BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 1685—1687 (1967)

Studies of Peptide Antibiotics. IX. Synthesis of *cyclo*-(L-Valyl-L-ornithyl-L-leucyl-D-phenylalanyl-glycyl)

Haruhiko AOYAGI, Michio KONDO, Tetsuo KATO, Satoru MAKISUMI and Nobuo IZUMIYA

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

(Received December 16, 1966)

It was previously observed that the cyclization reaction of L-valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanyl-glycine *p*-nitrophenyl ester in pyridine yields a large amount of the pyridine-insoluble product (IV), besides a small amount of the protected cyclic decapeptide by the dimerization. By the molecular weight determination of IV with a osmometer, it was proved that the product IV is the protected cyclic pentapeptide. Hydrogenolysis of IV in a mixture of acetic acid and dimethylformamide gave *cyclo*-(valyl-ornithyl-leucyl-D-phenylalanyl-glycyl) acetate which showed no antibacterial activity in relation to any of the microorganisms tested.

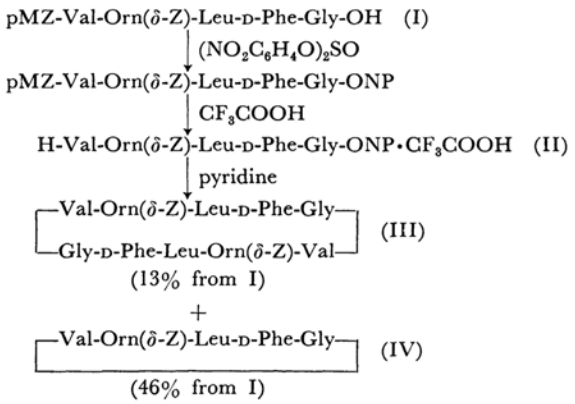

Fig. 1. Cyclization of linear pentapeptide active ester.

In a previous paper, we reported that the cyclization reaction of valyl-δ-benzyloxycarbonyl-ornithyl-leucyl-D-phenylalanyl-glycine active ester (II) in pyridine yields a large amount of the pyridine-insoluble product, besides a small amount of the dimerized material (III).[1] In this paper, we will describe the experiment in which the pyridine-insoluble product is proved to be the protected cyclic pentapeptide shown as IV, together the preparation of the cyclic pentapeptide acetate (V·AcOH).

As shown in Fig. 1, the cyclization reaction of II yielded a mixture of the benzyloxycarbonyl-substituted cyclic peptides which is separated to the two compounds by the way of fractional recrystallization. The compound III with mp 248—250°C, which is soluble in pyridine or methanol, was identified as the protected cyclic decapeptide previously.[1] Another compound showed the unusual physical characters compared with any of the benzyloxycarbonyl-substituted cyclic peptides prepared in this laboratory; its melting point is high as 324—326°C and its solubility in pyridine or methanol is extremely low. Its character as the protected cyclic peptide was shown by the fact that the very dilute methanolic solution of this compound passes through the columns of cation and anion ion-exchange resin. Furthermore, the elemental analysis of the compound and the amino acid analysis of its hydrogenated material indicated that the compound is represented as *cyclo*-(Val-Orn(δ-Z)-Leu-D-Phe-Gly)$_n$, in which n will be 1, 3 or a higher number. To estimate the degree of polymerization, the molecular weight determination was attempted. The micro Rast method using a solvent of hexahydro-*p*-aminobenzoic acid lactam,[2] which was used successfully for the molecular weight determination of the benzyloxycarbonyl-substituted Gly$^{1,1',5,5'}$-gramicidin S,[3] could not be applied because of insolubility of the compound in the solvent. The application of mass spectrometer, which had been

1) H. Aoyagi, T. Kato, M. Ohno and N. Izumiya, *J. Am. Chem. Soc.*, **86**, 5700 (1964); H. Aoyagi, T. Kato, M. Ohno, M. Kondo, M. Waki, S. Makisumi and N. Izumiya, This Bulletin, **38**, 2139 (1965).

2) G. Went, *Ber.*, **75**, 425 (1942).

3) M. Kondo, H. Aoyagi, T. Kato and N. Izumiya, This Bulletin, **39**, 2234 (1966).

successful for the molecular weight determination of destruxin, a natural cyclic hexadepsipeptide,[4] did not give reasonable value for the compound.[5] Finally, an osmometer was successfully applied for the molecular weight determination using dimethylformamide as a solvent, and the compound was proved to be the monomer shown as IV.[6]

The cyclization reaction of II yielded the protected cyclic pentapeptide (IV) predominantly; the molar ratio between IV and III is calculated as 88 : 12. It would be noteworthy that the cyclization reaction of H-Val-Orn(δ-Z)-Leu-D-Phe-Sar active ester affords also a mixture of the protected penta- and decapeptide with the molar ratio of 92 : 8.[7]

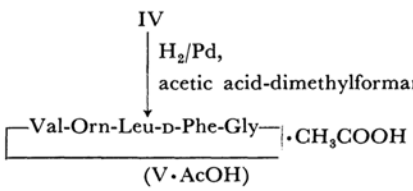

Fig. 2. Hydrogenation of the protected pentapeptide.

Attempts to obtain the hydrogenated product of IV with a solvent of methanol containing hydrogen chloride or acetic acid was unsuccessful because of insolubility of IV in these solvents. Finally, the desired product (V) was obtained as monoacetate by the hydrogenation of IV with a large amount of dimethylformamide containing acetic acid.

The antibacterial activity of V toward several microorganisms was examined. V had no retarding effect on the growth of the microorganisms, even at so high a concentration as 100 μg per ml of the assay medium. Gly[5,5']-gramicidin S, the dimeric product of V, or gramicidin S, however, showed substantial activity under these conditions. It would be of interest to note that Sar[5,5']-gramicidin S, a cyclic decapeptide, was active as gramicidin S, whereas Sar[5]-cyclosemigramicidin S,[8] a cyclic pentapeptide, showed no activity in relation to any of the microorganism tested.[7]

Experimental

All melting points are uncorrected.

***cyclo*-(L-Valyl-δ-benzyloxycarbonyl-L-ornithyl-L-leucyl-D-phenylalanyl-glycyl (II).** *p*-Methoxybenzyloxylcarbonyl pentapeptide (I) (1.694 g, 2 mmol) was converted to the pentapeptide active ester trifluoroacetate (II) as described before.[1] The ester (II) was added to pyridine (300 ml) at 55—60°C, and the crystals deposited were collected by the filtration and washed with pyridine. The crystals (0.567 g) with mp 324—326°C (decomp.) was pure enough for many purposes such as elemental analysis. The filtrate and the washings were combined and evaporated *in vacuo*. The residue was dissolved in a mixture of methanol (600 ml) and water (100 ml) and the solution was treated with the columns of Dowex 1 and 50. The effluent was evaporated to dryness *in vacuo*, and the product (0.31 g) remained was separated to the two compounds by the fractional recrystallization with methanol; the protected cyclic decapeptide (III) with mp 248—251°C (decomp.) was obtained in a yield of 0.173 g (13% from I), and the protected cyclic pentapeptide (IV) with mp 324–326°C (decomp.) in a yield of 0.061 g after recrystallization from dimethylformamide-ether. Total yield of IV, 0.628 g (46% from I); $[\alpha]_D^{20}$ −48.0° (*c* 0.3, dimethylformamide).

Found: C, 61.73; H, 7.50; N, 12.49%. Calcd for $C_{35}H_{48}O_7N_6 \cdot H_2O$: C, 61.61; H, 7.38; N, 12.32%.

Molecular Weight Determination of IV. The osmometer of Hitachi, type 115, was employed. The standard curve was obtained with the solutions (concentrations, 0.27—1.3×10^{-2} mmol per gram of solution) of BOC-D-Phe-L-Orn(δ-Z)-OEt[9] (molecular weight, 542) in dimethylformamide. The mole concentration of IV (1.518 mg of IV in 722 mg of dimethylformamide) was calculated as 0.32×10^{-2} mmol per gram of solution compared with the standard curve by the use of the osmometer.

Found: 660. Calcd for $C_{35}H_{48}O_7N_6 \cdot H_2O$: 683.

***cyclo*-(L-Valyl-L-ornithyl-L-leucyl-D-phenylalanylglycyl) Acetate (V·AcOH).** IV (80 mg), dissolved in dimethylformamide (30 ml) and acetic acid (1 ml), was subjected to hydrogenolysis in the presence of palladium black. The filtrate was evaporated *in vacuo* and the crystals remained were collected by the filtration with the aid of acetone. It decomposed gradually at 270°C. Yield, 55 mg (79%), $[\alpha]_D^{20}$ −56.6° (*c* 0.3, dimethylformamide). The amino acid analysis gave the molar ratio of 0.9: 0.9: 1.0: 1.1: 0.9 for Gly, Val, Leu, Phe, and Orn.

Found: C, 58.96; H, 7.85; N, 14.23%. Calcd for

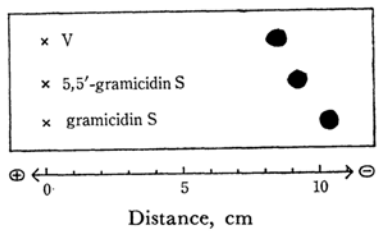

Fig. 3. Paper electrophoresis of the cyclic peptides.

4) S. Kuyama and S. Tamura, *Agr. Biol. Bull.*, **29**, 168 (1965).

5) We are indebted to Mr. K. Fujimori, Naka Works, Hitachi, Ltd., for the experiment by the mass spectrometer.

6) In this laboratory, the osmometer had been customarily applied for the molecular weight determination with methanol or dioxane solution of many benzyloxycarbonyl-substituted cyclic peptides. Since the compound IV was almost insoluble in methanol or dioxane, the use of dimethylformamide as a solvent was developed.

7) H. Aoyagi and N. Izumiya, This Bulletin, **39**, 1747 (1966).

8) Schröder suggests the name of cyclosemigramicidin S for *cyclo*-(Val-Orn-Leu-D-Phe-Pro); E. Schröder and L. Lübke, "The Peptides," Vol. II, Academic Press, New York (1966), p. 429.

9) N. Izumiya, T. Kato, Y. Fujita, M. Ohno and M. Kondo, This Bulletin, **37**, 1809 (1964).

$C_{29}H_{46}O_7N_6$: C, 59.01; H, 7.98; N, 14.31%.

Its homogeneity was also observed by paper electrophoresis as shown in Fig. 3. A small amount of V·AcOH was placed on a paper of Toyo Toshi No. 52, and 500 V/30 cm were applied for 3 hr at room temperature, using a formic acid-acetic acid-methanol-water (1 : 3 : 6 : 10 v/v) system, at pH 1.8. Figure 3 demonstrates that the mobility of V was slower than its dimerized analogue, $Gly^{5,5'}$-gramicidin S.