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Studies of Peptide Antibiotics. XXVI.^{1,2)} Syntheses of Cyclodipeptides containing N^δ-*p*-Aminobenzenesulfonyl Ornithine Residue

KUNIKI IKEDA,^{3a)} KENJI KUROMIZU,^{3b)} KOUJI OKAMOTO,
and NOBUO IZUMIYA³⁾

Faculty of Science, Kyushu University³⁾

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A cyclo-N^δ-*p*-aminobenzenesulfonyl-L-ornithyl-L-leucyl (LL-X) was prepared through the cyclization reaction of N^δ-*p*-aminobenzenesulfonyl-L-ornithyl-L-leucine ethyl ester with methanolic ammonia. Other three diastereomeric cyclodipeptides (DD-X, LD-X, and DL-X) and cyclo-N^δ-*p*-aminobenzenesulfonyl-ornithyl-glycyls (L and D form) were synthesized in the same manner. All *p*-aminobenzenesulfonyl cyclodipeptides showed no antibacterial activity against *Escherichia coli* and *Bacillus subtilis* even at the concentration of 1000 µg/ml.

Certain of polypeptide antibiotics such as gramicidin S possess several structural and chemical features in common. These include a basic character due to the presence of a diamino acid, a cyclic conformation, and the presence of at least one amino acid residue of the D-configuration. For a study of the relationship between chemical structure and biological activity of peptide antibiotics, some cyclodipeptides such as cyclo-L-Orn-D-Val which are the simplest compounds possessing the characteristics mentioned above, were prepared and subjected to the experiment of biological assay.⁴⁾ However, all of them were found to be devoid of the antibacterial activity; cyclo-L-Orn-D-Val did not exhibit the activity even at the concentration of 100 µg/ml, while the minimum inhibitory concentration of gramicidin S was 12.5 µg/ml for *Bacillus subtilis*.⁴⁾

In nature, however, there occur several amino acid and peptide antibiotics with small membered cyclic structure such as D-cycloserine and penicillins. It was reported that the minimum inhibitory concentrations of D-cycloserine and penicillin G toward *B. subtilis* were 25 and 0.02 µg/ml, respectively.⁵⁾

- 1) Part XXV: S. Makisumi, S. Matsuura, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Japan*, **44**, 210 (1971).
- 2) Symbols for amino acids used are those recommended in *Biochemistry*, **5**, 1445 (1966). Other abbreviations: Acbs=*p*-acetylaminobenzenesulfonyl, Acbs-Cl=*p*-acetylaminobenzenesulfonyl chloride, Ambs=*p*-aminobenzenesulfonyl, Nps=*o*-nitrophenylsulfonyl, Npsbs=*p*-(*o*-nitrophenylsulfonyl)-aminobenzenesulfonyl, Z=benzyloxycarbonyl, DCC=dicyclohexylcarbodiimide, DCHA=dicyclohexylamine, TEA=triethylamine, TFA=trifluoroacetic acid, TsOH=*p*-toluenesulfonic acid, DMF=dimethylformamide, THF=tetrahydrofuran.
- 3) Location: Hakozaki, Fukuoka-shi; a) Present address: Research Laboratory, Yoshitomi Pharmaceutical Industries, Ltd., Yoshitomi-cho, Fukuoka-ken; b) Children's Cancer Research Foundation, Boston, Mass., U.S.A.
- 4) N. Izumiya, T. Kato, Y. Fujita, M. Ohno, and M. Kondo, *Bull. Chem. Soc. Japan*, **37**, 1809 (1964).
- 5) A.C. Cuckler, B.M. Frost, L. McClelland, and M. Solotorovsky, *Antibiotics and Chemotherapy*, **5**, 191 (1955).

From the facts described above, we postulated that certain cyclodipeptides such as cyclo-L-Orn-D-Val might possess latent antibacterial ability even though such compounds did not exhibit apparent activity.⁴⁾ We anticipated that a combined compound of an antibacterial agent such as *p*-aminobenzenesulfonyl amide (sulfonamide)⁶⁾ with a cyclodipeptide might exhibit stronger activity than the parent agent. Shankman, *et al.* reported that *p*-(L-Val-L-Val)-aminobenzenesulfonyl amide was an effective inhibitor of growth of a microorganism, *P. cerevisiae*, which was not inhibited by the parent sulfonamide.⁷⁾ The present paper describes the syntheses and antibacterial properties of four diastereomers of *p*-aminobenzenesulfonyl cyclo-Orn-Leu and of several related amino acids and peptides.

It was attempted to prepare cyclo-L-Orn(δ -Ambs)-L-Leu as a model compound through preferential hydrolysis by hydrochloric acid on an acetyl amino linkage in cyclo-L-Orn(δ -Acbs)-L-Leu (LL-VI) which was synthesized *via* a reaction sequence shown in Chart 1. In a preliminary experiment, it was observed that an ornithine derivative, H-L-Orn(δ -Acbs)-OH (L-I), was completely converted to H-L-Orn(δ -Ambs)-OH (L-XVIII) by the treatment with 1N hydrochloric acid at 100° for 1—1.5 hr. However, cyclo-L-Orn(δ -Ambs)-L-Leu could not be isolated from a reaction mixture which was derived from cyclo-L-Orn(δ -Acbs)-L-Leu by the treatment with 1N hydrochloric acid at 100° for 0.5, 1 or 1.5 hr. By analysis of the reaction mixture with thin-layer and paper chromatography, it was observed that one pep-

Chart 1

Chart 2

tide bond (-Orn(δ -Acbs)-Leu- or -Leu-Orn(δ -Acbs)-) was hydrolyzed rapidly as the acetyl amino linkage was. Since it was well known that a peptide bond with valine residue was appreciably resistant to acid hydrolysis,⁸⁾ cyclo-L-Orn(δ -Acbs)-L-Val (LL-VII) was prepared and subjected to a similar treatment by hydrochloric acid. However, one peptide bond was again hydrolyzed rapidly, and consequently the desired cyclo-L-Orn(δ -Ambs)-L-Val could not be isolated.

- 6) E.H. Northey, "The Sulfonamides and Allied Compounds," Reinhold Pub. Co., New York, N.Y., 1948.
- 7) S. Shankman, S. Makineni, and V. Gold, *Arch. Biochem. Biophys.*, **100**, 431 (1963).
- 8) For example, R. Hirohata, Y. Kanda, M. Nakamura, N. Izumiya, A. Nagamatsu, T. Ono, S. Fujii, and M. Kimitsuki, *Z. Physiol. Chem.*, **295**, 368 (1953).

The desired cyclo-L-Orn(δ -Ambs)-L-Leu (LL-X) was obtained in crystalline state through the cyclization reaction of H-L-Orn(δ -Ambs)-L-Leu-OEt (LL-XX) with methanolic ammonia as shown in Chart 2. The trifluoroacetate of LL-XX was prepared from the corresponding di-*o*-nitrophenylsulfenyl dipeptide ester (LL-VIII) by the treatment of trifluoroacetic acid. Similarly other three diastereomers (DD-X, LD-X, and DL-X) of cyclo-Orn(δ -Ambs)-Leu and two antipodes (L-XI and D-XI) of cyclo-Orn(δ -Ambs)-Gly were prepared. Since the dipeptide ester LL-XX possessed two free amino groups, the formation of an assumed cyclopeptide (XXIII) could not be excluded though it was expected that a stable six membered cyclodipeptide (LL-X) was produced preferentially. Acetylation of the product which was derived from H-L-Orn(δ -Ambs)-L-Leu-OEt (LL-XX) with methanolic ammonia afforded a pure acetyl derivative, and its properties agreed well with the cyclodipeptide LL-VI which was derived from H-L-Orn(δ -Acbs)-L-Leu-OEt (XIX) possessing only one free amino group.

Chart 3

Chart 4

As related peptide for antibacterial assay, a dipeptide (LL-XIV) in which δ -amino function of ornithine residue was covered with *p*-aminobenzenesulfonyl group was prepared by either of two reaction sequences as shown in Chart 3. Another cyclopeptide, cyclo-L-Orn-L-Leu·HCl (LL-XVII·HCl), was prepared by a conventional reaction sequence as shown in Chart 4.

The antibacterial activity of *p*-aminobenzenesulfonyl cyclodipeptides (LL-X, DD-X, LD-X, DL-X, L-XI, and D-XI) and other related amino acids and dipeptides (I, VI, VII, XIII, XIV, and XVII) toward *E. coli* and *B. subtilis* was examined. These compounds showed no activity for either of the microorganisms even at 1000 $\mu\text{g/ml}$ of the assay media, whereas three reference compounds (sulfathiazole, gramicidin S and dihydrostreptomycin) showed strong bactericidal activity at the level of 4 $\mu\text{g/ml}$. From the finding that the compounds of two types, H-Orn(δ -Ambs)-OH and cyclo-Orn(δ -Ambs)-Leu or cyclo-Orn(δ -Ambs)-Gly, were inactive at the level of the concentrations (1000 $\mu\text{g/ml}$), it will be difficult to conclude that cyclic ornithyl-leucyls and ornithyl-glycyls possess latent antibacterial ability. To clarify further the properties of certain cyclodipeptides, an experiment on the preparation and properties of a derivative of natural gramicidin S, wherein two cyclodipeptide moieties are attached to two δ -amino groups in gramicidin S, are in progress in this laboratory.

Experimental

All the melting points are uncorrected. Thin-layer chromatography (TLC) was carried out on Merck silica gel G with the following solvent systems: Rf^1 , *n*-butanol-AcOH-pyridine-water (4:1:1:2, v/v); Rf^2 , chloroform-MeOH (5:1, v/v). Spot of a material on a plate was detected by spraying ninhydrin for a material with free amino group, by spraying 47% HBr and ninhydrin for that with Z or Nps group, or by spraying butyl hypochlorite reagent for that with peptide bond.⁹⁾

H-Orn(δ -Acbs)-OH (I)—(a) The L Form (L-I): A mixture of H-L-Orn-OH·HCl (11.8 g) and CuCO₃ (20 g) in water (200 ml) was heated at 100° for 30 min. The excess CuCO₃ was filtered off, and the filtrate was cooled to room temperature. To the solution, Acbs-Cl¹⁰⁾ (19.6 g) and NaHCO₃ (21.2 g) were added in portions under stirring over a period of 1 hr. After the mixture was stirred for additional 3 hr, the precipitate was collected by filtration, and washed with water, acetone and ether; yield of Cu salt of L-I, 19.3 g. The salt was suspended in water (500 ml) and H₂S was bubbled through the suspension. The mixture was heated at 100° and CuS was filtered off. The filtrate was evaporated *in vacuo* and the resulting solid was recrystallized from hot water; yield, 14.3 g (62%); mp 250–253° (decomp.); $[\alpha]_D^{25} +17.4^\circ$ ($c=1$, 1N HCl); Rf^1 0.54. Anal. Calcd. for C₁₃H₁₉O₅N₃S: C, 47.4; H, 5.8; N, 12.8; S, 9.7. Found: C, 47.2; H, 5.9; N, 12.7; S, 9.8.

(b) The D Form (D-I): This compound was prepared from H-D-Orn-OH·HCl¹¹⁾ by the same method as described above; yield, 65%; mp 251–253° (decomp.); $[\alpha]_D^{25} -17.6^\circ$ ($c=1$, 1N HCl); Rf^1 0.54 (Anal. Found: C, 47.1; H, 6.0; N, 12.5; S, 9.8).

Z-L-Orn(δ -Acbs)-OH DCHA Salt (L-II·DCHA)—To a solution of L-I (9.88 g, 30 mmole) in 1N NaOH (30 ml), Z-Cl (5.8 ml, 36 mmole) and 1N NaOH (45 ml) were added in portions over a period of 30 min at 0°. After being stirred for additional 2 hr at room temperature, the mixture was washed with ether. To the aqueous layer, 6N HCl (12 ml) was added and the resulting oil was extracted with ethyl acetate. To the organic layer which was dried over Na₂SO₄, DCHA (5.4 g, 30 mmole) was added, and the solution was evaporated. The resulting crystals were collected by filtration with the aid of ether. They were recrystallized from EtOH-ether; yield, 15.27 g (80%); mp 197–198°; Rf^1 0.82. Anal. Calcd. for C₃₃H₄₈O₇N₄S: C, 61.5; H, 7.5; N, 8.7. Found: C, 61.3; H, 7.6; N, 8.6.

Nps-Orn(δ -Npsbs)-OH DCHA Salt (III·DCHA)—(a) The L Form (L-III·DCHA): A solution of L-I (6.58 g, 20 mmole) in 1N HCl (800 ml) was heated in a bath at 100° (a pattern of TLC of the reaction mixture as a function of time is shown in Fig. 1). After 1.5 hr, the solution was evaporated *in vacuo* to dryness. The resulting hygroscopic crystals (L-XVIII·HCl) showed only one spot on TLC by ninhydrin reagent (Rf^1 0.48). The HCl salt of L-XVIII dissolved in dioxane (25 ml) was neutralized with 2N NaOH. To the solution were added 2N NaOH (35 ml) and Nps-Cl¹²⁾ (8.72 g, 46 mmole) which was dissolved in dioxane (25 ml) in portions over a period of 30 min at room temperature. After being stirred for additional 30 min, the reaction

Fig. 1. Thin-layer Chromatogram of a Reaction Mixture of H-L-Orn(δ -Acbs)-OH and 1N HCl at 100°

solvent, *n*-butanol-AcOH-pyridine-water=4:1:1:2 (v/v)

A: H-L-Orn (δ -Acbs)-OH

B: H-L-Orn-OH·HCl

C: H-L-Orn (δ -Ambs)-OH

mixture was acidified with 1M citric acid at 0°. The resulting oil was extracted with ethyl acetate. The organic layer was treated with DCHA (3.63 g, 20 mmole) as described for the preparation of L-II·DCHA; yield of L-III·DCHA, 12.35 g (79%); mp 139–140°; Rf^2 0.38. Anal. Calcd. for C₃₅H₄₆O₈N₆S₃·1/2H₂O: C, 53.6; H, 6.0; N, 10.7. Found: C, 53.7; H, 6.0; N, 10.6.

(b) The D Form (D-III·DCHA): This was prepared from D-I (6.58 g) by the same method as described above; yield, 12.83 g (82%); mp 140°; Rf^2 0.38 (Anal. Found: C, 53.6; H, 6.1; N, 10.8).

Z-L-Orn(δ -Acbs)-L-Leu-OEt (LL-IV)—A suspension of L-II·DCHA (9.48 g, 14.7 mmole) in 0.5M citric acid (45 ml) and ethyl acetate (120 ml) was shaken for 1 hr. The organic layer was washed with water, dried and evaporated. The resulting oil was dissolved in THF (35 ml) containing TEA (2.06 ml). To the solution was added isobutyl chloroformate (1.95 ml, 14.7 mmole) at –10°. After 10 min, to the reaction

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10) S. Smith and J. Stewart, "Organic Syntheses," Coll. Vol. I, 1956, p.8.

11) N. Izumiya, *Nippon Kagaku Zasshi*, **72**, 149 (1951).

12) L. Zervas, D. Borovas, and E. Gazis, *J. Am. Chem. Soc.*, **85**, 3660 (1963); L. Zervas and C. Hamalidis, *ibid.*, **87**, 99 (1965).

mixture was added a solution of H-L-Leu-OEt·TsOH¹³) (4.87 g, 14.7 mmole) and TEA (2.06 ml) in chloroform (35 ml) at 0°. The mixture was allowed to stand overnight at room temperature and then evaporated. After the residue was dissolved in ethyl acetate, the solution was washed with 2% HCl and 4% NaHCO₃, dried and evaporated. The resulting solid was recrystallized from ethyl acetate-petroleum ether; yield, 7.06 g (79%); mp 95–97°; *R*_f¹ 0.98. *Anal.* Calcd. for C₂₅H₄₀O₅N₄S: C, 57.6; H, 6.7; N, 9.3. Found: C, 57.4; H, 6.5; N, 9.1.

Z-L-Orn(δ-Acbs)-L-Val-OEt (LL-V)—This was prepared from L-II·DCHA (3.55 g, 5.5 mmole) and H-L-Val-OEt·TsOH¹³) (1.74 g, 5.5 mmole) by the same method as described above; yield, 2.28 g (71%); mp 178–179°; *R*_f¹ 0.98. *Anal.* Calcd. for C₂₈H₃₈O₅N₄S: C, 56.9; H, 6.5; N, 9.5. Found: C, 57.2; H, 6.4; N, 9.5.

Cyclo-[L-Orn(δ-Acbs)-L-Leu] (LL-VI)—(a) From LL-IV: A solution of LL-IV (605 mg, 1 mmole) in 25% HBr in AcOH (3 ml) was allowed to stand for 1 hr at room temperature. The solution was evaporated to dryness, and the residue (LL-XIX·HBr) was dissolved in MeOH (15 ml) saturated with NH₃.⁴⁾ After 2 days, the solution was evaporated, and the resulting solid was collected by filtration with the aid of water. It was recrystallized from hot MeOH; yield, 330 mg (77%); mp 241–243°; [α]_D¹⁵ –22.0° (*c*=1, DMF); *R*_f¹ 0.90. *Anal.* Calcd. for C₁₉H₂₈O₅N₄S: C, 53.8; H, 6.7; N, 13.2; S, 7.6. Found: C, 53.4; H, 6.5; N, 13.3; S, 7.4.

(b) From cyclo-[L-Orn(δ-Ambs)-L-Leu] (LL-X): LL-X (60 mg) which was prepared as described later was dissolved in Ac₂O (5 ml). The solution was allowed to stand for 2 days at room temperature and evaporated. The resulting solid was treated as described above; yield, 38.2 mg (57%); mp 240–243°; [α]_D¹⁵ –21.6° (*c*=1, DMF); *R*_f¹ 0.90 (*Anal.* Found: C, 52.5; H, 6.5; N, 13.1; S, 7.5). A mixture of this crystal with that obtained in (a) showed the same melting point and single spot on TLC, and both crystals gave identical IR patterns.

Cyclo-[L-Orn(δ-Acbs)-L-Val] (LL-VII)—LL-V (590 mg, 1 mmole) was treated with HBr in AcOH and methanolic NH₃ successively as described for the preparation of LL-VI from LL-IV; yield, 204 mg (50%); mp 243–245°; [α]_D¹⁵ –33.0° (*c*=1, DMF); *R*_f¹ 0.88. *Anal.* Calcd. for C₁₈H₂₆O₅N₄S: C, 52.7; H, 6.4; N, 13.7. Found: C, 52.6; H, 6.2; N, 13.9.

Nps-Orn(δ-Npsbs)-Leu-OEt (VIII)—(a) The LL Diastereomer (LL-VIII): To a solution of L-III·DCHA (7.75 g, 10 mmole) and H-L-Leu-OEt·TsOH (3.31 g, 10 mmole) in chloroform (40 ml) was added DCC (2.06 g, 10 mmole). After being stirred overnight at 0°, the resulting dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was dissolved in ethyl acetate, and the solution was washed with 1 M citric acid and 4% NaHCO₃, dried, and evaporated; yield of an oil, 7.15 g. The oil showed one main spot (*R*_f² 0.49) and several minor spots (*R*_f², 0.14, 0.43, 0.77 and 0.98). A part (0.5 g) of the oil was purified with a Sephadex LH-20 column (3×70 cm) with MeOH as a solvent. Fractions from 375 to 420 ml were evaporated and there remained a semi solid (LL-VIII); *R*_f¹ 0.98, *R*_f² 0.49. The same procedures were repeated more 13 times with each 0.5 g sample of the oil; total yield of LL-VIII, 3.22 g (44%).

(b) The DD, LD and DL Diastereomers (DD-VIII, LD-VIII and DL-VIII): Each of these compounds was prepared from III·DCHA (L or D form) and H-Leu-OEt (L or D form) by the DCC method, and the purification was carried out with a Sephadex LH-20 column as described above. All compounds were obtained as a semi solid with a single spot on TLC (*R*_f² 0.49).

Nps-Orn(δ-Npsbs)-Gly-OEt (IX)—(a) The L Form (L-IX): The oily Nps-L-Orn(δ-Npsbs)-OH (L-III) was isolated from L-III·DCHA (4.733 g, 6.1 mmole) as described for the preparation of Z-L-Orn(δ-Acbs)-OH (L-II). The L-III and H-Gly-OEt·HCl (0.851 g, 6.1 mmole) were condensed by the mixed anhydride method as described for the preparation of LL-IV; yield of an oil, 3.708 g. The oil showed one main spot (*R*_f² 0.74) and several minor spots (*R*_f², 0.32, 0.56, 0.84 and 0.98). Treatment of this oil with a Sephadex LH-20 column was not effective to isolate a pure L-IX. Therefore, the oily product was used directly to the step for the preparation of a cyclodipeptide (L-XI).

(b) The D Form (D-IX): This was prepared from D-III·DCHA (4.57 g) and H-Gly-OEt·HCl (0.823 g) by the same method as described above; yield of an oil, 3.41 g; *R*_f², main spot (0.74) and several minor spots.

Cyclo-[Orn(δ-Ambs)-Leu] (X)—(a) The LL Diastereomer (LL-X): The LL-VIII (766 mg, 1.04 mmole) was dissolved in TFA (10 ml).¹⁴⁾ The solution was allowed to stand for 1 hr at room temperature and evaporated, and the residue (LL-XX·2TFA) was dissolved in methanolic NH₃ (15 ml). After 2 days, the solution was evaporated to dryness. The resulting oil changed to solid by the trituration with ether, and the solid was collected by filtration. It was recrystallized from hot MeOH; yield, 168 mg (42%); mp 228–230°; [α]_D²⁰ –33.4° (*c*=1, DMF); *R*_f¹ 0.85, *R*_f² 0.55. *Anal.* Calcd. for C₁₇H₂₆O₄N₄S: C, 53.4; H, 6.9; N, 14.7. Found: C, 53.0; H, 6.9; N, 14.4.

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

14) It was observed that the removal of Nps group from N^δ-Npsbs portion in LL-VIII by 1 N HCl in dioxane was incomplete, while Nps group in N^α-Nps portion of LL-VIII removed easily. However, the action of TFA on LL-VIII resulted complete removal of both Nps groups.

(b) The DD Diastereomer (DD-X): This was prepared from DD-VIII in the same manner as described above; yield, 46%; mp 225—228°; $[\alpha]_D^{20} + 33.0^\circ$ ($c=1$, DMF); R_f^2 0.55 (Anal. Found; C, 52.9; H, 6.8; N, 14.4).

(c) The LD Diastereomer (LD-X): This was prepared from LD-VIII (367 mg) in the same manner as described above; yield, 125 mg (65%); mp 209—211°; $[\alpha]_D^{20} - 4.8^\circ$ ($c=1$, DMF); R_f^2 0.58 (Anal. Found; C, 53.5; H, 6.8; N, 14.7).

(d) The DL Diastereomer (DL-X): Yield, 58%; mp 208—211°; $[\alpha]_D^{20} + 4.2^\circ$ ($c=1$, DMF); R_f^2 0.58 (Anal. Found; C, 53.2; H, 6.8; N, 14.3).

Cyclo-[Orn(δ -Ambs)-Gly] (XI)—(a) The L Form (L-XI): The crude oily L-IX (679 mg) was treated with TFA (10 ml) and methanolic NH_3 (15 ml) successively as described for the preparation of LL-X; yield, 98 mg (30%); mp 199—202°; $[\alpha]_D^{20} + 1.8^\circ$ ($c=1$, DMF); R_f^2 0.20. Anal. Calcd. for $\text{C}_{13}\text{H}_{18}\text{O}_4\text{N}_4\text{S}$: C, 47.9; H, 5.6; N, 17.2. Found: C, 47.5; H, 5.7; N, 17.1.

(b) The D Form (D-XI): This was prepared from the oily D-IX; yield, 26%; mp 200—202°; $[\alpha]_D^{20} - 1.6^\circ$ ($c=1$, DMF); R_f^2 0.20 (Anal. Found; C, 47.5; H, 5.6; N, 17.0).

Z-L-Orn(δ -Acbs)-L-Leu-OH (LL-XII)—To a solution of LL-IV (1.21 g, 2 mmole) in EtOH (30 ml), 1N NaOH (3 ml) was added at room temperature. After 5 hr, 1N HCl (3 ml) was added, and the solution was evaporated. Ethyl acetate was added to the residue, and the mixture was washed with water. The organic layer was dried and evaporated. The resulting solid was collected by filtration with the aid of petroleum ether, and recrystallized from ethyl acetate-petroleum ether; yield, 1.06 g (92%); mp 95°; $[\alpha]_D^{20} - 6.3^\circ$ ($c=1$, MeOH); R_f^1 0.80. Anal. Calcd. for $\text{C}_{27}\text{H}_{36}\text{O}_8\text{N}_4\text{S}$: C, 56.2; H, 6.3; N, 9.7. Found: C, 56.0; H, 6.2; N, 9.5.

H-L-Orn(δ -Acbs)-L-Leu-OH (LL-XIII)—A solution of LL-XII (0.577 g, 1 mmole) in 25% HBr in AcOH (3 ml) was allowed to stand for 1 hr. The reaction mixture was evaporated to dryness, and the residue was dissolved in water. After the insoluble material in small amount was filtered off, the filtrate was put on a column (1.3 $\times$ 8 cm) of Dowex 50 (H^+ form). The column was washed with water, and then eluted with 2N NH_4OH (about 50 ml). The eluate was evaporated, and the resulting crystals were collected by filtration with the aid of water. They were recrystallized from hot water; yield, 0.312 g (71%); mp 236—237° (decomp.); $[\alpha]_D^{25} + 29.8^\circ$ ($c=1$, 1N HCl). Anal. Calcd. for $\text{C}_{19}\text{H}_{30}\text{O}_6\text{N}_4\text{S} \cdot 1/2\text{H}_2\text{O}$: C, 50.5; H, 6.9; N, 12.4. Found: C, 50.3; H, 6.9; N, 12.2.

H-L-Orn(δ -Ambs)-L-Leu-OH (LL-XIV)—(a) From LL-XIII: A solution of LL-XIII (0.228 g) in a mixture of 1N HCl (25 ml) and dioxane (12.5 ml) was heated in a bath at 100°. After 1.5 hr, it was evaporated to dryness and the residue was treated with a Dowex 50 column as described for the preparation of LL-XIII. The eluate with 2N NH_4OH was evaporated, and the resulting solid was recrystallized from water-EtOH; yield, 0.134 g (65%); mp 215—216° (decomp.); $[\alpha]_D^{25} + 13.0^\circ$ ($c=1$, 1N HCl); R_f^1 0.75. Anal. Calcd. for $\text{C}_{17}\text{H}_{28}\text{O}_5\text{N}_4\text{S}$: C, 51.0; H, 7.1; N, 14.0. Found: C, 50.9; H, 6.9; N, 14.2.

(b) From LL-VIII: LL-VIII (1.47 g, 2 mmole) was saponified as described for the preparation of LL-XII, 1M citric acid (3 ml) being used instead of 1N HCl. Thus, Nps-Orn(δ -Npsbs)-Leu-OH (LL-XXI) was obtained as a semi solid (1.33 g) with R_f^2 0.52, and it was dissolved in TFA (20 ml). After 1 hr, the solution was evaporated to dryness. The residue was triturated with ether, and the precipitate was collected by filtration. It was dissolved in a mixture of water (30 ml) and MeOH (15 ml), and the solution was treated with a Dowex 50 column. The eluate with 2N NH_4OH was treated as described above, and the crude solid was recrystallized from water-EtOH; yield, 0.56 g (70%); mp 214—215° (decomp.); $[\alpha]_D^{25} + 12.8^\circ$ ($c=1$, 1N HCl); R_f^1 0.75 (Anal. Found; C, 51.0; H, 6.9; N, 14.3).

Nps-L-Leu-L-Orn(δ -Z)-OEt (LL-XV)—This was prepared from Nps-L-Leu-OH $\cdot$ DCHA¹²⁾ (1.53 g, 3.3 mmole) and H-L-Orn(δ -Z)-OEt $\cdot$ TsOH¹³⁾ (1.54 g, 3.3 mmole) by the DCC method as described for the preparation of LL-VIII. The resulting solid was recrystallized from ethyl acetate-petroleum ether; yield, 1.48 g (80%); mp 88°; R_f^1 0.98. Anal. Calcd. for $\text{C}_{27}\text{H}_{36}\text{O}_7\text{N}_4\text{S} \cdot 1/2\text{H}_2\text{O}$: C, 56.9; H, 6.5; N, 9.8. Found: C, 56.7; H, 6.5; N, 10.0.

Cyclo-[L-Orn(δ -Z)-L-Leu] (LL-XVI)—A solution of LL-XV (0.56 g, 1 mmole) in 1N HCl in dioxane (5 ml) was allowed to stand for 30 min. The solution was evaporated to dryness, and the resulting solid was collected by filtration with the aid of ether; yield of a hygroscopic powder (LL-XXII $\cdot$ HCl), 0.42 g. The powder was treated with methanolic NH_3 (15 ml) as described for the preparation of LL-VI. The resulting solid was recrystallized from hot MeOH; yield, 0.21 g (58%); mp 202—203° (decomp.); R_f^1 0.97. Anal. Calcd. for $\text{C}_{19}\text{H}_{27}\text{O}_4\text{N}_3$: C, 63.1; H, 7.5; N, 11.6. Found: C, 63.0; H, 7.6; N, 11.6.

Cyclo-(L-Orn-L-Leu) HCl Salt (LL-XVII $\cdot$ HCl)—A solution of LL-XVI (0.181 g, 0.5 mmole) in 0.05N HCl in MeOH (12 ml) was hydrogenated in the presence of Pd black, and the catalyst was removed by filtration. The filtrate was evaporated, and the resulting solid was collected by filtration with the aid of acetone. It was recrystallized from MeOH-acetone; yield, 0.106 g (80%); mp 245—250° (decomp.); $[\alpha]_D^{25} - 27.2^\circ$ ($c=2$, MeOH); R_f^1 0.80. Anal. Calcd. for $\text{C}_{11}\text{H}_{21}\text{O}_2\text{N}_3 \cdot \text{HCl}$: C, 50.1; H, 8.4; N, 15.9. Found: C, 49.8; H, 8.2; N, 15.8.

Microbiological Assays—The microorganisms employed are *Escherichia coli* and *Bacillus subtilis*. An amount of compound for inhibition of growth was determined by a cup method with a bouillon agar medium and with a synthetic medium. Three reference compounds (sulfathiazole, gramicidin S and dihydrostreptomycin) exhibited strong bacteriostatic activity at the concentrations of 63, 16 and even 4 $\mu\text{g/ml}$.

On the other hand, the following 13 compounds were found to be devoid of any activity at the concentrations of 63, 250 and even 1000 $\mu\text{g/ml}$; amino acids (L-I, D-I), dipeptides (LL-XIII, LL-XIV), cyclodipeptide (LL-XVII), acetylamino benzenesulfonyl cyclodipeptides (LL-VI, LL-VII) and aminobenzenesulfonyl cyclodipeptides (LL-X, DD-X, LD-X, DL-X, L-XI, D-XI).

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