acidified (cold 4 *N* HCl). This was washed (C_6H_6) after which the aq layer was made basic at 0° (concd NH_4OH) and extd (C_6H_6). The C_6H_6 fractions were washed (satd NaCl) and dried (Na_2SO_4). Evapn of the solvent *in vacuo* yielded 0.8 g (30%) of **36** as dark yellow oil: ir (neat) 2970, 2800, 1450, 1350, 1180 cm^{-1}; nmr ($CDCl_3$) δ 4.5 (m, 2 H, $CH_2OSO_2CH_3$), 3 (s, 3 H, mesylate CH_3), 2–3 (m, 7 H).

2-Methyl-6-dimethylaminomethyl-2-azabicyclo[2.2.2]octane (37) was prepd in 43% yield as described for **23** and obtd as a yellow oil: ir (neat) 2970, 2780, 1450 cm^{-1}; nmr ($CDCl_3$) δ 3 (m, 2 H), 2.2 (s, 9 H), 1–2 (m, 11 H). Its mass spectrum showed peaks at *m/e* 184, 141, 112, 113, 114, 98, 85, 84, 70, 58, and 44.

2-Methyl-6-dimethylaminomethyl-2-azabicyclo[2.2.2]octane dimethiodide (16) was prepd in 50% yield as described for **10** and analyzed as the dipicrate: mp 180–182°. *Anal.* ($C_{25}H_{30}N_8O_{14}$) C, H, N.

Partition Coefficients. The dipicrate salt (0.005 g) (Table I) was dissolved in H_2O and made up to 250 ml. Two 30-ml aliquots were pipetted into flasks contg 170 ml of $CHCl_3$. The mixt was stirred for 24 hr at 25°. The system was allowed to stand for 20–30 min to assure complete phase sepn. The aqueous phase was transferred to a clean flask with care being taken to assure that no $CHCl_3$ phase contaminated it at this point. The amt of the dipicrate salt which partitioned into the $CHCl_3$ was detd spectrophotometrically at 352 mμ. Calcn of π values was carried out according to the equation[30] $\pi = \log P_X - \log P_{n=2}$ where P_X and $P_{n=2}$ are the measured partition coefficients of quat ammonium compounds with longer C chains and the parent, 2-C chain compd (1), respectively.

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Antistaphylococcal and Antifibrinolytic Activities of N^{α}-(ω-Aminoacyl)-L-lysines[1,2,†]

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A series of N^{α}-(ω-aminoacyl)-L-lysines (**1–5**) have been synthesized and tested for antistaphylococcal and antifibrinolytic activities. All of the N^{α}-L-lysine dipeptides had antistaphylococcal activity which was approximately equal to or slightly less than that of γ-aminobutyryl-L-histidine. These compounds were found to be effective antifibrinolytic agents and N^{α}-(ε-aminocaproyl)-L-lysine was the most active of the dipeptides investigated.

In previous publications,[1,3] it was found that a series of ω-amino acids and of ω-aminoacyl-L-histidines protected mice from death by *Staphylococcus aureus* infections. Of the peptides, ε-aminocaproyl-L-histidine possessed the highest antistaphylococcal activity.[1] The present investigation extends these studies to N^{α}-(ω-aminoacyl)-L-lysines which have been synthesized and tested for antistaphylococcal activity in Swiss albino mice. The antifibrinolytic activity of these dipeptides was also tested because of the interest described previously.[1]

The compounds discussed in this paper are: N^{α}-(ω-aminoacyl)-L-lysines, $H_2N(CH_2)_nCONHCH[(CH_2)_4NH_2]COOH$, where $n = 1$, N^{α}-glycyl-L-lysine (**1**); $n = 2$, N^{α}-(β-alanyl)-L-lysine (**2**); $n = 3$, N^{α}-(γ-aminobutyryl)-L-lysine (**3**); $n = 4$, N^{α}-(δ-aminovaleryl)-L-lysine (**4**); $n = 5$, N^{α}-(ε-aminocaproyl)-L-lysine (**5**).

Chemistry. The syntheses of **1**,[4-10] **2**,[11,12] and **3**[13,14] have been reported from several laboratories using the carbobenzoxy method. All of these methods are modifications of that reported by Bergmann, *et al.*,[4] who used N^{ϵ}-carbobenzoxy-L-lysine methyl ester and N^{α}-carbobenzoxyglycine as the starting materials for the N^{α}-glycyl-L-lysine preparation. The same method was employed by us in an endeavor to synthesize the higher ω-amino acid homologs of this N^{α}-L-lysine dipeptide. It was found to be very difficult to obtain pure N^{ϵ}-carbobenzoxy-L-lysine methyl ester by the method described.[4] On tlc the recrystallized product showed

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Table I. N^{α}-(ω-Aminoacyl)-L-lysines and Related Compounds

Compd	Yield, %[a]	Mp, °C[b]	$[\alpha]^{25}$D, deg[c]	Formula	Analyses[d]
1[e]	62.0	193–194 dec[f]	*g–k*	$C_8H_{17}N_3O_3$	C, H, N[l]
2[m]	79.8	184–185 dec[n]	+23.0 (*c* 1, H_2O)	$C_9H_{19}N_3O_3$	C, H, N
3[o]	82.3	203–205 dec[p]	+11.0 (*c* 1, H_2O)	$C_{10}H_{21}N_3O_3 \cdot H_2SO_4$	C, H, N, S
4[q]	88.6	183–184 dec	0.0 (*c* 1, H_2O)	$C_{11}H_{23}N_3O_3 \cdot HCl \cdot H_2O$	C,[r] H,[r] N, Cl
5[s]	92.7	162–163	+1.0 (*c* 2, H_2O)	$C_{12}H_{25}N_3O_3$	C,[t] H, N[t]
6	100.0	88–89[u]	+3.0 (*c* 2, H_2O)	$C_{23}H_{32}N_2O_7S$	C,[v] H, N, S

[a] Based in 1–5 on 6 as 100%. [b] Melting points were detd in capillaries and are uncorrected. [c] Specific opt rotations were taken with a Laurent Polarimeter. [d] Analytical results were within ±0.4% of the theoretical values, except where indicated. [e] Ir peaks (cm^{-1}) were 662, 714, 779, 818, 839, 872. [f] Lit.[11] 147° dec for 1·H_2SO_4. [g] The soln was not clear and rotation could not be obtd. [h] Grassmann and Wünsch[17] give $[\alpha]^{20}$D −13.0° (*c* 2, 0.5 *N* HCl) for 1·HCl. [i] Lit.[7] $[\alpha]^{25}$D −12.8° (*c* 2, 1 *N* HCl) for 1·HCl. [j] Lit.[11] [α]D −10.0° (*c* 5, H_2O) for 1·H_2SO_4. [k] Padayatty and Van Kley[18] give $[\alpha]^{24.5-26.5}$D +4.33° (*c* 0.5, H_2O) for 1·AcOH. [l] N: calcd, 20.68; found, 20.21. [m] Ir peaks (cm^{-1}) were 610, 710, 750, 810, 867. [n] Lit.[12] yield, 55.6%; mp 216–218.5° dec for 2·hydroxyazobenzenesulfonate·H_2O. [o] Ir peaks (cm^{-1}) were 592, 609, 720, 843, 881. [p] Lit.[13,14] yield, 36%; mp 231–232° for 3·hydroxyazobenzenesulfonate·2H_2O. [q] Ir peaks (cm^{-1}) were 640, 692, 936, 992, 1010. [r] C: calcd, 44.05; found, 45.26; H: calcd, 8.74; found, 9.33. [s] Ir peaks (cm^{-1}) were 550, 742, 890, 928, 976. [t] C: calcd, 55.56; found, 55.12; N: calcd, 16.21; found, 15.78. [u] Lit.[15] yield, 95%; mp 88°; $[\alpha]^{20}$D +5.9° (*c* 2, DMF). [v] C: calcd, 57.48; found, 56.45.

Table II. R_f Values of N^{α}-(ω-Aminoacyl)-L-lysines and Related Compounds (R_f × 100)

	Tlc[a]						Pc[b]	
Compd	Solv 1	Solv 2	Solv 3	Solv 4	Solv 5	Solv 6	Solv 7	Solv 8
1	13	14	21	00	44	41	51	13
2	14	14	17	00	20	36	67	17
3	18	14	20	00	13	38	73	18
4	24	15	20	00	15	39	76	19
5	25	16	21	00	20	41	83	26
6						87		
N^{ϵ}-Z-L-Lysine						81		
L-Lysine	12	14	17	01	19	45	57	16
L-Alanine	33	39	46	39	80	56	66	41

[a] Silica gel G (E. Merck) 250-μ plates (Analtech, Inc.) were used for tlc. Solvent 1, PhOH–H_2O (75:25, w/v), pH 2.0; 2, *n*-BuOH–AcOH–H_2O (60:20:20), pH 2.4; 3, *i*-PrOH–formic acid–H_2O (77:4:19), pH 2.7; 4, *sec*-BuOH–MeCOEt–dicyclohexylamine–H_2O (55:15:10:20), pH 10.3; 5, $CHCl_3$–MeOH–17% NH_4OH (40:40:20), pH 11.6; 6, *n*-BuOH–AcOH–pyridine–H_2O (30:6:30:24), pH 5.2. [b] Whatman No. 1 paper was used for pc. Solvent 7, PhOH–H_2O (80:20 w/v), pH 1.6; 8, *n*-BuOH–AcOH–H_2O (60:15:25), pH 2.7.

4 ninhydrin-positive substances, which were identified as N^{ϵ}-carbobenzoxy-L-lysine methyl ester, N^{ϵ}-carbobenzoxy-L-lysine, L-lysine methyl ester, and L-lysine. By purification with several solvent systems pure N^{ϵ}-carbobenzoxy-L-lysine was obtained in small yield. Preparation of N^{ϵ}-carbobenzoxy-L-lysine ethyl ester *p*-toluenesulfonate by the method reported by Kato, *et al.*,[15] was accomplished without much difficulty and in good yield.

The mixed acid anhydride method, almost identical with that described previously,[1] was employed for the coupling reaction. The products were purified by means of ion-exchange chromatography, using NH_4OH as the effluent solution, a modification of the method reported by Kumon[13] and Nakajima, *et al.*[14] The yields and physical and analytical data for the compounds are given in Table I. Table II gives the R_f values on tlc and paper chromatography (pc).

Biological Results and Discussion. Antistaphylococcal (*in vivo*) and antifibrinolytic activities (*in vitro*)[1] as related to the maximum possible distance between the COOH group and the ω-NH_2 group (calculated from models) are summarized in Figure 1. γ-Aminobutyryl-L-histidine was used as the positive control. All of N^{α}-(ω-aminoacyl)-L-lysines gave better protection for mice against *S. aureus* than the corresponding ω-amino acids. By probit analysis,[16] using 140 mice at 7 dosage levels, the ED_{50} of N^{α}-(β-alanyl)-L-lysine was determined to be 0.6 mg/mouse, or approximately 27 mg/kg body weight (Figure 2). This may be compared with an ED_{50} of 0.23 mg/mouse (approximately 10.4 mg/kg)

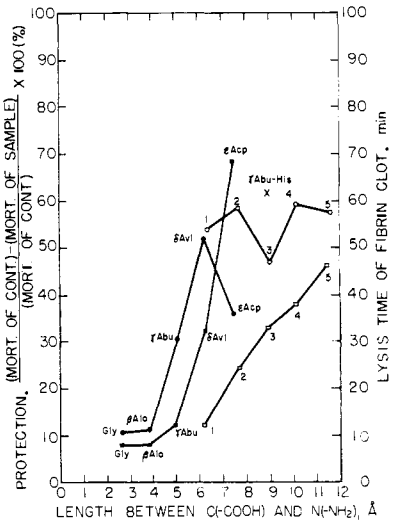

Figure 1. Antistaphylococcal and antifibrinolytic activities of ω-amino acids and their N^{α}-L-lysine dipeptides. Numbers refer to compounds. The antistaphylococcal activity of each compound was established at 5 mg/mouse with 10 mice in each of 4 experiments (40 mice per compound): ω-amino acid (●—●), N^{α}-(ω-aminoacyl)-L-lysines (○—○). The antifibrinolytic activity was determined in 0.05 *M* phosphate buffer–saline solution with 10^{-5} *M* sample: ω-amino acids (■—■), N^{α}-(ω-aminoacyl)-L-lysines (□—□). X indicates the antistaphylococcal activity of γ-aminobutyryl-L-histidine used as control.

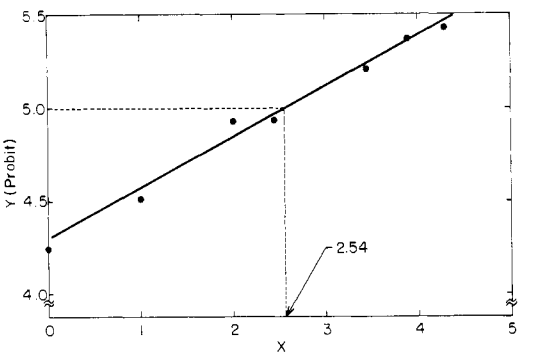

Figure 2. Determination of ED_{50} for N^{α}-(β-alanyl)-L-lysine. Dose = 0.01×5^{X}. $ED_{50} = 0.01 \times 5^{2.54} = 0.6$ mg/mouse (approx 27 mg/kg).

for δ-aminovaleryl-L-histidine. All of N^{α}-(ω-aminoacyl)-L-lysines showed some antifibrinolytic activity which increased with the distance between the COOH group and the ω-NH_2 group. All of the L-lysine dipeptides showed stronger antifibrinolytic activity than the corresponding

ω-amino acids on an equimolar basis with the exception of N^{α}-(ε-aminocaproyl)-L-lysine.

It is of interest that all of the N^{α}-L-lysine dipeptides gave similar degrees of protection against *S. aureus* infections, whereas the activity of the corresponding L-histidine dipeptides showed marked dependence upon length of the CH_2 chain.[1] Activity dependence upon chain length was found for the parent ω-amino acids in confirmation of the earlier report.[1] On the other hand, the lysine dipeptides were found to possess antifibrinolytic activity which is related to CH_2 chain length, although the corresponding histidine dipeptides were essentially lacking in this activity.[1] The reason for these differences is not known.

Experimental Section

N^{α}-Carbobenzoxyglycine and N^{γ}-carbobenzoxy-γ-aminobutyric acid were obtained from Nutritional Biochemicals. N^{β}-Carbobenzoxy-β-alanine and N^{ϵ}-carbobenzoxy-ε-aminocaproic acid were purchased from the Sigma Chemical Co. Since N^{δ}-carbobenzoxy-δ-aminovaleric acid was not available commercially, it was synthesized from δ-aminovaleric acid (Mann Research Laboratories, Inc.). N^{ϵ}-Carbobenzoxy-L-lysine anhydride was obtained from the same firm. All other solvents and reagents were obtained 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 infrared spectrophotometer, Model IR-20, at Matheson Coleman and Bell, Norwood, Ohio. Specific optical rotations were taken with a Laurent polarimeter. Elementary analyses were performed by the Crobaugh Laboratories, Cleveland, Ohio.

N^{ϵ}-Carbobenzoxy-L-lysine Ethyl Ester *p*-Toluenesulfonate (6). The equipment used for the reaction was described by Kato, *et al.*[15] A mixt of 14.5 g of N^{ϵ}-Z-L-lysine, 10.5 g of *p*-TsOH·H_2O, 40 ml of abs EtOH, and 200 ml of CCl_4 was boiled for 12 hr until a clear soln resulted. The azeotropic mixt of H_2O–EtOH–CCl_4 on the EtOH–CCl_4 layer was approx 13 ml after 24 hr. Et_2O (100 ml) and 200 ml of petr ether (bp 40–49°) were added to the concd syrupy reaction mixt. Crystals of 6 were formed immediately. After recrystn from hot Me_2CO–Et_2O, 24.0 g (100%) of product was obtained. The product included a small amt of unreacted N^{ϵ}-Z-lysine, confirmed by tlc.

Carbobenzoxy-δ-aminovaleric acid was synthesized as described.[1]

N^{α}-(δ-Aminovaleryl)-L-lysine (4). To a soln of 6.3 g of Z-δ-aminovaleric acid in 125 ml of CH_2Cl_2 was added 3.5 ml of Et_3N. After the resulting soln had been chilled to −5°, 2.4 ml of ethyl chloroformate was added and the mixt was kept at the temp of −5° for 10 min. A soln of 6 prepd by the addn of 10 ml of Et_3N to a soln of 12.0 g of 6 in 125 ml of CH_2Cl_2 which had been chilled to 0°, was added rapidly. The resulting mixt was stored at 25° for 2 days. It was then washed with 200 ml of H_2O and 200 ml of 1 *N* $NaHCO_3$, dried (Na_2SO_4), and concd to a syrupy consistency. This product was dissolved in 50 ml of EtOH and then 50 ml of 1 *N* NaOH was added. After 3 hr at 25°, the soln was adjusted to pH 5.0 with 2 *N* H_2SO_4 and concd to dryness *in vacuo*. The residue was extd with 2 portions of 50 ml each of hot EtOH, followed by 50 ml of H_2O. After the addn of 0.5 g of 10% Pd/C, the mixt was hydrogenated in an app with an outlet for excess H_2 gas. The formation of CO_2 gas was checked occasionally until it ceased after 8 hr. The soln was filtered and concd *in vacuo*. The remaining syrupy mixt was dissolved in 20 ml of H_2O and 2 *N* HCl was added to lower the pH below 5.0. For the sepn on a column (1.8 × 40 cm) of Amberlite CG 120 resin (which had been equilibrated with 2 *M* NH_4OH and washed until neutral with H_2O), H_2O, 0.1 *M* NH_4OH, and 0.3 *M* NH_4OH effluent solns were used.[12-14] The ninhydrin reaction was employed for the detection of the pure fractions of δ-aminovaleric acid, lysine, and 4. The peak quantity of 4 appeared in the effluent at approx 2900 ml. The pure 4 fractions, 2300–3400 ml, were pooled and concd *in vacuo*. The syrupy residue was treated with 1 *N* HCl in order to adjust the pH to approx 5.0. Fine white crystals sepd from this soln after the addn of abs EtOH. Acid hydrolysis (6 *N* HCl, 110°, 24 hr) of 4 produced δ-aminovaleric acid and lysine, confirmed by tlc.

N^{α}-(ε-Aminocaproyl)-L-lysine (5). Z-ε-Aminocaproic acid (6.7 g) and 12.0 g of 6 were used as the starting materials. The reaction and purification procedures were the same as described before. The pure 5 fractions, 2700–3700 ml, were pooled and concd *in vacuo*. The dry residue was crystd from hot H_2O–EtOH to form fine white crystals. Acid hydrolysis as described gave ε-aminocaproic acid and lysine.

N^{α}-(γ-Aminobutyryl)-L-lysine (3). Z-γ-Aminobutyric acid (5.9 g) and 12.0 g of 6 were used as the starting materials. The reaction and purification procedures were the same as described before. The pure 3 fractions, 1900–2800 ml, were pooled and concd *in vacuo*. The dry residue was cryst from aq EtOH after the treatment of 1 *N* HCl to adjust to approx pH 5.0. The white needles were very hygroscopic so that the mp was as low as 50–60°: $[\alpha]^{25}D$ +2.5° (*c* 2, H_2O). The stable 3 monosulfate was prepared by treating 3·$(HCl)_n$ with 2 *N* H_2SO_4. Acid hydrolysis as described gave γ-aminobutyric acid and lysine.

N^{α}-(β-Alanyl)-L-lysine (2). Z-β-Alanine (5.5 g) and 12.0 g of 6 were used as the starting materials. The reaction and purification procedures were the same as described before. The pure 2 fractions, 1300–2300 ml, were pooled and concd *in vacuo*. The dry residue was crystd from hot H_2O–EtOH to form fine white crystals. Acid hydrolysis as described gave β-alanine and lysine.

N^{α}-Glycyl-L-lysine (1). Z-Glycine (5.1 g) and 12.0 g of 6 were used as the starting materials. The reaction and purification procedures were the same as described before. The pure fractions of 1, 700–1100 ml, were pooled and concd *in vacuo*. The dry residue was crystd from hot H_2O–EtOH. Acid hydrolysis as described gave glycine and lysine.

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