Synthesis of [4-5,4′-5′-Bis(δ-Aminovaleric Acid)]-Gramicidin S[1]

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An analog of gramicidin S, [4-5,4′-5′-bis(δ-aminovaleric acid)]-gramicidin S, was synthesized, in which both of the two residues of D-Phe-L-Pro of gramicidin S (GS) were replaced with δ-aminovaleric acid residues (δAva). The spectra of optical rotatory dispersion (ORD) and circular dichroism (CD) of this analog suggest that its conformation in solution at room temperature is a random structure by comparison with that of GS. This analog has no antimicrobial activity. The linear peptide H-(Val-Orn-Leu-δAva-)$_2$-OH also has no activity and its ORD and CD are nearly the same as those exhibited by the random structure of the polypeptide. These results indicate the importance of the presence of D-Phe-L-Pro in GS for the maintenance of the specific conformation.

In order to investigate the contribution of amide bonds in peptides to their activity and conformation, we have synthesized analogs of gramicidine S (GS) containing δ-aminovaleric acid residue (δAva). In a previous paper,[1] it was reported that the synthetic analog, [δAva$^{4-5}$]-GS, had antimicrobial activity and showed a type of opitical rotatory dispersion (ORD) and circular dichroism (CD) spectra similar to that of GS. Proton magnetic resonance data[2] indicated that the secondary structure of this analog was similar to that of GS.

In this paper, we describe the synthesis of [δAva$^{4-5,4'-5'}$]-GS, in which both of the residues of D-Phe-L-Pro of GS were replaced with δAva, as well as the results of the antimicrobial assay and the ORD and CD measurements of this analog.

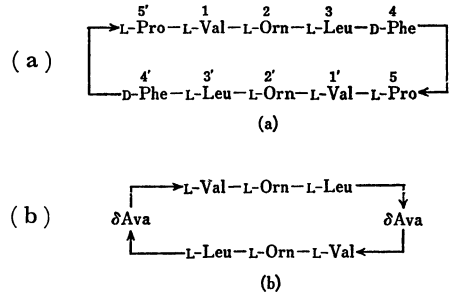
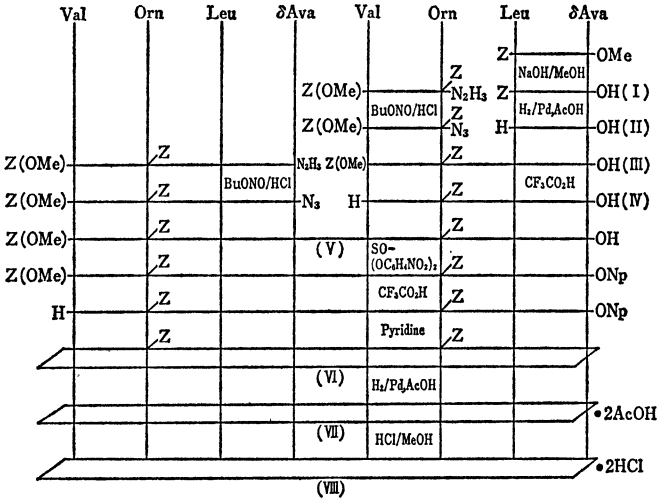


Fig. 1. Structures of gramicidin S (a), and [δAva$^{4-5,4'-5'}$]-GS (b).

The route of the synthesis of this analog is shown in Scheme 1. The synthesis of *p*-methoxybenzyloxycarbonyl-L-valyl-N^{δ}-benzyloxycarbonyl-L-ornithyl-L-leucyl-δ-aminovaleric acid hydrazide has been reported in the previous paper.[1] The azide derived from the hydrazide was coupled with IV. The resulting linear octapeptide (V) was used for cyclization reaction, which was carried out in the same manner as in the cyclization described in the previous paper. The cyclic peptide (VI) was obtained in 32% yield, which was inferior to that of the cyclization of the peptide containing one residue of δAva in the case of [δAva$^{4-5}$]-GS. This result might indicate that the yield of cyclization depends on whether the conformation of the active ester is such as to be favorable for the cyclization or not. [δAva$^{4-5,4'-5'}$]-GS diacetate (VII) was obtained by hydrogenolysis of the benzyloxycarbonyl groups on the ornithine residues of VI in the presence



Scheme 1.

of acetic acid. VII was coupled with 1-dimethylaminonaphthalene-5-sulphonyl chloride (DNS-Cl).[3] After the hydrolysis of this DNS derivative, only N^{δ}-DNS-ornithine was detected in thin layer chromatography.[1] This result proves that VII has a cyclic form. The antimicrobial activity of this analog toward several microorganisms was tested for, but none could be found.

The related linear octapeptide, H-(Val-Orn-Leu-δAva-)$_2$-OH·3HCl (IX),[4] was prepared from V by hydrogenolysis of its N-protecting groups in the presence of hydrogen chloride. This peptide also did not exhibit any antimicrobial activity.

ORD and CD spectra of the cyclic peptide dihydrochloride (VIII) were measured. These spectra are different from those of GS and some of its biologically active analogs[1,5] as shown in Figs. 2 and 3. The minimum value of ORD of the analog (VIII) is observed at 208 nm, this represents a conspicuous difference from the spectrum of GS (232 nm). The CD spectrum of this peptide has a trough at 198 nm. In methanol also the trough of the CD at room temperature appeared at 199 nm.

ORD and CD spectra of the linear peptide (IX) in Figs. 2 and 3 show that it has a random structure.

Thus, the conformation of VIII in solution at room temperature is presumed to be a random structure,[6,7] while those of GS[8] and [δAva$^{4-5}$]-GS[1] are ordered.

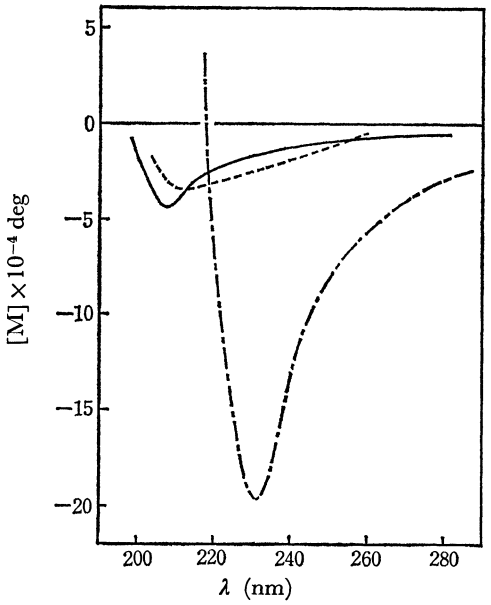


Fig. 2. Optical rotatory dispersion spectra of [δAva$^{4-5,4'-5'}$]-GS·2HCl (VIII), linear peptide (IX), and gramicidin S·2HCl (GS) in water; VIII (——), IX (----), and GS (——). Measurments were made using a 1 mm quartz cell at room temperature.

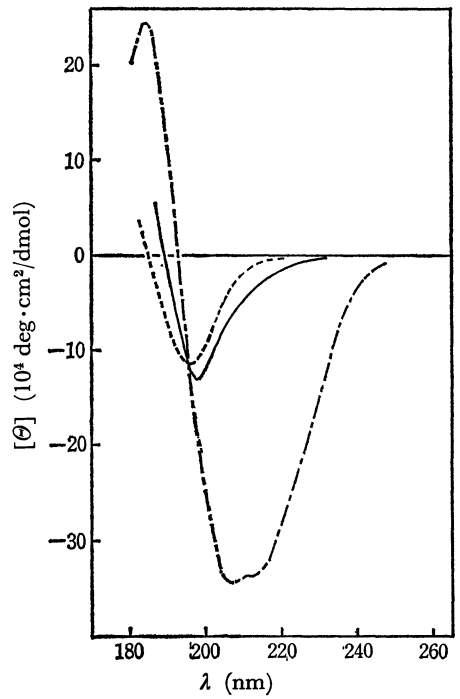


Fig. 3. Circular dichroism spectra of [δAva$^{4-5,4'-5'}$]-GS·2HCl (VIII), linear peptide (IX), and gramicidin S·2HCl (GS) in water; VIII (——), IX (----), and GS (——). Measurments were made using a 0.5 mm quartz cell at room temperature.

These results indicate that the cyclic structure of GS is retained not only by the formation of hydrogen bonds but also by the presence of D-phenylalanyl-L-prolyl residue in the β-turn.[9]

Experimental[10]

ORD and CD spectra were measured with a JASCO model J-20 spectrometer and represented by the values of molar optical rotation and molar ellipticity, respectively. The molecular weight was determined with a Hitachi molecular weight apparatus model 115, using methanol. Thin layer chromatography (tlc) was carried out on silica gel and the spot was detected with ninhydrin reagent. N-protected compounds were also detected with ninhydrin after deblocking with concd hydrobromic acid. The following solvent systems (V/V) were used in tlc: Solv. 1, $CHCl_3$-MeOH-AcOH (95 : 5 : 3); Solv. 2, $CHCl_3$-MeOH (9 : 1); Solv. 3, *n*-BuOH-AcOH-pyridine-H_2O (4 : 1 : 1 : 2); Solv. 4, *n*-BuOH-EtOH-concd NH_3 (6 : 1 : 3). Amino acid analyses were performed with a JEOL automatic amino acid analyzer, after hydrolyzing samples with 6 N HCl in evacuated sealed ampoules for 20 hr at 110 °C.

*Benzyloxycarbonyl-*L*-leucyl-δ-aminovaleric Acid (I).* Z-L-Leu-δAva-OMe (12.5 g, 33 mmol)[1] was saponified in methanol (90 ml) with 1 M NaOH (34 ml) at 38 °C for 2 hr. The reaction solution was acidified with 1 M HCl on cooling and evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate and extracted with 5% sodium bicarbonate. This solution was acidified with 1 M HCl and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and evaporated *in vacuo*. Upon recrystallization from ethyl acetate and *n*-hexane the product (10.18 g) was obtained in 84.6% yield. Mp 70—72 °C; $[\alpha]_D^{20.5}$ −15.4° (*c* 1.0, ethanol); tlc; R_f 0.42 (Solv. 1).

Found: C, 62.35; H, 7.80; N, 7.57%. Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69%.

L-Leucyl-δ-aminovaleric Acid Acetate (II). I (4.38 g, 12 mmol) in ethanol (50 ml) and glacial acetic acid (2.5 ml) was hydrogenolyzed in the presence of palladium black for 4 hr. After removal of the catalyst and concentration of the filtrate *in vacuo*, an oily product was obtained in the theoretical yield.

*p-Methoxybenzyloxycarboxyl-*L*-valyl-*N$^{\delta}$*-benzyloxycarbonyl-*L*-ornithyl-*L*-leucyl-δ-aminovaleric Acid(III).* Z(OMe)-L-Val-L-Orn(δ-Z)-$NHNH_2$[11] (6.53 g, 12 mmol) was suspended in DMF (100 ml), followed by the addition of 1.81 M dry HCl in THF (26.51 ml) at −30 °C. After the addition of *n*-butyl nitrite[12] (1.45 ml) the reaction mixture was stirred at −20——30 °C for 15 min. To this azide solution, Et_3N (6.68 ml) and a solution of II(12 mmol) and Et_3N (1.67 ml) in DMF (20 ml) were added dropwise at −60 °C. The stirring was continued at −20 °C for 1.5 hr and at 0 °C for 2 days. After the addition of water (1 l) into the reaction mixture, the precipitate was filtered and washed with 5% citric acid and water. The crude product was recrystallized from DMF and ethyl ether. Yield, 6.24 g (70.1%); mp 177—180 °C; $[\alpha]_D^{24}$ −14.6° (*c* 1.0, DMF); tlc: R_f 0.72 (Solv. 2).

Found: C, 61.33; H, 7.56; N, 9.39%. Calcd for $C_{38}H_{55}N_5O_{10}$: C, 61.52; H, 7.47; N, 9.44%.

*L-Valyl-*N$^{\delta}$*-benzyloxycarbonyl-*L*-ornithyl-*L*-leucyl-δ-aminovaleric Acid Trifluoroacetate(IV).* III (5.94 g, 8.0 mmol) was dissolved in trifluoroacetic acid (30 ml) containing anisole (2.56 ml) in an ice-salt bath. After 20 min, the reaction solution was concentrated *in vacuo* at 0 °C. White crystals produced by trituration of the oily residue with ethyl ether were collected by filtration, and after drying weighed 5.30 g (95.8%). Mp 88—90 °C; $[\alpha]_D^{20}$ −17.0° (*c* 1.0, ethanol); tlc: R_f 0.8 (Sov. 3).

*p-Methoxybenzyloxycarbonyl-*L*-valyl-*N$^{\delta}$*-benzyloxycarbonyl-*L*-

ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-N$^{\delta}$-benzyloxycarbonyl-L-ornithyl-L-leucyl-δ-aminovaleric Acid (V). Z(OMe)-L-Val-L-Orn(δ-Z)-L-Leu-δAva-$NHNH_2$[1] (5.30 g, 7.0 mmol) and IV (4.92 g. 7.1 mmol) were subjected to the same treatment as was used for III. After the reaction mixture was stirred for 3 days at 0 °C, water was added to it. The resulting precipitate was separated by centrifugation and washed with 5% citric acid and water. Recrystallization of the dried crude product from DMF and ethyl ether gave 4.96 g (54.4%) of the pure product. Mp 254—257 °C (decomp.); $[\alpha]_D^{20}$ +17.7 °C (*c* 0.1, DMF); tlc: R_f 0.81 (Solv. 1).

Found:,C, 61.22; H, 7.66; N, 10.74%. Calcd for $C_{67}H_{100}N_{10}O_{16}$: C, 61.83; H, 7.74; N, 10.76%.

cyclo-L-*Valyl-N$^{\delta}$-benzyloxycarbonyl-L-ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-N$^{\delta}$-benzyloxycarbonyl-L-ornithyl-L-leucyl-δ-aminovaleryl (VI).* The reaction of V (1.96 g, 1.5 mmol) and di-*p*-nitrophenyl sulfite (4.87 g, 15 mmol) in a mixture of DMF (200 ml) and pyridine (50 ml) at room temperature for 45 hr gave the *p*-nitrophenyl ester of V. In the same manner as described in Ref. 1, the removal of Z(OMe)-group of this ester and the cyclization (1.5×10^{-3} M in pyridine, at 58—60 °C) were carried out. After evaporation of the reaction solution and treatment through columns, Dowex-1 (OH^- form) and Dowex-50 (H^+ form), the effluent was concentrated to dryness *in vacuo* and a white crystalline product was obtained in 33.4% yield. It was recrystallized from aqueous methanol. Yield, 540 mg (32.2%); mp 209—210 °C; $[\alpha]_D^{20}$ −71.7° (*c* 0.3, methanol); tlc: R_f 0.35 (Solv. 1), 0.6 (Solv. 2); mol wt. Found: 1090 (Calcd for $C_{58}H_{90}N_{10}O_{12}$:1119).

Found: C, 61.68; H, 8.45; N, 12.22%. Calcd for $C_{58}H_{90}N_{10}O_{12}\cdot1/2\ H_2O$: C, 61.73; H, 8.13; N, 12.41%.

cyclo-L-*Valyl-L-ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-L-ornithyl-L-leucyl-L-δ-aminovaleryl Diacetate (VII).* VI (400 mg, 0.36 mmol) was hydrogenolyzed in ethanol containing a few drops of glacial acetic acid in the presence of palladium black (0.5 g) for 2 hr. After removal of the catalyst, the filtrate was concentrated to an oily residue *in vacuo*. Its aqueous solution was filtered through active charcoal. The product was obtained by lyophilization of the filtrate. Yield, 220 mg (63.5%); mp 282—285 °C (decomp.); $[\alpha]_D^{20}$ −86.4° (*c* 0.22, ethanol); tlc: R_f 0.7 (Solv. 3), 0.4 (Solv. 4). Paper electrophoresis: migration distance −11.8 cm, *cf.* −11.0 cm for GS diacetate [HCOOH : AcOH : H_2O= 4 : 15 : 180 (pH 1.9), 600 V, 11.5—14.5 mA, 2 hr, Toyo No. 50 (15×40 cm)]. Amino acid ratios; Val, 0.96; Orn, 1.00; Leu, 1.14; δAva, 0.89.

Found: C, 57.33; H, 9.44; N, 14.14%. Calcd for $C_{46}H_{86}N_{10}O_{12}$: C, 56.88; H, 8.93; N, 14.42%.

cyclo-L-*Valyl-L-ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-L-ornithyl-L-leucyl-δ-aminovaleryl Dihydrochloride (VIII).* The acetate (VII) was dissolved in excess dry methanolic hydrogen chloride (*ca.* 1M) at room temperature. After the solution had been evaporated *in vacuo*, the residue was lyophilized in water. The product was obtained in the theoretical yield. Mp 200—202 °C (decomp.); $[\alpha]_D^{28}$ −63.1 °C (*c* 0.15, ethanol).

Found: C, 50.62; H, 8.93; N, 13.60%. Calcd for $C_{62}H_{80}O_8N_{10}Cl_2\cdot4H_2O$: C, 50.64; H, 8.90; N, 14.06%.

L-*Valyl-L-ornithyl-L-leucyl-δ-aminovaleryl-L-valyl-L-ornithyl-L-leucyl-δ-aminovaleric Acid Trihydrochloride (IX).* V (1.0 g, 0.77 mmol) was hydrogenolyzed in DMF (120 ml) containing concd HCl (1.0 ml) in the presence of palladium black (610 mg) for 22 hr. After removal of the catalyst, the filtrate was concentrated to dryness *in vacuo*. The product was isolated from this crude preparation by countercurrent distribution (0.01 M HCl/*n*-butanol = 10/10 ml, ten transfers). The aqueous layers (No. 3—7) were concentrated to dryness *in vacuo*. The pure product was obtained by recrystallization from methanol and ethyl ether in 60.3% yield (470.3 mg). Mp 221—225 °C; $[\alpha]_D^{26}$ −44.3° (*c* 0.3, ethanol). Paper electrophoresis: migration distance −13.5 cm, *cf.* −10.8 cm for GS dihydrochloride (the same conditions in the case of VII). Amino acid ratios: Val, 0.86; Orn, 1.08; Leu, 1.07; αAva, 0.98.

Found: C, 49.58; H, 9.02; N, 14.17; Cl, 12.66%. Calcd for $C_{42}H_{83}N_{10}Cl_3\cdot H_2O\cdot1/2HCl$: C, 49.71; H, 8.49; N, 13.80; Cl, 12.22%.

Peptides VIII and IX did not exhibit antimicrobial activity toward the microorganisms *Bacillus subtilis, Staphylococcus aureus, Micrococcus flavus, and Sarcina lutea* in concentrations below 100 mcg/ml by the agar dilution method.

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4) Abbreviations with no prefix show L-amino acid residue.
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10) The following abbreviations are from *J. Biol. Chem.*, **247**, 977 (1972): Z-, benzyloxycarbonyl; Z(OMe)- *p*-methoxybenzyloxycarbonyl; -ONp, *p*-nitrophenoxy; -OMe, methoxy. Other abbreviations: DMF, dimethylformamide; THF, tetrahydrofuran; Et_3N, triethylamine.
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