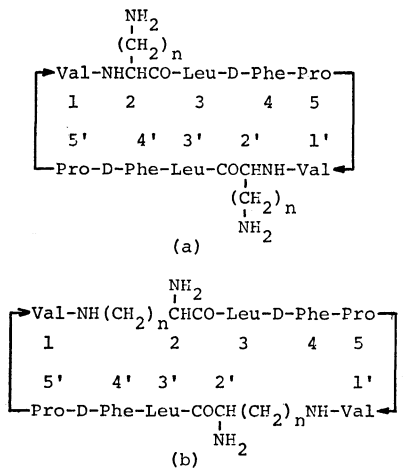
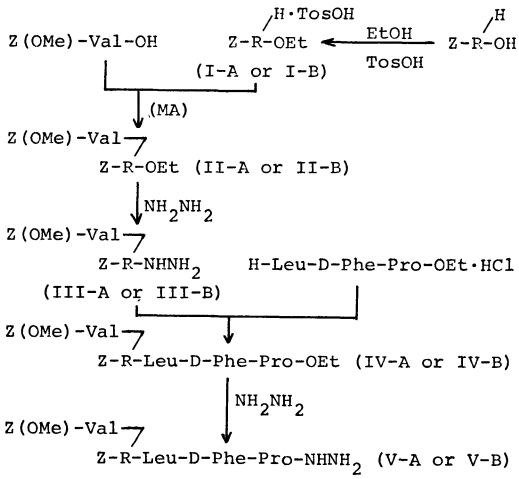


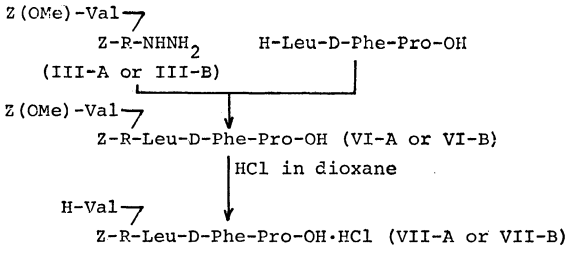
Fig. 1. Structure of gramicidin S (GS) and related peptides.
(a) GS (n=3) and [Lys$^{2,2'}$]-GS (n=4).
(b) *iso*-GS (n=3) *iso*-[Lys$^{2,2'}$]-GS (n=4).

Ressler and her collaborators presented a deduced structure of bacitracin; the antibiotic possesses a cyclic heptapeptide moiety as a partially sequence which involves a peptide bond between α-carboxyl group of asparagine residue and ε-amino group of lysine residue.[11] The present investigation would be worth as a model study to synthesize a natural antibiotic with peptide bond participating ω-amino group of α,ω-diamino acid.

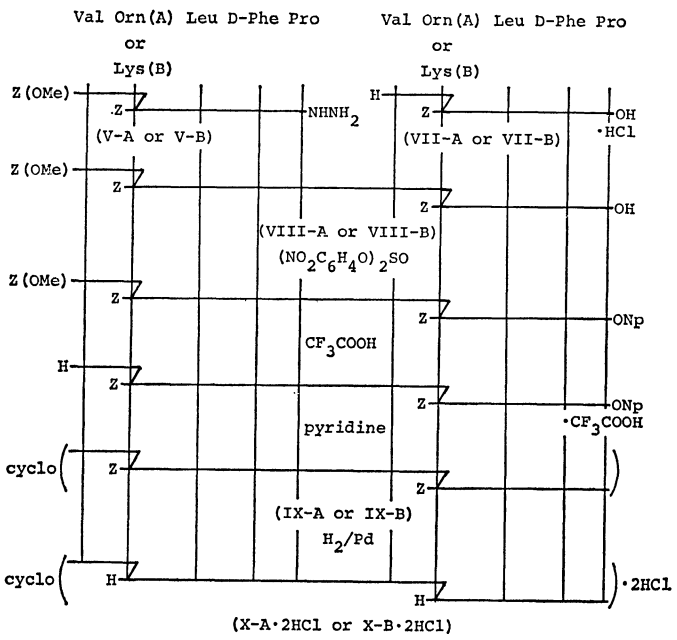
Scheme 1 indicates a route for syntheses of intermediate acylpentapeptide hydrazides (V-A and V-B). Acrylpentapeptide ethyl ester (IV) was prepared without any difficulties by condensation of the azide derived from acyldipeptide hydrazide (III) and a tripeptide ester. The ester (IV) was converted into the



Scheme 1. Syntheses of acylpentapeptide hydrazides. V-A (R=Orn), V-B (R=Lys).



Scheme 2. Syntheses of neutral pentapeptides. VII-A (R=Orn), VII-B (R=Lys).



Scheme 3. Syntheses of *iso*-GS (X-A) and *iso*-[Lys$^{2,2'}$]-GS (X-B).

hydrazide (V) by the usual way. Scheme 2 indicates a sequence of reaction employed for the syntheses of another intermediates, namely free pentapeptides (VII-A and VII-B).

Scheme 3 indicates a route for syntheses of the desired cyclic decapeptides (X-A and X-B). The azide derived from Z(OMe)-pentapeptide hydrazide (V) was condensed with a neutral pentapeptide (VII), and the resulting acyldecapeptide acid (VIII) was converted into Z(OMe)-decapeptide *p*-nitrophenyl ester by the action of di-*p*-nitrophenyl sulfite. Its Z(OMe) group was removed with trifluoroacetic acid, and the resulting decapeptide ester trifluoroacetate was treated with pyridine for the cyclization reaction. The reaction mixture yielded a pure Z-substituted cyclic decapeptide (IX-A or IX-B) which was hydrogenated to afford *iso*-GS (X-A) or *iso*-[Lys$^{2,2'}$]-GS (X-B) as a crystalline dihydrochloride. The homogeneity of these cyclic decapeptides was ascertained by paper and thin layer chromatography, paper electrophoresis, and elemental analysis.

The antibacterial activities toward several microorganisms were examined. Both synthetic isomers (X-A and X-B) showed no activity for any of the microorganisms even at 100 μg/ml of the assay medium, whereas minimum concentration of growth inhibition for *Bacillus subtilis* was found to be 1—2 μg/ml with GS as a reference compound.

The ORD curves measured in a solvent of ethanol are shown in Fig. 2-a. The synthetic isomers have similarly shaped curves with a negative trough at 234 nm as GS possesses the same. In 6 M urea solution in 50% ethanol which causes denaturation of some polypeptide, the troughs of two isomers were moved to 225 nm whereas that of GS remained constant (Fig. 2-b). The results indicate that a conformation of the synthetic isomers is unstable, whereas that of GS has the rigid β-pleated sheet structure with four

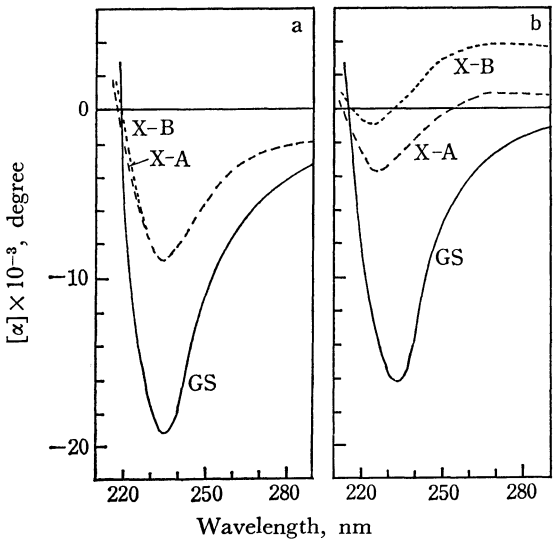


Fig. 2. ORD curves of GS and its isomers.
Solvent: a, ethanol; b, 6 M urea in 50% ethanol.

hydrogen bondings.[3] The isomers may have similar conformation as GS in a mild condition such as a solution in ethanol, but the conformation is transformed in such a urea solution. The fact that the isomers possess no antibacterial activity may be correlated to the unstableness of conformation in a certain medium.

Experimental

All 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 the cyclic peptides (IX-A, IX-B, X-A, and X-B). Thin layer chromatography was carried out on Merck silica gel G with the following solvent system; R_f^1, *n*-butanol-acetic acid-pyridine-water (4 : 1 : 1 : 2, v/v). Paper chromatography was carried out on Toyo Roshi No. 52 with the same solvent, R_f^2 being used.

α-Z-Orn-OEt·TosOH (I-A). This compound was prepared according to the general procedure of Kato *et al.*[12] A solution of α-Z-Orn-OH[13] (6.66 g, 25 mmol) and *p*-toluenesulfonic acid monohydrate (6.09 g, 32 mmol) in a mixture of ethanol (12 ml) and carbon tetrachloride (80 ml) was refluxed, and the water thus liberated was removed as an azeotropic mixture. The reaction mixture was concentrated *in vacuo*, and the residual oil was washed with ether and petroleum ether by decantation. The product was obtained as an oil; yield, 11.7 g (100%); R_f^1 0.77.

α-Z-Lys-OEt·TosOH (I-B). This compound was preparated from α-Z-Lys-OH[14] (5.60 g, 20 mmol) as described above. The product was recrystallized from acetone-ether; yield, 8.84 g (92%); mp 113—115 °C; $[\alpha]_D^{20}$ −7.8° (*c* 2, DMF); R_f^1 0.78.

Found: C, 57.23; H, 6.82; N, 5.77%. Calcd for $C_{23}H_{32}O_7N_2S$: C, 57.48; H, 6.71; N, 5.83%.

δ-[Z(OMe)-Val]-Orn(α-Z)-OEt (II-A). To a solution of Z(OMe)-Val-OH[15] (6.28 g, 22.3 mmol) and TEA (3.12 ml, 22.3 mmol) in tetrahydrofuran (70 ml) at −5 °C, was added isobutyl chloroformate (2.92 ml, 22.3 mmol). After 15 min, to the solution was added a mixture of I-A (10.4 g, 22.3 mmol) and TEA (3.12 ml) in chloroform (70 ml) at 0 °C. The reaction mixture was allowed to stand overnight at room temperature, and evaporated *in vacuo*. The residue was triturated with water and petroleum ether, and the

precipitate was collected by filtration. The product was washed successively with 4% sodium bicarbonate, 10% citric acid, and water. It was recrystallized from methanol-ether-petroleum ether; yield, 9.45 g (76%); mp 155—156 °C; $[\alpha]_D^{30}$ +2.4° (*c* 2, DMF); R_f^1 0.88.

Found: C, 62.83; H, 7.21; N, 7.41%. Calcd for $C_{29}H_{39}O_8N_3$: C, 62.46; H, 7.05; N, 7.54%.

ε-[Z(OMe)-Val]-Lys(α-Z)-OEt (II-B). This compound was obtained from Z(OMe)-Val-OH (4.50 g, 16 mmol) and I-B (7.69 g, 16 mmol) as described above; yield, 7.77 g (85%); mp 148—150 °C; $[\alpha]_D^{20}$ −2.1° (*c* 2, DMF); R_f^1 0.94.

Found: C, 62.72; H, 7.27; N, 7.16%. Calcd for $C_{30}H_{41}O_8N_3$: C, 63.03; H, 7.23; N, 7.35%.

δ-[Z(OMe)-Val]-Orn(α-Z)-NHNH$_2$ (III-A). A solution of II-A (7.81 g, 14 mmol) and hydrazine hydrate (13.6 ml, 280 mmol) in DMF (28 ml) was allowed to stand for 2 days at room temperature. The solution was concentrated *in vacuo* to a small volume. The hydrazide precipitated upon the addition of water (140 ml) was collected by filtration; yield, 5.81 g (76%); mp 162—165 °C; $[\alpha]_D^{31}$ −11.0° (*c* 1, AcOH).

Found: C, 59.40; H, 7.09; N, 13.29%. Calcd for $C_{27}H_{37}O_7N_5$: C, 59.66; H, 6.86; N, 12.88%.

ε-[Z(OMe)-Val]-Lys(α-Z)-NHNH$_2$ (III-B). II-B (5.15 g, 9 mmol) was converted to hydrazide (III-B) as described above; yield, 3.92 g (78%); mp 194—196 °C; $[\alpha]_D^{21}$ −13.2° (*c* 1, AcOH).

Found: C, 60.14; H, 7.32; N, 12.49%. Calcd for $C_{28}H_{39}O_7N_5$: C, 60.31; H, 7.05; N, 12.56%.

δ-[Z(OMe)-Val]-Orn(α-Z)-Leu-D-Phe-Pro-OEt (IV-A). III-A (2.17 g, 4 mmol), in a mixture of glacial acetic acid (48 ml) and N hydrochloric acid (8.8 ml), was treated with N sodium nitrite (4.4 ml) at −5 °C. After 6 min, cold water (200 ml) was added to the mixture. The azide was extracted with ethyl acetate, and the organic layer was washed with 4% sodium bicarbonate and water, and dried over sodium sulfate. The filtered solution was added to a solution of H-Leu-D-Phe-Pro-OEt·HCl[16] (1.76 g, 4 mmol) and TEA (0.56 ml) DMF (20 ml). The mixture was stirred for 3 days at 0 °C and evaporated *in vacuo* to a small volume. The precipitate which formed upon the addition of water was collected, washed with 4% sodium bicarbonate, 10% citric acid, and water, and dried. The product was recrystallized from dioxane-ether-petroleum ether; yield, 2.12 g (58%); mp 118—122 °C; $[\alpha]_D^{30}$ −18.0° (*c* 1, DMF); R_f^1 0.88.

Found: C, 64.01; H, 7.30; N, 9.16%. Calcd for $C_{49}H_{66}O_{11}N_6$: C, 64.31; H, 7.27; N, 9.18%.

ε-[Z(OMe)-Val]-Lys(α-Z)-Leu-D-Phe-Pro-OEt (IV-B). This compound was prepared from III-B (1.67 g, 3 mmol) and H-Leu-D-phe-Pro-OEt·HCl (1.32 g, 3 mmol) as described above; yield, 1.90 g (68%); mp 132—136 °C; $[\alpha]_D^{24}$ −23.6° (*c* 1, DMF); R_f^1 0.96.

Found: C, 64.42; H, 7.30; N, 9.20%. Calcd for $C_{50}H_{68}O_{11}N_6$: C, 64.64; H, 7.38; N, 9.05%.

δ-[Z(OMe)-Val]-Orn(α-Z)-Leu-D-Phe-Pro-NHNH$_2$ (V-A). A solution of IV-A (1.10 g, 1.2 mmol) and hydrazine hydrate (1.2 ml) in DMF (4.5 ml) was allowed to stand for 7 days at room temperature, and evaporated *in vacuo*. The hydrazide which precipitated upon the addition of water (40 ml) was collected and recrystallized from dioxane-ether; yield, 0.873 g (81%); mp 112—115 °C; $[\alpha]_D^{27}$ −31.4° (*c* 1, DMF).

Found: C, 62.24; H, 7.50; N, 12.13%. Calcd for $C_{47}H_{64}O_{10}N_8 \cdot 1/2H_2O$: C, 62.03; H, 7.20; N, 12.31%.

ε-[Z(OMe)-Val]-Lys(α-Z)-Leu-D-Phe-Pro-NHNH$_2$ (V-B). IV-B (1.47 g, 1.58 mmol) was converted to the hydrazide (V-B) as described above; yield, 1.23 g (85%); mp 118—120 °C $[\alpha]_D^{20}$ −28.2° (*c* 1, DMF).

Found: C, 63.25; H, 7.35; N, 12.50%. Calcd for $C_{48}H_{66}O_{10}N_8$: C, 63.00; H, 7.27; N, 12.24%.

δ-[Z(OMe)-Val]-Orn(α-Z)-Leu-D-Phe-Pro-OH (IV-A). III-A (1.63 g, 3 mmol) was converted to the azide as described for the preparation of IV-A. The azide solution in ethyl acetate was added to a solution of H-Leu-D-Phe-Pro-OH[17] (1.13 g, 3 mmol) and TEA (0.42 ml) in DMF (24 ml). The mixture was stirred for 3 days at 0 °C and evaporated *in vacuo*. To the concentrated solution was added 10% citric acid, and the oil separated was extracted with ethyl acetate. The organic layer was washed with 10% citric acid and water, and dried. The filtered solution was evaporated, and the precipitate which formed upon the addition of ether and petroleum ether was collected. It was recrystallized from ethyl acetate-petroleum ether; yield, 1.20 g (45%); mp 112—115 °C; $[\alpha]_D^{27}$ −27.8° (*c* 1, DMF); R_f^1 0.82.

Found: C, 63.34; H, 7.16; N, 9.17%. Calcd for $C_{47}H_{62}O_{11}N_6$: C, 63.64; H, 7.05; N, 9.47%.

ε-[Z(OMe)-Val]-Lys(α-Z)-Leu-D-Phe-Pro-OH (VI-B). This compound was prepared from III-B (1.95 g, 3.5 mmol) and H-Leu-D-Phe-Pro-OH (1.31 g, 3.5 mmol) as described above. The product was recrystallized from methanol-ether-petroleum ether; yield, 2.24 g (71%); mp 126—129 °C; $[\alpha]_D^{25}$ −14.6° (*c* 1, DMF); R_f^1 0.94.

Found: C, 63.53; H, 7.20; N, 9.55%. Calcd for $C_{48}H_{64}O_{11}N_6$: C, 63.98; H, 7.16; N, 9.33%.

δ-(H-Val)-Orn(α-Z)-Leu-D-Phe-Pro-OH·HCl (VII-A). To a solution of VI-A (887 mg, 1 mmol) in dioxane (10 ml), 4.8 N hydrogen chloride in dioxane (6.2 ml) was added at room temperature. After 3 hr, the solution was evaporated to dryness and the product was collected with the aid of ether; yield of a hydroscopic powder, 683 mg (90%); mp 157—164 °C; $[\alpha]_D^{20}$ −12.6° (*c* 1, DMF); R_f^1 0.71.

Found: C, 59.85; H, 7.54; N, 11.37%. Calcd for $C_{38}H_{55}O_8N_6Cl$: C, 60.11; H, 7.30; N, 11.07%.

ε-(H-Val)-Lys(α-Z)-Leu-D-Phe-Pro-OH·HCl (VII-B). VI-B (723 mg, 0.802 mmol) was converted to VII-B as described above; yield of a hygroscopic powder, 564 mg (91%); mp 137—140 °C; $[\alpha]_D^{28}$ +4.0° (*c* 0.5, DMF); R_f^1 0.73.

Found: C, 60.36; H, 7.65; N, 10.98%. Calcd for $C_{39}H_{57}O_8N_6Cl$: C, 60.57; H, 7.43; N, 10.87%.

δ-{δ-[Z(OMe)-Val]-Orn(α-Z)-Leu-D-Phe-Pro-Val}-Orn(α-Z)-Leu-D-Phe-Pro-OH (VIII-A). V-A (808 mg, 0.897 mmol) was converted to corresponding azide as described for the preparation of the azide from III-A. The azide in ethyl acetate was coupled with VII-A (681 mg, 0.897 mmol) in TEA (0.25 ml) and DMF (20 ml) as described for the preparation of VI-A. The product was recrystallized from dioxane-ether-petroleum ether; yield, 871 mg (61%); mp 150—153 °C; $[\alpha]_D^{17}$ −50.0° (*c* 0.5, DMF); R_f^1 0.80.

Found: C, 64.38; H, 7.42; N, 10.56%. Calcd for $C_{85}H_{114}O_{18}N_{12}$: C, 64.13; H, 7.22; N, 10.56%.

ε-{ε-[Z(OMe)-Val]-Lys(α-Z)-Leu-D-Phe-Pro-Val}-Lys(α-Z)-Leu-D-Phe-Pro-OH (VIII-B). This compound was prepared from V-B (641 mg, 0.7 mmol) and VII-B (541 mg, 0.7 mmol) as described above. The product was recrystallized from ethyl acetate-petroleum ether; yield, 952 mg (84%); mp 130—132 °C; $[\alpha]_D^{34}$ −34.6° (*c* 0.5, DMF); R_f^1 0.83.

Found: C, 64.35; H, 7.57; N, 10.63%. Calcd for $C_{87}H_{118}O_{18}N_{12}$: C, 64.51; H, 7.34; N, 10.38%.

cyclo-{δ-[δ-(-Val)-Orn(α-Z)-Leu-D-Phe-Pro-Val]-Orn(α-Z)-Leu-D-Phe-Pro-} (IX-A). To a solution of VIII-A

(637 mg, 0.4 mmol) in pyridine (8 ml), di-*p*-nitrophenylsulfite[18] (1.30 g, 4 mmol) was added. The reaction mixture was allowed to stand for 3 days at room temperature. After the solvent had been evaporated, the oily product was triturated with petroleum ether, washed repeatedly with a mixture of ether and petroleum ether (1 : 1) by decantation, and the residual solid was collected. To the solid (the *p*-nitrophenyl ester) were added anisole (0.4 ml) and trifluoroacetic acid (4 ml) at −5 °C. After 30 min, the solution was evaporated at 0 °C, and the residue was triturated with ether. A decapeptide *p*-nitrophenyl ester trifluoroacetate was collected in a cold room, washed with ether, and dried. The trifluoroacetate was dissolved in DMF (10 ml) containing acetic acid (0.4 ml), and the solution added to pyridine (200 ml) which had been kept at 60 °C over a period of 1 hr. The stirring was continued for additional 2 hr. After the solvent had been evaporated, the residue was dissolved in a mixture of methanol (150 ml) and water (50 ml). The solution was passed successively through the columns (2.5 × 12 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (600 ml), and the effluent was evaporated to dryness. The residual product was collected with the aid of water, and recrystallized from methanol–water; yield, 121 mg (21% from VIII-A); mp 160—163 °C; $[\alpha]_D^{10}$ −68.7° (*c* 0.3, DMF); R_f^1 0.96.

Found: C, 62.86; H, 7.75; N, 11.28%; mol wt, 1420.[19] Calcd for $C_{76}H_{104}O_{14}N_{12}\cdot 2H_2O$: C, 63.14; H, 7.53; N, 11.63%; mol wt, 1446.

cyclo-{ε-[ε-(−*Val*)–*Lys*(α-*Z*)–*Leu*–D-*Phe*–*Pro*–*Val*]–*Lys*(α-*Z*)–*Leu*–D–*Phe*–*Pro*–} *(IX-B).* VIII-B (648, 0.4 mmol) was converted to the cyclic diZ-decapeptide (IX-B) as described above; yield, 147 mg (25% from VIII-B); 222—224 °C; $[\alpha]_D^{17}$ −41.3° (*c* 0.3, DMF); R_f^1 0.98.

Found: C, 63.96; H, 7.72; N, 11.40%; mol wt, 1480.[19] Calcd for $C_{78}H_{108}O_{14}N_{12}\cdot 2H_2O$: C, 63.57; H, 7.66; N, 11.40%; mol wt, 1474.

cyclo-{δ-[δ-(−*Val*)–*Orn*–*Leu*–D-*Phe*–*Pro*–*Val*]–*Orn*–*Leu*–D-*Phe*–*Pro*–}·*2HCl* (iso-*GS Dihydrochloride) (X-A*·*2HCl).* A solution of IX-A (72 mg, 0.05 mmol) in 0.05 M methanolic hydrogen chloride (2.2 ml) was subjected to hydrogenolysis in the presence of palladium black. The filtrate from the catalyst was evaporated to dryness, and the residual solid was collected with the aid of ether; yield of an air-dried product, 44 mg (71%); mp 197—199 °C (decomp.); $[\alpha]_D^{24}$ −77° (*c* 0.2, DMF); R_f^1 0.86 and R_f^2 0.93.

Found: C, 58.02; H, 7.73; N, 13.71%. Calcd for $C_{60}H_{94}O_{10}N_{12}Cl_2\cdot 2H_2O$: C, 57.63; H, 7.90; N, 13.44%.

cyclo-{ε-[ε-(−*Val*)–*Lys*–*Leu*–D-*Phe*–*Pro*–*Val*] –*Lys*–*Leu*–D-*Phe*–*Pro*}·*2HCl* (iso-[*Lys*$^{2,2'}$]-*GS Dihydrochloride) (X-B*·*2HCl).* IX-B (64 mg, 0.0445 mmol) was converted to X-B-2HCl as described above; yield, 46 mg (78%); mp 201—203 °C (decomp.); $[\alpha]_D^{24}$ −73° (*c* 0.2, DMF); R_f^1 0.85 and R_f^2 0.95.

Found: C, 56.48; H, 8.31; N, 12.61%. Calcd for $C_{62}H_{98}O_{10}N_{12}Cl_2\cdot 4H_2O$: C, 56.65; H, 8.13; N, 12.79%.

Electrophoresis. Electrophoresis on Toyo Roshi No. 50 paper was carried out with a solvent system, formic acid–acetic acid–methanol–water (1 : 3 : 6 : 10, v/v; pH 1.8) for 3 hr at 500 V/30 cm. Figure 3 shows that mobilities of X-A and X-B were comparable with that of GS.

ORD Measurement. The measurements were performed with a Jasco Model ORD/UV 5 spectropolarimeter. Cell of path length 0.2 cm was used and the runs were made at ambient temperature. In Fig. 2 are reported the ORD curves (range 220—300 nm) of the isomers (X-A and X-B) and GS in ethanol or 6 M urea solution in 50% ethanol[20] (*c* 0.1, each).

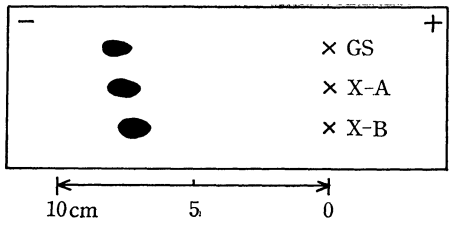


Fig. 3. Paper electrophoresis of GS and its isomers.
GS, gramicidin S; X-A, *iso*-GS; X-B, *iso*-[Lys$^{2,2'}$]-GS.

Microbiological Assays.[21] The microorganisms employed are *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Bacillus subtilis*. The minimum amount of the compounds necessary for the complete inhibition of growth was determined by a dilution method in both Bouillon agar medium and synthetic medium. GS was examined as a reference compound. X-A and X-B exhibited no antibacterial activity against the microorganisms tested even at 100 μg/ml, whereas minimum concentration of GS for inhibition were 2—5 μg/ml on *S. aureus* and 1—2 μg/ml on *B. subtilis*.

The authors wish to express their thanks to Drs. T. Kato, M. Waki, and S. Matsuura of Kyushu University for helpful discussions and ORD measurements.

Rerferences

1) Part XXX: H. Sugano, H. Abe, M. Miyoshi, T. Kato, and N. Izumiya, This Bulletin, submitted.

2) Presented at the 28th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1973.

3) T. Kato and N. Izumiya, "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins", Vol. 2, ed. by B. Weinstein, Marcel Dekker, New York (1973), in press.

4) Abbreviations: Z, benzyloxycarbonyl; Z(OMe), *p*-methoxybenzyloxycarbonyl; ONp, *p*-nitrophenoxy; MA, mixed anhydride method; TEA, triethylamine; TosOH, *p*-toluenesulfonic acid; DMF, dimethylformamide; ORD, optical rotatory dispersion. Amino acid symbols except D-Phe denote the L-configuration.

5) S. Matsuura, M. Waki, S. Makisumi, and N. Izumiya, This Bulletin, **43**, 1197 (1970).

6) S. Matsuura, M. Waki, T. Kato, and N. Izumiya, *ibid.*, **46**, 977 (1973).

7) An α-isomer (H–Glu–Cys–Gly–OH) of natural glutathione (H–γ-Glu–Cys–Gly–OH) was synthesized and named as *iso*-glutathione [V. du Vigneaud, H. S. Loring, and G. L. Miller, *J. Biol. Chem.*, **118**, 391 (1937)]. In analogy with this case, we introduce the naming of *iso*-GS for X-A in Fig. 1. A structure of *iso*-GS will be described as *cyclo*{δ-[δ-(−Val)–Orn–Leu–D-Phe–Pro–Val]-Orn–Leu–D-Phe-Pro–}.

8) M. Waki, O. Abe, R. Okawa, T. Kato, S. Makisumi, and N. Izumiya, This Bulletin, **40**, 2904 (1967).

9) T. Kato, M. Waki, S. Matsuura, and N. Izumiya, *J. Biochem.* (Tokyo), **68**, 751 (1970).

10) R. Myokei, A. Sakurai, C. F. Chang, Y. Kodaira, N. Takahashi, and S. Tamura, *Agr. Biol. Chem.*, **33**, 1491 (1969).

11) E. Ratti, C. Lauinger, and C. Ressler, *J. Org. Chem.*, **33**, 1309 (1968).

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

13) E. Schröder, H. S. Petras, and E. Klieger, *Ann. Chem.*, **679**, 221 (1964).
14) B. Benzas and L. Zervas, *J. Amer. Chem. Soc.*, **83**, 719 (1961).
15) T. Kato, M. Kondo, M. Ohno, and N. Izumiya, This Bulletin, **38**, 1202 (1965).
16) M. Waki and N. Izumiya, *ibid.*, **40**, 1687 (1967).
17) N. Mitsuyasu, S. Matsuura, M. Waki, M. Ohno, S. Makisumi, and N. Izumiya, *ibid.*, **43**, 1829 (1970).
18) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1760 (1960).
19) Molecular weight was determined on a Hitachi Osmometer, type 115, using DMF as a solvent.
20) X-A and X-B are sparingly soluble in 8 M aqueous urea which is an usual solvent for denaturation.
21) We are indebted to the staffs of Takeda Chemical Industries, Ltd. for the assay.