

Fig. 2. Synthetic scheme of des-Val¹, Leu^{3'}-GS.

idine at 25 °C for 1 d. The final concentration of the active esters was 3 mM (1 M=1 mol dm⁻³). High-performance liquid chromatography (HPLC) profiles of the crude cyclic products are shown in Fig. 3. The molecular weight of each cyclic product isolated by semipreparative HPLC was determined by FAB mass spectrometry. The cyclic products from the tetrapeptide active esters have widely different molecular

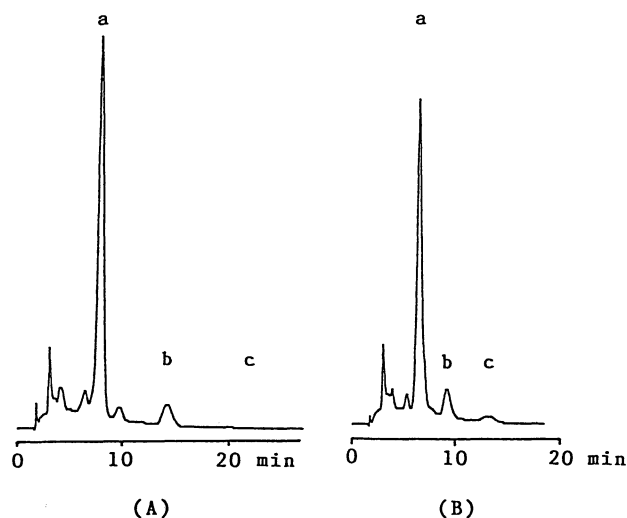


Fig. 3. HPLC profiles^{a)} of products in cyclization of H-Orn(Z)-Leu-D-Phe-Pro-ONSu (A) and H-Val-Orn(Z)-D-Phe-Pro-ONSu (B).

(a: cyclic dimer, b: cyclic trimer, c: cyclic tetramer)

a) Elution solvent used in these HPLC analysis was MeOH-H₂O (6:1).

weights. Compound **1b'** produced the cyclic dimer (30%), trimer (4%), and tetramer (0.5%); Compound **2b'** produced the cyclic dimer (35%), trimer (6%), and tetramer (0.5%). These active esters, however, did not produce a cyclic monomer. The numbers in parentheses are the yields of each cyclic product calculated from **1b** and **2b**. Recently, regarding the cyclization of H-Val-Orn(Z)-Leu-D-Phe-Pro-ONSu and H-Tyr(Bzl)-Val-Orn(Z)-Leu-D-Phe-Pro-ONSu containing the D-Phe-Pro sequence at the C-terminal, we reported that the former yields exclusively the cyclic dimer, while the latter produces the cyclic monomer and dimer.¹⁰⁾ The present results indicate that the lengths of these active esters greatly affect their mode of cyclization.

The yields, physical properties and analytical data of intermediary products and GS analogs are summarized in Tables 1 and 2.

Des-Val^{1,1'}-GS, des-Leu^{3,3'}-GS and des-Val¹, Leu^{3'}-GS showed no antibiotic activity against all of the microorganisms tested. We also found that cyclo(-Orn-D-Phe-Pro)₂ shows no activity.¹¹⁾ On the other hand, we have reported that some cyclododecapeptides related to GR with various sequences at the β -turn part show strong activity,^{7,8)} and that several cyclotetradecapeptides related to GS possess 1/4–1/8 activity of GS.⁹⁾ These results indicate that removing either two or four of the Val and/or Leu residues at positions 1, 1', 3, and 3' from GS results in a loss of activity, while the addition of two or four amino acid residues to GS makes it feasible to take a suitable conformation for exhibiting antibiotic activity.

The CD spectra of these synthetic peptides and GS in an aqueous solution are shown in Fig. 4. These CD

Table 1. Yields and Analytical Data of Intermediary Products of GS Analogs

	Yield %	Mp °C	[α] _D ²⁵ / (c 1, DMF)		Elemental analysis/%					
					C	H	N	C	H	N
1a.	60 ^{a)}	89–92	–35.4	C ₄₅ H ₅₉ O ₉ N ₅ · 0.5H ₂ O	Found: 65.79	7.34	8.70	Calcd: 65.67	7.35	8.51
1b.	92	94–96	–40.7	C ₃₈ H ₅₃ O ₉ N ₅ · 0.5H ₂ O	Found: 62.59	7.70	9.02	Calcd: 62.28	7.43	9.35
1c.	30	120–126	–6.7 ^{b)}	C ₆₆ H ₈₆ O ₁₂ N ₁₀ · H ₂ O	Found: 64.18	7.24	11.13	Calcd: 64.47	7.21	11.39
2a.	67 ^{a)}	72–75	–21.7	C ₄₄ H ₅₇ O ₉ N ₅ · 0.5H ₂ O	Found: 65.27	7.20	8.82	Calcd: 65.33	7.23	8.66
2b.	97	73–78	–27.3	C ₃₇ H ₅₁ O ₉ N ₅	Found: 62.25	7.28	9.38	Calcd: 62.61	7.24	9.87
2c.	35	131–137	+8.7 ^{b)}	C ₆₄ H ₈₂ O ₁₂ N ₁₀ · 1.5H ₂ O	Found: 63.12	6.97	11.65	Calcd: 63.51	7.08	11.57
3b.	87	116–122	–36.0	C ₇₀ H ₉₄ O ₁₅ N ₁₀ · 1.5H ₂ O	Found: 62.74	7.22	10.42	Calcd: 62.62	7.28	10.43
3c.	64	120–125	–0.3 ^{b)}	C ₆₅ H ₈₄ O ₁₂ N ₁₀ · 2H ₂ O	Found: 63.37	7.03	11.49	Calcd: 63.29	7.19	11.36

a) The yields of **1a** and **2a** were calculated on the basis of the amount of Pro-OBzl · HCl as a starting material.

b) c 0.5.

Table 2. Yields and Analytical Data of GS Analogs

1d.	Yield, 80%; mp, 232–235 °C; [α] _D ²⁵ –97.1° (c 0.5, EtOH); MS(FAB) <i>m/z</i> 944 (MH ⁺). Amino acid analysis: Orn, 1.96; Leu, 2.07; Phe, 1.97; Pro, 2.00. Found: C, 55.82; H, 7.60; N, 12.96%. Calcd for C ₅₀ H ₇₄ O ₈ N ₁₀ · 2HCl · 3.5 H ₂ O: C, 55.65; H, 7.75; N, 12.98%.
2d.	Yield, 80%; mp, 251–254 °C; [α] _D ²⁵ –42.2° (c 0.5, EtOH); MS(FAB); <i>m/z</i> 915 (MH ⁺). Amino acid analysis: Val, 2.09; Orn, 2.03; Phe, 1.95; Pro, 1.92. Found: C, 53.31; H, 7.29; N, 12.80%. Calcd for C ₄₈ H ₇₀ O ₈ N ₁₀ · 2HCl · 5H ₂ O: C, 53.47; H, 7.67; N, 12.99 %.
3d.	Yield, 90%; mp, 226–230 °C; [α] _D ²⁵ –108.8° (c 0.5, EtOH); MS(FAB) <i>m/z</i> 929 (MH ⁺). Amino acid analysis: Val, 1.05; Orn, 2.00; Leu, 1.07; Phe, 1.90; Pro, 1.99. Found: C, 54.30; H, 7.41; N, 12.89 %. Calcd for C ₄₉ H ₇₂ O ₈ N ₁₀ · 2HCl · 4.5H ₂ O: C, 54.34; H, 7.72; N, 12.93%.

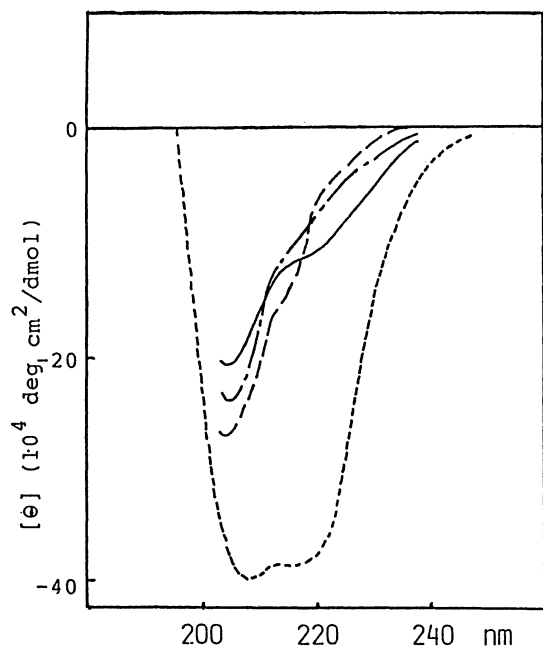


Fig. 4. CD spectra of GS and its analogs in aqueous solution.
des-Val^{1,1'}-GS, —; des-Leu^{3,3'}-GS, — — —; des-Val¹,
Leu^{3'}-GS, - - - -; GS, ·····.

spectra resemble each other; although a trough is observed at 204 nm and a shoulder near 217 nm, their CD patterns differ from that of GS, even though these peptides possess the D-Phe-Pro sequence. We recently reported that the CD spectra of cyclo(-Val-Orn-Leu-D-Phe-Pro-Tyr)₂ and cyclo(-Val-Orn-Leu-Leu-D-Phe-Pro-Leu)₂ having the D-Phe-Pro sequence are similar to that of GS.^{7,9)} On the other hand, the CD spectrum of cyclo(-Orn-D-Phe-Pro)₂ resembles those of the synthetic cyclooctapeptides, rather than GS; a trough was observed near 200 nm and a shoulder near 220 nm.¹²⁾ Kopple et al. reported from NMR studies that this cyclohexapeptide possesses a β -turn caused by a D-Phe-Pro sequence as GS.¹³⁾ Further, the same β -turn was also found in cyclo(-Ala-Gly-D-Phe-Pro)₂, which possesses the Ala-Gly sequence in place of the Orn-Leu or Val-Orn sequence of the cyclooctapeptides synthesized in our studies.¹⁴⁾ These facts suggest that the synthetic cyclooctapeptides have a β -turn similar to that of GS, and that the difference between these synthetic cyclooctapeptides and GS in CD spectra may result from removing two of Val and /or Leu residue from GS, in other words, a distinction of the ring size.

Experimental

All melting points are uncorrected. The CD spectra were measured by a JASCO J-500 spectropolarimeter at a concentration of $1.5\text{--}2.0 \times 10^{-4}$ M. The molecular weights of these

synthetic peptides were determined by FAB mass spectrometry using a JEOL JMS-D-300 mass spectrometer. Amino acid analyses were carried out using a Hitachi 835 amino acid analyzer after hydrolysis in 6 M HCl at 110 °C for 24 h. HPLC analysis was carried out using an 800 series (JASCO). A Finepak SIL C18 column (10 μ m, 250 $\times$ 4.6 mm I.D.; or 250 $\times$ 6.7 mm I.D., JASCO) was used: flow rate, 1 ml min⁻¹; solvent, MeOH-H₂O (6:1) or MeOH-5%-NaClO₄ (4:1); monitoring wavelength, 220 nm.

Microbiological Assays. The microorganisms employed were *Staphylococcus aureus* ATCC 6538, *Streptomyces pyogenes* N.Y.5, *Corynebacterium diphtheriae* P.W.8, *Micrococcus pyogenes* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ-JC2, and *Proteus vulgaris* OX 19. The minimum concentration of compounds necessary to completely inhibit the growth of these microorganisms was determined by an agar dilution method with 10^6 organisms per milliliter.

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References

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