Studies of Peptide Antibiotics. XXXIX.[1] Syntheses of Gramicidin S Analogs Containing L-Histidine in Place of L-Ornithine

Kazuhiko NONAKA,† Kazuki SATO, Shigeyuki TERADA, Tetsuo KATO, and Nobuo IZUMIYA*

Laboratory of Biochemistry, Faculty of Science 33, Kyushu University, Higashi-ku, Fukuoka 812

(Received February 10, 1978)

Two analogs of gramicidin S, [2-L-histidine]-gramicidin S (**14**) and [2,2′-di-L-histidine]-gramicidin S (**19**), were synthesized to compare the biological role of δ-amino groups in L-ornithine residues in the antibiotic with that of weakly basic imidazolyl groups in L-histidine residues. Compound **14** exhibited identical activity to that of gramicidin S, but **19** was only 1/8 as active. The optical rotatory dispersions of these analogs and gramicidin S were nearly the same: molecular conformation of these peptides must be very similar. Compounds **14** and **19** showed esterolytic activity against *p*-nitrophenyl acetate. The activity of **14** was higher than that of imidazole, **14** being more active than **19**.

There is ample evidence that the presence of charged amino groups in ornithine residues is essential to the antibacterial activity of gramicidin S (GS). Change in the length of the methylene chain in basic amino acid residues does not alter the activity.[2,3] Conversion of the amino group into strongly basic guanidino group afforded similarly active [Arg$^{2,2'}$]-GS.[4] On the other hand, acylation of the amino groups caused drastic decrease in activity.[5] However, no attempt seems to have been made to substitute the ornithine residues with weakly basic histidine residues and to examine the role of amino and imidazolyl groups for antimicrobial activity. This paper deals with the syntheses, antibacterial properties, and ORD measurements of [His^2]-GS (namely, [His^2]-hybrid GS) and [His$^{2,2'}$]-GS, in which the L-ornithyl residues in 2 and 2′ positions are replaced, singly or together, by L-histidine residues (Fig. 1). Possible esterolytic activity of these analogs was tested, because histidine is known to constitute a part of the catalytic site in several esterolytic enzymes such as α-chymotrypsin and subtilisin.

The syntheses of [His^2]-GS and [His$^{2,2'}$]-GS are outlined in Figs. 2 and 3.[6] The principle of the syntheses is to locate the leucyl residue at the *C*-terminal.[7]

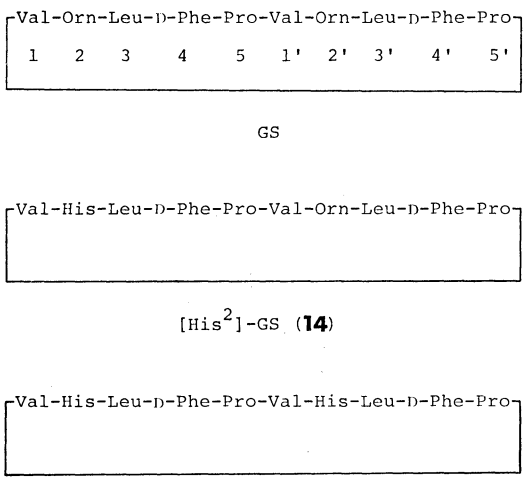

Fig. 1. Structure of GS and its analogs.

† Present address: Teikoku Hormone Mfg. Co., Ltd., Takatsu-ku, Kawasaki 213.

The imidazolyl function of histidine is protected by the benzyloxycarbonyl group only in the first coupling step, and unprotected in later steps, because azide coupling is known to proceed without affecting imidazolyl function, and because there is no suitable and conveniently cleavable protecting group for imidazolyl function.

For the synthesis of [His^2]-GS (**14**), Boc-pentapeptide ester (**7**) was perpared by the coupling of Boc-tripeptide hydrazide (**6**) with dipeptide ester (**2**) which was obtained by mixed anhydride coupling of Z–His(Z)–OH and H–Leu–OEt and subsequent hydrogenation. Boc–Pro–Val–OEt (**3**) and Boc–D-Phe–Pro–Val–OEt (**5**) were obtained similarly. A part of **7** was converted into Boc-pentapeptide hydrazide (**8**). Coupling of **8** and **9**, which was derived from Boc–D-Phe–Pro–Val–Orn(Z)–Leu–OEt,[8] afforded Boc-decapeptide ester (**10**). This was converted into the corresponding hydrazide (**11**) which was then subjected

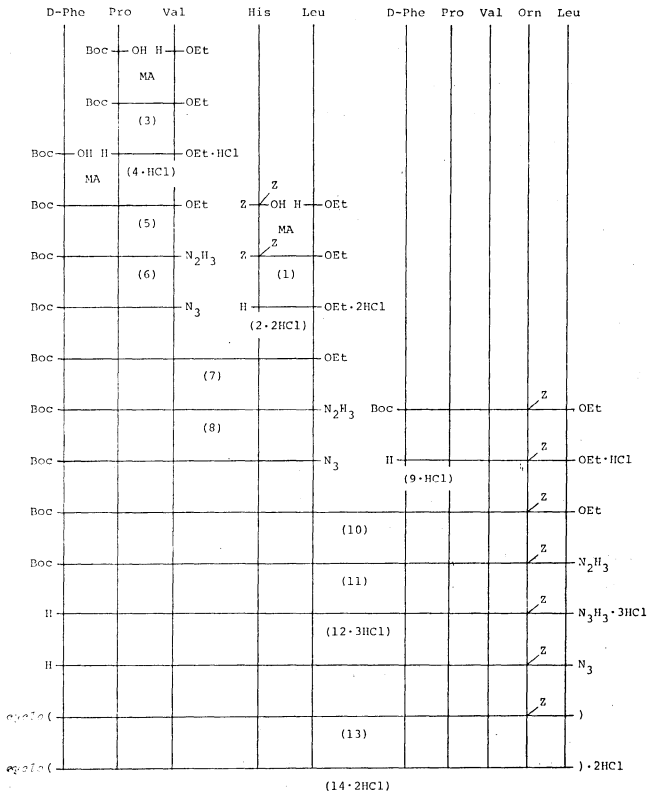

Fig. 2. Synthesis of [His^2]-GS.

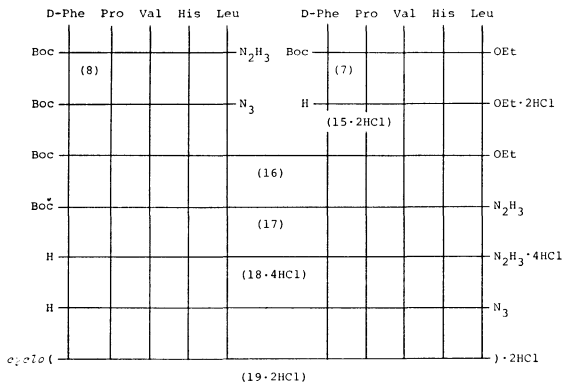

Fig. 3. Synthesis of [His$^{2,2'}$]-GS.

TABLE 1. ANTIBACTERIAL ACTIVITY OF GS AND ITS ANALOGS
(Minimum inhibitory concentration, μg/ml)

Strain	GS	[His^2]-GS (**14**)	[His$^{2,2'}$]-GS (**19**)
Staphylococcus aureus	3.12	3.12	50
Staphylococcus epidermidis	3.12	3.12	25
Bacillus subtilis	1.56	1.56	12.5
Escherichia coli	>100	>100	>100

to cyclization. The protected cyclodecapeptide (**13**) was obtained in 54% yield. A product **14** was obtained by hydrogenation of **13** in 75% yiled. Another product **19** was synthesized from cyclization of linear decapeptide precursor (**16**) instead of doubling cyclization with use of pentapeptide precursor (**7**). Synthesis of **19** was carried out thus because the results of previous experiments suggested that cyclization from **7** could give a mixture of cyclic penta- and decapeptides and, furthermore, the content of the cyclic pentapeptide might be considerably high.[9]

The homogeneity of **14** and **19** was confirmed by paper and thin-layer chromatography, paper electrophoresis and elemental analysis. Intermediates containing histidine such as **7** and **13** and the final product **19** were purified by silica gel column chromatography, and intermediates **10** and **16** by Sephadex LH-20, instead of the usual acid washing in order to avoid loss of the basic peptides. Satisfactory results were obtained for elemental analyses of all the crystalline compounds.

Table 1 shows antibacterial activities of **14**, **19**, and GS. Activity of **14** was exactly the same as that of GS, the presence of one amino and additional one imidazolyl groups apparently being enough to exhibit full activity. In contrast, **19** was only 1/8 or 1/16 times active as compared with GS. This result is interesting but somewhat unusual, because many amino acid-substituted analogs gradually change their activity from mono- to di-substitution. We tentatively explain the apparent discrepancy as follows: [His^2]-GS can gain access to bacterial membrane by ionic interaction between amino group and phospholipid.[5] A weakly basic imidazolyl group also can make a salt-like linkage

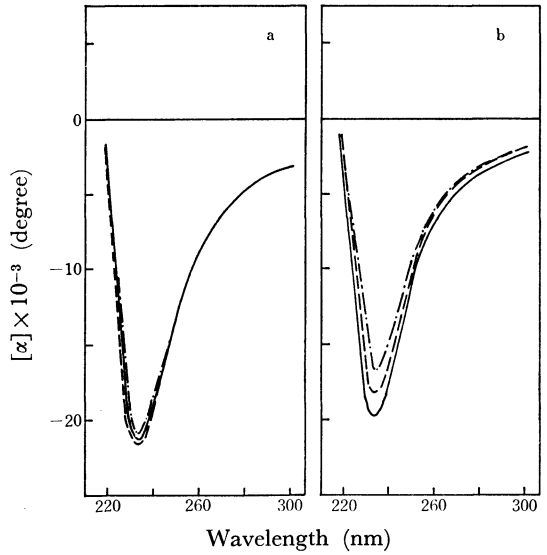

Fig. 4. ORD curves of GS and its analogs.
Solvent: a, EtOH; b, 8 M urea. Curve: ——, GS; ----, [His^2]-GS; —•—, [His$^{2,2'}$]-GS.

TABLE 2. HYDROLYSIS OF *p*-NITROPHENYL ACETATE WITH CATALYSTS[a]

Catalyst	Second order rate constant ($M^{-1} \cdot min^{-1}$)	
	20% EtOH	50% EtOH
[His^2]-GS (**14**)	38	12
[His$^{2,2'}$]-GS (**19**)	22	7
Boc-pentapeptide-OEt (**7**)	10	10
GS	0	0
Imidazole	22	13

a) Buffer, 1/30 M phosphate at pH 7.86 containing EtOH. Temp, 25 °C. Concentrations, 1.4×10^{-5} M and 6×10^{-5} M for *p*-nitrophenyl acetate and each catalyst, respectively.

with the phospholipid, when the antibiotic sufficiently approaches the membrane. However, ionic interaction between [His$^{2,2'}$]-GS and the membrane may be so weak that it is difficult for the antibiotic to come near the lipid membrane, thus causing weakening of its antimicrobial activity.

Figure 4-a shows the ORD curves of **14**, **19**, and GS measured in ethanol, all the curves being superimposable. In 8 M urea, which causes denaturation of various polypeptides, a slight deviation was observed, the trough at 233 nm being slightly shallower in the case of **14** and **19** (Fig. 4-b). The results of ORD measurements indicate that the conformation of the analogs must be very similar to that of GS but the stability of the analogs decreases with the number of the substitution. Dygert *et al.* proposed the presence of intramolecular hydrogen bondings between δ-amino groups in ornithine and carbonyl groups in D-phenylalanine.[10] Loss of these hydrogen bondings might cause slight instabilization in the peptide backbone of the analogs.

Table 2 shows catalytic activities of **14** and **19** on

the hydrolysis of *p*-nitrophenyl acetate. Experimental conditions are similar to those described by Katchalski *et al.*[11] In 1/30 M phosphate buffer, pH 7.86, containing 20% ethanol, catalytic activity of **14** was enhanced, but the enhancement disappeared in a more hydrophobic solvent, 50% ethanol. This suggests that the enhancement was caused by the hydrophobic interaction between the peptides and the substrate. Nakajima and Okawa reported a similar enhanced catalytic activity of cyclo(–L-His–L-Glu–L-Cys–D-Phe–Gly–)$_2$ on the hydrolysis of *p*-nitrophenyl acetate.[12] Imanishi *et al.* observed strongly enhanced catalytic activity of cyclo(–L-His–D-Leu–) on *p*-nitrophenyl laurate.[13] Since **7** shows no enhancement on the catalysis, the mere presence of an imidazolyl group in a peptide does not give high catalytic activity. Rigid β-sheet backbone conformation of **14** and **19** and hydrophobic environment derived from this conformation should be responsible for the activity. The high activity of **14** is interesting because it contains only one histidine residue. The presence of a free amino group in ornithine might cause additional accelerating effect.

Experimental

All the melting points are uncorrected. TLC was carried out on silica gel G (Merck) with the following solvent systems, the ratio in parentheses after each solvent system being indicated by vol: R_f^1, $CHCl_3$–MeOH (5:1); R_f^2, $CHCl_3$–MeOH–AcOH (95:5:1); R_f^3, *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). Optical rotations were measured on a Union high sensitivity polarimeter PM-71. Amino acid analyses were performed with a Hitachi amino acid analyzer KLA-5.

*Z-His(Z)-Leu-OEt (**1**).* To a chilled solution of Z-His(Z)-OH·EtOH (5.84 g, 12.4 mmol) and NMM (1.36 ml, 12.4 mmol) in THF (10 ml) was added isobutyl chloroformate (1.36 ml, 12.4 mmol) at −15 °C. After 10 min, a chilled solution of H-Leu-OEt·TsOH (4.10 g, 12.4 mmol) and NMM (1.62 ml, 12.4 mmol) in CH_2Cl_2 (10 ml) was added. The mixture was stirred at 0 °C for 1 h and at room temperature overnight, evaporated *in vacuo*, and the residue was dissolved in EtOAc. The solution was washed successively with 4% $NaHCO_3$, 10% citric acid and water, dried (Na_2SO_4), and evaporated. The resulting solid was recrystallized from EtOH–ether; yield, 5.60 g (80%); mp 116—118 °C; $[\alpha]_D^{20}$ −6.4° (*c* 0.5, EtOH); R_f^1 0.75, R_f^2 0.46.

Found: C, 63.78; H, 6.39; N, 9.96%. Calcd for $C_{30}H_{36}O_7N_4$: C, 63.81; H, 6.43; N, 9.92%.

*H-His-Leu-OEt·2HCl (**2**·2HCl).* Compound **1** (3.95 g, 7 mmol) dissolved in 0.2 M hydrogen chloride in EtOH (70 ml) was hydrogenated using palladium black as a catalyst. After 5 h, the filtrate from the catalyst was evaporated. Addition of ether to the oily residue gave hygroscopic crystals, which were recrystallized from EtOH–ether; yield, 2.09 g (81%); R_f^1 0.17.

*Boc-Pro-Val-OEt (**3**).* This was prepared from Boc-Pro-OH (6.46 g, 30 mmol) and H-Val-OEt·TsOH (9.52 g, 30 mmol) as described for **1**. The product was recrystallized from EtOH–ether–petroleum ether; yield, 7.50 g (73%); mp 72—75 °C; $[\alpha]_D^{20}$ −70.1° (*c* 0.5, EtOH); R_f^1 0.88, R_f^2 0.66.

Found: C, 59.35; H, 8.80; N, 8.06%. Calcd for $C_{17}H_{30}O_5N_2$: C, 59.62; H, 8.83; N, 8.18%.

*H-Pro-Val-OEt·HCl (**4**·HCl).* Compound **3** (3.60 g, 10.5 mmol) was dissolved in 2.8 M hydrogen chloride in EtOAc (75 ml). The solution was allowed to stand at room temperature for 30 min and then evaporated to leave an oil; yield, 2.93 g (100%); R_f^1 0.32.

*Boc-D-Phe-Pro-Val-OEt (**5**).* This was prepared from Boc-D-Phe-OH (2.79 g, 10.5 mmol) and **4**·HCl (2.93 g, 10.5 mmol) as described for **1**. The product was obtained as an oil; yield, 4.63 g (90%); R_f^1 0.82.

*Boc-D-Phe-Pro-Val-N_2H_3 (**6**).* A solution of **5** (4.63 g, 9.46 mmol) and hydrazine hydrate (9.18 ml, 189 mmol) in MeOH (40 ml) was allowed to stand at room temperature for 3 days. The solution was evaporated and the residue was dissolved in $CHCl_3$ (200 ml). The solution was washed with water, dried (Na_2SO_4), and evaporated. The residual oil was solidified by the addition of ether, and the product was recrystallized from EtOH–ether; yield 3.59 g (80%); mp 158—160 °C; $[\alpha]_D^{20}$ −86.8° (*c* 0.5, EtOH); R_f^1 0.65, R_f^2 0.31.

Found: C, 60.40; H, 7.82; N, 14.58%. Calcd for $C_{24}H_{37}O_5N_5$: C, 60.61; H, 7.84; N, 14.73%.

*Boc-D-Phe-Pro-Val-His-Leu-OEt (**7**).* To a solution of **6** (1.76 g, 3.7 mmol) in DMF (70 ml) were added 2.8 M hydrogen chloride in EtOAc (3.96 ml) and isopentyl nitrite (0.57 ml, 4.07 mmol) at −60 °C. After being left to stand at −20 °C for 10 min, the solution was cooled again to −60 °C and neutralized with TEA (1.55 ml, 11.1 mmol). To this solution was added a chilled solution of **2**·2HCl (2.05 g, 5.55 mmol) and TEA (1.55 ml, 11.1 mmol) in DMF (30 ml). The reaction mixture was stirred at 0 °C for 48 h and then evaporated. The residue was dissolved in EtOAc and the solution was washed successively with 4% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The oily product was applied to a column (5×50 cm) of silica gel (Merck) and the column was washed with $CHCl_3$–EtOH–AcOH (95:5:1) (3200 ml). The product was then eluted with a mixture (1500 ml) of $CHCl_3$–EtOH (5:1). Each fraction was assayed by TLC, and the fractions containing the desired product were evaporated to leave an oil which was crystallized by the addition of ether and petroleum ether; yield, 2.14 g (78%); mp 89—92 °C; $[\alpha]_D^{20}$ −74.5° (*c* 0.5, EtOH); R_f^1 0.66, R_f^2 0.07.

Found: C, 58.83; H, 7.61; N, 12.48%. Calcd for $C_{38}H_{57}O_8N_7\cdot 2H_2O$: C, 58.82; H, 7.92; N, 12.64%.

*Boc-D-Phe-Pro-Val-His-Leu-N_2H_3 (**8**).* Compound **7** (601 mg, 0.81 mmol) was treated with hydrazine hydrate (1.58 ml, 32.5 mmol) as described for **6**; yield, 498 mg (84%); mp 170—175 °C; $[\alpha]_D^{20}$ −69.4° (*c* 0.5, EtOH); R_f^1 0.53, R^2 0.04.

Found: C, 58.15; H, 7.58; N, 16.92%. Calcd for $C_{36}H_{55}O_7N_9\cdot H_2O$: C, 58.13; H, 7.72; N, 16.95%.

*H-D-Phe-Pro-Val-Orn(Z)-Leu-OEt·HCl (**9**·HCl).* This was prepared from Boc-D-Phe-Pro-Val-Orn(Z)-Leu-OEt[8] (2.21 g, 2.6 mmol) as described for **4**·HCl. The oily product was crystallized by the addition of ether and petroleum ether; yield, 1.85 g (91%); mp 111—120 °C; $[\alpha]_D^{20}$ −120° (*c* 0.5, EtOH); R_f^1 0.67, R_f^2 0.14.

Found: C, 60.80; H, 7.71; N, 10.45%. Calcd for $C_{40}H_{58}O_8N_6\cdot HCl$: C, 61.01; H, 7.55; N, 10.67%.

*Boc-D-Phe-Pro-Val-His-Leu-D-Phe-Pro-Val-Orn(Z)-Leu-OEt (**10**).* This was prepared from **8** (327 mg, 0.45 mmol) and **9**·HCl (425 mg, 0.54 mmol) as described for **7**. The crude product dissolved in MeOH (5 ml) was applied to a column (2.8×156 cm) of Sephadex LH-20. The column was developed with MeOH, the fractions with the desired product were evaporated, and the oily residue was crystallized by the addition of ether; yield, 408 mg (63%); mp 135—138 °C; $[\alpha]_D^{20}$ −106° (*c* 0.3, EtOH); R_f^1 0.69, R_f^2 0.13.

Found: C, 62.01; H, 7.63; N, 12.42%. Calcd for $C_{76}H_{109}O_{15}N_{13}\cdot 3/2H_2O$: C, 62.02; H, 7.67; N, 12.37%.

Boc-D-Phe-Pro-Val-His-Leu-D-Phe-Pro-Val-Orn(Z)-Leu-

*N_2H_3 (**11**).* Compound **10** (387 mg, 0.27 mmol) was treated with hydrazine hydrate (0.26 ml, 5.4 mmol) as described for **6**; yield, 359 mg (94%); mp 140—145 °C; $[\alpha]_D^{20}$ −82.1° (*c* 0.5, MeOH); R_f^1 0.49, R_f^2 0.05.

Found: C, 60.45; H, 7.55; N, 14.38%. Calcd for $C_{74}H_{107}O_{14}N_{15}\cdot 2H_2O$: C, 60.60; H, 7.63; N, 14.32%.

*H–D-Phe–Pro–Val–His–Leu–D-Phe–Pro–Val–Orn(Z)–Leu–N_2H_3·3HCl (**12**·3HCl).* Compound **11** (341 mg, 0.24 mmol) was treated with 0.1 M hydrogen chloride in formic acid (7.4 ml) as described for **4**·HCl and the oily product was crystallized by the addition of ether; yield, 330 mg (96%); mp 174—178 °C; $[\alpha]_D^{20}$ −154° (*c* 0.5, MeOH); R_f^1 0.22.

Found: C, 56.81; H, 7.17; N, 14.51%. Calcd for $C_{69}H_{99}O_{12}N_{15}\cdot 3HCl\cdot H_2O$: C, 56.84; H, 7.19; N, 14.41%.

*cyclo(–D-Phe–Pro–Val–His–Leu–D-Phe–Pro–Val–Orn(Z)–Leu–) (**13**).* To a solution of **12**·3HCl (310 mg, 0.22 mmol) in DMF (4 ml) were added 2.8 M hydrogen chloride in EtOAc (0.24 ml) and isopentyl nitrite (0.031 ml, 0.22 mmol) in DMF (0.3 ml) at −30 °C. After 15 min, the reaction mixture was added to pyridine (66 ml) at 0 °C. After being stirred at 5 °C for 4 days, the solution was evaporated and the addition of water afforded a white precipitate, which was filtered and washed with water. The crude product was purified with a column (3.3×42 cm) of silica gel using a mixture of $CHCl_3$–EtOH (5:1) as a developing agent as described for **6**; yield, 151 mg (54%); mp 238—240 °C; $[\alpha]_D^{20}$ −295° (*c* 0.5, MeOH); R_f^1 0.66, R_f^2 0.05.

Found: C, 61.07; H, 7.28; N, 13.25%. Calcd for $C_{65}H_{95}O_{12}N_{13}\cdot 3H_2O$: C, 61.26; H, 7.53; N, 13.46%.

*cyclo(–D-Phe–Pro–Val–His–Leu–D-Phe–Pro–Val–Orn–Leu–)·2HCl ([His^2]-GS·2HCl) (**14**·2HCl).* Compound **13** (78 mg, 0.06 mmol) dissolved in 0.05 M hydrogen chloride in MeOH (2.7 ml) was hydrogenated as described for **2**·2HCl. The product was recrystallized from EtOH–ether; yield, 56 mg (75%); mp 274—275 °C (dec); $[\alpha]_D^{20}$ −283° (*c* 0.5, EtOH); R_f^1 0.35, R_f^3 0.81. Amino acid ratios in acid hydrolyzate; Phe 2.10, Pro 2.05, Val 1.96, His 1.06, Orn 1.08, Leu 2.00.

Found: C, 56.37; H, 7.40; N, 13.55%. Calcd for $C_{61}H_{89}O_{10}N_{13}\cdot 2HCl\cdot 4H_2O$: C, 55.95; H, 7.62; N, 13.91%.

*H–D-Phe–Pro–Val–His–Leu–OEt·2HCl (**15**·2HCl).* This was prepared from **7** (592 mg, 0.8 mmol) as described for **4**·HCl. The oily product was crystallized by the addition of ether and petroleum ether; yield, 520 mg (91%); mp 190—200 °C; $[\alpha]_D^{20}$ −147° (*c* 0.5, EtOH); R_f^1 0.26.

Found: C, 54.64; H, 7.44; N, 13.16%. Calcd for $C_{33}H_{49}O_6N_7\cdot 2HCl\cdot H_2O$: C, 54.24; H, 7.31; N, 13.42%.

*Boc–(D-Phe–Pro–Val–His–Leu)$_2$–OEt (**16**).* This was prepared from **8** (363 mg, 0.5 mmol) and **15**·2HCl (428 mg, 0.6 mmol) as described for **7**. The crude product was purified by a column (2.8×156 cm) of Sephadex LH-20 as described for **10**; yield, 213 mg (32%); mp 150—156 °C; $[\alpha]_D^{20}$ −123° (*c* 0.3, EtOH); R_f^1 0.61.

Found: C, 59.45; H, 7.37; N, 13.97%. Calcd for $C_{69}H_{100}O_{13}N_{14}\cdot 3H_2O$: C, 59.72; H, 7.70; N, 14.13%.

*Boc–(D-Phe–Pro–Val–His–Leu)$_2$–N_2H_3 (**17**).* Compound **16** (200 mg, 0.15 mmol) was treated with hydrazine hydrate (0.15 ml, 3 mmol) as described for **6**; yield, 181 mg (91%); mp 190—192 °C (dec); $[\alpha]_D^{20}$ −111° (*c* 0.4, MeOH); R_f^1 0.30.

Found: C, 59.16; H, 7.41; N, 16.31%. Calcd for $C_{67}H_{99}O_{12}N_{16}\cdot 2H_2O$: C, 59.31; H, 7.65; N, 16.52%.

*H–(D-Phe–Pro–Val–His–Leu)$_2$–N_2H_3·4HCl (**18**·4HCl).* Compound **17** (166 mg, 0.13 mmol) was treated with 0.1 M hydrogen chloride in formic acid (5.2 ml) as described for **4**·HCl and the oily product was crystallized by the addition of ether; yield, 158 mg (92%); mp 239—240 °C; $[\alpha]_D^{20}$ −164° (*c* 0.5, MeOH); R_f^1 0.06.

Found: C, 53.36; H, 7.04; N, 15.60%. Calcd for $C_{62}H_{90}O_{10}N_{16}\cdot 4HCl\cdot 2H_2O$: C, 53.13; H, 7.05; N, 15.99%.

*cyclo(–(D-Phe–Pro–Val–His–Leu)$_2$–)·2HCl ([$His^{2,2'}$]-GS·2HCl) (**19**·2HCl).* Compound **18**·4HCl (137 mg, 0.1 mmol) dissolved in DMF (3 ml) was treated with 2.8 M hydrogen chloride in EtOAc (0.14 ml) and isopentyl nitrite (0.014 ml, 0.1 mmol) in DMF (0.1 ml) as described for **13**. The crude product was purified with a column (3.3×30 cm) of silica gel as described for **13**; yield, 41 mg (32%); mp 262—263 °C (dec); $[\alpha]_D^{20}$ −276° (*c* 0.5, EtOH); R_f^1 0.28, R_f^3 0.85. Amino acid ratios in acid hydrolyzate; Phe 1.04, Pro 0.98, Val 0.96, His 1.10, Leu 1.00.

Found: C, 57.53; H, 7.05; N, 14.73%. Calcd for $C_{62}H_{86}O_{10}N_{14}\cdot 2HCl\cdot 2H_2O$: C, 57.44; H, 7.15; N, 15.12%.

Paper Electrophoresis. This was carried out with Toyo Roshi No. 52 paper and with a solvent system of HCOOH–AcOH–MeOH–H_2O (1:3:6:10, pH 1.8) for 2.5 h at 500 V/30 cm. Each of the peptides (**14** and **19**) revealed a single spot, the mobility being the same as that of GS.

Microbiological Assays and ORD Measurements. Measurements were carried out as described in a previous paper,[7] the results being shown in Table 1 and Fig. 4.

Kinetic Measurements. The esterolytic reaction was initiated by the addition of an ethanolic solution of *p*-nitrophenyl acetate (10 μl) to an aqueous ethanolic solution of catalyst (2.99 ml). Hydrolysis by the action of the catalyst was monitored by the increase in absorbance at 400 nm on a Hitachi 124 spectrophotometer; light path, 1 cm.

References

1) Part XXXVIII of this series: K. Sato, K. Ueda, M. Kondo, H. Aoyagi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **51**, 1830 (1978).

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

3) M. Waki, O. Abe, R. Okawa, T. Kato, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **40**, 2904 (1967).

4) A. B. Silaev and V. M. Stepanov, *Dokl. Akad. Nauk SSSR*, **112**, 297 (1957).

5) A. S. Kaprel'yants, V. V. Nikiforov, A. I. Miroshnikov, L. G. Sneshkova, V. A. Eremin, and O. N. Ostrovski, *Biochemistry (translated from Russian)*, **42**, 252 (1977).

6) Abbreviations recommended by the IUPAC-IUB Commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used throughout. Additional abbreviations: DMF, *N*,*N*-dimethylformamide; MA, mixed anhydride; NMM, *N*-methylmorpholine; TEA, triethylamine; THF, tetrahydrofuran.

7) M. Ohno, K. Kuromizu, H. Ogawa, and N. Izumiya, *J. Am. Chem. Soc.*, **93**, 5251 (1971).

8) H. Abe, K. Sato, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **49**, 3113 (1976).

9) K. Nonaka, K. Kamekawa, K. Sato, M. Waki, T. Kato, and N. Izumiya, "Peptide Chemistry 1977," ed by T. Shiba, Protein Res. Foundation, Osaka, pp. 135—138.

10) M. Dygert, N. Go, and H. A. Scheraga, *Macromolecules*, **8**, 750 (1975).

11) E. Katchalski, G. D. Fasman, E. Simons, E. R. Blout, F. R. N. Gurd, and W. L. Koltun, *Arch. Biochem. Biophys.*, **88**, 361 (1960).

12) K. Nakajima and K. Okawa, *Bull. Chem. Soc. Jpn.*, **46**, 1811 (1973).

13) Y. Imanishi, M. Tanihara, T. Sugihara, and T. Higashimura, *Biopolymers*, **16**, 2203 (1977).