tion was dried (Na_2SO_4), freed of solvent, and vacuum distilled. The yield of product was 12 g (65% based on the cyclohexylamine salt and 6.4% based on 2-hexenoic acid).

2-Bromo-3-fluoroheptanoic Acid. 2-Heptenoic acid (91 g, 0.7 mol) was bromo fluorinated in 150 ml of liquid HF with NBA (104 g, 0.75 mol), and the product was purified in the same manner as 2-bromo-3-fluorohexanoic acid except that a dicyclohexylamine salt was used for purification. The yield of salt was 226 g (79%), and an analytical sample was crystallized from an ether-petroleum ether mixture: mp 117.5–118°. *Anal.* ($C_{19}H_{35}BrFNO_2$) C, H, Br, N.

Dicyclohexylammonium 2-bromo-3-fluoroheptanoate (226 g, 0.55 mol) was dissolved in 200 ml of concentrated H_2SO_4, and 50 ml of H_2O was added dropwise with cooling and stirring. The mixture was extracted twice with 100-ml portions of $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4), freed of solvent, and vacuum distilled. The yield of product was 47.7 g (38% based on the dicyclohexylamine salt and 30% based on 2-heptenoic acid).

3-Fluoroalanine. 2-Bromo-3-fluoropropionic acid (30.8 g, 0.18 mol) was dissolved in 75 ml of liquid NH_3 and sealed in a stainless steel pressure vessel. After remaining at room temperature for 3 days, the NH_3 was removed, and the residue was dissolved in a small volume of H_2O and brought to pH 5 with HBr. The solution was evaporated under vacuum below 40°, and the residue was slurried repeatedly with MeOH until a negative test was obtained with $AgNO_3$. A yield of 5.4 g of product was obtained.

2-Amino-3-fluorobutyric Acid. 2-Bromo-3-fluorobutyric acid (52 g, 0.28 mol) was treated with 100 ml of liquid NH_3 in the same manner as for the preparation of 3-fluoroalanine. A yield of 12.2 g of product was obtained.

3-Fluorovaline was prepared in the same manner as 3-fluoroalanine except that the amination was carried out for 3 days at 65°. 2-Bromo-3-fluoro-3-methylbutyric acid (31 g, 0.31 mol) in 80 ml of liquid NH_3 yielded 10 g of product.

3-Fluoronorvaline was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluoropentanoic acid (39 g, 0.2 mol) in 150 ml of liquid NH_3 yielded 4.4 g of product.

3-Fluoronorleucine was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluorohexanoic acid (10.8 g, 0.05 mol) was aminated in 50 ml of liquid NH_3. The yield of product was 2.7 g.

2-Amino-3-fluoroheptanoic Acid was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluoroheptanoic acid (30 g, 0.13 mol) was aminated in 100 ml of liquid NH_3, and the yield of product was 2.2 g.

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Supplementary Material Available. Infrared spectra will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-1407.

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Probiotics.† Antistaphylococcal and Antifibrinolytic Activities of ω-Guanidino Acids and ω-Guanidinoacyl-L-histidines[2,‡]

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Following the discovery of the antistaphylococcal activity of a series of ω-amino acids,[3] ω-aminoacyl-L-histidines,[3b] N^{α}-(ω-aminoacyl)-L-lysines,[4] and ω-amino-β-hydroxyacyl-L-histidines,[5] we have now prepared a series of ω-guanidino acids and ω-guanidinoacyl-L-histidines in order to compare their antistaphylococcal activity. The antifibrinolytic activity of both ω-guanidino acids and ω-guanidinoacyl-L-histidines was also determined because of the relationship discussed previously.[3b,4]

The compounds described in this paper are ω-guanidino acids, $H_2NC(=NH)NH(CH_2)_nCOOH$ [where n = 1, guanidinoacetic acid (**1**); n = 2, β-guanidinopropionic acid (**2**); n = 3, γ-guanidinobutyric acid (**3**); n = 4, δ-guanidinovaleric acid (**4**); n = 5, ε-guanidinohexanoic acid (**5**)], and ω-guanidinoacyl-L-histidines, $H_2NC(=NH)NH(CH_2)_nCO$-His [where n = 1, guanidinoacetyl-L-histidine (**6**); n = 2, β-guanidinopropionyl-L-histidine (**7**); n = 3, γ-guandinobutyryl-L-histidine (**8**); n = 4, δ-guanidinovaleryl-L-histidine (**9**); n = 5, ε-guanidinohexanoyl-L-histidine (**10**)].

Chemistry. Compounds **4**–**10** were prepared from the corresponding ω-amino acids and ω-aminoacyl-L-histidines[3b] by treating with *S*-ethylisothiourea. This preparative procedure is a modification of the one described by Takahashi, *et al.*[6] Compounds **4** and **5** were synthesized under strong basic conditions,[6] but **6**–**10** were formed at mild basic pH of 8–9. Previous references indicated the use of strong basic conditions, such as concentrated NH_4OH,[7,8] 1 *N* MeONa,[9] and 2 *N* NaOH.[6] We found that weak basic conditions (pH 8–9) are also applicable, an advantage for the synthesis of the compounds which are not stable under strong basic conditions. The reaction products were purified by means of ion-exchange chromatography, using aqueous pyridine for **4** and **5** and pyridine–NH_4OH for **6**–**10**. The yields and physical and analytical data for the compounds are given in Table I. R_f values on tlc were determined in the five solvents used previously.[3b] All compounds were homogeneous by tlc.

Testing Procedure. *In vivo* antistaphylococcal activity was determined by the method described before.[3b,4] γ-Aminobutyryl-L-histidine was used as the positive control. Antistaphylococcal activity *in vitro* was determined by the paper disk method. Approximately 0.2 mg of the sample was placed on the paper disk which rested upon the surface of a plate of Bacto Staphylococcus Medium 110 (Difco) which had been inoculated with *Staphylococcus aureus*. The plates were examined after incubation for 24 hr at 37°. The lysis time procedure for antifibrinolytic activity *in vitro* was described previously[3b] with ε-aminohexanoic acid as a positive control. The lysis area procedure for antifibrinolytic activity was as follows.[10],§ Bovine fibrinogen (4%, 10 ml) in pH 7.4 Palitzsch's buffer was poured into a 10-cm (i.d.) petri dish. Bovine thrombin (5

†The term probiotics has been proposed to designate these compounds and those described previously which build resistance to infection in the host but do not inhibit the growth of microorganisms *in vitro*. The term first was used in 1953.[1a] Sperti[1b] describes the earlier history and particularly the biological aspects. The isolation and identification of probiotics from natural sources was recently summarized by Cook and Tanaka.[1c]

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§A. Fujii, Ph.D. Dissertation, St. Thomas Institute, Cincinnati, Ohio, 1970.

Table I. ω-Guanidino Acids and ω-Guanidinoacyl-L-histidines

Compd	Yield, %[a]	Mp, °C[b]	Solvents	Formula	Analyses[c]	Color reaction[d] P	S	N
4[e]	28.0	267–271	H_2O–EtOH–Et_2O	$C_6H_{13}N_3O_2$	C, H, N	−	+	−
5[f]	68.0	274–275	Aqueous NH_4OH[g]	$C_7H_{15}N_3O_2 \cdot HCl$	C, H, N, Cl	−	+	−
6[h]	69.2	108–111 dec	H_2O–EtOH	$C_9H_{14}N_6O_3 \cdot H_2O$	C, H, N	+	+	−
7[i]	38.8	121–124 dec	H_2O–EtOH	$C_{10}H_{16}N_6O_3 \cdot H_2O$	C, H, N	+	+	−
8[j]	51.7	119–124 dec	H_2O–EtOH[k]	$C_{11}H_{18}N_6O_3 \cdot H_2SO_4$	C, H, N, S	+	+	−
9[l]	50.6	80–82 dec	H_2O–EtOH[k]	$C_{12}H_{20}N_6O_3 \cdot H_2SO_4 \cdot H_2O$	C, H, N, S	+	+	−
10[m]	56.1	102–103 dec	H_2O–EtOH	$C_{13}H_{22}N_6O_3$	C, H, N	+	+	−

[a] Based on ω-amino acids for **4** and **5** and ω-aminoacyl-L-histidines for **6**–**10**. [b] Melting points were determined by the capillary tube method and were uncorrected. [c] Analytical results for C, H, N, Cl, and S were within ±0.4% of the theoretical value. [d] P, S, and N indicate Pauli, Sakaguchi, and ninhydrin reactions, respectively. [e] Ir peaks (cm^{-1}) were 632, 716, 828, 934, 981, 1094, 1151. [f] Ir peaks (cm^{-1}) were 615, 658, 690, 742, 818, 845, 911, 977, 1013, 1081, 1150. [g] At pH 8.0. [h] Ir peaks (cm^{-1}) were 620, 660, 818, 935, 984, 1090, 1105, 1190, 1268, 1332, 1398, 1441. [i] Ir peaks (cm^{-1}) were 618, 661, 935, 986, 1086, 1102, 1181, 1250, 1322, 1393, 1431. [j] Ir peaks (cm^{-1}) were 620, 808, 918, 990, 1127, 1266, 1398, 1440. [k] At pH 5.0 (2 *N* H_2SO_4). [l] Ir peaks (cm^{-1}) were 615, 820, 918, 980, 1045, 1114, 1256, 1391, 1435. [m] Ir peaks (cm^{-1}) were 670, 808, 930, 980, 1043, 1082, 1100, 1176, 1260, 1320, 1400, 1430.

Table II. Antistaphylococcal and Antifibrinolytic Activities of ω-Guanidino Acids and ω-Guanidinoacyl-L-histidines

Compd	Antistaphylococcal activity: No. of mice	No. of trials	% protection[a] Mean	SD	Antifibrinolytic activity[b]: Lysis time, min[c] Mean	SD	Lysis area, % inhibition[d] Mean	SD
1	42	5	50	9	13	2	5	4
2	42	5	32	7	16	2	17	11
3	42	5	44	13	23[e]	3	49	17
4	32	4	66	8	40	2	50	20
5	32	4	57	8	15[f]	2	12	4
6	30	3	45	9	14	2	0	
7	30	3	58	18	14	2	0	
8	30	3	78	10	15	1	5	2
9	30	3	65	4	15	1	0	
10	31	3	71	2	13	2	0	
L-Arg	22	3	6	5	12	2	5	3
γ-Abu-His[g]	72	8	65	11				
ε-Ahx[h]					90	8	90	18
Negative control[i]	72	8			12	1		

[a] Per cent protection = [(mortality of control) − (mortality of experimental)] × 100/(mortality of control). [b] Sample concentration was 10^{-5} *M* in all experiments. [c] Number of minutes required for the clot to flow out from the test tubes. [d] Per cent inhibition = [(lysis area of control) − (lysis area of experimental)] × 100/(lysis area of control). [e] M. Mangyo [*Seikagaku*, **36**, 735 (1964)] gave the minimal inhibitory dose as 2×10^{-3} mol/ml when ε-Ahx was 10^{-5} *M*. [f] K. Lohmann, F. Markwardt, and H. Landmann, *Throm. Diath. Haem.*, **10**, 424 (1964), gave the minimal inhibitory dose as 100 μmol/ml when ε-Ahx was 1.4 μmol/ml. [g] γ-Aminobutyryl-L-histidine was used as a positive control for antistaphylococcal experiments. [h] ε-Aminohexanoic acid was used as a positive control for antifibrinolytic tests. [i] Control for antifibrinolytic test, lysis time method, 0.05 *M* phosphate buffer–saline solution.

ml of 10 units/ml) was added and the dish was rotated for 3 sec. The fibrin was allowed to harden. Each dish served for four tests: negative control (human blood serum plus streptokinase), positive control (same plus ε-aminohexanoic acid), and two experimentals (serum, streptokinase, test compound). After incubation for 2 hr at 37° the lysis area was obtained by measuring the longest and shortest dimensions of the clear zone.

Biological Results and Discussion. Antistaphylococcal and antifibrinolytic activities of ω-guanidino acids and ω-guanidinoacyl-L-histidines are summarized in Table II. All of the ω-guanidino acids produced better protection for mice against *S. aureus* infections than the corresponding ω-amino acids.[3] Most of the histidine compounds were more potent antistaphylococcal agents than the corresponding component ω-guanidino acids. The gain in potency of the ω-guanidino-L-histidines was essentially similar to that of ω-aminoacyl-L-histidines.[3b] Compound **1** provided unexpectedly high protection. Compound **4** was the best antistaphylococcal agent among the ω-guanidino acids and was much more protective against *S. aureus* infection than L-arginine which differs only in having an α-NH_2 group. Compounds **8** and **10** were shown to have greater antistaphylococcal activity than homocarnosine. None of the compounds inhibited the growth of *S. aureus in vitro*.

All of the ω-guanidino acids showed some antifibrinolytic activity and **4** was the most active agent. No antifibrinolytic activity was obtained in ω-guanidinoacyl-L-histidines. This is also essentially similar to the action of ω-aminoacyl-L-histidines.[3b] ω-Amino acids were more active antifibrinolytic agents than the corresponding ω-guanidino acids.[3b,4]

In summary, a series of ω-guanidino acids and ω-guanidinoacyl-L-histidines was found to possess antistaphylococcal activity *in vivo* but did not inhibit the growth of *S. aureus in vitro*. The results confirm the previously reported relationship between biological activity and carboxy-amino distance.[3b]

Experimental Section

Compounds **1**, **2**, and **3** were purchased from Sigma Chemical Co. δ-Aminovaleric acid and ε-aminohexanoic acid were obtained from Schwartz and Mann. ω-Aminoacyl-L-histidines were prepared previously in our laboratory.[3b] *S*-Ethylisothiourea sulfate was purchased from K & K Laboratories, Inc. All other solvents and chemicals were purchased from Matheson Coleman and Bell. Melting points were taken by the capillary tube method and are uncorrected. Ir spectra (KBr) were taken with a Beckman in

frared spectrophotometer, Model IR-20, at Matheson Coleman and Bell, Norwood, Ohio. Elementary analyses were made by Galbraith Laboratories, Inc., Knoxville, Tenn.

δ-Guanidinovaleric Acid (4). To the solution of *S*-ethylisothiourea dihydrogen sulfate (10.0 g, 0.05 mol) dissolved in 25 ml of 2 *N* NaOH was added 6.0 g (0.05 mol) of δ-aminovaleric acid dissolved in 10 ml of H_2O under ice cooling and vigorous stirring. The mixture stood for 5 hr in the ice bath and was kept overnight at room temperature. The turbid solution was filtered and acidified (pH 4) with concentrated HCl. Absolute EtOH was added to the solution and the precipitate of NaCl was filtered off. The filtrate was concentrated and subjected to ion-exchange chromatography, using a 2.0 × 40 cm column of Amberlite CG-120, 200-400 mesh, pyridine form. For elution, first 500 ml of H_2O and then 0.1 *M* pyridine were used. The peak quantity of 4 appeared in the effluent at approximately 1800 ml. The 4 fractions, 1600-2100 ml, were pooled and concentrated *in vacuo*. The dry residue was treated with H_2O-EtOH-Et_2O to produce crystals: yield, 28.0%; mp 267-271°.

ε-Guanidinohexanoic Acid (5). *S*-Ethylisothiourea dihydrogen sulfate (10.0 g, 0.05 mol) and 6.5 g (0.05 mol) of ε-aminohexanoic acid were treated in the same way as 4 except for the following modifications. After the reaction was completed the precipitate was filtered and washed with cold H_2O. The crystals were then dissolved in 20 ml of 2 *N* HCl. The pH was adjusted to 8.0 by the addition of 2 *N* NH_4OH. After standing at 5° overnight, the white crystals were filtered and washed with cold H_2O and EtOH and then dried in a vacuum desiccator: yield, 68.0%; mp 274-275°.

Guanidinoacetyl-L-histidine Hydrate (6). The solutions of 1.50 g (0.007 mol) of *S*-ethylisothiourea dihydrogen sulfate in 5 ml of H_2O and 1.74 g (0.007 mol) of Gly-His·HCl in 3 ml of H_2O were adjusted to pH 8-9 with 4 *N* NaOH. They were then mixed and kept at room temperature for a week. The cloudy suspension was filtered and the filtrate concentrated to dryness *in vacuo*. The residue, after addition of a small amount of EtOH, was dried *in vacuo*. A small amount of H_2O was added to dissolve the dry residue and resulting solution was poured onto the column (2.5 cm i.d. × 30 cm Amberlite CG-120, 200-400 mesh, pyridine form). H_2O, 1.0 *M* pyridine, 2.0 *M* pyridine, and 1.0 *M* pyridine-0.5 *M* NH_4OH were used as the effluent solutions. Each fraction was tested for ninhydrin, Pauli, and Sakaguchi reactions. The 6 fractions were pooled and concentrated to dryness *in vacuo*: crystals from H_2O-EtOH; yield, 69.2%; mp 108-111° dec. Hydrolysis (6 *N* HCl, 110°, 24 hr) gave 1 and His, confirmed by tlc.

β-Guanidinopropionyl-L-histidine Hydrate (7). *S*-Ethylisothiourea dihydrogen sulfate (5.0 g, 0.025 mol) and β-Ala-His (5.3 g, 0.025 mol) were treated in the same way as 6: yield, 38.8%; mp 121-124° dec. Hydrolysis as described above gave 2 and His (tlc).

γ-Guanidinobutyryl-L-histidine Dihydrogen Sulfate (8). *S*-Ethylisothiourea dihydrogen sulfate (10.0 g, 0.05 mol) and γ-Abu-His (11.0 g, 0.05 mol) were treated in the same way as 6 except for the following changes. For the final crystallization, the aqueous solution was adjusted to pH 5 with 2 *N* H_2SO_4 and absolute EtOH was added to form fine white crystals; yield, 51.7%; mp 119-124° dec. Hydrolysis as described gave 3 and His (tlc).

δ-Guanidinovaleryl-L-histidine Dihydrogen Sulfate Hydrate (9). *S*-Ethylisothiourea dihydrogen sulfate (0.8 g, 0.0037 mol) and δ-Avl-His (1.0 g, 0.0037 mol) were treated in the same way as 8: yield, 50.6%; mp 80-82° dec. Hydrolysis as above gave 4 and His (tlc).

ε-Guanidinohexanoyl-L-histidine (10). *S*-Ethylisothiourea dihydrogen sulfate (1.4 g, 0.0063 mol) and ε-Ahx-His (1.7 g, 0.0063 mol) were treated in the same way as 6: yield, 56.1%; mp 102-103° dec. Hydrolysis as above gave 5 and His (tlc).

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Synthetic Trypanocides. 3. Structure-Activity Relationships†

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We have previously reported the trypanocidal activity of several substituted 1,2,3,4-tetrahydrocarbazoles[1] (THC) and dihydro[c]benzocarbazoles.[2] Continuing with the search for more active compounds and the establishment of a relation between chemical structure and activity, we have prepared several new compounds active against *Trypanosoma cruzi*, the parasite responsible for Chagas's disease.

In the study on structure-activity relationships, the biological effect produced by chemical modification at three regions of the compounds containing an N-substituted indole nucleus was considered, *i.e.*, the benzene ring, the alicyclic ring, and the heterocyclic ring. Previously,[2] we have found that in compounds containing an N-substituted indole nucleus, a methoxy or chlorine substituent in the benzenoid portion of the aromatic ring provides compounds with higher activity. From the several substituents checked on the aromatic nitrogen, the 2-piperidinoethyl has proven to enhance the activity both on the epimastigote and trypomastigote stage of *T. cruzi*.

Maintaining the most active structure, when the alicyclic ring was dehydrogenated, the fully aromatic compounds **6-8**, although with good activity on the epimastigote stage, lose completely their activity on the hematic form.

The amount required of the more active compound **4** to prevent the transmission of the disease by an average blood transfusion is slightly below that of Crystal Violet,[3,4] with the advantage of being 20-fold less toxic and colorless. Compounds **9** and **10** and the 8-substituted 5,6-dihydrobenzo[*a*]carbazoles (DHBC) are water insoluble and cannot be checked *in vitro*. Their *in vivo* activity is low even at a high dose.

Biological Results. The activities reported in Table I were measured on the epimastigote stage of the Tulahuen strain of *T. cruzi* in a liquid culture medium.[5] When the same test was performed in blood, on the trypomastigote stage, the activity of **4** is 200 μg/ml and therefore only 100 mg will be required for an average transfusion. In the same conditions the activity for Crystal Violet is 250 μg/ml.

The toxicity of **4** was tested on Rockland mice weighing 18-20 g and the LD_{50} was 200-220 mg/kg (ip), whereas

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