ON PROTEOLYTIC ENZYMES

VII. THE SYNTHESIS OF PEPTIDES OF *l*-LYSINE AND THEIR BEHAVIOR WITH PAPAIN

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Lysine is coupled in the long peptide chains of proteins with its α-amino and carboxyl groups, leaving the ϵ-amino group free. For the study of proteolytic enzymes and of protein structure in general, a method was required to synthesize such lysine peptides with a single amino group, the ϵ, free. Lysylhistidine (1), lysylaspartic acid (2), and similar compounds have been prepared previously, but in each of these lysine occupies the terminal amino position with both its basic groups free. We have now developed a new application of the carbobenzoxy method which makes it possible to form lysine peptides with the lysine coupled as it is in natural proteins.

The key substance in this method is ϵ-carbobenzoxylysine (IV), obtained from dicarbobenzoxylysyl chloride (II), through the intermediate ϵ-carbobenzoxy-α-carboxyllysine anhydride (III). Formulæ (I) to (V) represent the series of reactions involved.

Dicarbobenzoxylysyl chloride (II) has been prepared from (I) with phosphorus pentachloride. After the chlorination, the products have been heated for a time at 50–60° in order to convert the chloride (II) into the anhydride (III) with the splitting off of benzyl chloride.

ϵ-Carbobenzoxy-α-carboxyllysine anhydride is analogous to the N-carboxyl anhydrides of glycine and other amino acids studied by Leuchs and Geiger (3) and by Wessely (4). Like the latter substances, its ring is readily opened in aqueous hydrochloric acid

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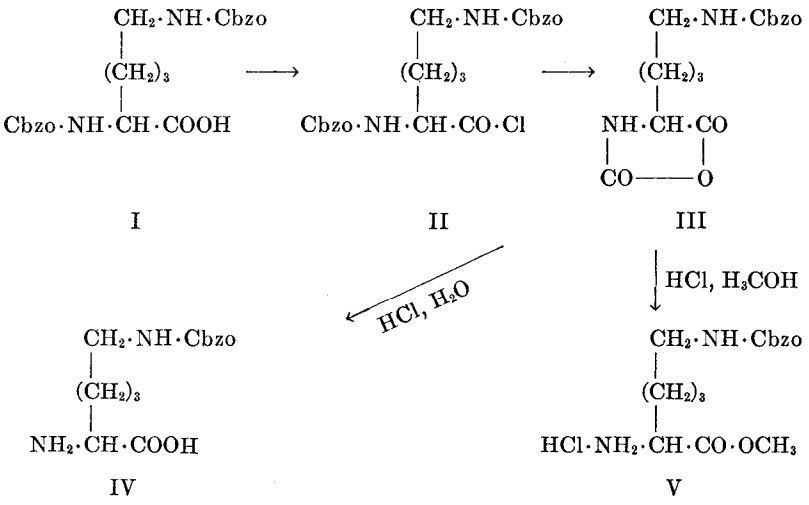
to give ε-carbobenzoxylysine (IV), or in methyl alcoholic hydrochloric acid to give the corresponding methyl ester hydrochloride (V).[1]

This ester may be coupled through its α-amino group with carbobenzoxyglycyl chloride (any chloride or carbobenzoxypeptide-azide) to give a dicarbobenzoxypeptide ester. This, after conversion into the azide, may be condensed further with an amino acid ester or peptide ester to increase the length of the peptide chain. In this manner several series of modified lysine peptides have been first synthesized, for they were of interest in the inves-



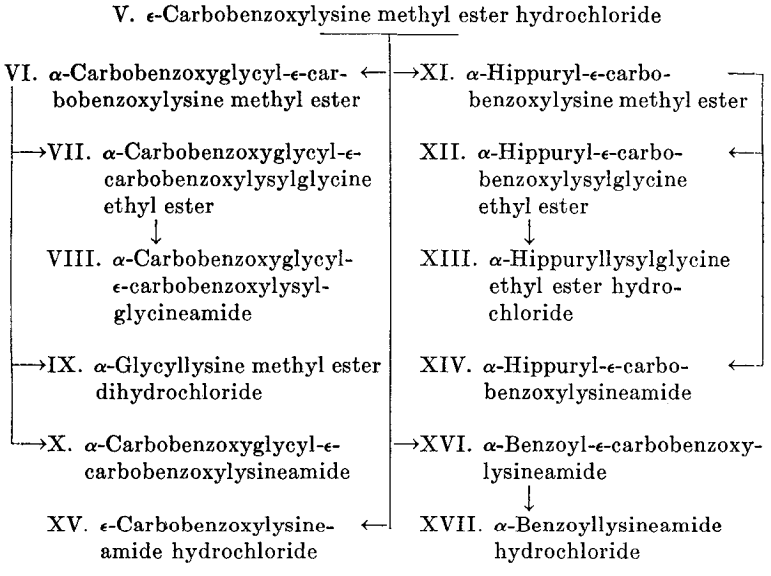
tigation of papain specificity. For simplicity's sake, these are presented in tabular form (Table I).

The carbobenzoxy radical is especially well adapted for protecting the ε-amino group of lysine during the synthesis of lysine peptides with a free ε-amino group. In the preparation of simple

[1] The carbobenzoxy derivatives of the other amino acids may be converted into anhydrides in a similar way. The conversion of carbobenzoxy-amino acid chlorides into carboxyl anhydrides occurs so readily that the anhydrides may be obtained in an especially pure state, without any polymerization. Such anhydrides have been frequently used in this laboratory to prepare amino acid benzyl esters, which have the advantage that they can be split by hydrogenation without the use of saponifying reagents.

peptides with a free terminal α-amino group, such as α-glycyllysine methyl ester dihydrochloride (IX), the ε-carbobenzoxy group can be removed simultaneously with the terminal α-carbobenzoxy group from (VI). In the preparation of peptides with an acylated terminal α-amino group such as α-hippuryllysylglycine ethyl ester hydrochloride (XIII), the removal of the ε-carbobenzoxy group of compound (XII) does not interfere with the acyl group.

TABLE I

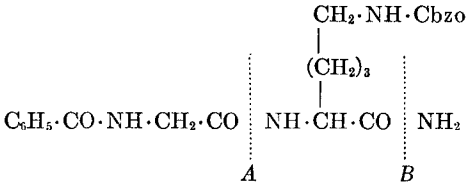


In order to prove that it is the α-amino group which forms the anhydride ring when benzyl chloride is split off (II → III), α-benzenesulfonyllysine was prepared from our ε-carbobenzoxylysine methyl ester (V). Gurin and Clarke (5) obtained this compound from the partial hydrolysis of ε-benzoyl-α-benzenesulfonyllysine. Through the kindness of these authors we were able to compare the two preparations and identify them as the same compound.

When acylated lysine peptides were subjected to the action of papain, it was found that those with the ε-amino group free behaved like the simpler peptide derivatives, such as benzoylglycyl-

l-leucylglycine, reported in the preceding paper (6). In α-hippuryllysylglycine ethyl ester hydrochloride (XIII) one peptide linkage was split, and the isolation of hippuric acid from the products proved that this one was that linkage between the hippuryl and lysylglycine ester residues. The simplest lysine derivative of this nature, α-benzoyllysineamide hydrochloride (XVII), was also split, forming α-benzoyllysine. These examples showed that in simple acylated lysine peptides the splitting occurs next to the acylamino group, exactly as in the simple monoamino acid peptides, described in the preceding paper, which have no free ε-amino group. Lysine is combined in these peptides exactly as it is in natural proteins.

When, however, the ε-amino group of the lysine residue within the peptide is also acylated, the behavior of the peptide with papain becomes quite different. Such a compound, α-hippuryl-ε-



XIV

carbobenzoxylysineamide (XIV), is split in two places: the amide linkage (*B*) and the peptide linkage (*A*). ε-Carbobenzoxylysine and hippuric acid were isolated as products. The very similar compound, α-carbobenzoxyglycyl-ε-carbobenzoxylysineamide (X) was split in the same manner, and α,ε-dicarbobenzoxylysineamide was hydrolyzed by papain, too. In contrast, ε-carbobenzoxylysineamide was not attacked by the enzyme. It thus appears that the ε-carbobenzoxy group is responsible for an additional hydrolysis at the amide linkage (*i.e.*, *B*) and that it is effective only when the α-amino group of lysine is acylated.

Furthermore, it becomes apparent that in (XIV) and similar compounds the presence of the acylated ε-amino group makes the rate of hydrolysis much greater at *B* than at *A*. Thus, from (XIV) there is first formed α-hippuryl-ε-carbobenzoxylysine which is split again by papain at *A*. If the rate of hydrolysis were greater

at A than at B, ε-carbobenzoxylysineamide would result and no further splitting would occur.

The influence of the acylation of the basic lysine side chain on the enzymic hydrolysis seems not to be restricted to papain. Gurin and Clarke (5) found that the benzenesulfonylation of gelatin, blocking the free amino groups of lysine, inhibits its digestion by pepsin.

The experiments reported in this and the preceding paper make it possible to draw some preliminary conclusions with regard to papain specificity, and to compare the latter with the specificity of peptidases. Aminopeptidase, carboxypeptidase, and dipeptidase need in addition to a peptide bond a free amino group or a free carboxyl group or both. Thus, their action is restricted to the ends of peptide chains. Papain (*i.e.*, the component of natural papain which is effective on our peptide substrates) does not split a peptide linkage next to a free α-amino group and does not need a free α-carboxyl group. What it does need is another peptide linkage besides the one which is to be hydrolyzed. These two necessary peptide linkages are to be found throughout a peptide chain; thus, hydrolysis with papain is not restricted to the ends of the chains.

On the other hand, our experiments show that each peptide linkage does not have the same behavior towards our papain component, the splitting rate of each being decisively influenced by the nature of the side chains of the amino acid residues involved.

Since this influence of side chains should be effective not only on our papain component but on all proteinases, it is necessary to continue its investigation.

We wish to thank Dr. A. Elek who kindly performed the elementary microanalyses reported in this paper.

EXPERIMENTAL

α,ε-Dicarbobenzoxy-l (+)-Lysine—25 gm. of *l*-lysine dihydrochloride were dissolved in 171 cc. of 2 N NaOH, and coupled with 60 gm. of carbobenzoxy chloride with 142 cc. of 4 N NaOH. All reagents were thoroughly cooled and the coupling done at 0°, with shaking, and in four portions. The resulting liquid (the sodium salt of dicarbobenzoxylysine often formed as an oil) was acidified

with hydrochloric acid and the product taken up with ether. It was purified by extracting with aqueous potassium bicarbonate and transferring to fresh ether. An almost colorless syrup was left when the solvent was removed *in vacuo*. This was quite soluble in ether and was therefore the most convenient form when the chloride was next to be made. Yield, 45 gm.

By further purification, a repeated extraction, and transfer to ether, dicarbobenzoxylysine could be obtained as minute needles forming spongy masses. The yield in both cases was almost the theoretical. The substance was recrystallized from ethyl acetate for analysis. M.p., 150°.

$C_{22}H_{26}N_2O_6$.	Calculated.	C 63.7, H 6.3, N 6.8
414.2	Found.	" 63.8, " 6.4, " 6.7

ε-Carbobenzoxy-α-Carboxyl-l-Lysine Anhydride—45 gm. of α,ε-dicarbobenzoxy-*l*-lysine (syrup) were dissolved in 175 cc. of dry ether and cooled to 0°. 26 gm. of powdered phosphorus pentachloride were added and the resulting mixture was shaken, with cooling (about 10°), for 20 to 30 minutes, when most of the solid had disappeared. After being filtered, the solution was quickly concentrated *in vacuo*, 40–50°, with careful exclusion of moisture. Ethyl acetate was twice added and distilled off *in vacuo* (50°). The product, crystallized from ethyl acetate and petroleum ether, weighed 28.1 gm. Yield, 85 per cent of the theoretical (calculated from *l*-lysine dihydrochloride). M.p., 100° with decomposition. After standing several months, the melting point may rise even above 250°; we believe this to be due to polymerization as the composition remains unchanged.

$C_{15}H_{18}N_2O_5$.	Calculated.	C 58.8, H 5.9, N 9.2
306.1	Found.	" 58.8, " 5.9, " 9.1

ε-Carbobenzoxy-l-Lysine—15.7 gm. of ε-carbobenzoxy-α-carboxyl-*l*-lysine anhydride were dissolved in 75 cc. of acetone. 20 cc. of 5 N HCl were added and the solution was allowed to stand overnight at room temperature. It was then concentrated *in vacuo*, the crystalline mass dissolved in 75 cc. of N HCl, and the solvent again removed *in vacuo*. The ε-carbobenzoxylysine was finally dissolved in hot water and precipitated as needles by making the solution slightly ammoniacal. Yield, almost quantitative.

For analysis it was recrystallized from hot 50 per cent ethyl alcohol. M.p., near 255°.

$[\alpha]_D^{25} = (+0.40° \times 2.500)/(1 \times 1.02 \times 0.070) = +14.0°$ (in water with 2 molecular equivalents of HCl)

$C_{14}H_{20}N_2O_4$.	Calculated.	C 60.0, H 7.2, N 10.0
280.1	Found.	" 59.9, " 7.1, " 10.1

ε-Carbobenzoxy-l-Lysine Methyl Ester Hydrochloride—23.1 gm. of ε-carbobenzoxy-α-carboxyl-*l*-lysine anhydride were dissolved in 15 cc. of absolute methyl alcohol, and 151 cc. of N HCl in methyl alcohol were added. On warming to 50°, evolution of carbon dioxide began. The reacting solution was allowed to stand overnight, after which it was concentrated *in vacuo*, 40–50°. Crystallization occurred when the resulting oil was scratched in the presence of a little ether. Yield, 24.5 gm., 98 per cent of theory.

After recrystallization from hot acetone, ε-carbobenzoxy-*l*-lysine methyl ester hydrochloride formed long prisms melting at 117°.

$C_{15}H_{23}N_2O_4Cl$.	Calculated.	C 54.4, H 7.0, N 8.5
330.7	Found.	" 54.4, " 7.1, " 8.4

ε-Carbobenzoxylysineamide Hydrochloride—2 gm. of the above ester were converted into the amide by standing 2 days in ammoniacal methyl alcohol which was saturated at 0°. After recrystallization from methyl alcohol, the product formed fine needles melting at 203°.

$C_{14}H_{22}N_3O_3Cl$.	Calculated.	C 53.2, H 7.0, N 13.3
315.7	Found.	" 53.2, " 7.2, " 13.4

α-Hippuryl-ε-Carbobenzoxy-l-Lysine Methyl Ester—The ether solution of the free ester from 6.0 gm. of ε-carbobenzoxylysine methyl ester hydrochloride prepared with potassium carbonate (7) was dried over Na_2SO_4 and diluted with an equal volume of ethyl acetate. Then a suspension in dry ether of 2.0 gm. of hippuryl chloride was added in two portions. On shaking, much of the chloride went into solution, followed by immediate formation of ε-carbobenzoxylysine methyl ester hydrochloride as long needles. After 1 to 2 hours the crystalline ε-carbobenzoxylysine ester hydrochloride was recovered by filtering, several cc. of pyridine

were added to decompose any excess hippuryl chloride, and the solution was washed with dilute HCl, water, aqueous $KHCO_3$, and water, and dried. On concentrating *in vacuo*, the product formed as white and fluffy crystalline aggregations. The yield of the coupling product was 2.2 gm. (52 per cent of theory), and of recovered ester hydrochloride, about 50 per cent of the starting product.

α-Hippuryl-ε-carbobenzoxylysine methyl ester may be recrystallized from ethyl acetate for analysis. M.p., 145°.

$C_{24}H_{29}O_6N_3$.	Calculated.	C 63.3, H 6.4, N 9.2
455.2	Found.	" 63.5, " 6.4, " 9.1

α-Hippuryl-ε-Carbobenzoxy-l-Lysine Hydrazide—3.5 gm. of α-hippuryl-ε-carbobenzoxy-*l*-lysine methyl ester were dissolved in 15 cc. of hot methyl alcohol, and 0.96 cc. of hydrazine hydrate was at once added. After standing overnight, a heavy precipitate of the hydrazide had formed; yield, 3.4 gm. (97 per cent of theory). When the product was recrystallized from methyl alcohol, the melting point was 195°.

$C_{23}H_{29}O_5N_5$.	Calculated.	C 60.6, H 6.4, N 15.4
455.2	Found.	" 60.6, " 6.4, " 15.4

α-Hippuryl-ε-Carbobenzoxy-l-Lysylglycine Ethyl Ester—3.7 gm. of α-hippuryl-ε-carbobenzoxylysylglycine hydrazide were dissolved in 35 cc. of water and 25 cc. of acetic acid with gentle warming. The resulting solution was cooled to 4°, whereupon it became very cloudy, although no crystals formed. There was then slowly added an aqueous solution of 0.61 gm. of sodium nitrite. The oily masses of azide which formed were extracted into about 50 cc. of ice-cold ethyl acetate. This solution was now quickly washed several times with ice water, with cold aqueous $KHCO_3$, and with water, and dried over Na_2SO_4. Previously an ethyl acetate solution of the free ester from 4.0 gm. of glycine ethyl ester hydrochloride had been prepared and dried with Na_2SO_4. The ester and azide solutions were combined and concentrated *in vacuo*. After standing overnight, 1.8 gm. of very fine needles were obtained.

For analysis, α-hippuryl-ε-carbobenzoxylysylglycine ethyl ester was twice recrystallized from acetone. M.p., about 163°.

$C_{27}H_{34}N_4O_7$.	Calculated.	C 61.5, H 6.5, N 10.6
526.2	Found.	" 61.5, " 6.4, " 10.5

α-Hippuryl-l-Lysylglycine Ethyl Ester Hydrochloride—1.25 gm. of α-hippuryl-ε-carbobenzoxylysylglycine ethyl ester, recrystallized from acetone, were dissolved in ethyl alcohol and hydrogenated in the customary manner with palladium black in the presence of at least 1 molecular equivalent of HCl (ethyl alcoholic). After the hydrogenation was complete, the filtered solution was concentrated *in vacuo* (40–45°) to an oil which was redissolved in ethyl alcohol, the solution being again concentrated. The resulting syrup, after standing overnight under ether, could be crystallized into indiscriminate forms by scratching with a glass rod. The product could not be further purified by recrystallization; it melted very unsharply between 123–133°.

$[\alpha]_D^{25} = (-0.67° \times 1.0503)/(0.5 \times 1.02 \times 0.0452) = -30.5°$ (in water)

$C_{19}H_{29}N_4O_5Cl$.	Calculated.	C 53.2, H 6.8, N 13.1
428.7	Found.	" 53.1, " 6.9, " 13.2

Papain Hydrolysis—From the hydrolysate solution, 93 per cent split, 140 mg. (87 per cent of the theoretical) of hippuric acid were isolated. M.p., 187°.

$C_9H_9NO_3$.	Calculated.	C 60.3, H 5.0, N 7.8
179	Found.	" 60.4, " 5.2, " 7.8

α-Hippuryl-ε-Carbobenzoxy-l-Lysineamide—2.2 gm. of α-hippuryl-ε-carbobenzoxy-*l*-lysine methyl ester were converted into the amide with methyl alcoholic ammonia. After two recrystallizations from methyl alcohol, the yield was 1.5 gm. and the product melted at 209°.

$C_{23}H_{28}N_4O_5$.	Calculated.	C 62.7, H 6.4, N 12.7
440.2	Found.	" 62.7, " 6.5, " 12.7

Papain Hydrolysis—As this substrate was insoluble in water, the hydrolysis mixture was shaken throughout the experiment, and representative samples for analysis were taken before settling had occurred. After 190 hours there was no apparent decrease in the amount of solid phase present. However, there had been an increase in titration corresponding to two carboxyl groups and in nitrogen, by the Van Slyke procedure, equal to more than one amino group (Table II), the excess being attributable to liberated ammonia. At the end of the experiment the solid was allowed to settle; in the clear supernatant liquid there was a decrease in

TABLE II

Papain Hydrolysis of Lysine Peptides

Substrate	Time at 40°	Titration	Van Slyke	Product isolated
	hrs.	*per cent*	*per cent*	
α-Hippuryllysylglycine ethyl ester hydrochloride	5.0		50	
	24		77	
	48		93	Hippuric acid, 87%
α-Benzoyllysineamide hydrochloride	4.6	55		
	22	83	25	
	45	104		
α-Hippuryl-ε-carbobenzoxylysineamide	5.3	26		
	22	77	56	
	48	123	88	
	96	168	109	ε-Carbobenzoxylysine and hippuric acid
	111	180		
	141	208		
	187	220	155	
α-Carbobenzoxyglycyl-ε-carbobenzoxylysineamide	3.2	30	20	
	22	130	92	
	46	189	135	ε-Carbobenzoxylysine and carbobenzoxyglycine
	70	206		
	93	214	153	
	117	222	157	
ε-Carbobenzoxylysineamide hydrochloride				
Sample 1	2.0	−5		
	24	−3		
	47	+1		
" 2	50	4		
	98	2		
Dicarbobenzoxylysineamide hydrochloride				
Sample 1	2.3	9		
	19	12		
	44	27		
	72	46		
	117	52		
	170	48		
" 2	5.0	7		
	28	18		
	75	40		
	167	49		

In every experiment the solution contained for each cc. of volume 0.05 cc. of substrate, 0.1 cc. of 0.2 M disodium citrate buffer (pH 5.0), and 0.2 cc. of an HCN-activated papain solution prepared according to Grassmann (8). The liberated carboxyl was determined by the method of Grassmann and Heyde (9) and the amino nitrogen after Van Slyke with the Van Slyke-Neill apparatus (10). For each substrate a blank was performed under the same conditions but with no enzyme to insure absence of hydrolysis without papain. The experimental treatment of the hydrolysates and the isolation of the products are presented along with the synthesis of the substrate in the experimental section.

titration equal to 91 per cent (220 − 129) of one carboxyl group and nitrogen (Van Slyke) equal to 97 per cent (155 − 58) of one amino group.

The solid was filtered off and crystallized by making its solution ammoniacal in N HCl. Its melting point was 250° with sintering (known melting point of ε-carbobenzoxylysine, 255°), and the yield (127 mg.) was 70 per cent of the theoretical for ε-carbobenzoxylysine.

$$[\alpha]_D^{25} = (+0.21° \times 1.7493)/(0.5 \times 1.02 \times 0.0482) = +14.5°$$
$$[\alpha]_D^{25} = (+0.44° \times 1.7493)/(1 \times 1.02 \times 0.0482) = +15.6°$$

$C_{14}H_{20}N_2O_4$.	Calculated.	C 60.0, H 7.2, N 10.0
280.1	Found.	" 59.9, " 7.2, " 10.3

The filtrate was concentrated *in vacuo* and acidified with hydrochloric acid. Formation of long needles began at once; m.p., 187° (known for hippuric acid, 187°; mixed melting point, 186–187°). The yield (96 mg.) corresponded to 68 per cent of the theoretical for hippuric acid.

$C_9H_9NO_3$.	Calculated.	C 60.3, H 5.0, N 7.8
179	Found.	" 60.2, " 5.1, " 8.0

α-Carbobenzoxyglycyl-ε-Carbobenzoxy-l-Lysine Methyl Ester—To the ethyl acetate-ether solution of the free ester from 3.2 gm. of ε-carbobenzoxylysine methyl ester hydrochloride was added with shaking 1 gm. of recrystallized carbobenzoxyglycyl chloride. The formation of crystalline ε-carbobenzoxylysine methyl ester hydrochloride occurred, and the mixture was allowed to stand 5 minutes. Then it was shaken simultaneously with another 1 gm. portion of carbobenzoxyglycyl chloride and an aqueous solution of 1.22 gm. of K_2CO_3, whereupon the solid ester hydrochloride disappeared. When it had been shaken 15 minutes, 1 cc. of pyridine was added to the solution to decompose any excess chloride, and then it was washed with HCl, aqueous $KHCO_3$, and water. On concentrating *in vacuo*, clusters of long needles formed. These were recrystallized from methyl alcohol and ether. The yield, 3.8 gm., corresponded to 88 per cent of that calculated for the carbobenzoxyglycyl chloride used.

An alternate procedure involved the coupling of carbobenzoxyglycyl chloride with ε-carbobenzoxylysine in an aqueous alkaline

medium. The acid thus obtained was methylated with diazomethane and gave an ester identical with that from the first method. The yield was about 50 per cent.

The methyl ester of α-carbobenzoxyglycyl-ε-carbobenzoxylysine was recrystallized from ethyl acetate and ether; it formed needles melting at 97°.

$C_{25}H_{31}N_3O_7$.	Calculated.	C 61.8, H 6.4, N 8.7
485.2	Found.	" 61.8, " 6.4, " 8.6

α-Glycyl-l-Lysine Methyl Ester Dihydrochloride—2 gm. of α-carbobenzoxyglycyl-ε-carbobenzoxylysine methyl ester were hydrogenated in the usual manner with palladium black in the presence of 5.5 cc. of 2 N HCl in methyl alcohol. The resinous oil which resulted from evaporating the solution *in vacuo* crystallized as long needles after standing 10 days under ether. The yield was 1 gm. M.p., about 177° with sintering.

$[\alpha]_D^{25} = (-0.97° \times 2.0476)/(1 \times 1.02 \times 0.0816) = -23.9°$ (in water)

$C_9H_{21}N_3O_3Cl_2$.	Calculated.	C 37.2, H 7.3, N 14.5
290.2	Found.	" 37.1, " 7.3, " 14.4

α-Carbobenzoxyglycyl-ε-Carbobenzoxy-l-Lysineamide—A solution of 1.5 gm. of α-carbobenzoxyglycyl-ε-carbobenzoxylysine methyl ester in methyl alcohol was saturated with ammonia at 0° and allowed to stand 2 days at room temperature. Then it was concentrated *in vacuo*, and the product crystallized from methyl alcohol with ether. Yield, 1.3 gm. For analysis this amide was dissolved in methyl alcohol and slowly thrown out by ether as spongy, crystalline masses. The melting point was unsharp, 130–134°.

$C_{24}H_{30}N_4O_6$.	Calculated.	C 61.3, H 6.4, N 11.9
470.2	Found.	" 61.3, " 6.3, " 11.9

Papain Hydrolysis—This substrate was treated in exactly the same manner as α-hippuryl-ε-carbobenzoxy-*l*-lysineamide. The removal of the solid phase after hydrolysis resulted in a decrease of 104 per cent (222 − 118) in liberated carboxyl and of 100 per cent (157 − 57) in amino nitrogen.

The recovered ε-carbobenzoxylysine melted at 254° after recrystallization from N HCl and ammonium hydroxide.

$[\alpha]_D^{25} = (+0.10° \times 0.1750)/(0.5 \times 1.02 \times 0.00226) = +15.2°$

$C_{14}H_{20}N_2O_4$.	Calculated.	C 60.0, H 7.2, N 10.0
	Found.	" 59.9, " 7.2, " 10.1

In the hydrolysate after concentration colorless needles formed, which melted at 116° (known melting point of carbobenzoxyglycine, 120°) and gave no depression in a mixed melting point determination with the latter.

α-Carbobenzoxyglycyl-ε-Carbobenzoxy-l-Lysine Hydrazide—To a warm methyl alcoholic solution of 4.9 gm. of α-carbobenzoxyglycyl-ε-carbobenzoxylysine methyl ester was added 0.8 cc. of hydrazine hydrate. After standing overnight, crystalline aggregations of the corresponding hydrazide had formed. Yield, 4.5 gm. This hydrazide was recrystallized from methyl alcohol. M.p., 167°.

$C_{24}H_{31}N_5O_6$.	Calculated.	C 59.4, H 6.4, N 14.4
485.2	Found.	" 59.6, " 6.5, " 14.3

α-Carbobenzoxyglycyl-ε-Carbobenzoxy-l-Lysylglycine Ethyl Ester—This reaction was carried out similarly to the preparation of ε-carbobenzoxy-α-hippuryl-*l*-lysylglycine ethyl ester. 1.9 gm. of α-carbobenzoxyglycyl-ε-carbobenzoxylysine hydrazide, dissolved in 10 cc. of acetic acid and 20 cc. of water, were treated with a solution of 0.3 gm. of sodium nitrite. The oily azide which formed was taken up with ethyl acetate, and after washing and drying was combined with an ether solution of the ester from 2.2 gm. of glycine ethyl ester hydrochloride. The formation of flocculent crystalline aggregates soon began. After standing overnight, these were removed and the solution washed with HCl, aqueous $KHCO_3$, and water, and concentrated *in vacuo*. The crystalline deposit from this, similar to the solid above, was combined with it, making a total yield of 1 gm. After recrystallization from ethyl acetate and petroleum ether, this product melted at 146°, beginning to sinter at 140°.

$C_{28}H_{36}N_4O_8$.	Calculated.	C 60.4, H 6.5, N 10.1
556.3	Found.	" 60.8, " 6.6, " 9.8

α-Carbobenzoxyglycyl-ε-Carbobenzoxy-l-Lysylglycineamide—This amide was prepared from the corresponding ester in the usual manner; it formed a spongy crystalline mass melting at 90–95°.

$C_{26}H_{33}N_5O_7$.	Calculated.	C 59.3, H 6.3, N 13.2
529.3	Found.	" 59.1, " 6.5, " 12.9
		" 13.2

α-Benzoyl-ε-Carbobenzoxy-l-Lysineamide—The ester corresponding to 3.3 gm. of ε-carbobenzoxylysine methyl ester hydrochloride was coupled with 1.2 gm. of benzoyl chloride in two portions, K_2CO_3 being used to remove the liberated HCl. This method has been described for α-carbobenzoxyglycyl-ε-carbobenzoxy-*l*-lysine methyl ester. The product, α-benzoyl-ε-carbobenzoxylysine methyl ester, was a resinous oil. The yield was 3.3 gm. Without further purification, this oil was converted into the amide by the usual procedure; *i.e.*, a solution in methyl alcohol was allowed to stand 24 hours with excess ammonia. Upon removing the solvent *in vacuo*, colorless crystals formed. These were recrystallized from methyl alcohol with a little ether. M.p., 172–173°; yield, 3.0 gm.

$C_{21}H_{25}N_3O_4$.	Calculated.	C 65.8, H 6.6, N 11.0
383.2	Found.	" 65.9, " 6.6, " 11.2

α-Benzoyl-l-Lysineamide Hydrochloride—2 gm. of the recrystallized α-benzoyl-ε-carbobenzoxy-*l*-lysineamide were hydrogenated with palladium black in methyl alcohol. 6.4 cc. of N HCl in methyl alcohol were present in the solution. The product, after concentration, was an oil which resisted crystallization. Finally, it was left in a good vacuum over P_2O_5. After 24 hours it had formed an inflated mass of thin films, which on being broken up gave the illusion of being crystalline plates. This form of the product also would not crystallize; it was very hygroscopic and therefore did not give a good analysis after being washed with ether and dried.

$C_{13}H_{20}N_3O_2Cl$.	Calculated.	C 54.6, H 7.1, N 14.7
285.7	Found.	" 53.2, " 7.2, " 14.0

α-Benzenesulfonyl-ε-Carbobenzoxy-l-Lysine Methyl Ester—An ether solution of the ester from 10.0 gm. of ε-carbobenzoxylysine methyl ester hydrochloride was diluted with an equal volume of ethyl acetate, and 2.5 gm. of benzenesulfonyl chloride were added. Soon the formation of needles of the ester hydrochloride began. After 24 hours this precipitate was filtered off, and the solution was

washed with HCl, aqueous $KHCO_3$, and water, and dried. On evaporating *in vacuo*, an oil formed which readily crystallized. Yield, 5.0 gm.

This product formed beautiful clusters of long hair-like needles when it was recrystallized from ether-petroleum ether. M.p., 80°.

$C_{21}H_{26}N_2O_6S$.	Calculated.	C 58.0, H 6.0, N 6.5
434.2	Found.	" 58.1, " 6.1, " 6.5

α-Benzenesulfonyl-l-Lysine—3.5 gm. of α-benzenesulfonyl-ε-carbobenzoxy-*l*-lysine methyl ester were hydrolyzed with the theoretical amount of N NaOH. After standing overnight, the alkaline solution was acidified; it deposited an oil which was taken up with ether. The product was extracted from the ether with aqueous $KHCO_3$, transferred to a fresh solution, and concentrated *in vacuo* to a resinous oil. Yield, 3.2 gm.

This oil was at once hydrogenated in a water-methyl alcohol solution acidified with HCl; palladium black was used as catalyst. When the hydrogenation was over, the solution was concentrated *in vacuo*, dissolved twice in water, and the water distilled off. A thick, aqueous solution resulted; it deposited well formed, wedge-shaped prisms when made barely ammoniacal. The yield was 1.8 gm., corresponding to 80 per cent of the theoretical calculated from 3.5 gm. of ester.

This α-benzenesulfonyllysine, recrystallized from 50 per cent alcohol, melted at 277° with decomposition. The product of Gurin and Clarke, we found, melted also at 277° with decomposition; a mixed melting point with the two gave no depression.

$C_{12}H_{18}O_4N_2S$.	Calculated.	C 50.3, H 6.3, N 9.8
286.1	Found.	" 50.3, " 6.4, " 9.9

The specific rotation of α-benzenesulfonyllysine depends upon the relative amount of base present. Since 0.0990 N NaOH was used as solvent in the following determinations, there was 1 molecular equivalent of sodium hydroxide in the first, and 2 in the second.

(1) $[\alpha]_D^{25} = (-0.27° \times 3.8584)/(1 \times 1.02 \times 0.1060) = -9.6°$ (1 mole NaOH)
(2) $[\alpha]_D^{25} = (-0.32° \times 3.7993)/(1 \times 1.02 \times 0.0530) = -22.5°$ (2 moles NaOH)

Dicarbobenzoxy-l-Lysineamide—4.1 gm. of *l*-lysine dihydrochloride were converted into dicarbobenzoxylysine, methylated with diazomethane, and allowed to stand overnight in a methyl alcoholic solution saturated with ammonia. The product formed aggregations of long needles which melted at 155°.

$C_{22}H_{27}N_3O_5$.	Calculated.	C 63.9, H 6.6, N 10.2
413.2	Found.	" 64.0, " 6.5, " 10.2

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