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Studies on Hydroxy Amino Acids. III.*1 The Synthesis of Cyclic Peptide Containing L-Serine

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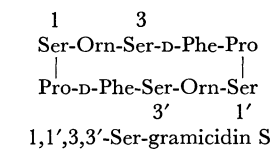
Modified gramicidin S peptides, *cyclo*(Ser-Orn-Ser-D-Phe-Pro)$_2$ and *cyclo*(Ser-Orn-Ser-D-Phe-Pro), were synthesized in order to clarify the correlation between the component amino acids and the antibiotic activity. To obtain the cyclic peptides, we examined several sets of conditions for cyclization reactions. The antibiotic activity of these peptides could not be found against some microorganisms *in vitro*. It was concluded that the lipophilic amino acids residues are the essential groups contributing to gramicidin S activity.

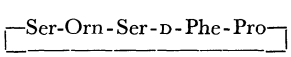
Many works concerning the synthesis of gramicidin S analogues have been investigated by several workers, especially Izumiya *et al.*, in order to study the correlation between the component amino acids and their biological activities.[1)]

We have been studying the synthesis of modified gramicidin S peptides, in which the lipophilic amino acids are replaced by hydrophilic amino acid, such as serine or threonine.

In the present work, the authors have studied the synthesis of a cyclic decapeptide containing L-serine, 1,1′,3,3′-L-seryl gramicidin S, and also cyclic pentapeptide, *cyclo* Ser-Orn-Ser-D-Phe-Pro (Fig. 1).*5



1,1′,3,3′-Ser-gramicidin S
(XIX)



(XXIV)

Fig. 1

The synthetic scheme of 1,1′,3,3′-L-seryl gramicidine S is shown in Fig. 2. The blocking groups were used as follows: the δ amino group of the ornithine was protected by the Z group, the carboxyl groups of the C terminus amino acids were blocked by the methyl ester, while the hydroxyl group of serine was protected by the benzyl and the BOC group was used as a protecting group for the amino group of serine.

The BOC-pentapeptide methyl ester (XI) was prepared by the usual coupling methods, DCCD, mixed anhydride, and the azide method, mp 138.5—139°C, $[\alpha]_D^{24}$ −40.8° (*c* 1.0, AcOH). The ester of pentapeptide (XI) was converted into the hydrazide (XII) by hydrazinolysis in methanol, while XI was also converted into the free ester (XIII) by the acid-catalyzed cleavage of the BOC group. These two components could be coupled by means of the azide method to form the BOC-decapeptide methyl ester (XIV) as a white powder in a 75.5% yield; $[\alpha]_D^{24}$ −47.8° (*c* 0.5, AcOH).

The BOC-decapeptide ester (XIV) was converted into the BOC-decapeptide *p*-nitrophenyl ester (XVI) by hydrolysis with sodium hydroxide in a methanol solution, followed by esterification with *p*-nitrophenol and *N*,*N*′-dicyclohexylcarbodiimide. The active ester (XVI) was confirmed by thin-layer chromatography on silica gel and by a study of its IR spectra at 1760 cm^{-1} (ester) and 1350 cm^{-1} (C–NO_2); it was used immediately for the next reaction. The *N*-terminal protecting group (BOC) of the decapeptide active ester (XVI) was removed by treatment with anhydrous trifluoroacetic acid. The

*1 Part II: K. Okawa, K. Hori, K. Hirose, and Y. Nakagawa, This Bulletin, **42**, 2720 (1969).

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1) H. Aoyagi, T. Kato, M. Ohno, M. Waki, S. Makizumi and N. Izumiya, This Bulletin, **38**, 2139 (1965); H. Aoyagi and N. Izumiya, *ibid.*, **39**, 1747 (1966); H. Aoyagi, M. Kondo, T. Kato, S. Makizumi and N. Izumiya, *ibid.*, **40**, 1685 (1967).

*5 The abbreviations used for the amino acid residues, peptides and other abbreviations are based on the proposals by: E. Schröder and K. Lübke "The Peptides." Academic Press, New York, 1965. E. Bricas (ed.) "Peptides 1968" Proceedings of the Ninth European Peptide Symposium, Orsay, France, April 1968., North-Holland Publishing Company, Amsterdam.

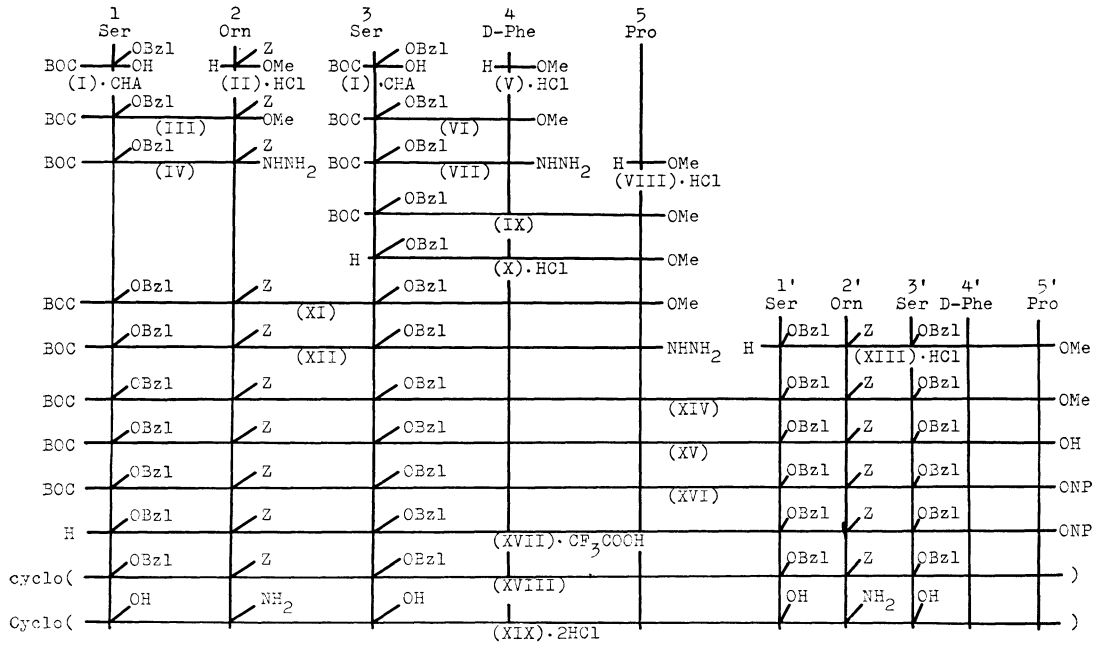

Fig. 2. Synthetic route of 1,1′,3,3′-Ser-gramicidin S (XIX).

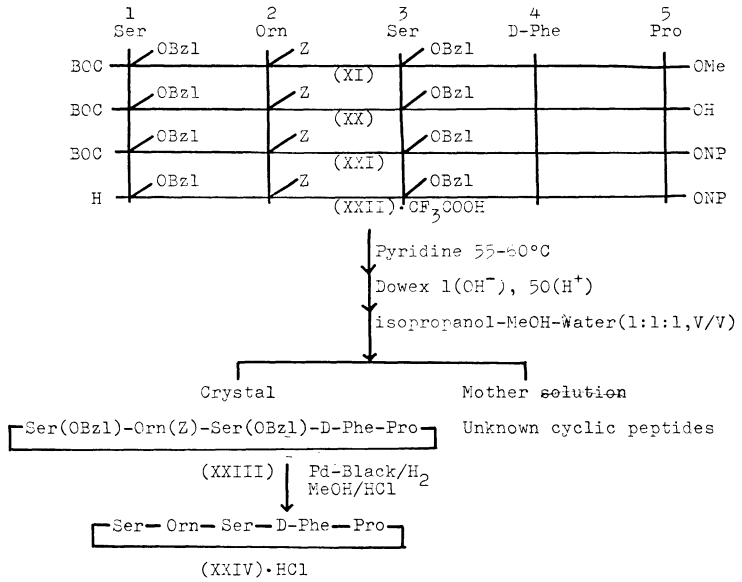

Fig. 3. Cyclization of linear pentapeptide.

cyclization of the free decapeptide *p*-nitrophenyl ester (XVII) trifluoroacetate was accomplished by means of Schwyzer's high-dilution method.[2] It was carried out by the drop-by-drop addition (4 hr 57°C) of XVII trifluoroacetate in DMF to a large amount of anhydrous pyridine (5×10^{-4}M solution). The crude product in methanol-water (10:1 V/V) was treated with Dowex 1(OH^-) and 50(H^+) to remove the linear peptide fractions. From the neutral part, *cyclo* (Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro)$_2$ (XVIII) was obtained as crystal in a 44.6% yield (mp 246—247°C, $[\alpha]_D^{24}$ −185.0° (*c* 0.2, AcOH)); it was confirmed by molecular-weight measurements. The protecting groups of XVIII were removed by catalytic hydrogenation, and the resulting 1,1′,3,3′-L-seryl gramicidin S (XIX) dihydrochloride was crystallized from methanol-ether

2) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).

in a 75.7% yield; mp 320°C (dec.), $[\alpha]_D^{25}$ −126° (*c* 0.1, AcOH). This analogue (XIX) of gramicidin S has almost the same mobility on paper electrophoresis as the native gramicidin S (Fig. 4).

As an alternative approach to the synthesis of gramicidin S, Schwyzer and Sieber have reported that gramicidin S can be synthesized by the dimeric cyclization of the linear pentapeptide active ester.[3] Izumiya and Waki reported, however, that the linear pentapeptide active ester does not cyclize in the dimeric form exclusively, but that monomeric cyclization occurs.[4] The present authors have also at tempted the dimeric cyclization reaction of our pentapeptide (Fig. 3).

The BOC-pentapeptide methyl ester (XI) was saponified and then converted into the BOC-pentapeptide *p*-nitrophenyl ester (XXI) by estirification using DCCD. After the cleavage of the BOC group by treatment with anhydrous trifluoroacetic acid, the cyclization reaction were carries out as has been described above in the case of anhydrous pyridine. The molar concentrations of the pentapeptide active ester (XXII) trifluoroacetate in pyridine, 0.26×10^{-3} mol, 1.3×10^{-3} mol and 6.5×10^{-3} mol, were employed for the cyclization reactions. The resulting crude product was treated in methanol-water (5 : 1 v/v) with Dowex 1 (OH^-) and Dowex 50(H^+) to remove any linear peptide fractions. Cyclic pentapeptide (XXIII), *cyclo* Ser(OBzl)-Orn-(Z)-Ser(OBzl)-D-Phe-Pro, could be isolated as crystals from a solution of isopropanol-methanol-water (1 : 1 : 1 v/v) and was confirmed by molecular-weight measurements; mp 118—119°C, $[\alpha]_D^{24}$ −93.4° (*c* 0.7, AcOH). The resulting *cyclo* Ser-Orn-Ser-D-Phe-Pro (XXIV) hydrochloride was crystallized from methanol-ether in an 82.3% yield, mp 250°C (dec.), $[\alpha]_D^{24}$ −156.8° (*c* 0.5, AcOH), and was shown to have a smaller mobility than either the native gramicidin S or the cyclic decapeptide (XIX) on paper electrophoresis, as is shown in Fig. 4.

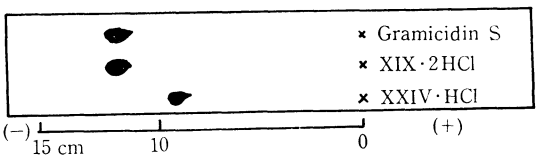

Fig. 4. Paper electrophoresis: solvent system AcOH 1M HCOOH 0.6M (pH 1.8) 600 V/30 cm 2 hr Toyo Roshi No. 50.

In the mother solution of XXIII, the presence of cyclic decapeptide (XVIII) and two other products was confirmed on thin-layer chromatography in each case, but no crystalline cyclic peptides other than cyclic decapeptide could be isolated. Under different concentrations of the cyclization, the yields of the cyclic pentapeptides were 76.3% (0.26×10^{-3} mol), 50.2% (1.3×10^{-3} mol), and 13.4% (6.5×10^{-3} mol) respectively.

The above results confirmed that the yield of the cyclic pentapeptide decreases proportionally to the increase in the reaction concentration, and that the monomeric cyclization proceeded predominantly more than the dimeric cyclization when the high-dilution method was used.

The biological activities of cyclic pentapeptide (XXIV) hydrochloride and 1,1′,3,3′-L-seryl-gramicidin S (XIX) dihydrochloride against several microorganisms were investigated by the paper-disk method. No antibiotic activity could be found against *B. subtilis*, *St. albus*, *M. lysodakius*, and *E. coli*. From the above results, it may be concluded that the lipophilic amino acid residues are essential groups in contributing to gramicidin S activities.

Experimental

All the melting points are uncorrected.

***N*-*t*-Butyloxycarbonyl-*O*-benzyl-L-serine (I) Cyclohexylamine salt.** I was prepared from *O*-benzyl-L-serine[5] according to Anderson's method[6] in an 80% yield; mp 156—157°C, $[\alpha]_D^{24}$ +29.5° (*c* 1.3, MeOH).

Found: C, 63.78; H, 8.62; N, 7.14%. Calcd for $C_{21}H_{34}O_5N_2$: C, 63.93; H, 8.69; N, 7.10%.

N^{δ}-Benzyloxycarbonyl-L-ornithine Methyl Ester (II) Hydrochloride. II hydrochloride was prepared by the method of Boissonnas[7] in a 92.4% yield. N^{α},N^{δ}-dibenzyloxycarbonyl-L-ornithine[8] was treated with thionyl chloride in anhydrous ether-tetrahydrofuran (9 : 1 v/v), and with anhydrous methanol; mp 140—142°C, $[\alpha]_D^{24}$ +2.2° (*c* 1.0, AcOH).

Found: C, 53.30; H, 6.63; N, 8.86; Cl, 11.32%. Calcd for $C_{14}H_{21}O_4N_2Cl$: C, 53.08; H, 6.68; N, 8.84; Cl, 11.19%.

BOC-Ser(OBzl)-Orn(Z)-OMe(III). II (from the hydrochloride; 9.5 g, 0.03 mol) was treated with *N*,*N*′-dicyclohexylcarbodiimide (6.8 g, 0.03 mol) and I (from cyclohexylamine salt 11.8 g, 0.03 mol) in methylene chloride (200 ml) at 5°C for 1 hr. After the reaction mixture had then been allowed to stand overnight at room temperature, acetic acid (3 ml) was added and the reaction mixture was stirred for 15 min. The precipitate thus produced was filtered off; after the filtrate had been washed with 0.1N hydrochloric acid, N sodium bicarbonate, and water successively, it was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. III was obtained from ethyl acetate - petroleum ether in a 90% (15 g) yield. The product was recrystallized from ethyl acetate - petroleum ether; mp 86—88°C, $[\alpha]_D^{24}$ +2.8° (*c* 1.1, AcOH).

3) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186 (1958).

4) M. Waki and N. Izumiya, This Bulletin, **40**, 1687 (1967).

5) K. Okawa, *ibid.*, **29**, 486 (1956).

6) G. W. Anderson and A. C. Mc Gregon, *J. Amer. Chem. Soc.*, **79**, 6180 (1957).

7) R. A. Boissonnas, S. Guttmann, R. L. Huguenin, P. A. Jaquenoud and E. Sandrin, *Helv. Chim. Acta*, **41**, 1867 (1958).

8) R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948).

Found: C, 62.47; H, 6.79; N, 7.44%. Calcd for $C_{29}H_{39}O_8N_3$: C, 62.46; H, 7.04; N, 7.53%.

BOC-Ser(OBzl)-Orn(Z)-NHNH$_2$ (IV). To III (54.64 g, 1.00 mol) in ethanol (200 m*l*), we added 80% hydrazine hydrate (63 g, 1.25 mol) in ethanol (100 m*l*) and then stirred the mixture for 1 hr in an ice-bath. After the reaction mixture had been kept at room temperature for 3 days, white crystals were formed; these were collected (45.50 g), and another crop of the hydrazide (8.29 g) was obtained from the mother liquor. The total yield was 53.79 g (98%). They were recrystallized from ethanol - water; mp 152—154°C, $[\alpha]_D^{24}$ −9.5° (*c* 1.0, AcOH).

Found: C, 60.39; H, 7.14; N, 12.60%. Calcd for $C_{28}H_{39}O_7N_5$: C, 60.31; H, 7.03; N, 12.56%.

BOC-Ser(OBzl)-D-Phe-OMe (VI). I (from cyclohexylamine salt (7.89 g, 0.02 mol)) was treated with ethyl chloroformate (2.17 g, 0.02 mol) and triethylamine in THF at −5°C. After the solution had stood for 20 min, D-phenylalanine methyl ester (V) (from hydrochloride[9] (4.31 g, 0.02 mol)) was added, drop by drop, to the above solution at −5°C. After the reaction mixture had then been allowed to stand overnight at room temperature, the precipitate thus produced was filtered off. The solvent was replaced by ethyl acetate (70 m*l*); after the solution had then been washed with water, 4% sodium bicarbonate, 0.5N hydrochloric acid, and water successively, it was dried over anhydrous sodium sulfate and concentrated under reduced pressure. VI was obtained from ethyl acetate – *n*-hexane in a 76.8% (7.03 g) yield. Recrystallization from ethyl acetate–*n*-hexane gave a pure material; mp 81.5—82°C, $[\alpha]_D^{13}$ −18.7° (*c* 1.1, AcOH).

Found: C, 65.82; H, 6.97; N, 6.13%. Calcd for $C_{25}H_{32}O_6N_2$: C, 65.77; H, 7.06; N, 6.14%.

BOC-Ser(OBzl)-D-Phe-NHNH$_2$ (VII). VI (6.7 g, 0.01 mol) was treated with 80% hydrazine hydrate (1.5 g, 0.03 mol) in anhydrous methanol (12 m*l*). After the reaction mixture had then been allowed to stand for 46 hr at room temperature, it was concentrated to dryness. The precipitate was recrystallized from ethyl acetate–*n*-hexane. The yield was 6.0 g (89.5%). Mp 114—115°C, $[\alpha]_D^{13}$ −6.3° (*c* 1.1, AcOH).

Found: C, 63.17; H, 7.04; N, 12.28%. Calcd for $C_{24}H_{32}O_5N_4$: C, 63.14; H, 7.06; N, 12.28%.

BOC-Ser(OBzl)-D-Phe-Pro-OMe (IX). Into a solution of VII (2.29 g, 5 mmol) in acetic acid (25 m*l*) and N hydrochloric acid (10 m*l*), we added N sodium nitrite (5 m*l*) drop by drop under cooling at −5——10°C. After stirring for 10 min, sodium bicarbonate (1.5 g) and ice-cold water (50 m*l*) were added to the above solution and the product was extracted with ice-cold ethyl acetate (50 m*l*). The organic layer was washed with ice-cold 0.5N sodium bicarbonate, and then immediately cooled to under −5°C. To this, the L-proline methyl ester (VIII) (from hydrochloride;[9] 0.94 g, 5.65 mmol) was stirred in, drop by drop; the stirring was thereafter continued for 3 hr at 0°C. After the reaction mixture had been allowed to stand overnight in a refrigerator, it was washed with 0.1N hydrochloric acid, N sodium bicarbonate, and water. The solution was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. IX was obtained from ether–*n*-hexane in the theoretical yield; mp 62—63°C, $[\alpha]_D^{24}$ −46.6° (*c* 1.0, AcOH).

Found: C, 64.79; H, 7.22; N, 7.56%. Calcd for $C_{30}H_{39}O_7N_3$: C, 65.08; H, 7.10; N, 7.59%.

H-Ser(OBzl)-D-Phe-Pro-OMe (X) Hydrochloride. Through an ethyl acetate (45 m*l*) solution of IX (5.54 g, 0.01 mol) dry hydrogen chloride was bubbled for 30 min in an ice-bath. After the reaction mixture had then been allowed to stand at room temperature for 30 min, the solution was concentrated under reduced pressure. The addition of ether gave 4.65 g (95%) of a white powder; mp 189.5—190°C.

Found: C, 61.20; H, 6.52; N, 8.53; Cl, 7.32%. Calcd for $C_{25}H_{32}O_5N_3Cl$: C, 61.28; H, 6.58; N, 8.58; Cl, 7.24%.

BOC-Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro-OMe (XI). IV (12.27 g, 22 mmol) (5 m*l*) was coupled with X (from hydrochloride (10.47 g, 22 mmol)) in ethyl acetate (300 m*l*) by the azide method described in connection with compound IX. The subsequent crystallization from ethyl acetate gave 19.1 g of XI (88.8%); mp 138.5—139°C, $[\alpha]_D^{24}$ −40.8° (*c* 1.0, AcOH).

Found: C, 64.72; H, 6.92; N, 8.43%. Calcd for $C_{53}H_{66}O_{12}N_6$: C, 65.01; H, 6.79; N, 8.58%.

BOC-Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro-NHNH$_2$ (XII). Hydrazine hydrate (80%) (5.1 g, 102 mmol) in anhydrous methanol (10 m*l*) was added to an anhydrous methanol solution (40 m*l*) of XI (5 g, 5.1 mmol). The reaction mixture was then kept at 35—40°C for 3 days. After the excess hydrazine had then been removed under reduced pressure, a large amount of cold water was added. The product was soon solidfied; it was collected as a white powder, and dried in a desiccator over sulfuric acid to yield 4.80 g (96%). $[\alpha]_D^{23}$ −37.3° (*c* 0.7, AcOH).

Found: C, 63.85; H, 6.92; N, 11.21%. Calcd for $C_{52}H_{66}O_{11}N_8$: C, 63.78; H, 6.80; N, 11.45%.

H-Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro-OMe (XIII) Hydrochloride. Through an anhydrous dioxane (20 m*l*) solution of XI (2 g, 2.04 mmol), dry hydrogen chloride was bubbled for 20 min under cooling in an ice-bath. The reaction mixture was then allowed to stand room temperature for 1 hr. After the evaporation of the solvent and hydrogen chloride under reduced pressure, ether was added to the residual oil. The crude product was recrystallized from methanol–ether; yield, 1.72 g (92.4%); mp 168°C.

Found: C, 62.67; H, 6.78; N, 9.05; Cl, 4.11%. Calcd for $C_{48}H_{59}O_{10}N_6Cl$: C, 62.97; H, 6.50; N, 9.18; Cl, 3.87%.

BOC-(Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro)$_2$-OMe (XIV). XII (2.94 g, 3 mmol) and XIII (from hydrochloride (2.75 g, 3 mmol)) were coupled by the azide method in the same way as was used in the case of the synthesis of compound IX. The resulting oily material was chromatographed over silica gel using ethy acetate as a solvent. A white powder was subsequently obtained by the addition of *n*-hexane. Yield, 4.14 g (75.5%); $[\alpha]_D^{23}$ −47.8° (*c* 0.5, AcOH).

Found: C, 65.50; H, 6.67; N, 9.29%; mol wt,*6 1875. Calcd for $C_{100}H_{120}O_{21}N_{12}$: C, 65.77; H, 6.62; N, 9.20%; mol wt, 1826.

9) B. F. Erlanger, H. Sachs and E. Brand, *J. Amer. Chem. Soc.*, **76**, 1806 (1954).

*6 Knauer Vapour Pressure Osmometer; solvent: methanol, standard curve; the solution of BOC-Ser-(OBzl)-D-Phe-OMe (VI) in methanol.

BOC-(Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro)$_2$-OH (XV). XIV (1.83 g, 1 mmol) in anhydrous methanol (20 ml) was treated with 2N sodium hydroxide (2 ml, 4 mmol) at room temperature. The solution was kept at 37°C until no turbidity appeared upon the addition of one drop of water (8 hr) After the solution had then been adjusted to pH 2 with N hydrochloric acid (about 4 ml) in an ice-bath, water (100 ml) was added to give a precipitate; yield, 1.54 g. Recrystallization from methanol - water yielded 1.32 g (73%) of pure crystals; mp 113—115°C, R_f 0.15*7 $[\alpha]_D^{23}$ −39.3° (*c* 0.9, AcOH).

Found: C, 65.66; H, 6.54; N, 9.34%; mol wt, 1820.*6 Calcd for $C_{99}H_{118}O_{21}N_{12}$: C, 65.62; H, 6.56; N, 9.28%; mol wt, 1820.

BOC-(Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro)$_2$-ONP (XVI). XV (500 mg, 0.27 mmol) and *p*-nitrophenol (70 mg, 0.5 mmol) were dissolved in methylene chloride (5 ml), and then a methylene chloride solution of *N,N'*-dicyclohexylcarbodiimide (62 mg, 0.30 mmol) was added, drop by drop, to the solution at 0—5°C. The reaction mixture was allowed to stand overnight in a refrigerator. After the addition of acetic acid (one drop), the dicyclohexylurea thus produced was filtered off and washed with cold ethyl acetate. The filtrate was concentrated under reduced pressure, and the resulting oily material was dissolved in a cold ethyl acetate solution. It was washed with N sodium bicarbonate and water to remove the excess *p*-nitrophenol, and then dried over anhydrous sodium sulfate. XVI was obtained from ethyl acetate–ether in the yield of 460 mg (88%); mp 110—112°C, R_f 0.89*7 ν_{max}^{Nujol} 1760 (ester), 1350 cm^{-1} (C–NO$_2$).

Found: C, 63.98; H, 6.26; N, 9.42%. Calcd for $C_{105}H_{121}O_{23}N_{13}\cdot 2H_2O$: C, 64.04; H, 6.39; N, 9.25%.

***Cyclo* (Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro)$_2$ (XVIII).** The BOC-decapeptide active ester (XVI) (200 mg, 0.1 mmol) was dissolved in anhydrous trifluoroacetic acid (3 ml), after which the solution was kept at 0°C for 1 hr. The trifluoroacetic acid was subsequently evaporated under reduced pressure at room temperature. The residual decapeptide *p*-nitrophenyl ester (XVII) trifluoroacetate was immediately dissolved in dimethylformamide (10 ml) without purification. A DMF solution containing 5 drops of acetic acid was added, drop by drop, to pyridine (200 ml) at 57°C over a 4 hr period, after which the stirring was continued for an additional 2 hr. The solution was then evaporated under reduced pressure and water was added to give a crude product. This colored product was collected, washed with water, and dissolved in a mixed solvent of methanol and water (5 : 1 v/v). After the insoluble substance had been filtered off, the filtrate was treated with a Dowex 1 (OH^-) column (1.5×20 cm) and a Dowex 50 (H^+) column (1.5×20 cm). The effluent (300 ml) was evaporated; the subsequent addition of water yielded a white powder which gave a negative ninhydrin reaction; 75.5 mg (44.6%). Purification with Sephadex LH-20 and recrystallization from ethanol - water yielded 50.7 mg (30% from XVI) of XVIII; mp 246—247°C, R_f 0.88,*7 $[\alpha]_D^{24}$ −185.0° (*c* 0.2, AcOH).

Found: C, 65.64; H, 6.46; N, 9.90%. Calcd for $C_{94}H_{112}O_{20}N_{12}$: C, 65.26; H, 6.53; N, 9.72%; mol wt,*6 1710, Calcd for $C_{94}H_{108}O_{18}N_{12}$; mol wt, 1694.

*7 Thin-layer chromatography with Merck silica gel; solvent system; water saturated ethyl acetate.

***Cyclo* (Ser-Orn-Ser-D-Phe-Pro)$_2$ (XIX) Dihydrochloride.** A methanol solution (5 ml) of XVIII (84.7 mg, 0.05 mmol) containing N hydrochloric acid (0.05 ml) was hydrogenated, by the use of palladium black (300 mg) as the catalyst, at room temperature for 42.5 hr. After the catalyst had been filtered off and the solvent had been evaporated under reduced pressure, the addition of methanol - ether gave needle crystals (43.1 mg (75.7%)); mp 320°C (dec.), $[\alpha]_D^{25}$ −126° (*c* 0.1, AcOH), R_f 0.55.*8

Found: C, 47.69; H, 6.36; N, 13.58%. Calcd for $C_{50}H_{83}O_{19}N_{12}Cl_2$: C, 48.89; H, 6.89; N, 13.69%.

Amino acid ratios in acid hydrolyzate: Ser 1.94, Orn 0.89, Phe 1.00, Pro 1.03.

BOC-Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro-OH (XX). XI (4.9 g, 5 mmol) in dioxane (5 ml) was treated with N sodium hydroxide (5.25 ml) at room temperature for 2 hr. The reaction mixture was then adjusted to pH 2 with N hydrochloric acid, the reaction products were extracted with methylene chloride, and the organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the residuals were crystallized from ethyl acetate–*n*-hexane (4.35 g (90.1%)). Recrystallization from methanol–water gave a pure material; mp 135.5—136°C, R_f 0.10,*7 $[\alpha]_D^{24}$ −35.2° (*c* 1.0, AcOH).

Found: C, 64.82; H, 6.94; N, 8.54%. Calcd for $C_{52}H_{64}O_{12}N_6$: C, 64.71; H, 6.68; N, 8.71%.

BOC-Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro-ONP (XXI). A solution of XX (9.7 g, 10 mmol) and *p*-nitrophenol (2.8 g, 20 mmol) in methylene chloride (30 ml) was treated with *N,N'*-dicyclohexylcarbodiimide (2.27 g, 11 mmol) at 0°C for 4 hr with stirring. After the dicyclohexylurea thus produced had been filtered off, the reaction mixture was washed with 10% citric acid, N sodium bicarbonate, and water successively, and then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residual crystals were recrystallized from ethyl acetate–ether (8.99 g (82.6%)); mp 151—152°C, $[\alpha]_D^{24}$ −35.8° (*c* 1.0, AcOH), ν_{max}^{Nujol} 1770 (ester), 1345 cm^{-1} (C–NO$_2$).

Found: C, 63.35; H, 6.41; N, 9.23%. Calcd for $C_{58}H_{67}O_{14}N_7$: C, 64.13; H, 6.22; N, 9.03%.

***Cyclo* Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro (XXIII).** XXI (1.09 g, 1 mmol) was suspended in anhydrous trifluoroacetic acid (2.5 ml) containing anisole (0.25 ml), after which the mixture was allowed to stand at 0°C for 1 hr. After the solvent had been removed under reduced pressure at room temperature, the addition of absolute ether gave a white powder of XXII trifluoroacetate (1.03 g (93.6%)). This active ester was used immediately in the following cyclization procedure.

XXII trifluoroacetate (768 mg, 0.698 mmol), dissolved in DMF (10 ml) containing 6 drops of acetic acid, was stirred, drop by drop, into anhydrous pyridine (2685 ml, 0.26×10^{-3} molar concentration of XXII·CF_3COOH) at 55—60°C for 4 hr. After the stirring had been continued for 2 hr, the solvent was evaporated under reduced pressure. The residual oil was dissolved in methanol–water (5 : 1 v/v) and then treated with Dowex 1 (OH^-) and 50 (H^+). After elution with the same solvent (300 ml), the effluent was concentrated

*8 Thin-layer chromatography with Merck silica gel; solvent system; *n*-butanol: acetic acid: water: pyridine 4 : 1 : 2 : 1 (v/v).

under reduced pressure and the residuals were treated with isopropanol–methanol–water (1 : 1 : 1 v/v). *Cyclo* Ser(OBzl)-Orn(Z)-Ser(OBzl)-D-Phe-Pro (XXIII) was thus obtained as fine crystals; yield, 451 mg (76.3%), R_f 0.53*[7], mp 118—119°C $[\alpha]_D^{24}$ −93.4° (*c* 0.7, AcOH).

Found: C, 65.52; H, 6.45; N, 10.17%; mol wt*[6], 867. Calcd for $C_{47}H_{56}O_{10}N_6$: C, 65.24; H, 6.53; N, 9.72%; mol wt, 847.

In other cases using different concentrations of the active ester (1.3×10^{-3} mol and 6.5×10^{-3} mol), XXIII was also obtained as crystals, but in different yields (50.2% and 13.4% respectively). From the mother solution, cyclic decapeptide (XVIII), R_f 0.88*[7] and two other cyclic peptides, R_f 0.77*[7] and 0.85*[7] confirmed in each case on thin-layer chromatography, but no crystalline cyclic peptides have yet been isolated except for cyclic decapeptide (XVIII).

***Cyclo* Ser-Orn-Ser-D-Phe-Pro (XXIV) Hydrochloride.** XXIII (169 mg, 0.2 mmol) was hydrogenated in methanol (10 m*l*) with N hydrochloric acid (0.2 m*l*), using palladium black (200 mg) as the catalyst at room temperature for 13.5 hr. After the catalyst had then been filtered off and the solvent removed under reduced pressure, the addition of methanol–ether gave needle crystals (80 mg (75%)); mp 250° (dec.), $[\alpha]_D^{25}$ −156.8° (*c* 0.6, AcOH), R_f 0.55.*[8]

Found: C, 52.75; H, 6.61; N, 14.63; Cl, 6.47%. Calcd for $C_{25}H_{37}O_7N_6Cl$: C, 52.76; H, 6.55; N, 14.77; Cl, 6.23%. Amino acid ratios in acid hydrolyzate: Ser 1.93, Orn 0.87, Phe 1.00, Pro 0.94.

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