dibenzoyl(*o*-sulfonic acid)desformylgramicidin [R_f 0.41 (A); desformylgramicidin A 0.12 (A); gramicidin A 0.41 (A)]. This product, when dissolved in a few drops of ethanol, is not precipitated by a large excess of water; such a solution is not degraded by pronase.[51]

(51) We are indebted to Dr. N. M. Green for this test.

Gramicidin A. VI. The Synthesis of Valine- and Isoleucine-gramicidin A

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Contribution from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland. *Received October 16, 1964*

Coupling of the octapeptide derivative Z-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-N_3 with the heptapeptide ethanolamide L-(H)Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-$NHCH_2CH_2OH$ or, more conveniently, of the pentapeptide Z-L-Val(or L-Ileu)-Gly-L-Ala-D-Leu-L-Ala-OH with the decapeptide ethanolamide D-(H)Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-$NHCH_2CH_2OH$ gave the decapentapeptide ethanolamide R-L-Val(or L-Ileu)-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-$NHCH_2CH_2OH$, having all the properties of N-carbobenzyloxydesformyl (R = Z), desformyl- (R = H), and (R = CHO) valine- or isoleucine-gramicidin.

The structures HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-$NHCH_2CH_2OH$ and HCO-L-Ileu-Gly-. . . were proposed for valine- and isoleucine-gramicidin A.[2] This paper describes the synthesis of these two formyl decapentapeptide ethanolamides and the comparison of their properties with those of the natural antibiotics.

No particular difficulties were expected in the synthesis of these linear peptides since the building stones are neutral amino acids. However, the acid sensitivity of tryptophan put certain limitations on the procedures to be used. The decapentapeptide was to be constructed from two moieties since the over-all yield in a stepwise synthesis of the entire decapentapeptide would be expected to fall off rapidly. There is always the danger of racemization in joining two peptides[3] except when the azide method is employed. Therefore, the peptide moiety containing COOH-terminal glycine would be convenient for such a connection and would remove the issue of racemization. Unfortunately, this approach is not feasible because glycine happens to be the second amino acid in the sequence of gramicidin A.

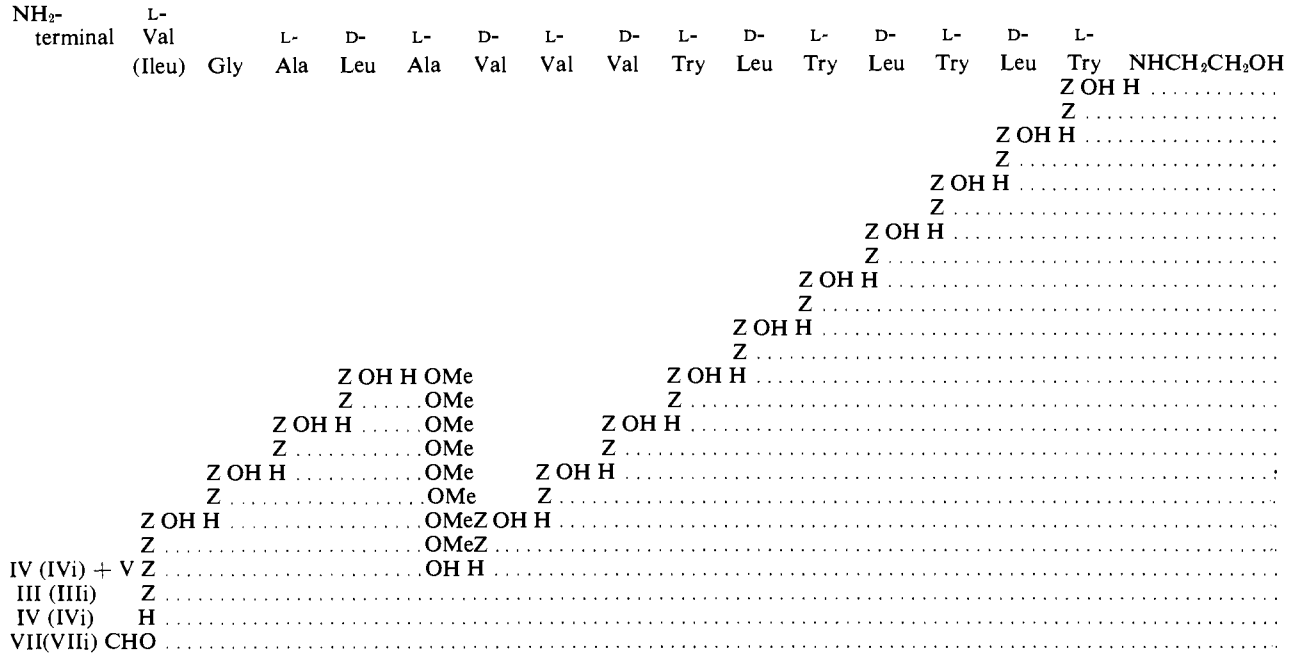
We planned on joining the octapeptide derivative Z-(or For-)L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-R (I) and the heptapeptide ethanolamide L-(H)Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-AE (II) (Z = carbobenzyloxy, For = HCO, AE = $NHCH_2CH_2OH$) either by the azide method or by coupling with dicyclohexylcarbodiimide in tetrahydrofuran at low temperature in the absence of salts.[4] When the formyl peptides were found to have undesirable solubility properties the carbobenzyloxy group was used throughout this work as the protecting group of choice. If successful, the coupling of I and II should lead to a carbobenzyloxy decapentapeptide ethanolamide (III), identical with N-carbobenzyloxydesformylgramicidin A. Removal of the blocking group should then yield desformylgramicidin A whose reformylation to gramicidin A has been described previously.[2]

The heptapeptide II was prepared by stepwise synthesis from the carboxyl end by the mixed anhydride method, which is fast, convenient, and, when used properly, minimizes the danger of racemization.[5] The hydroxyl group of ethanolamine was not protected. The octapeptide derivative Z-L-Val-. . . .D-Val-OMe (Ia) was prepared in an analogous manner. Unfortunately saponification of this ester to the required fragment I was impossible. The ester was always recovered unchanged after treatment with base. The ester Ia was therefore converted to the hydrazide and coupled, *via* the azide, with the heptapeptide ethanolamide II to a product with the properties of N-carbobenzyloxydesformylgramicidin A where a new difficulty arose: all attempts to remove the protecting group by hydrogenolysis in acetic acid over palladium on charcoal failed, and it was not possible to isolate any tryptophan-containing peptide with the small amount of material available. The same was true for authentic N-carbobenzyloxydesformylgramicidin A. It was found subsequently that hydrogenolysis over palladium black proceeds very smoothly to give desformylgramicidin A when methanol is used as the solvent.

A second more convenient synthetic approach consisted in coupling the pentapeptide derivative Z-L-Val-Gly-L-Ala-D-Leu-L-Ala-OH (IV) with the decapeptide ethanolamide D-(H)Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-AE (V, Chart I). The pentapeptide IV, synthesized by stepwise coupling from the carboxyl end, was obtained as the methyl ester, which was saponified without difficulty. The decapeptide V was prepared by stepwise prolongation of II. With dicyclohexylcarbodiimide, IV and V were

(1) Visiting Fellow, U. S. Public Health Service, 1962–1964.
(2) R. Sarges and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2011 (1965).
(3) T. Wieland and H. Determann, *Angew. Chem.*, **75**, 539 (1963).
(4) F. Weygand, A. Prox, L. Schmidhammer, and W. Konig, *ibid.*, **75**, 282 (1963).
(5) H. Determann and T. Wieland, *Ann.*, **670**, 136 (1963).

Chart I. Schematic Representation of the Synthesis of Valine- (and Isoleucine-) gramicidin A by the Coupling of the Pentapeptide IV with the Decapeptide Fragment V



joined to the carbobenzyloxy decapentapeptide ethanolamide, Z-L-Val-Gly-. . . .L-Try-AE (III), which on thin layer chromatography was identical with authentic N-carbobenzyloxydesformylgramicidin A. Hydrogenolysis of III in methanol over palladium black led to the peptide ethanolamide H-L-Val-. . . .L-Try-AE (VI), which on thin layer chromatography was identical with desformylgramicidin A. Formylation of VI with formic acid–acetic anhydride[2,12] followed by saponification of the O-formyl ester led to the peptide derivative For-L-Val-Gly-. . . .L-Try-AE (VII) which had all the properties of valine-gramicidin A (Table I).

In an analogous fashion the pentapeptide Z-L-Ileu-Gly-L-Ala-D-Leu-L-Ala-OH (IVi) was coupled with the decapeptide V to give the carbobenzyloxy decapentapeptide ethanolamide IIIi, which after hydrogenolysis and formylation gave a product identical with isoleucine-gramicidin A (Table I).

Table I. Comparison of the Properties of Natural and Synthetic Gramicidin A

	Natural			Synthetic	
	Valine-gramicidin A + isoleucine-gramicidin A			Valine-gramicidin A	Isoleucine-gramicidin A
K value (countercurrent distribution) (CH_3OH–H_2O–$CHCl_3$–benzene)[3]	(0.70)[a]	0.93	(0.59)[a]	0.97 (t = 26°)	0.81 (t = 26°)
K(Val)/K(Ile), g./g.		1.19		1:20	
$\epsilon_{282\ m\mu}^{EtOH}$		24,000		22,100 ± 1000	22,450 ± 1000
$[\alpha]^{20}D$ (CH_3OH)		+24.5 ± 1°		+27.3 ± 2.5° (c 1.3)	+26.5 ± 3° (c 0.45)
R_f t.l.c., AcOH–$CHCl_3$ (2:1) (only one spot)		0.43		0.42	0.45
Butanone–2–pyridine (7:3) (fastest moving, strongest spot)		0.65		0.65	0.66

Amino acid analyses[b]	Desformylgramicidin A[c]	Gramicidin A[d]		
Gly	0.90	0.99	0.86	0.96
Ala	2.00	2.01	1.79	1.89
Val	2.80	3.50	3.39	2.46
Ileu	0.18	0.27	0.00	0.92
Val + Ileu	2.98	3.77	3.39	3.38
Leu	4.00	4.00	4.00	4.00
Aminoethanol	0.97	0.79	0.86	0.75
NH_3	0.44	0.13	0.26	0.21

[a] K values of 0.59 (J. D. Gregory and L. C. Craig, *J. Biol. Chem.*, **172**, 839 (1948)) and up to 0.70 (L. K. Ramachandran, *Biochemistry*, **2** 1138 (1963)) have been observed. Apparently these coefficients depend on the temperature. [b] The samples were hydrolyzed in a mixture of 0.3 ml. of acetic acid and 1.5 ml. of 6.0 N hydrochloric acid. [c] Hydrolyzed for 22 hr. (110°). [d] Hydrolyzed for 23 hr.

The antibiotic activities of the synthetic formyl decapentapeptide ethanolamide (valine series) was indistinguishable from that of gramicidin A (Table II). In addition the effects of synthetic valine- and isoleucine-gramicidin A on succinate oxidation, swelling, and H^+ production of mitochondrial protein were

Table II. *In Vitro* Comparison of Natural Gramicidin A and Synthetic Valine-gramicidin A (Twofold Tube Dilution Assay)[a]

Organisms	Inoculum size, cells/ml.	M.I.C. in mcg./ml. Natural gramicidin A	M.I.C. in mcg./ml. Synthetic valine-gramicidin A
Staphylococcus aureus (209P)	10^6	6.3 (4.7)[b]	9.4 (4.7)
Staphylococcus aureus (209P)	10^3	0.003 (0.06)	0.002 (0.05)
Streptococcus lactis (SC 1783)	10^3	0.38 (0.79)	0.79 (1.2)
Streptococcus faecalis (SC 1648)	10^3	0.01 (0.02)	0.01 (0.01)
Staphylococcus aureus (SC 2406)	10^6	2.4	9.4
Salmonella schottmuelleri (SC 3850)[c]	10^3	>50.0	>50.0
Proteus vulgaris (SC 3855)[c]	10^3	>50.0	>50.0
Escherichia coli (SC 2927)[c]	10^3	>50.0	>50.0

[a] Both samples were diluted in 50% aqueous methanol prior to their assay in a twofold tube dilution test, and neither was completely in solution when assayed. Under the conditions of the test procedures, both samples demonstrated the same spectrum and approximate level of activity against most of the organisms tested. (We are greatly indebted to Dr. James D. Dutcher, The Squibb Institute for Medical Research, for arranging these tests). [b] Numbers in parentheses represent repeat assays. [c] These microbes are Gram-negative and would not be expected to be inhibited by gramicidin A.

indistinguishable from those of natural gramicidin A.[6] The distribution pattern on countercurrent distribution is pictured in Figure 1. The very characteristic behavior of natural and synthetic gramicidin

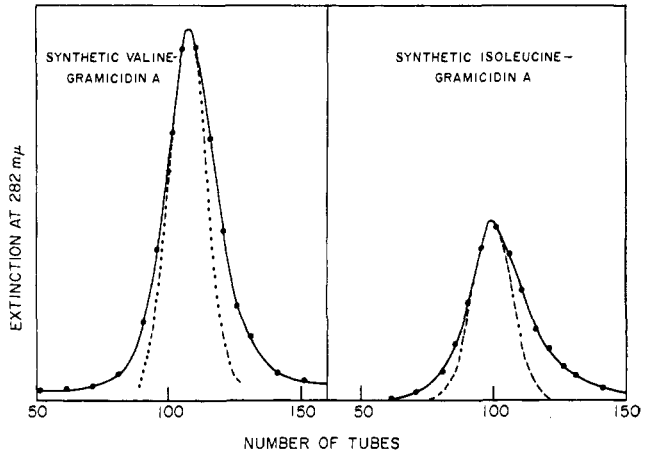


Figure 1. Countercurrent distribution pattern of synthetic valine- and isoleucine-gramicidin A. The broken line signifies the theoretical curve.

samples on thin layer chromatography in solvent C is shown in Figure 2. The number and positions of individual spots are dependent on the nature of the solvents (S. Ishii, unpublished observations). The reversible equilibrium of labile components is independent of their valine or isoleucine contents. This phenomenon is best explained by the assumption of a monomeric gramicidin A in equilibrium with various dimers or higher states of association.[2]

Experimental

The purity of the peptides after each synthetic step was ascertained by thin layer chromatography on silica gel (Merck) as well as by high voltage paper electrophoresis (40 v./cm.). The solvent systems for thin layer chromatography are abbreviated as follows: A, AcOH–$CHCl_3$ (2:1); B, *s*-BuOH–HCOOH–H_2O (75:15:10); C, butanone-2-pyridine (7:3). The R_f values in these solvent systems are difficult to reproduce and should only be considered as relative guide lines (spot detection on t.l.c., ninhydrin for unprotected peptides, Ehrlich reaction for all tryptophan-containing peptides, charring with sulfuric acid for N-protected tryptophan-free peptides).

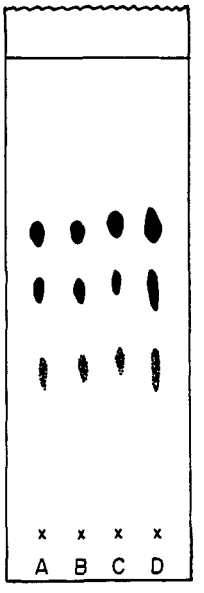


Figure 2. Typical multiple spots of natural and synthetic gramicidin A samples on thin layer chromatography in pyridine–butanone-2 (3:7). A = natural pure valine-gramicidin A; B = synthetic valine-gramicidin A; C = synthetic isoleucine-gramicidin A; D = commercial sample of the mixture of valine- and isoleucine-gramicidin A.

*Carbobenzyloxy-*L*-tryptophyl-2-hydroxyethylamide.* To a solution of 16.9 g. (50 mmoles) of carbobenzyloxy-L-tryptophan (Mann) and 7.1 ml. (50 mmoles) of triethylamine in 300 ml. of tetrahydrofuran, cooled to −20°, was added 4.75 ml. (50 mmoles) of ethyl chloroformate, and the mixture was stirred at −20° for 15 min. Then 3 ml. (50 mmoles) of freshly distilled 2-aminoethanol was added, the cooling bath removed, and the mixture stirred for 1 hr. The solvent was removed by evaporation and the residue dissolved in ethyl acetate and washed, in turn, with 1.0 *N* hydrochloric acid, water, 5% aqueous bicarbonate, and water. The

(6) J. B. Chappel and A. R. Crofts, unpublished results. We are indebted to Dr. Chappel for these tests.

ethyl acetate extract was dried over magnesium sulfate and evaporated, and the residue was recrystallized from ethyl acetate–petroleum ether (b.p. 40–60°) to give 11.9 g. (62%) of colorless needles, m.p. 124–125°, $[\alpha]^{20}D$ −4.6 ± 1.5° (*c* 0.86, methanol), R_f 0.72 (A).

Anal. Calcd. for $C_{21}H_{23}N_3O_4$: C, 66.12; H, 6.08; N, 11.02. Found: C, 65.86; H, 6.31; N, 11.11.

L-*Tryptophyl-2-hydroxyethylamide.* A solution of 9.5 g. of Z-L-Try-AE in 40 ml. of acetic acid was hydrogenolyzed over palladium black catalyst[7] until the evolution of CO_2 ceased (3 hr.). The catalyst was removed by filtration and the filtrate evaporated. The oily residue (acetate) was dissolved in methanol and filtered through a Dowex 1-X2 column (200/400 mesh, OH^- form, equilibrated with methanol). The methanol filtrate was evaporated and the residue recrystallized from methanol–ether to give 4.85 g. (79%) of the free peptide in rhombic crystals, m.p. 144°, $[\alpha]^{20}D$ 18.2 ± 1.0° (*c* 1, methanol), R_f 0.35 (B); EPM (electrophoretic mobility): 5.05×10^{-5} cm.^{2} v.$^{-1}$ sec.$^{-1}$.

Anal. Calcd. for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.38; H, 6.69; N, 17.26.

Carbobenzyloxy-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide.* By the mixed anhydride method as above 5.3 g. of Z-D-Leu-OH (prepared from D-leucine (Mann)) was allowed to react with a solution of 4.94 g. of H-L-Try-AE in tetrahydrofuran. The product was purified as above to give 8.29 g. (84%) of colorless crystals, m.p. 169–170° (from ethyl acetate), R_f 0.77 (A).

Anal. Calcd. for $C_{27}H_{34}N_4O_5$: C, 65.57; H, 6.93; N, 11.33. Found: C, 64.90; H, 7.31; N, 10.69.

D-*Leucyl*-L-*tryptophyl-2-hydroxyethylamide.* Hydrogenolysis of 8.0 g. of Z-D-Leu-L-Try-AE in 40 ml. of acetic acid over palladium black followed by filtration, evaporation, and filtration over Dowex 1-X2 as above gave 5.68 g. (96.5%) of a homogeneous noncrystalline product, R_f 0.39 (B); EPM 4.3.

Carbobenzyloxy-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide.* By the mixed anhydride method as described above there was obtained from 5.23 g. of Z-L-Try-OH and 5.56 g. of D-(H)Leu-L-Try-AE 8.51 g. (81%) of the tetrapeptide which was obtained in solid form from ethyl acetate–petroleum ether (b.p. 40–60°), $[\alpha]^{20}D$ 24.6 ± 1.0° (*c* 0.9, methanol), R_f 0.82 (A).

Anal. Calcd. for $C_{38}H_{44}N_6O_6$: C, 67.04; H, 6.51; N, 12.35. Found: C, 66.89; H, 6.81; N, 12.15.

L-*Tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide.* By hydrogenolysis of 8.5 g. of Z-L-Try-D-Leu-L-Try-AE in acetic acid there was obtained 6.3 g. (92%) of the free tetrapeptide after purification on Dowex 1-X2, R_f 0.50 (B); EPM 3.3.

Carbobenzyloxy-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide.* By the coupling reaction of 3 g. of Z-D-Leu-OH with 6.15 g. of L-(H)-Try-D-Leu-L-Try-AE 8.03 g. (90%) of the protected pentapeptide was obtained, which crystallized from ethyl acetate–petroleum ether (b.p. 40–60°), m.p. 195–202°, $[\alpha]^{20}D$ −3.0 ± 1.0° (*c* 0.8, methanol), R_f 0.86 (A).

Anal. Calcd. for $C_{44}H_{55}N_7O_7$: C, 66.56; H, 6.98; N, 12.35. Found: C, 66.49; H, 6.96; N, 12.44.

From the pentapeptide level on upward, with molecular weights >700, elemental analyses no longer adequately characterized the product. The uniformity of the mostly noncrystalline products was ascertained by thin layer chromatography and paper electrophoresis. The carbobenzyloxy peptides were obtained as solids from ethyl acetate–petroleum ether and the free peptides from methanol–ether. Hydrogenolysis of 8 g. of Z-D-Leu-L-Try-D-Leu-L-Try-AE in acetic acid gave 6.6 g. (100%) of the free pentapeptide after the customary purification on a column of Dowex 1-X2, R_f 0.63 (A), 0.66 (B).

Carbobenzyloxy-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide* was obtained by coupling 3.1 g. of Z-L-Try-OH with 6 g. of H-[D-Leu-L-Try-]$_2$-AE in a yield of 8.3 g. (92%), R_f 0.87 (A).

L-*Tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide* was obtained by hydrogenolysis of 8.2 g. of Z-L-Try-[D-Leu-L-Try-]$_2$-AE in 88% yield after purification on Dowex 1-X2, R_f 0.72 (A), 0.73 (B); EPM 3.2.

Carbobenzyloxy-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide* was obtained by coupling 1.77 g. of Z-D-Leu-OH with 5.76 g. of L-(H)Try-[D-Leu-L-Try-]$_3$-AE in a yield of 6.8 g. (93%), R_f 0.90 (A).

D-*Leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide.* Hydrogenolysis of 6.7 g. of Z-[D-Leu-L-Try-]$_3$-AE in acetic acid for 20 hr. led to a product which contained some neutral material probably because of incomplete hydrogenolysis. The crude product was dissolved in methanol and filtered over a column of Dowex 50W-X2 (H^+ form, equilibrated with methanol). The column was washed with methanol and the desired basic material was eluted with 2.0 *N* ammonia in methanol prepared by mixing 80 ml. of 12.0 *N* aqueous ammonia with 400 ml. of methanol. There was obtained 4.5 g. (77%) of the heptapeptide, R_f 0.78 (A), 0.77 (B). This peptide and the following larger peptides showed no electrophoretic mobility.

Carbobenzyloxy-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide.* The protected octapeptide was obtained by coupling 1.32 g. of Z-L-Try-OH with 3.75 g. of H-[D-Leu-L-Try-]$_3$-AE in a yield of 4.1 g. (82%), R_f 0.89 (A).

L-*Tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl-2-hydroxyethylamide* (II). Hydrogenolysis of 4 g. of Z-L-Try-[D-Leu-L-Try-]$_3$-AE in methanol with a few drops of acetic acid for 20 hr. gave, after purification on Dowex 1-X2, 3.6 g. (100%) of the free octapeptide, R_f 0.80 (A), 0.85 (B).

Carbobenzyloxy-D-*valine.* Carbobenzyloxylation of 11.7 g. of D-valine (Mann) in the usual manner[8] gave 18.1 g. (72%) of colorless plates, m.p. 54–57° after recrystallization from ethyl acetate–petroleum ether (b.p. 40–60°), $[\alpha]^{20}D$ +5.0 ± 1.0° (*c* 1.3, methanol). An analytical sample melted at 60–62°.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.27; H, 6.96; N, 5.65.

Carbobenzyloxy-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-

(7) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p. 1233.

(8) (a) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932); (b) J. R. Vaughan and J. A. Eichler, *J. Am. Chem. Soc.*, **75**, 5556 (1953).

2-*hydroxyethylamide*. This peptide was prepared by coupling 0.81 g. of Z-D-Val-OH with 3.68 g. of L-(H)-Try-[D-Leu-L-Try-]$_3$-AE to yield 3.6 g. (81%), R_f 0.90 (A).

D-*Valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide*. Hydrogenolysis of 3.6 g. of Z-D-Val-L-Try-[D-Leu-L-Try-]$_3$-AE in methanol containing a few drops of acetic acid was complete after 10 hr. and gave, after purification on Dowex 50W-X2; 2.4 g. (74%) of the free peptide, R_f 0.79 (A).

Carbobenzyloxy-L-*valyl*-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide*. This peptide was obtained in the coupling reaction of 0.47 g. of Z-L-Val-OH[8] (prepared from L-valine (Mann)) with 2.35 g. of D-(H)Val-L-Try-[D-Leu-L-Try-]$_3$-AE in a yield of 2.51 g. (89%), R_f 0.91 (A).

L-*Valyl*-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide*. Hydrogenolysis of 2.5 g. of Z-L-Val-D-Val-L-Try-[D-Leu-L-Try-]$_3$-AE in methanol containing a few drops of acetic acid was complete after 10 hr. and gave, after purification on Dowex 1-X2, 2 g. (88%) of the free peptide, R_f 0.81 (A).

Carbobenzyloxy-D-*valyl*-L-*valyl*-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide*. The coupling reaction of 375 mg. of Z-D-Val-OH with 2 g. of L-(H)Val-D-Val-L-Try-[D-Leu-L-Try-]$_3$-AE gave 1.95 g. (83%) of the protected peptide, R_f 0.92 (A).

D-*Valyl*-L-*valyl*-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-2-*hydroxyethylamide*. Hydrogenolysis of 1.9 g. of Z-D-Val-L-Val-D-Val-L-Try-[D-Leu-L-Try-]$_3$-AE in methanol for 10 hr. gave, after purification on Dowex 50W-X2, 1.04 g. (60%) of free peptide, R_f 0.82 (A).

L-*Valyl*-D-*valine Methyl Ester Hydrochloride*. Hydrogenolysis of 6.3 g. of Z-L-Val-D-Val-OMe,[9] R_f 0.70 (A), m.p. 161°, prepared by the mixed anhydride method from Z-L-Val-OH and D-(H)Val-OMe·HCl,[10] in 2-propanol–acetic acid over palladium on charcoal for 4 hr., followed by filtration, evaporation, addition of methanolic hydrochloric acid, evaporation, and crystallization from methanol–ether, gave 3.7 g. (82%) of dipeptide ester in colorless prisms, m.p. 214°, $[\alpha]^{20}D$ +59 ± 1.0° (*c* 1, methanol), R_f 0.45 (B); EPM 5.45.

Anal. Calcd. for $C_{11}H_{23}N_2O_3Cl$: C, 49.52; H, 8.69; N, 10.50. Found: C, 49.57; H, 8.74; N, 10.58.

Carbobenzyloxy-D-*valyl*-L-*valyl*-D-*valine Methyl Ester*. By the mixed anhydride method 3.3 g. of Z-D-Val-OH was coupled with 3.46 g. of L-(H)Val-D-Val-OMe·HCl to yield 4.98 g. (83%) of protected tripeptide ester, m.p. 205–206°, after recrystallization from ethyl acetate–petroleum ether, $[\alpha]^{20}D$ 6.0 ± 0.5° (*c* 1.3, methanol), R_f 0.71 (A).

Anal. Calcd. for $C_{24}H_{37}N_3O_6$: C, 62.18; H, 8.05; N, 9.07. Found: C, 62.47; H, 7.75; N, 8.89.

Carbobenzyloxy-L-*alanyl*-D-*valyl*-L-valyl-D-*valine Methyl Ester* (*V*). Coupling, by the mixed anhydride method, of 1.95 g. of Z-L-Ala-OH (Mann) and 3.2 g. of (oily) D-(H)Val-L-Val-D-Val-OMe·HCl,[11] R_f 0.46 (B) (prepared by hydrogenolysis of the carbobenzyloxy compound), gave 3.6 g. (77%) of the tetrapeptide, which crystallized in needles from ethyl acetate–petroleum ether (b.p. 40–60°), m.p. 215°, $[\alpha]^{20}D$ 3.0 ± 1.0° (*c* 1.3, methanol), R_f 0.69 (A).

Anal. Calcd. for $C_{27}H_{42}N_4O_7$: C, 60.65; H, 7.92; N, 10.48. Found: C, 60.82; H, 7.81; N, 10.44.

L-*Alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester Hydrochloride*. By hydrogenolysis of 3.2 g. of Z-L-Ala-D-Val-L-Val-D-Val-OMe in acetic acid over palladium on charcoal the free peptide ester was obtained, which could not be crystallized as the acetate or hydrochloride; yield of the hydrochloride: 2.6 g. (100%), R_f 0.48 (B); EPM 4.3.

Carbobenzyloxy-D-*leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester*. This pentapeptide was obtained from 1.6 g. of Z-D-Leu-OH and 2.6 g. of L-(H)Ala-D-Val-L-Val-D-Val-OMe·HCl by the mixed anhydride method in a yield of 2.17 g. (56%), R_f 0.72 (A).

D-*Leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester Acetate*. Hydrogenolysis of 2.15 g. of Z-D-Leu-L-Ala-D-Val-L-Val-D-Val-OMe in acetic acid over palladium on charcoal gave 1.6 g. (84%) of a product as a noncrystalline solid from methanol–ether, R_f 0.65 (B); EPM 3.65.

Carbobenzyloxy-L-*alanyl*-D-*leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester*. The coupling of 580 mg. of Z-L-Ala-OH with 1.5 g. of D-(H)Leu-L-Ala-D-Val-L-Val-D-Val-OMe·AcOH yielded 1.6 g. (86%) of the hexapeptide, R_f 0.69 (A).

L-*Alanyl*-D-*leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester Acetate*. By hydrogenolysis of 1.5 g. of Z-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-OMe in acetic acid over palladium–charcoal 1.1 g. (81%) of the hexapeptide ester was obtained in solid form from methanol–ether, R_f 0.60 (B); EPM 3.45.

Formyl-L-*valyl-glycine*. Formyl-L-valyl-glycine ethyl ester was prepared by the mixed anhydride method from formyl-L-valine and ethyl glycinate in tetrahydrofuran at −20° as described above. The yield was only 36%, which is due to loss of the water-soluble peptide during the normal washing procedure of the ethyl acetate solution. The melting point was 153–155° after crystallization from ethyl acetate (reported 156–157°[12]). Saponification of the ester in dioxane with 2.0 *N* alkali followed by evaporation and acidification of the water solution gave the free peptide in 30% yield. After recrystallization from water, it had m.p. 206–208° dec., $[\alpha]^{20}D$ −49.1 ± 1.0° (*c* 1.4, 70% ethanol), reported m.p. 207–208°, $[\alpha]^{28}D$ −50.9° (*c* 1.3, 70% ethanol).[12] For comparison the formyl dipeptide ester was also prepared by the carbodiimide method. After saponification of this ester crystalline formyl-L-valyl-glycine was obtained, m.p. 208–209° dec., $[\alpha]^{20}D$ −50.2 ± 1.0° (*c* 1.1, 70% ethanol). The synthesis of optically pure formyl peptides by the mixed anhydride method is feasible provided that the proper solvent and temperature are used.[4,12,13]

Formyl-L-*valyl-glycyl*-L-*alanyl*-D-*leucyl*-L-*alanyl*-D-

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valyl-L-*valyl*-D-*valine Methyl Ester.* By the mixed anhydride method 202 mg. of formyl-L-valyl-glycine, dissolved in a mixture of tetrahydrofuran and acetonitrile, were coupled with 645 mg. of L-(H)Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-OMe to give 546 mg. (74%) of the formyl octapeptide ester, R_f 0.52 (A). This ester was not very soluble in ethyl acetate and was washed as a suspension. The ester was recovered unchanged after several saponification attempts with 3.0 *N* sodium hydroxide in methanol and dioxane at room temperature.

Carbobenzyloxy-L-*valyl-glycyl*-L-*alanyl*-D-*leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valine Methyl Ester* (Ia). Ethyl carbobenzyloxy-L-valyl-glycinate was prepared by the mixed anhydride method in 75% yield, m.p. 168°, (reported m.p. 166°[14]). Saponification of this ester with 1.0 *N* alkali in dioxane gave Z-L-Val-Gly in 59% yield, m.p. 147° after recrystallization from ethyl acetate–petroleum ether (reported m.p. 146°[13]). By the mixed anhydride method 93 mg. of Z-L-Val-Gly-OH and 194 mg. of L-(H)Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-OMe gave 210 mg. of the octapeptide ester (m.p. 264–265°), R_f 0.68 (A). All saponification attempts failed.

Anal. Calcd. for $C_{43}H_{70}N_8O_{11}$: C, 59.2; H, 8.07; N, 12.80. Found: C, 58.40; H, 8.10; N, 12.56.

Carbobenzyloxy-L-*valyl-glycyl*-L-*alanyl*-D-*leucyl*-L-*alanyl*-D-*valyl*-L-*valyl*-D-*valyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryptophyl*-D-*leucyl*-L-*tryprophyl*-*2-hydroxyethylamide* (III). To a solution of 80 mg. of Z-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-OMe in 5 ml. of methanol was added 3 ml. of 98% hydrazine and the mixture was kept at 50° for 4 hr.[15] The product was precipitated with 100 ml. of water, washed with water, and dried to give 60 mg. (75%) of the hydrazide, R_f 0.70 (A). *Anal.* Calcd. N, 16.08%. Found: N, 16.49%. To a solution of 53.5 mg. of the hydrazide in 3 ml. of acetic acid containing 0.2 ml. of 1.0 *N* hydrochloric acid, cooled to 0°, was added 0.1 ml. of 1.0 *N* sodium nitrite solution, and the mixture was stirred for 15 min. at 0°. Addition of 10 ml. of ice–water precipitated the azide, which was filtered, washed with water and bicarbonate solution, and dried in the desiccator at −10°. The azide was dissolved in 4 ml. of dimethylformamide and allowed to react with 46 mg. of L-(H)Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-AE at 4° for 5 days. The reaction product was precipitated with water, collected, dissolved in methanol, and filtered over a column of Dowex 50W-X2 (H^+ form). Evaporation of the filtrate gave 24 mg. (30%) of material which was, on thin layer chromatography, identical with carbobenzyloxydesformylgramicidin A; R_f values 0.57 (A), 0.81 (C). Hydrogenolysis of this compound in acetic acid over palladium on charcoal leads to complete destruction of the tryptophan residues. The successful hydrogenolysis in methanol is described later.

Carbobenzyloxy-D-*leucyl*-L-*alanine Methyl Ester.* L-Alanine methyl ester hydrochloride was prepared by the thionyl chloride method[16]: 70 ml. of methanol was cooled to −10° and 10 ml. of thionyl chloride was added. To this mixture was added slowly 8.9 g. of L-alanine and the mixture was kept at −10° for 5 min. and at room temperature for 30 min. The mixture was evaporated, but the residue did not crystallize from methanol–ether due to incomplete esterification (electrophoresis). Therefore the esterification procedure was repeated, this time keeping the mixture for 12 hr. at room temperature. After evaporation the residue crystallized readily from methanol–ether and was electrophoretically pure, m.p. 109–110° (reported m.p. 109–110°,[17,18] 154–155°), yield 12.6 g. (90%). By the mixed anhydride method 9.0 g. of carbobenzyloxy-D-leucine was coupled with 4.75 g. of methyl L-alaninate hydrochloride to give 6.9 g. (58%) of the dipeptide ester in colorless needles; after recrystallization from ethyl acetate–petroleum ether (b.p. 40–60°), m.p. 130–131°, analytical sample m.p. 131–132°, $[\alpha]^{20}D$ 4.8 ± 1.5° (*c* 0.63, methanol), R_f 0.72 (A).

Anal. Calcd. for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 8.00. Found: C, 61.85; H, 7.73; N, 8.25.

D-*Leucyl*-L-*alanine Methyl Ester Hydrochloride.* Hydrogenolysis of Z-D-Leu-L-Ala-OMe in acetic acid over palladium black gave a considerable amount of crystalline material, m.p. 265°, which was, according to analysis and infrared spectrum (only one band at 1680 $cm.^{-1}$),[19] the diketopiperazine. *Anal.* Calcd. for $C_9H_{16}N_2O_2$: C, 58.67; H, 8.75; N, 15.21. Found: C, 58.40; H, 8.55; N, 15.40. Apparently the acetic acid insufficiently protected the amino group. Therefore, 6.0 g. of Z-D-Leu-L-Ala-OMe was catalytically debenzylated in methanol containing 1.5 ml. of 15.0 *N* hydrochloric acid in methanol in the presence of palladium black to give 4.3 g. (99%) of the hydrochloride, which did not crystallize but was pure on paper electrophoresis, R_f 0.51 (B); EPM 5.45.

Carbobenzyloxy-L-*alanyl*-D-*leucyl*-L-*alanine Methyl Ester.* By the mixed anhydride method 3.8 g. of carbobenzyloxy-L-alanine was coupled with 4.3 g. of D-(H)Leu-L-Ala-OMe·HCL to give 5.0 g. (71%) of the peptide ester, colorless prisms from ethyl acetate–petroleum ether (b.p. 40–60°), m.p. 152–153°, $[\alpha]^{20}D$ 5.4 ± 1.0° (*c* 0.85, methanol), R_f 0.68 (A).

Anal. Calcd. for $C_{21}H_{31}N_3O_6$: C, 59.84; H, 7.44; N, 9.97. Found: C, 60.06; H, 7.54; N, 9.72.

L-*Alanyl*-D-*leucyl*-L-*alanine Methyl Ester Acetate.* The tripeptide ester was obtained in 100% yield as an oil by hydrogenolysis of the carbobenzyloxy derivative in methanol containing acetic acid, R_f 0.47 (B); EPM 4.65.

Carbobenzyloxy-glycyl-L-*alanyl*-D-*leucyl*-L-*alanine Methyl Ester.* By the mixed anhydride method 2.8 g. of carbobenzyloxyglycine was coupled with 4.6 g. of L-(H)Ala-D-Leu-L-Ala-OMe·AcOH to yield 4.48 g. (71%) of tetrapeptide ester in colorless needles; after recrystallization from ethyl acetate–petroleum ether (b.p. 40–60°), m.p. 116–119°; on reheating the compound melted at 166°, $[\alpha]^{20}D$ −13 ± 1.0° (*c* 1.1, methanol); R_f 0.59 (A).

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Anal. Calcd. for $C_{23}H_{34}N_4O_7$: C, 57.72; H, 7.16; N, 11.71. Found: C, 57.68; H, 6.88; N, 11.74.

Glycyl-L-alanyl-D-leucyl-L-alanine Methyl Ester Acetate. The tetrapeptide ester was obtained as a noncrystalline solid from methanol–ether after hydrogenolysis of Z-Gly-L-Ala-D-Leu-L-Ala-OMe in methanol containing acetic acid in 100% yield, R_f 0.36 (B); EPM 4.4.

Carbobenzyloxy-L-valylglycyl-L-alanyl-D-leucyl-L-alanine Methyl Ester. When 630 mg. of carbobenzyloxy-L-valine was coupled with 990 mg. of Gly-L-Ala-D-Leu-L-Ala-OMe there was obtained 1.06 g. (74%) of the pentapeptide ester in noncrystalline, solid form, R_f 0.61 (A).

Carbobenzyloxy-L-valyl-glycyl-L-alanyl-D-leucyl-L-alanine (IV). To a solution of 850 mg. of Z-L-Val-Gly-L-Ala-D-Leu-L-Ala-OMe in 5 ml. of dioxane was added 2 ml. of 1.0 *N* alkali and later 20 ml. of water. After 1 hr. at room temperature the mixture was evaporated, the residue dissolved in water, the solution washed with ethyl acetate, the water layer acidified to pH 3 at 0°, and the precipitated crystals were collected and recrystallized from ethyl acetate–methanol–petroleum ether (b.p. 40–60°) to give 400 mg. (49%) of colorless prisms, m.p. 222–223°, $[\alpha]^{20}D$ 11.2 ± 1.5° (*c* 0.8, methanol), R_f 0.56 (A).

Anal. Calcd. for $C_{27}H_{41}N_5O_8$: C, 57.53; H, 7.33; N, 12.43. Found: C, 57.61; H, 7.31; N, 12.36.

Carbobenzyloxy-L-isoleucyl-glycyl-L-alanyl-D-leucyl-L-alanine Methyl Ester. When 0.4 g. of carbobenzyloxy-L-isoleucine was coupled with 0.6 g. of H-Gly-L-Ala-D-Leu-L-Ala-OMe·AcOH by the mixed anhydride method there was obtained 712 mg. (80%) of the pentapeptide ester in a solid, noncrystalline form, R_f 0.63 (A).

Carbobenzyloxy-L-isoleucyl-glycyl-L-alanyl-D-leucyl-L-alanine (IVi). Saponification of 0.7 g. of Z-L-Ileu-Gly-L-Ala-D-Leu-L-Ala-OMe in 40 ml. of dioxane with 1.4 ml. of 1.0 *N* alkali, as described above, gave 0.35 g. (51%) of the pentapeptide in colorless prisms; after recrystallization from ethyl acetate–methanol–petroleum ether (b.p. 40–60°), m.p. 215–216°, $[\alpha]^{20}D$ 11.6 ± 1.5° (*c* 1.1, methanol), R_f 0.57 (A).

Anal. Calcd. for $C_{28}H_{43}N_5O_8$: C, 58.21; H, 7.50; N, 12.12. Found: C, 58.29; H, 7.38; N, 11.93.

Formyl-L-valylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryprophyl-2-hydroxyethylamide (VII). To a solution of 113 mg. of Z-L-Val-Gly-L-Ala-D-Leu-L-Ala-OH (IV) and 288 mg. of D-(H)Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-AE (V) in 15 ml. of tetrahydrofuran and 0.2 ml. of dimethylformamide, cooled to −20°, was added 50 mg. of dicyclohexylcarbodiimide, and the mixture was kept at −20° for 24 hr. and at room temperature for 3 days. The solvent was evaporated and the residue dissolved in methanol and filtered over a column of Dowex 50W-X2 (H^+ form) and a Dowex 1-X2 (OH^- form) to give, after evaporation, 475 mg. of neutral product, containing some dicyclohexylurea (characteristic crystals), which on thin layer chromatography gave R_f values 0.57 (A) and 0.81 (C) identical with N-carbobenzyloxydesformylgramicidin A. This product was decarbobenzyloxylated in methanol containing a few drops of acetic acid over palladium black, the solution was filtered and evaporated, and the residue was dissolved in methanol and filtered over a column of Dowex 50W-X2 (H^+ form). The column was washed with methanol and then eluted with 2.0 *N* ammonia in methanol to give 180 mg. (50%) of a basic product (VI), which on thin layer chromatography showed R_f values 0.68 (B) and 0.23 (A), identical with desformylgramicidin A. To a solution of 150 mg. of this basic peptide VI in 2 ml. of 98% formic acid, cooled to 0°, was added 0.6 ml. of acetic anhydride, and the mixture was kept at 0° for 30 min. and at room temperature for 4 hr. The solvent was removed *in vacuo* and the residue (R_f 0.47 (A) and 0.79 (C)) was dissolved in 2 ml. of methanol and treated with 0.4 ml. of 1.0 *N* alkali for 1 hr. at room temperature. The reaction mixture was filtered over a Dowex 50W-X2 column and after evaporation gave 125 mg. (82%) of a product which was purified by countercurrent distribution. After 203 transfers in the system methanol–water–chloroform–benzene (23:7:15:15)[20] at 26° one major peak (yield 65 mg., optical density at 282 mμ) was obtained with a *K* value of 0.97 (Figure 2).[20] In the literature *K* values of 0.59,[20] 0.58,[21] and up to 0.70[21] for valine-gramicidin A have been reported, but with more transfers a value of 0.87 has also been found.[22] For comparison commercial gramicidin was distributed under the same conditions and the major peak was found to have a *K* value of 0.93. The temperature has considerable influence on the *K* values. This material was collected and was found on thin layer chromatography to have R_f values 0.42 (A) (only one spot) and 0.65 (C) for the fastest moving and strongest spot, identical with valine-gramicidin A. The product shows the characteristic multiplicity of spots in solvent system C, which is characteristic of gramicidin, and had $[\alpha]^{20}D$ 27.3 ± 2.5° (*c* 1.3, methanol), and ε 22,100 ± 1000 in ethanol at λ 282 mμ.

Attempts at crystallization have so far not been successful. However, crystallization of commercial or purified gramicidin A[23] in our hands proved very difficult on a small scale (10–50 mg.).

Formyl-L-isoleucyl-glycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryptophyl-2-hydroxyethylamide (VIIi). This peptide derivative was prepared in an analogous manner by coupling 58 mg. of Z-L-Ileu-Gly-L-Ala-D-Leu-L-Ala-OH (IVi) with 144 mg. of D-(H)Val-L-Val-D-Val-L-Try-D-Leu-L-Try-D-Leu-L-Try-D-Leu-L-Try-AE (V) in the presence of 25 mg. of dicyclohexylcarbodiimide. Hydrogenolysis of the carbobenzyloxy peptide ethanolamide (IIIi), R_f 0.58 (A), gave 118 mg. (62%) of basic material, whose R_f 0.25 (A) was identical with desformylgramicidin A. Formylation of this material as above gave 90 mg. (75%) of a product which on countercurrent distribution gave one major peak (42 mg.) with a *K* value of 0.81 and R_f values of 0.45 (A) and 0.66 (C) (for the fastest moving spot), $[\alpha]^{20}D$ 26.5 ± 3.0° (*c* 0.45, methanol), ε 22,450 ± 1000 in ethanol.

N-Carbobenzyloxydesformylgramicidin A. To a solution of 54 mg. of desformylgramicidin A (prepared

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from natural gramicidin A) in 5 ml. of ethyl acetate was added 2 ml. of 5% aqueous bicarbonate solution and 2 drops (20 mg.) of carbobenzyloxy chloride. The heterogeneous mixture was stirred vigorously at room temperature for 1 hr. The layers were separated and the ethyl acetate extract was washed with water, dried, and evaporated. The residue was dissolved in 2 ml. of dioxane and treated for 1 hr. at room temperature with 0.5 ml. of 1.0 *N* alkali. The mixture was evaporated, the residue was dissolved in ethyl acetate, washed with water, and dried, and the solvent was removed by evaporation. The residue was precipitated from ethyl acetate–petroleum ether (b.p. 40–60°) to give 50 mg. (86%) of carbobenzyloxydesformylgramicidin A (ninhydrin negative, R_f 0.57 (A) and 0.81 (C)). Hydrogenolysis in methanol containing a few drops of acetic acid over palladium black gave desformylgramicidin A.

Gramicidin. VII. The Structure of Valine- and Isoleucine-gramicidin B

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The volatile acidic fragment in the hydrolysate of gramicidin B was identified as formic acid which, after reduction to formaldehyde, was assayed by the chromotropic acid test. The primary sequence of gramicidin B, which in the sample examined consisted of 89% of valine-gramicidin B and 11% of isoleucine-gramicidin B, was determined by a 14-step Edman degradation on desformylgramicidin B (obtained from gramicidin B by mild methanolysis) as HCO-L-*Val*(L-*Ileu*)-*Gly*-L-*Ala*-D-*Leu*-L-*Ala*-D-*Val*-L-*Val*-D-*Val*-L-*Try*-D-*Leu*-L-*Phe*-D-*Leu*-L-*Try*-D-*Leu*-L-*Try*-$NHCH_2CH_2OH$.

The peptide antibiotic gramicidin, isolated by Hotchkiss and Dubos,[2] was shown by Gregory and Craig[3] to be heterogeneous. By countercurrent distribution in the solvent system methanol–water–benzene–chloroform (23:7:15:15) three fractions were obtained, the major fraction designated gramicidin A (distribution coefficient K = 0.61), and two minor fractions designated gramicidin B (K = 0.31) and gramicidin C (K = 1.32).[3-5]

The structure of gramicidin A, a mixture of valine- (I) and isoleucine-gramicidin A (II),[5,6] was recently elucidated[7-9] and confirmed by synthesis.[10] Gramicidin B is composed of very similar acids except that one tryptophan is replaced by phenylalanine. Analogously, in gramicidin C one tryptophan is substituted by tyrosine.[5,11]

Gramicidin B shows the same multiplicity of spots on thin layer chromatography as gramicidin A in the solvent system methyl ethyl ketone–pyridine (7:3), and the two congeners cannot be distinguished in this system. However, in the system acetic acid–chloroform (2:1) the R_f value of gramicidin B (one spot, 0.42) is higher than that of gramicidin A (one spot, 0.35).

First we established that gramicidin B, too, is a formyl-peptide ethanolamide. On hydrolysis of gramicidin B with 50% sulfuric acid formic acid is released, which after distillation and reduction to formaldehyde was identified by the chromotropic acid reaction.[7,9,12] By treatment with hydrogen chloride in methanol under the same mild conditions that lead to deformylation of gramicidin A,[7,9,13] neutral gramicidin B is converted to a ninhydrin-positive basic peptide, desformylgramicidin B, in a yield of 95% after purification on Dowex 50W-X2. By N-bromosuccinimide oxidation[14] with 6 moles of NBS per 1845 g. of gramicidin B, *i.e.* 2 moles of NBS per mole of tryptophan, ethanolamine is liberated to the same extent as in gramicidin A. The liberation of ethanolamine was followed by paper electrophoresis and gas chromatography. In gramicidin B the carboxyl group of COOH-terminal tryptophan is connected to 2-aminoethanol in the fashion . . . .-Try-$NHCH_2CH_2OH$, as is the case in gramicidin A.[7,9]

An improved and extended 14-step Edman degradation[15] was carried out with desformylgramicidin B in the same way as described earlier for desformylgramicidin A.[8,9] Although this time the cyclization step with trifluoroacetic acid was carried out under nitrogen and at room temperature for only 1 hr., the gradual destruction of tryptophan could not be prevented even under such mild conditions. However, the yield in each cyclization step was increased to such an extent that a 14-step degradation was possible.

The phenylthiohydantoins of the amino acids were

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