Relationship between the Cyclization and Conformation of Pentapeptide Active Esters Related to Gramicidin S Having No Protecting Group on the Side Chain of the Ornithine Residue

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(Received November 8, 1996)

To investigate the contribution of the D-Phe–Pro–Val sequence in the direct formation of gramicidin S (GS) by the dimerization–cyclization of pentapeptide-active esters having no protecting group on the side chain of the Orn residue, the cyclization of four H–X–Pro–Y–Orn–Leu–ONSu's (X = L- or D-Phe, Y = L- or D-Val, –ONSu = succinimide ester) was examined. Only H–D-Phe–Pro–Val–Orn–Leu–ONSu gave semi-GS (cyclic monomer) and GS (cyclic dimer) in yields of 15 and 38%, respectively. The active ester with a D-Phe–Pro–D-Val sequence produced exclusively [D-Val]–semi-GS in 58% yield. On the other hand, the active esters having Phe–Pro–Val and Phe–Pro–D-Val sequences did not yield any amount of cyclic monomer and cyclic dimer. The change in the configurations of the Phe and Val residues around the Pro residue greatly affected the CD spectra in ethanol and the ^{1}H NMR spectra in DMSO-d_6 of the pentapeptide ethyl esters corresponding to four H–X–Pro–Y–Orn–Leu–ONSu's. A good correlation among the CD spectra, NMR spectra of the pentapeptide ethyl esters, and the main products in the cyclization of the active esters was found.

Gramicidin S (GS)[1] is an antibiotic cyclodecapeptide consisting of two identical pentapeptide sequences (Fig. 1).[2] In 1957, Schwyzer and Sieber reported that the cyclization of the H–Val–Orn(Tos)–Leu–D-Phe–Pro–*p*-nitrophenyl ester yielded the cyclic decapeptide (the ditosyl derivative of GS) as a main product, but not the cyclic pentapeptide.[3] Since then, various analogs of GS have been synthesized by this dimerization–cyclization of linear pentapeptide precursors related to GS with a protecting group on the side chain of the Orn residue.[4] Recently, we reported on the direct formation of GS by the cyclization of pentapeptide active esters having no protecting group on the side chain of the Orn residue.[5] Among the five succinimide esters (–ONSu) having Val, Orn, Leu, D-Phe, or Pro residues at each C-terminus, only H–D-Phe–Pro–Val–Orn–Leu–ONSu (peptide **1** shown in Fig. 2), having a sequence identical with that of the linear precursor pentapeptide in the biosynthesis of GS,[6] gave semi-GS (cyclic monomer) and GS (cyclic dimer) in yields of 15 and 38%, respectively. Other pentapeptide esters did not give GS. The process of the cyclization of peptide **1** is proposed as follows. In the intramolecular reaction, the active ester of the Leu residue slowly couples with the α-amino group of the D-Phe residue to give the semi-GS, but not with the δ-amino group of the Orn residue. In GS formation, the active ester dimerizes to a decapeptide active ester, which takes the GS like β-pleated sheet conformation and cyclized to afford GS. In addition, the conversion of the Orn and Leu residues into Lys and Ala residues, respectively, did not affect the reaction mode of peptide **1**.

However, the effect of the alteration of the configurations of the constituent amino acid residue for the cyclization of active ester **1** has not been yet established.

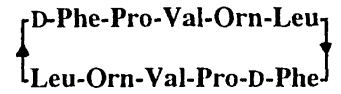

Fig. 1. Primary structure of GS.

H-D-Phe-Pro-Val-Orn-Leu-ONSu (1)
H-Phe-Pro-Val-Orn-Leu-ONSu (2)
H-Phe-Pro-D-Val-Orn-Leu-ONSu (3)
H-D-Phe-Pro-D-Val-Orn-Leu-ONSu (4)

H-D-Phe-Pro-Val-Orn-Leu-OEt (1E)
H-Phe-Pro-Val-Orn-Leu-OEt (2E)
H-Phe-Pro-D-Val-Orn-Leu-OEt (3E)
H-D-Phe-Pro-D-Val-Orn-Leu-OEt (4E)

Fig. 2. Pentapeptide active esters **1**—**4** and ethyl esters **1E**—**4E** related to GS.

On the other hand, concerning synthetic studies of various GS analogs by the cyclic dimerization of pentapeptide precursors having a Z-group on the side chain of the Orn residue, it has been reported that the configurations of the Phe and Val residues around the Pro residue in the active esters greatly affect the formation of the cyclic dimers (the diZ derivatives of GS analogs).[4a,7b]

In this paper, the cyclization of four linear pentapeptide–ONSu's **1** —**4** (Fig. 2) containing partial sequence, D-

X–Pro– L-Y, L-X–Pro– L-Y, L-X–Pro– D-Y, or D-X–Pro– D-Y (X = Phe, Y = Val), respectively, and having no protecting group on the side chain of the Orn residue was examined. In addition, the CD spectra in ethanol and the ^{1}H NMR spectra in DMSO-d_6 of pentapeptide ethyl esters **1E**—**4E** (Fig. 2) corresponding to peptides **1**—**4** were measured, in order to investigate the contribution of the D-Phe–Pro–Val sequence for a direct formation of GS by the dimerization-cyclization of H–D-Phe–Pro–Val–Orn–Leu–ONSu (peptide **1**).

Results and Discussion

The pentapeptide–ONSu's **2**—**4** were prepared by a similar method to those described in the case of peptide **1**.[5] These active esters (**1**—**4**) were cyclized in pyridine for 1 d at 25 °C (concentration of peptide in solution; 3 mM, M = mol dm^{-3}). Purification of the main products in the reaction mixture was performed by gel filtration using sephadex LH-20 and semipreparative high-performance liquid chromatography (HPLC). The primary structure of the products was supposed by amino acid analyses and fast-atom bombardment (FAB) mass spectra, which was confirmed by a direct comparison with authentic samples[7] synthesized according to conventional methods.

Peptide **1** gave semi-GS and GS in yields of 15 and 38%, respectively.[5] On the other hand, peptide **4** produced exclusively [D-Val]– semi-GS (cyclic monomer)[8] in 58% yield. Although peptides **2** and **3** gave many kinds of products, the formation of a cyclic monomer and a cyclic dimer by coupling between the ester group of the Leu residue and the α-amino group of the Phe residue could not be observed. These results indicate that the D-Phe–Pro sequence in peptide **1** is essential for the formation of GS and semi-GS, and the configuration of the Val residue following Pro residue significantly affects the yield of GS due to dimerization–cyclization.

The effect of the concentration of active esters **1**—**4** (0.3, 3, and 30 mM) in the cyclization yield was examined. Along with an increase in the concentration of peptide **1** having the D-Phe–Pro–Val sequence, the ratios of the GS to semi-GS in products increased, indicating that cyclic dimer (GS) formation competes with cyclic monomer (semi-GS) formation.[5] On the other hand, active ester **4** also gave exclusively [D-Val]–semi-GS, even at a concentration of 30 mM. Further, active esters **2** and **3** did not give any amount of cyclic monomer and dimer, even at a concentration of 0.3 mM. These results suggest that active ester **4**, having the D-Phe–Pro–D-Val sequence, possesses a very suitable conformation required for cyclic monomer formation by intramolecular cyclization, while peptides **2** and **3**, having Phe–Pro–Val or Phe–Pro–D-Val sequences at the N-terminus, respectively, have a conformation unsuitable for cyclization.

Next, the direct cyclization of peptides **1**—**4** was performed in ethanol and dimethyl sulfoxide (DMSO) at 25 °C for 1 d (concentration of peptides in solvent; 0.3, 3, and 30 mM). Triethylamine (10 molar amounts) was used as a base. Although the total yields were lower than those in pyridine, they gave cyclic products similar to those formed in pyridine, expect for the exclusive production of semi-GS by the cyclization of peptide **1** in DMSO at a concentration of 0.3 and 3 mM. That is, the modes of the cyclization of peptides **1**—**4** in these solvents seem to be similar.

In order to investigate the relationship between the cyclization mode and the conformations of peptides **1**—**4**, the CD spectra in ethanol and the ^{1}H NMR spectra in DMSO-d_6 of pentapeptide ethyl esters **1E**—**4E**, corresponding to peptides **1**—**4**, were measured.

The CD spectra of **1E**—**4E** in ethanol (concentration of peptides in solution; 3 mM) are shown in Fig. 3. The spectra are invariant over the concentration range from 0.3 to 15 mM, assuming that these peptides hold a monomeric state throughout this range.[9] The spectra of four ethyl esters (**1E**—**4E**) can be classified into two types (Fig. 3). Since all of peptides **1E**—**4E** are constituted with the same amino acid sequence, the difference among these spectra must reflect the distinction of their backbone conformations. The CD spectra of **2E** and **3E**, possessing a L-Phe residue at the N-terminus, were characterized by a peak at 219 nm and two troughs at 197 and 236 nm. On the other hand, the CD spectra of **1E** and **4E**, having a D-Phe residue at the N-terminus, showed a trough or a shoulder near to 217 nm, and a negative trough at 195 nm. To investigate the contribution of the constituent amino acid residue for the CD spectra, di-, tri-, and tetrapeptide esters related to peptides **1E** and **2E** having D-Phe or Phe residues at the N-terminus, respectively, were examined in ethanol (Fig. 4). D-Phe–Pro–OEt showed double troughs at 217 and 195 nm, while Phe–Pro–OMe showed two peaks at similar positions to that of D-Phe–Pro–OEt. Its features

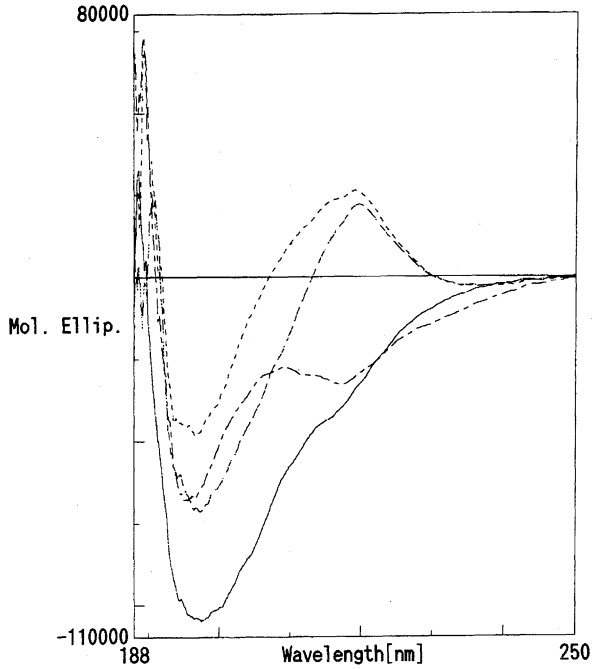

Fig. 3. CD spectra of **1E**—**4E** in ethanol at room temperature. peptide **1E**, —; peptide **2E**, – – –; peptide **3E**, - - -; peptide **4E**, – · –. *Data was obtained with a JASCO spectropolarimeter (model J-720w) using a 0.1 mm cell at room temperature. The peptide concentration is 3 mM.

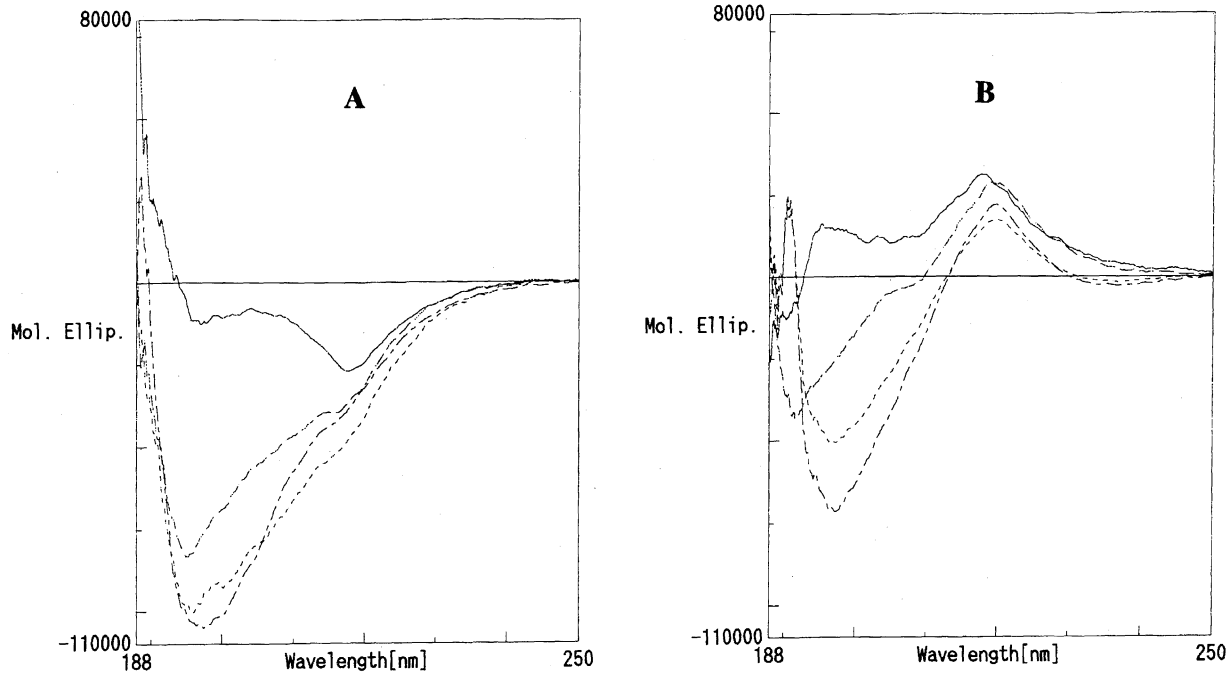

Fig. 4. CD spectra of the partial esters of **1E** and **2E** in ethanol at room temperature. [A] H–D-Phe–Pro–OEt, —; H–D-Phe–Pro–Val–OEt, – – –; H–D-Phe–Pro–Val–Orn–OEt, - - -; peptide **1E**, – – –. [B] H–Phe–Pro–OEt, —; H–Phe–Pro–Val–OEt, – – –; H–Phe–Pro–Val–Orn–OEt, - - -; peptide **2E**, – – –.

were a mirror image of each other. With an increase in the chain length, the depth of the trough at 195 nm increased, but the pattern near to 217 nm did not appreciably change. These results show that the major contributor to the positive or negative bands near to 217 nm is ordered structures formed by the Phe–Pro and D-Phe–Pro sequences, while the trough near to 195 nm reflects mainly the contribution of the random conformation formed by other peptide parts. The trough near to 195 nm of peptide **4E**, containing the D-Phe–Pro–D-Val sequence, is appreciably shallower than that of peptide **1E**, containing D-Phe–Pro–Val sequence (Fig. 3). Similar differences were also observed in the CD spectra of peptides **2E** and **3E**, having the Phe–Pro–Val or Phe–Pro–D-Val sequences, respectively (Fig. 3). These results suggest that the exchange of the Val residue to its antipode brings about a conformation change of the peptide parts at the C-terminus. Thus, these findings in CD studies suggest that the configurations of the Phe and Val residues around the Pro residue greatly affect both conformations of the N- and C-terminal parts, and the cyclization reaction of active ester **1**—**4**.

Next, the NMR spectra of peptides **1E**—**4E** were measured by 250 MHz ^{1}H NMR in DMSO-d_6, and are shown in Fig. 5. A small amount of a second component (<5% in peptide) in peptide **1E** and **4E** was observed in the 1D NMR spectra. This minor component was not conformationally analyzed in detail. The assignments of all protons were performed by means of COSY and HOHAHA. The chemical shift of the amide protons of peptide **1E**—**4E** are almost independent of the concentration of these peptides (3, 20, and 30 mM). In addition, the temperature coefficients of the chemical shifts of all amide protons of peptides **1E**—**4E** are $\geqq -4.6$ ppb/°C. These results suggest that peptides **1E**—**4E** are monomeric over the entire concentration range, and that the amide protons of the Val, Orn and Leu residues in these molecules do not involve an intramolecular hydrogen bond. The influences of an exchange of the Phe residue to its antipode appeared mainly in the chemical shifts of the protons of the Pro residue. The chemical shifts of the protons of the Pro residue in **1E**, having D-Phe residue at N-terminus, were similar to those of **4E**. The proton resonances of Pro residue in **2E** and **3E**, having the L-Phe residue at the N-terminus, were also observed at similar positions. However, appreciable differences among the proton chemical shifts of the Pro residues in these two groups were found. The proton resonances of Pro $^{\alpha}$CH in **2E** and **3E** fairly shifted to downfield compared with those of **1E** and **4E**. In **2E** and **3E**, the chemical shifts of the proton resonances for the diasterotopic Pro $^{\beta}$CH$_2$'s are separated by 0.30 and 0.26 ppm, respectively, while those of Pro $^{\gamma}$CH$_2$'s shared approximately a singlet at 1.80 ppm. On the contrary, in **1E** and **4E**, the chemical shifts of proton resonances for the diasterotopic Pro $^{\gamma}$CH$_2$'s are separated by 0.32 and 0.40 ppm, respectively, while those of the Pro $^{\beta}$CH$_2$'s shared approximately a singlet near to 1.75 ppm. In addition, the one proton of the Pro $^{\delta}$CH$_2$'s in peptides **1E** and **4E** shifted fairly upfield (−0.5 ppm) compared with those of peptides **2E** and **3E**. The splitting patterns of Phe $^{\beta}$CH$_2$ and D-Phe $^{\beta}$CH$_2$ in **1E**—**4E** showed two multiplets, indicating that they are unequivalent and have a hindered rotation in certain arrangements.[10,11] The differences among the proton chemical shifts of the Pro residues in **1E**—**4E** suggest that in **2E** and **3E** the aromatic ring of the Phe residue is closer to Pro $^{\alpha}$CH and Pro $^{\beta}$CH, and farther from Pro $^{\delta}$CH and Pro $^{\gamma}$CH on the NMR time scale. On the other hand, in

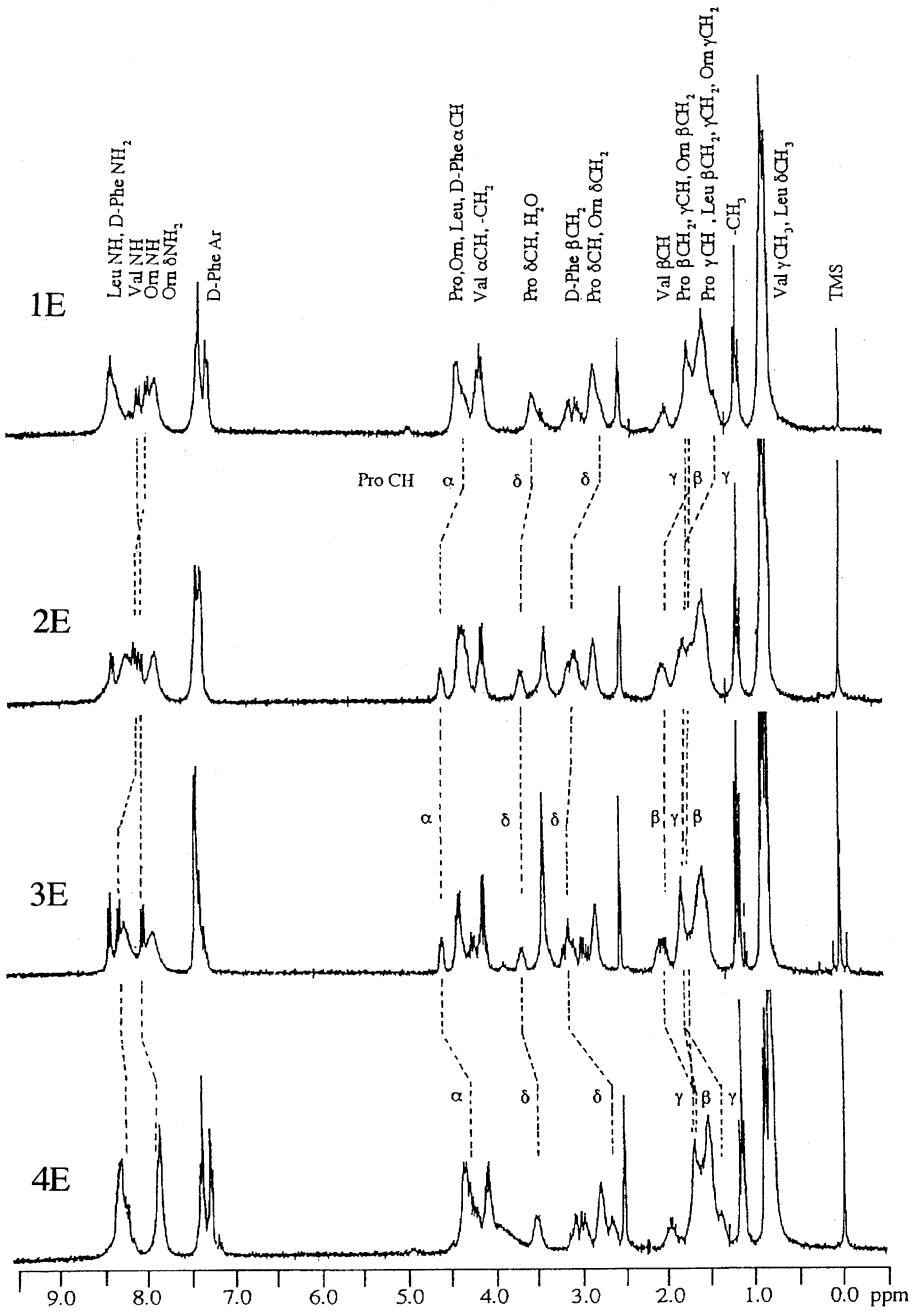

Fig. 5. The 250-MHz ^{1}H NMR spectra of **1E**—**4E** in DMSO-d_6 at 25 °C and changes in chemical shifts of protons of Pro residues. Data were obtained on a Bruker AM-250 instrument. Reference is trimethylsilane for the DMSO-d_6. Peptide concentration is about 20 mM.

1E and **4E**, the aromatic ring of the D-Phe residue is closer to Pro $^{\delta}$CH and Pro $^{\gamma}$CH, and further from Pro $^{\alpha}$CH and Pro $^{\beta}$CH.

Rae et al. reported concerning conformational studies of ^{1}H NMR of GS in DMSO-d_6 that the aromatic side chain of the D-Phe residue in the GS molecule is close to the Pro ring, and that one Pro $^{\delta}$CH strongly shielded by the aromatic ring.[11] These results indicate that the conformation of the N-terminus part held by the D-Phe–Pro sequence in H–D-Phe–Pro–Val–Orn–Leu–ONSu is similar to that of the D-Phe–Pro sequence in GS.

In addition, Brady et al. reported concerning studies of the cyclization of linear hexapeptides containing a D-Phe residue that the orientation of the N-terminal side chain, along with the consequent orientation of the amino group, is a significant factor governing the success or failure of a cyclization, and that the best linear precursor of a cyclic hexapeptide containing a D-amino acid residue is a sequence with the D residue at the N-teminus.[12] The present studies suggest that the presence of the D-Phe–Pro sequence in peptide 1 has an important role for formation of an ordered conformation, which may be favorable for the production of GS by dimerization–cyclization.

The influence of an exchange of the Val residue to its

antipode mainly affects the chemical shifts of the Val $^{\alpha}$NH and Orn $^{\alpha}$NH protons. The $^{\alpha}$NH proton resonances of the D-Val residues in **3E** and **4E** fairly shifted upfield in comparison with those of the L-Val residues in **1E** and **2E**. On the other hand, the $^{\alpha}$NH proton resonances of the Orn residues in **3E** and **4E** fairly shifted downfield in comparison with those of **1E** and **2E**. However, the chemical shifts of the $^{\alpha}$NH protons of the Leu residue at the C-terminus in **1E**—**4E** were little affected. These results indicate that a change in the configuration of the Val residue greatly influence the conformation of the –Val–Orn– sequence, and suggest that the conformational changes have an effect on the cyclization mode of these precursors.

Thus, the change in the configurations of the Phe and Val residue around the Pro residue greatly affected the conformations of pentapeptide ethyl esters corresponding to four H–X–Pro–Y–Orn–Leu–ONSu's. A good correlation among the CD spectra in ethanol, the NMR spectra in DMSO-d_6 of the pentapeptide ethyl esters, and the cyclization of the active esters was found. Consequently, the conformation of H–D-Phe–Pro–Val–Orn–Leu–ONSu in reaction solvents, such as ethanol and DMSO, is the most suitable for the direct formation of GS by a dimerization–cyclization reaction; also, it is very interesting that the sequence is identical with the linear precursor in the biosynthesis of GS.[6]

For a better understanding of the mode of those cyclizations, further detailed conformation analyses of the precursor pentapeptides and cyclic products is needed.

Experimental

All of the melting points are uncorrected. An amino acid analysis of each hydrolysate of the peptides was carried out with a Hitachi 835 amino acid analyzer. The molecular weights of the cyclic products were determined by using fast-atom bombardment (FAB) mass spectrometry on a JEOL JMS-D-300 mass spectrometer (in Asahi Chemical Industry Company).

Syntheses of *t*-Butoxycarbonyl (Boc)-pentapeptides. Boc-

Table 1. Physical Properties and Analytical Data for Intermediary Products and [D-Val]–semi-GS.[a,b,c,d]

No.	Compound	Mp °C	$[\alpha]_D^{25}$ (*c* 0.5 in DMF)	Formula	Found (%) (Calcd) C H N
1.	H–Phe–Pro–OEt·TFA	Oil	−3.6 (*c* 1.0 in EtOH)	—	—
2.	H–Phe–Pro–Val–OEt·TFA	Oil	−31.4 (*c* 1.0 in EtOH)	—	—
3.	H–Phe–Pro–Val–Orn–OEt·2HBr	177—180	−42.4 (*c* 1.0 in EtOH)	$C_{26}H_{41}N_5O_5\cdot 2HBr\cdot 2H_2O$	44.50 6.60 10.02 (44.52 6.75 9.98)
4.	H–D-Phe–Pro–OEt·TFA	122—124	−20.6 (*c* 1.0 in EtOH)	$C_{16}H_{22}N_2O_3\cdot TFA\cdot H_2O$	51.07 5.78 6.72 (51.18 5.97 6.63)
5.	H–D-Phe–Pro–Val–OEt·TFA	Oil	−110.8 (*c* 1.0 in EtOH)	—	—
6.	H–D-Phe–Pro–Val–Orn–OEt·2TFA	111—115	−104.2 (*c* 1.0 in EtOH)	$C_{26}H_{41}N_5O_5\cdot 2TFA\cdot 0.5H_2O$	48.70 5.70 9.43 (48.65 5.99 9.46)
7.	Boc–Phe–Pro–Val–Orn(Boc)–Leu–OEt	94—96	−40.1	$C_{42}H_{68}N_6O_{10}\cdot H_2O$	60.60 8.43 10.12 (60.41 8.45 10.06)
8.	Boc–Phe–Pro–D-Val–Orn(Boc)–Leu–OEt	85—88	−22.8	$C_{42}H_{68}N_6O_{10}\cdot 0.5H_2O$	61.20 8.32 10.21 (61.07 8.42 10.17)
9.	Boc–D-Phe–Pro–D-Val–Orn(Boc)–Leu–OEt	93—97	−32.5	$C_{42}H_{68}N_6O_{10}\cdot 0.5H_2O$	60.95 8.14 9.85 (61.07 8.42 10.17)
10.	Boc–Phe–Pro–Val–Orn(Boc)–Leu–OH	110—115	−33.2	$C_{40}H_{64}N_6O_{10}\cdot 0.5H_2O$	60.05 8.03 10.75 (60.21 8.21 10.53)
11.	Boc–Phe–Pro–D-Val–Orn(Boc)–Leu–OH	108—110	−21.8	$C_{40}H_{64}N_6O_{10}\cdot 0.5H_2O$	60.03 8.51 10.65 (60.21 8.21 10.53)
12.	Boc–D-Phe–Pro–D-Val–Orn(Boc)–Leu–OH	110—112	−25.6	$C_{40}H_{64}N_6O_{10}$	61.07 8.12 10.90 (60.89 8.18 10.65)
13.	H–Phe–Pro–Val–Orn–Leu–OEt·2TFA	115—116	−34.1 (*c* 0.2 in EtOH)	$C_{32}H_{52}N_6O_6\cdot 2TFA\cdot 0.5H_2O$	50.51 6.24 9.65 (50.64 6.49 9.84)
14.	H–Phe–Pro–D-Val–Orn–Leu–OEt·2TFA	113—114	−5.4 (*c* 0.2 in EtOH)	$C_{32}H_{52}N_6O_6\cdot 2TFA\cdot 0.5H_2O$	50.43 6.26 9.68 (50.64 6.49 9.84)
15.	H–D-Phe–Pro–D-Val–Orn–Leu–OEt·2TFA	114—116	−57.6 (*c* 0.2 in EtOH)	$C_{32}H_{52}N_6O_6\cdot 2TFA\cdot H_2O$	50.13 6.40 9.58 (50.11 6.54 9.74)
16.	Cyclo(–D-Phe–Pro–D-Val–Orn–Leu–)[e]	193—195	−29.8 (*c* 0.5 in EtOH)	$C_{30}H_{46}N_6O_5\cdot H_2O$	61.45 8.03 14.50 (61.20 8.22 14.27)

a) Compounds 10—12 were linear precursors used in the cyclization of pentapeptide–ONSu having no protecting group on the δ-amino group of Orn residue. b) Compounds 13—15 were pentapeptide ethyl esters used in the measurements of CD and NMR spectra. c) Compounds 3, 4, 6, and 10—16 were recrystallized from MeOH–ether. Compounds 7—9 were recrystallized from AcOEt–ether. d) Compound 16 was cyclic monomer produced by the cyclization of H–D-Phe–Pro–D-Val–Orn–Leu–ONSu in pyridine. e) Amino acid analysis Phe, 0.98; Pro, 1.00; Val, 0.97; Orn, 1.02; Leu, 0.97. MS (FAB), *m/z* 571 ($C_{30}H_{46}N_6O_5$, M+H$^+$, 48%).

pentapeptides, in which the δ-amino group of the Orn residue and the N-terminal amino group were protected by the Boc group, were prepared by a conventional method. In the synthesis of Boc–D-Phe–Pro–D-Val–Orn(Boc)–Leu–OH, as an example, Boc–D-Phe–Pro–D-Val–OBzl and Z–Orn(Boc)–Leu–OEt were prepared by a stepwise elongation using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCD·HCl) and 1-hydroxybenzotriazole (HOBt). Boc–D-Phe–Pro–Val–OBzl was converted into the corresponding acid by saponification. After the Z-group of Z–Orn(Boc)–Leu–OEt was removed by hydrogenolysis, the resulting ester was coupled with Boc–D-Phe–Pro–D-Val–OH to give Boc–D-Phe–Pro–D-Val–Orn(Boc)–Leu–OEt. This pentapeptide was saponified to afford Boc–D-Phe–Pro–D-Val–Orn(Boc)–Leu–OH. Other Boc-pentapeptides were synthesized in a similar manner. The peptides were characterized by elemental analyses, thin-layer chromatography, HPLC, and amino acid analyses of their hydrolysates. The physical properties and analytical data of these peptides are shown in Table 1.

Reaction of Pentapeptide-ONSu. Boc-pentapeptides (50—100 mg) were converted into the corresponding succinimide esters using HONSu and WSCD·HCl. Boc-pentapeptide–ONSu's were treated with trifluoroacetic acid (TFA) to remove all Boc groups. Pentapeptide–ONSu trifluoroacetates were dissolved in small amounts of *N*,*N*′-dimethylformamide, and the solutions were added dropwise into pyridine at 25 °C (concentration of the active esters was 3 mM). After the mixture was stirred for 1 d at 25 °C, the solvent was evaporated. The residues were dissolved in methanol and analyzed by HPLC. The main products from the reaction mixtures of D-Phe–Pro–D-Val–Orn–Leu–ONSu were purified by gel filtration on a Sephadex LH-20 column (1.5×150 cm) using methanol as the elution solvent and by reprecipitation from methanol-ether. The physical properties and analytical data for [D-Val]–semi-GS are shown in Table 1.

Determination of the Free Amino Group in Cyclic Peptide. A cyclic peptide isolated from reaction mixture in the synthesis of D-Phe–Pro–D-Val–Orn–Leu–ONSu was treated with 2, 4-dinitrofluorobenzene. The resulting dinitrophenyl cyclic peptide was hydrolyzed in 6 M HCl for 24 h at 110 °C. The free amino group of the peptide was confirmed by comparing the results of the amino acid analyses of the hydrolysates of both the DNP treated peptide and the nontreated peptide.

Syntheses of Di-, Tri-, Tetra-, and Pentapeptide Ethyl Esters. Di-, tri-, tetra-, and pentapeptide ethyl esters used in the measurements of CD and NMR spectra were prepared by a similar method as that described in the syntheses of Boc-pentapeptides. The analytical data for the esters are given in Table 1.

CD Spectroscopy. CD spectra were obtained with a JASCO sepectropolarimeter (model J-720) using 0.1, 1, and 10 mm cells at room temperature. The CD spectra of GS and its analogs were measured in ethanol solutions at a concentration of 0.3, 3, and 15 mM (1 M = 1 mol dm^{-3}).

NMR Spectroscopy. NMR spectra were measured in DMSO-d_6 at 25 °C (peptide concentration: ca. 3, 20, and 30 mM) on a Bruker AC-250 using standard pulse sequences and software. COSY and HOHAHA spectra with 1 K points in F2 and 256 points in F1 were recorded with a sweep width of 2500 Hz in the phase-sensitive mode using time-proportional phase incrementation. HOHAHA spectra were obtained with a mixing time of 130 ms. The temperature coefficients of the chemical shifts of the amide protons were obtained from least-squares fits to the data of 25, 35, 45, and 55 °C.

This work was supported by a Grant-in-Aid for Scientific Research No. 06640702 from the Ministry of Education, Science and Culture. We are grateful to the staff of the Research Laboratories of Asahi Chemical Industry Co., Ltd. for element analyses and measurements of the FAB mass spectra.

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