Studies of Peptide Antibiotics. XXX.[1] Syntheses of Gramicidin S Analogs Containing *N*-Methylleucine in Place of Leucine

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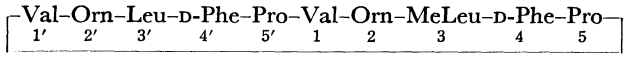
Two analogs of gramicidin S, *cyclo*(–Val–Orn–Leu–D-Phe–Pro–Val–Orn–MeLeu–D-Phe–Pro–), *i.e.*, [3-*N*-Methylleucine]–gramicidin S, and *cyclo*(–Val–Orn–MeLeu–D-Phe–Pro–Val–Orn–MeLeu–D-Phe–Pro–), *i.e.*, [3,3′-di-*N*-Methylleucine]–gramicidin S, were prepared in an attempt to investigate the contribution of the NH group of the leucine residue with regard to antibacterial activity. These analogs were almost as active as gramicidin S in the antibacterial test in reaction to microorganisms. The optical rotatory dispersions of these two analogs and of gramicidin S were measured in solvents of ethanol and 8 M aqueous urea. These studies suggested that both of the analogs containing *N*-methylleucine have a molecular conformation very similar to that of gramicidin S, and that the NH group of the leucine residues is not necessary for stabilizing the conformation in order to exhibit the biological properties.

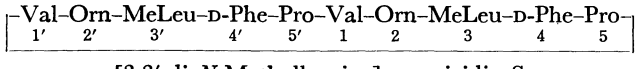
In studies of the structure-activity relationship of gramicidin S, various analogs have been synthesized.[2] From these works and the optical rotatory dispersion (ORD) studies, it has been suggested that the biological activity of gramicidin S and its analogs has a close correlation to their conformation, and that a specific conformation may be necessary for a molecule to exhibit antibacterial activity.[3]

For the conformation of gramicidin S in the solid- and the solution-state, several models have been proposed. A possible model which was first suggested on the basis of X-ray studies by Hodgkin and Oughton is the intramolecular antiparallel β-form, with four hydrogen bondings between the valyl and the leucyl residues.[4] A similar structure was proposed by Schwyzer,[5] by Craig's group,[6] and by Urry's group.[7] These proposals prompted us to synthesize analogs which lack some of the four intramolecular hydrogen bondings in order to investigate to what extent these hydrogen bondings will stabilize the conformation.

The present paper will deal with the syntheses, antibacterial properties, and ORD measurements of two analogs of gramicidin S, [3-*N*-Methylleucine]- and [3,3′-di-*N*-Methylleucine]-gramicidin S, in which the leucyl residues in positions 3 and 3′ are replaced, singly or together, by *N*-methylleucine residues. These analogs are lacking in one or two of the four hydrogen bondings of the proposed model.



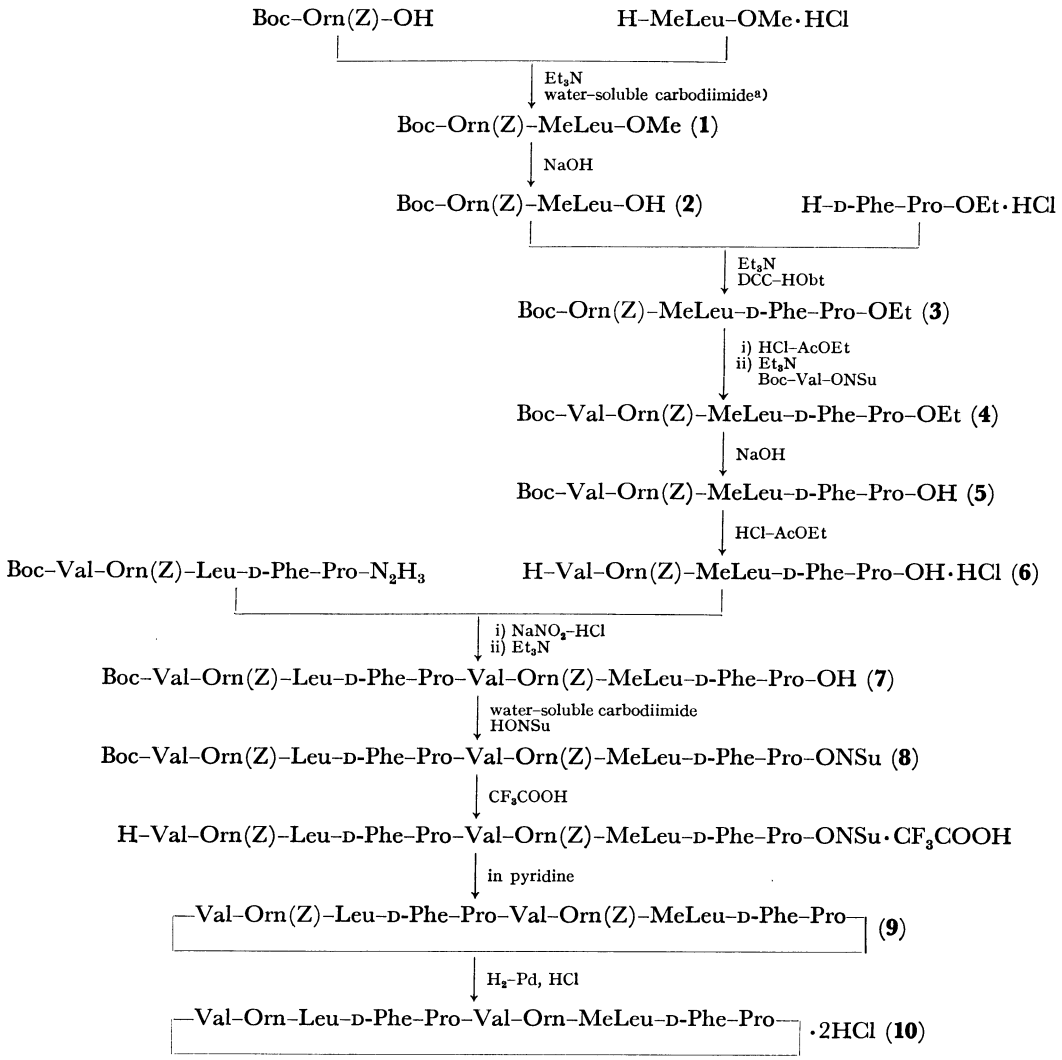
[3-*N*-Methylleucine]-gramicidin S



[3,3′-di-*N*-Methylleucine]-gramicidin S

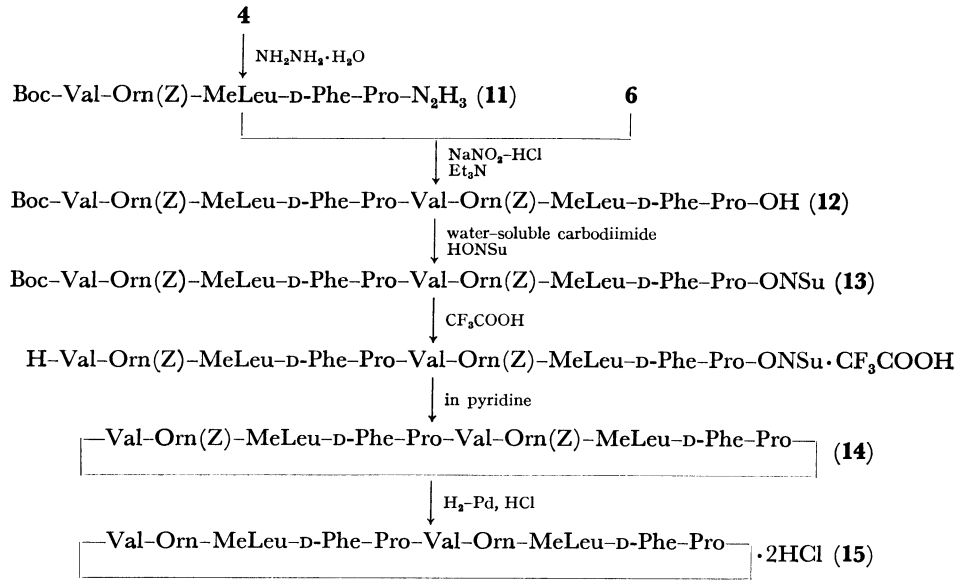
The synthesis of [3-*N*-Methylleucine]-gramicidin S is outlined in Scheme 1.[8] The strategy of the synthesis was chosen specifically to preclude the *N*-methylamino acid from the *N*- and *C*-terminus of the decapeptide, since acylation of the secondary amino group in *N*-methylamino acid residue and acylation by the activated carboxyl group in *N*-methylamino acid residue seems not to be favored.[9,10] Proline was chosen as the *C*-terminal residue in order to avoid racemization in the final cyclization step.

α-*t*-Butoxycarbonyl (Boc)-δ-benzyloxycarbonyl (Z)-ornithine was coupled with *N*-methylleucine methyl ester in the presence of dicyclohexylcarbodiimide (DCC) to yield the protected dipeptide, **1**, as an oil, together with the *N*-acylurea derived from the carboxyl component. All attempts at purification, such as crystallization and column chromatography, were unsuccessful. The corresponding reaction using the water-soluble carbodiimide gave the protected dipeptide, **1**, in a pure state in a 50% yield. The NMR evidence of the three *N*-methyl protons supported the proposed structure. Saponification of **1** easily gave the corresponding acid, **2**, as fine crystals. It was reported previously that, when the *C*-terminal amino acid residue of a carboxyl component was an *N*-methylamino acid residue, a system of DCC plus 1-hydroxybenzotriazole (HOBt)[11] was a useful coupling agent;[10] therefore α-Boc-δ-Z-ornithyl-*N*-methylleucine was coupled with D-phenylalanylproline ethyl ester by the aid of DCC and HOBt. The consequent yield of α-Boc-δ-Z-ornithyl-*N*-methylleucyl-D-phenylalanylproline ethyl ester (**3**) was nearly quantitative. After removal of the Boc group of **3**, the resulting tetrapeptide ester was coupled with Boc-valine *N*-hydroxysuccinimide ester to yield Boc-valyl-δ-Z-ornithyl-*N*-methylleucyl-D-phenylalanylproline ethyl ester (**4**) in an 80% yield; this ester was then converted to the corresponding acid **5** by saponification and subsequently to hydrochloride of valyl-δ-Z-ornithyl-*N*-methylleucyl-D-phenylalanylproline (**6**) by the action of hydrogen chloride in ethyl acetate. Condensation of the azide derived from Boc-valyl-δ-Z-ornithylleucyl-D-phenylalanylproline hydrazide with the pentapeptide, **6**, gave the acyldecapeptide acid, **7**, which was then isolated by column chromatography on silica gel. The acid, **7**, was then transformed into the Boc-decapeptide *N*-hydroxysuccinimide ester, **8**, by the reaction of *N*-hydroxysuccinimide (HONSu) and the water-soluble carbodiimide. After removal of the Boc group of **8** with trifluoroacetic acid, the decapeptide ester trifluoroacetate thus obtained was cyclized in pyridine at room temperature by the high-



a) 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide

Scheme 1.



Scheme 2.

dilution method.[12] Purification through ion-exchange columns, followed by fractionation on silica gel, gave the Z-substituted cyclic decapeptide, **9**, as colorless needles in a 56% yield. Hydrogenolysis of **9** afforded the desired [3-*N*-Methylleucine]-gramicidin S as a crystalline dihydrochloride, **10**.

[3,3'-Di-*N*-Methylleucine]-gramicidin S was prepared in a similar manner (Scheme 2). The Z-substituted cyclic derivative, **14**, was obtained in a 67.5% yield. These results indicate that the cyclization reaction is not affected by the *N*-methyl group. These two synthetic analogs gave satisfactory results in elemental and amino acid analyses, and were homogeneous by the criteria of paper electrophoresis and paper chromatography with various solvent systems.

Table 1. Antibacterial activity of the compounds
(Minimum inhibitory concentration, μg/ml)

Strain	Gramicidin S	[MeLeu³]-Gramicidin S (**10**)	[MeLeu³,³']-Gramicidin S (**15**)
Staphylococcus aureus	6.25 (1.56)[a]	6.25 (3.12)[a]	6.25 (6.25)[a]
Bacillus subtilis	3.12	3.12	3.12
Escherichia coli	100	50	50
Shigella flexneri	100	100	100
Candida albicans	50	50	25

a) ( ): Knight modified semisynthetic medium.

The results of the biological comparison of the two analogs to gramicidin S are shown in Table 1. In the antibacterial activities toward microorganisms, both of the analogs were almost identical with gramicidin S in potency. This indicates that the NH's of the leucine residues are not necessary for exhibiting the biological activity. Izumiya *et al.* have reported that the biologically-active gramicidin S analogs possess conformations similar to one another, irrespective of their primary structures.[3] Accordingly, we assume that these two

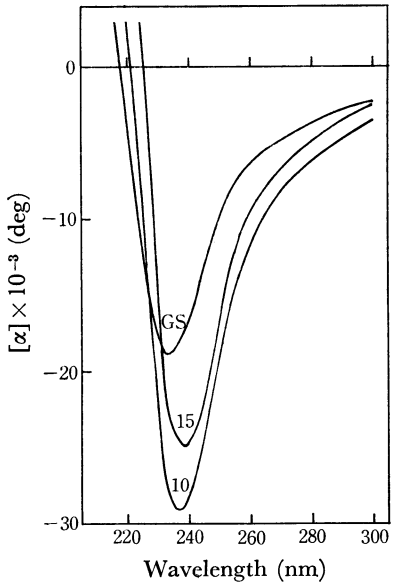


Fig. 1. ORD curves of [MeLeu³]-gramicidin S (**10**), [MeLeu³,³']-gramicidin S (**15**), and gramicidin S (GS) in ethanol, concentration 0.24 mg/ml.

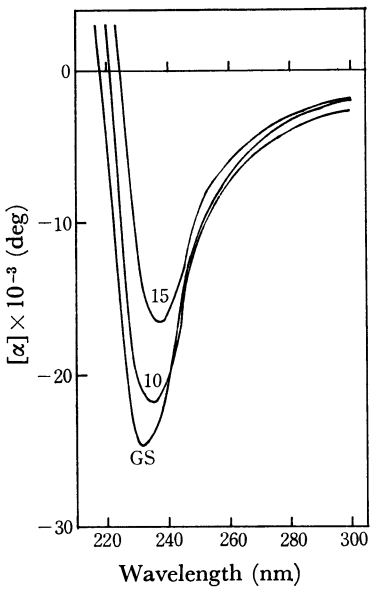


Fig. 2. ORD curves of [MeLeu³]-gramicidin S (**10**), [MeLeu³,³']-gramicidin S (**15**), and gramicidin S (GS) in 8 M urea, concentration 0.24 mg/ml.

analogs containing *N*-methylleucine also have conformations similar to that of gramicidin S. To confirm our assumption, ORD measurements were undertaken.

The ORD's of [3-*N*-methylleucine]- and of [3,3'-di-*N*-methylleucine]-gramicidin S in ethanol are shown in Fig. 1, along with that of gramicidin S. The shapes of the three ORD curves are very similar to each other. The troughs of the former two compounds are at 236—238 nm, whereas that of gramicidin S is at 232 nm. In a solvent of 8 M aqueous urea, which causes the denaturation of some polypeptides (*e.g.*, the biologically-inactive analogs of gramicidin S[12]), the positions of the troughs of the analogs (**10** and **5**) remained unchanged, as does that of gramicidin S (Fig. 2). In both solvents, their dispersions are different in their minimum rotations, and the positions of the troughs are slightly different, but the shapes of the curves are very similar even in 8 M urea, suggesting that all these three molecules have very similar and stable conformations. Judging from these results, two of the four hydrogen bondings in the β-type structure of gramicidin S proposed by several authors are not necessary for stabilizing the conformation. Furthermore, another model in which the NH's of the leucine residues do not participate in the intramolecular hydrogen bondings can be put forward. In this respect, as has been mentioned by Balasubramanian,[13] the Hodgkin-Oughton mixed α,β-structure and the Scheraga model can not be disregarded.[4,14] Further studies of this question are in progress.

Experimental

Thin-layer chromatography (tlc) was carried out on silica gel G (Merck) with the solvent systems: R_f^1, chloroform–methanol–acetic acid (95: 5: 3), R_f^2, acetone —28% aqueous ammonia (100: 5). The NMR spectra were recorded at 60 MHz, with tetramethylsilane as the internal standard and using a Hitachi R-20A apparatus. The optical rotations

were measured with a Perkin-Elmer polarimeter 141.

Boc-Orn(Z)-MeLeu-OMe (1). Into a solution of Boc-Orn(Z)-OH[15] (7.3 g, 20 mmol), H-MeLeu-OMe·HCl[10] (4 g, 20 mmol), and triethylamine (2.8 ml) in tetrahydrofuran (100 ml), was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide[16] (3.1 g, 20 mmol) at −5—0 °C over a period of 10 min. After the mixture had been stirred overnight at room temperature, the solvent was distilled off under reduced pressure and the residue was taken up in ethyl acetate. The ethyl acetate solution was then washed successively with M hydrochloric acid, 4% sodium bicarbonate, and water, and then dried over magnesium sulfate. The solvent was evaporated under reduced pressure to leave a colorless oil; yield, 5 g (50%); $[\alpha]_D^{25}$ −38.0° (*c* 1.5, methanol). NMR δ ($CDCl_3$): 1.45 (s, 9H, $(CH_3)_3C$–), 2.93 (s, 3H, CH_3N–), 3.65 (s, 3H, CH_3OCO–), 5.07 (s, 2H, $-C\underline{H}_2C_6H_5$), 7.30 (s, 5H, phenyl protons).

Boc-Orn(Z)-MeLeu-OH (2). A solution of compound **1** (4.7 g, 9.4 mmol) and 4M sodium hydroxide (2.5 ml) in methanol (18 ml) was stirred for 6 hr at 20—25 °C. The solution was then evaporated under reduced pressure below 40 °C to remove methanol, diluted with water (300 ml), and washed with ether. The aqueous solution was acidified with 2M hydrochloric acid, and the oil separated was repeatedly extracted with ether. The extract was washed with M hydrochloric acid and water. After drying over magnesium sulfate, the solution was evaporated to leave an oil which was subsequently crystallized in a refrigerator. The product was recrystallized from ether–*n*-hexane; yield, 3.4 g (73%); mp 95—97 °C; $[\alpha]_D^{25}$ −32.0° (*c* 1, methanol). Found: C, 60.62; H, 7.61; N, 8.50%. Calcd for $C_{25}H_{39}N_3O_7$: C, 60.83; H, 7.96; N, 8.51%.

Boc-Orn(Z)-MeLeu-D-Phe-Pro-OEt (3). To a stirred solution of **2** (9 g, 18 mmol), HOBt (2.4 g, 18 mmol), HCl·H-D-Phe-Pro-OEt[17] (6 g, 18 mmol), and triethylamine (2.5 ml) in tetrahydrofuran (90 ml), DCC was added at −10—−5 °C. After the stirring had been continued for 2 hr at −5—0 °C, and for a further 2 hr at room temperature, the mixture was left to stand overnight. The mixture was then evaporated under reduced pressure, and the residue was diluted with ethyl acetate (500 ml). The solution which was separated from the dicyclohexylurea by filtration was washed successively with water, 4% sodium bicarbonate, and water, and then dried over magnesium sulfate. The solvent was distilled off under reduced pressure to leave an oil; yield, 13.4 g (98%); R_f^1, 0.44; $[\alpha]_D^{25}$ −82.1° (*c* 0.55, methanol). NMR δ ($CDCl_3$): 1.45 (s, 9H, $(CH_3)_3C$–), 2.87 (s, 3H, CH_3-N–), 5.10 (s, 2H, $-OC\underline{H}_2C_6H_5$), 7.17—7.35 (m, 10H, phenyl protons).

Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-OEt (4). Compound **3** (7.65 g, 10 mmol) was dissolved in 3M hydrogen chloride in ethyl acetate (100 ml) and allowed to react for 30 min at room temperature. The solvent was distilled off under reduced pressure to dryness. The tetrapeptide ethyl ester hydrochloride thus obtained was dissolved in chloroform (30 ml), neutralized with triethylamine (1.4 ml), and subjected to a reaction with Boc-Val-ONSu[18] (3.45 g, 11 mmol) overnight at room temperature. To this solution, a few drops of *N*-2-aminoethylpiperazine were added, and the mixture was allowed to stand for 30 min. The solution was then diluted with chloroform (200 ml), washed successively with M hydrochloric acid and 4% sodium bicarbonate, and dried over magnesium sulfate. The solvent was distilled off under reduced pressure, and the residue was crystallized by adding dry ether; yield, 7.7 g (80%); mp 88—99 °C; $[\alpha]_D^{25}$ −91.2° (*c* 0.25, methanol). Found: C, 63.74; H, 7.85; N, 9.73%. Calcd for $C_{46}H_{68}N_6O_{10}$: C, 63.86; H, 7.92; N, 9.71%.

Boc-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (5). To a solution of **4** (6.5 g, 7.5 mmol) in methanol (70 ml), M sodium hydroxide (15 ml) was added; the solution was then allowed to stand for 6 hr at room temperature. The solution was evaporated under reduced pressure to remove methanol, diluted with water (100 ml), and acidified with 2M hydrochloric acid. The oil thus separated was extracted with ethyl acetate, and the extract was washed with water and dried over magnesium sulfate. The solvent was distilled off under reduced pressure to yield an oily product; 6.4 g (100%); $[\alpha]_D^{25}$ −80.5° (*c* 1, methanol). Found: C, 61.71; H, 7.72; N, 9.37%. Calcd for $C_{44}H_{64}N_6O_{10}\cdot H_2O$: C, 61.80; H, 7.78; N, 9.82%.

H-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH·HCl (6). Compound **5** (6.4 g, 7.5 mmol) was treated with 3M hydrogen chloride in ethyl acetate (80 ml) for 30 min at room temperature. The solution was then evaporated under reduced pressure, and the residue was triturated with dry ether. The product was collected by filtration, washed well with ether, and dried *in vacuo* over sodium hydroxide; yield, 5.8 g (100%).

Boc-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-OH (7). To a solution of Boc-Val-Orn(Z)-Leu-D-Phe-Pro-$NHNH_2$[12] (3.3 g, 4 mmol) and 3M hydrochloric acid (4 ml) in dimethylformamide (100 ml), was added a chilled solution of sodium nitrite (300 mg, 4.4 mmol) in water (1 ml) at −15 °C. After 15 min, the reaction mixture was neutralized with triethylamine (0.56 ml), and a solution of **6** (3 g, 4 mmol) and triethylamine (1.2 ml, 8 mmol) in dimethylformamide (40 ml) was added to the mixture. After 2 days at 3 °C, the solvent was evaporated under reduced pressure below 40 °C. The residue was added to 10% citric acid (50 ml), and the resulting oil was extracted with ethyl acetate. The ethyl acetate solution was washed successively with 10% citric acid and water and dried over magnesium sulfate. After removal of the solvent, the residue was subjected for purification to chromatography on a column of silica gel 60 (Merck), first with a mixture of acetone and 28% aqueous ammonia (100: 5). In this solvent system, the by-products (R_f^2, 0.9 and 0.8) were eluted; the main product (R_f^2, 0.1) was not eluted. The desired product was then removed with methanol. The fractions containing the product, as detected by tlc (R_f^1, 0.53), were pooled and evaporated to dryness. The residue was dissolved in ethyl acetate (200 ml), and the ethyl acetate solution was washed with 10% citric acid until the washings became acidic, and then with water. After drying over magnesium sulfate, the solvent was distilled off; yield of oil, 3.3 g (53%); $[\alpha]_D^{25}$ −96.9° (*c* 1, methanol). Found: C, 62.67; H, 7.77; N, 9.97%. Calcd for $C_{82}H_{116}N_{12}O_{17}\cdot 2H_2O$: C, 62.41; H, 7.66; N, 10.65%.

Boc-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-Phe-Pro-ONSu (8). To a solution of **7** (2.1 g, 1.3 mmol) in dimethylformamide (6 ml), HONSu (300 mg, 2.6 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (400 mg, 2.6 mmol) were added at 0 °C. After 12 hr at room temperature, the mixture was diluted with ethyl acetate (200 ml). The solution was washed with M hydrochloric acid, 4% sodium bicarbonate, and water. After drying over magnesium sulfate, the solvent was distilled off under reduced pressure. The crude product was reprecipitated from ethyl acetate–petroleum ether; yield, 2.0 g (95%); mp 105—111 °C; $[\alpha]_D^{25}$ −125.0° (*c* 0.6, ethyl acetate). Found: C, 62.21; H, 7.20; N, 10.92%. Calcd for $C_{86}H_{119}N_{13}O_{19}\cdot H_2O$: C, 62.33; H, 7.36; N, 10.98%.

cyclo(*-Val-Orn(Z)-Leu-D-Phe-Pro-Val-Orn(Z)-MeLeu-D-*

Phe-Pro-) (9). The decapeptide *N*-hydroxysuccinimide ester, **8** (400 mg), was dissolved in trifluoroacetic acid (15 ml). After 30 min, the solution was evaporated, and the residual powder was collected by filtration with the aid of dry ether and dried over sodium hydroxide *in vacuo*; yield, 340 mg. The *N*-hydroxysuccinimide ester trifluoroacetate thus obtained (340 mg) was dissolved in dimethylformamide (10 ml), and the solution was added, drop by drop, to pyridine (100 ml) at 30—40 °C over a period of 2 hr; stirring was then continued for an additional 2 hr at room temperature. The solvent was removed, and the residue was dissolved in a mixture of methanol (50 ml) and water (10 ml). The solution was passed through columns (1.9×40 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (500 ml), the combined effluent was evaporated, and the oil was extracted with ethyl acetate. The extract was dried over magnesium sulfate and evaporated to dryness. The oily product was further purified by a column (1.5×15 cm) with silica gel 60 (Merck). After the column had been washed with a mixture of ethyl acetate and benzene (1:1) (200 ml), the desired product, as determined by tlc, was eluted with ethyl acetate (100 ml). The solvent was evaporated to leave an oil, which was subsequently crystallized by the addition of a mixture of ethyl acetate and petroleum ether; yield, 163 mg (56%); mp 212—214 °C; $[\alpha]_D^{25}$ −248° (*c* 0.31, methanol). Found: C, 63.45; H, 7.46; N, 11.71%; molecular weight 1450.[19] Calcd for $C_{77}H_{106}N_{12}O_{14}\cdot 2H_2O$: C, 63.35; H, 7.59; N, 11.51%; molecular weight 1460.

cyclo*(-Val-Orn-Leu-*D*-Phe-Pro-Val-Orn-MeLeu-*D*-Phe-Pro-)·2HCl ([3-*N*-Methylleucine]-gramicidin S Dihydrochloride) (10).* The protected cyclic decapeptide **9** (100 mg) was dissolved in 0.01M hydrogen chloride in methanol (20 ml), after which hydrogenation was carried out for 4 hr, using palladium black as a catalyst. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The syrup which remained was treated with M hydrochloric acid (2 ml), and the crystals formed were filtered, washed in portions with M hydrochloric acid (2 ml), and dried *in vacuo* over sodium hydroxide; yield, 60 mg. Recrystallization from ethanol–M hydrochloric acid gave colorless crystals; yield, 50 mg; mp 237—239 °C; $[\alpha]_D^{25}$ −272° (*c* 0.05, ethanol). Amino acid ratios in acid hydrolyzate: Val 2.11, Orn 1.82, Leu 0.98, Pro 2.08, MeLeu 1.00, Phe 2.12.[10] Found: C, 57.89; H, 7.82; N, 13.23%. Calcd for $C_{61}H_{94}N_{12}O_{10}\cdot 2HCl\cdot 2H_2O$: C, 57.94; H, 7.97; N, 13.29%.

*Boc-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-N_2H_3 (11).* A solution of **4** (8.56 g, 10 mmol) and hydrazine hydrate (15 ml, 300 mmol) in dimethylformamide (20 ml) was allowed to stand at 30 °C for 7 days. The solution was evaporated below 40 °C, and then ethyl acetate (200 ml) was added to the residue. The solution was washed with M hydrochloric acid, 4% sodium bicarbonate, and water. After drying over magnesium sulfate, the solution was evaporated to dryness; yield of oil, 8.6 g (100%); $[\alpha]_D^{25}$ −88.6° (*c* 1.5, ethyl acetate). Found: C, 60.74; H, 7.72; N, 12.44%. Calcd for $C_{44}H_{66}N_8O_9\cdot H_2O$: C, 60.80; H, 7.88; N, 12.89%.

*Boc-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-OH (12).* Compound **11** (2.9 g, 3.3 mmol), in a mixture of 3M hydrochloric acid (3.5 ml) and dimethylformamide (100 ml), was treated with sodium nitrite (280 mg, 4 mmol) as in the case of **7**. To the azide solution was added a solution of **6** (2.6 g, 3.3 mmol) in dimethylformamide (40 ml) containing triethylamine (1.8 ml). After the mixture had been stirred for 24 hr at 3 °C, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (500 ml), and the ethyl acetate solution was washed with 10% citric acid and water and dried over magnesium sulfate. After removal of the solvent, the residue was triturated with petroleum ether. The product was reprecipitated from ethyl acetate–petroleum ether; yield, 2.0 g (39%), mp 94—106 °C; $[\alpha]_D^{25}$ −115.0° (*c* 1, methanol). Found: C, 62.58; H, 7.30; N, 10.76%. Calcd for $C_{83}H_{118}N_{12}O_{17}\cdot 2H_2O$; C, 62.62; H, 7.72; N, 10.55%.

*Boc-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-ONSu (13).* The decapeptide acid **12** (1.55 g, 1 mmol) was converted to the active ester, **13**, by the method described for the preparation of **8**; yield, 1.27 g (77%); mp 95—110 °C; $[\alpha]_D^{25}$ −111.7° (*c* 0.5, ethyl acetate). Found: C, 63.01; H, 7.18; N, 10.88%. Calcd for $C_{87}H_{121}N_{13}O_{19}$: C, 63.21; H, 7.37; N, 11.01%.

cyclo*(-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-Val-Orn(Z)-MeLeu-*D*-Phe-Pro-) (14).* Compound **13** (1.27 g, 0.77 mmol) was treated with trifluoroacetic acid (10 ml) as in the case of **9**. The decapeptide *N*-hydroxysuccinimide ester trifluoroacetate thus obtained (1.2 g) was dissolved in dimethylformamide (10 ml), and the solution was added to pyridine (700 ml). The solvent was removed, and the residue was dissolved in a mixture of methanol (50 ml) and water (10 ml). The solution was passed through columns (1.9×40 cm, each) of Dowex 1 (OH^- form) and Dowex 50 (H^+ form). The columns were washed with the same solvent (700 ml), the combined effluent was evaporated, and the oil was extracted with ethyl acetate. The extract was dried and evaporated. The oily product was further purified by column chromatography with silica gel 60 (Merck). After the column had been washed with a mixture of ethyl acetate and benzene (1:1) (50 ml), the desired product, as determined by tlc (R_f^1, 0.5), was eluted with a mixture of chloroform, methanol, and acetic acid (95:5:3). The fractions (105—240 ml) containing the product were pooled and evaporated to an amorphous powder; yield, 770 mg (67.5%); mp 105—115 °C; $[\alpha]_D^{25}$ −173° (*c* 0.4, methanol). Found: C, 63.49; H, 7.18; N, 11.17%; molecular weight 1465.[19] Calcd for $C_{78}H_{108}N_{12}O_{14}\cdot 2H_2O$: C, 63.56; H, 7.65; N, 11.40%; molecular weight 1474.

cyclo*(-Val-Orn-MeLeu-*D*-Phe-Pro-Val-Orn-MeLeu-*D*-Phe-Pro-)·2HCl ([3,3'-di-*N*-Methylleucine]-gramicidin S Dihydrochloride) (15).* The protected cyclic derivative, **14** (340 mg, 0.23 mmol), was converted to **15** by the method described for the preparation of **10**; yield, 255 mg (85%); mp 233—235 °C; $[\alpha]_D^{25}$ −235.7° (*c* 0.05, ethanol). Amino acid ratios in acid hydrolyzate: Val 1.10, Orn 0.89, MeLeu 1.00, Phe 0.98, Pro 1.05.[10] Found: C, 57.47; H, 7.68; N, 12.78%. Calcd for $C_{62}H_{96}N_{12}O_{10}\cdot 2HCl\cdot 3H_2O$: C, 57.43; H, 8.08; N, 12.96%.

Paper Electrophoresis. To ascertain further the purities of the analogs of gramicidin S synthesized here, paper electrophoresis was carried out as has been described before.[20] Each peptide revealed a single spot, and the mobilities were comparable with that of gramicidin S.

Microbiological Assays. The minimum amounts of the compounds necessary for the complete inhibition of growth were determined by a dilution method, using a bouillon agar and a semisynthetic medium; the results are shown in Table 1.

ORD Measurements. The measurements were performed with a JASCO spectropolarimeter model ORD/UV-5, over the wavelength range from 210 to 300 nm. A cell with a path length of 0.2 cm was used, and the runs were made at an ambient temperature. Patterns in the solvents of ethanol and 8M aqueous urea are shown in Figs. 1 and 2 respectively.

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