TABLE II

LYSINE DIPEPTIDES

ANALYTICAL DATA AND SPECIFIC ROTATION IN 0.5 *N* HCl (BASIS: FREE PEPTIDES)

Number	Compound[a]	Molecular formula	Mol. wt.	Nitrogen, % Calcd.	Nitrogen, % Found	Amino N, % Calcd.	Amino N, % Found	HCl, % Calcd.	HCl, % Found	Neut. equiv.[b] Calcd.	Neut. equiv.[b] Found	$[\alpha]^{24}D$ (*c*, 2)
26	H·Lys-Gly·OH·HCl (L)[c]	$C_8H_{17}O_3N_3$·HCl	239.7	17.5	17.3	11.7	11.7	15.2	14.9	120	119	+40.7[d]
27	H·Gly-Lys·OH·HCl (L)	$C_8H_{17}O_3N_3$·HCl	239.7	17.5	17.5	11.7	..	15.2	15.2	120	121	−12.8[d]
28	H·Ala-Lys·OH·HCl (L-L)	$C_9H_{19}O_3N_3$·HCl	253.7	16.6	16.5	11.0	10.9	14.4	14.3	127	127	− 7.4
29	H·Ala-Lys·OH·HCl (D-L)	$C_9H_{19}O_3N_3$·HCl	253.7	16.6	16.5	11.0	11.0	14.4	14.3	127	125	−30.4
30	H·Lys-Ala·OH·HCl (L-L)	$C_9H_{19}O_3N_3$·HCl	253.7	16.6	16.4	11.0	10.9	14.4	14.5	127	129	+ 2.7
31	H·Lys-Ala·OH·HCl (L-D)	$C_9H_{19}O_3N_3$·HCl	253.7	16.6	16.3	11.0	11.0	14.4	14.5	127	126	+80.4
32	H·Lys-Lys·OH·2HCl (L-L)[g]	$C_{12}H_{26}O_3N_4$·2HCl	347.3	16.1	15.8	12.1	12.2	21.0	21.3	115	116	
33	H·Lys-Lys·OH·3HCl·H_2O (L-L)[e,f]	$C_{12}H_{26}O_3N_4$·3HCl·H_2O	401.8	13.9	14.0	10.5	10.6	27.2	27.0	100	100	+ 8.2
34	H·Lys-Lys·OH·2HCl (L-D)	$C_{12}H_{26}O_3N_4$·2HCl	347.3	16.1	15.8	12.1	11.7	21.0	20.7	115	116	+44.7

[a,b] See Table I, Footnotes *a* and *b*. [c] $[\alpha]^{24}D$ +69.1° (2% in H_2O). This peptide was previously prepared (Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 26 (1934)) as the sulfate ($C_8H_{17}O_3N_3 \cdot H_2SO_4$) with $[\alpha]^{22}D$ +30.0° (1.5% in H_2O). We find that the specific rotation of dipeptides of the general formula H·R-Gly·OH is abnormally high in H_2O (ring formation?) as compared with their rotation in dilute acid. This explains, at least to some extent, the low value for $[\alpha]D$ reported by Bergmann, because his rotation was measured in a solution more acid than ours. (While this paper was in press, the sulfate was also prepared by Brenner and Burckhardt, *Helv. chim. acta*, **34**, 1070 (1951), however without reporting any properties of the peptide). [d] At 25°. [e] In order to determine if racemization took place during synthesis of H·Lys-Lys·OH·3HCl·H_2O (L-L), a sample was hydrolyzed at 135° for 17 hours in 6 *N* HCl. The specific rotation of the hydrolysate, calculated for lysine, was found to be +23.7°, indicating absence of racemization during synthesis. [f] The synthesis of this dipeptide was recently reported by Waley and Watson *Nature*, **167**, 360 (1951). [g] NOTE ON PROOF: Compound 32 has now been prepared in a much simpler way by the new, elegant method of R. A. Boissonnas, *ibid.*, **34**, 874 (1951).

methyl ester (Compound 11) is somewhat more complicated. The dry, cold, ethereal solution of ε-carbobenzoxy-L-lysine methyl ester (from 0.03 mole of the hydrochloride) is mechanically stirred in a 3-neck flask at −5°. A cold, ethereal solution of dicarbobenzoxy-L-lysine azide (prepared[5] from 0.02 mole of the hydrazide) is added over a period of five minutes. Almost immediately an oil begins to form. After 20–30 minutes stirring at −5°, a solid starts to precipitate, which carries down the oil. At this point filtration becomes feasible, so that the solution can be transferred with the aid of an immersion filter into another 3-neck flask, equipped with stirrer. Stirring is continued at 25° for about four hours, during which time a gelatinous solid precipitates. The flask is cooled to −5° and the crude product collected and washed with ether (m.p. 110–114°). After recrystallization from ethanol–ether, 6.5 g. of the compound is obtained; m.p. 115–117°; yield 45% of the hydrazide used. For the preparation of the corresponding hydrazide (Compound 15) the crude ester can be satisfactorily used.

The preparation of the corresponding L-D ester (Compound 12) presents no such difficulties and is carried out in the regular manner.

We have not as yet been able to prepare tricarbobenzoxy-L-lysyl-L-lysine benzyl ester.

Carbobenzoxy-dipeptide Hydrazides (Compounds 13–17).—The hydrazides were prepared as described previously.[2,3]

Carbobenzoxy Dipeptides (Compounds 18–25).—The carbobenzoxy dipeptide methyl and ethyl esters are saponified in methanol–2 *N* NaOH (about 15–20% excess of NaOH) at 37° for about two hours; the reaction is complete when a drop of the solution added to water no longer gives a turbidity. The solution is then filtered from any suspended matter and poured into three times its volume of water. Acidification with 2 *N* HCl immediately precipitates a filterable solid or an oil which solidifies on standing overnight at 5°. Recrystallization from ethyl acetate–petroleum ether yields 70–80% of the pure compounds.

Dipeptides (Compounds 26–34).—Lysine dipeptides are isolated as hydrochlorides, which are all more or less hygroscopic.

Hydrogenolysis of 0.01 mole of a carbobenzoxy dipeptide is carried out in 100 cc. of methanol, containing the amount of *N* HCl required for the ε-amino groups, with palladium black[2] as catalyst in a rapid stream of hydrogen. Hydrogenation is complete after about two hours, as indicated by cessation of CO_2 evolution. For carbobenzoxy dipeptide benzyl esters, 80% acetic acid plus the calculated amount of *N* HCl is used as solvent and hydrogenolysis continued for an additional two hours after cessation of CO_2 evolution. Concentration of the filtrates *in vacuo* results in oils, which are dried over P_2O_5 in high vacuum to glass-like solids. Crystallization is induced by dissolving the solids in warm methanol and judiciously adding anhydrous ethanol or ether or both. By recrystallization from the same solvents the pure peptide hydrochlorides are obtained in 60–80% yield. L-Lysyl-L-lysine was also prepared as trihydrochloride-monohydrate (Compound 33). For analysis and rotation measurements the dipeptide hydrochlorides are dried at 56° in high vacuum.

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Optical Rotation of Peptides. IV. Lysine Tripeptides[1]

BY ERWIN BRAND, BERNARD F. ERLANGER, JEROME POLATNICK, HOWARD SACHS AND DONALD KIRSCHENBAUM

Previous papers in this series dealt with the synthesis and specific rotation of dipeptides of alanine[2] and of lysine.[3] In this paper the syntheses and specific rotations (in 0.5 *N* HCl) of seven lysine tripeptides are presented. Detailed data on their specific rotations, on the *residue rotations*[4] of lysine and alanine residues in these peptides and on the hydrolytic as well as the synthetic action of trypsin and chymotrypsin on some of these peptides will be reported subsequently.

Experimental

The synthesis and properties of most of the starting materials have been previously described: L- and D-alanine,[2] L- and D-lysine,[3] methyl ester hydrochloride of ε-carbobenzoxy-L-lysine (ref. 3, footnote 6), benzyl ester hydrochlorides of glycine and of L- and D-alanine (ref. 2, Cmpds. 4–6), and of L- and D-ε-carbobenzoxylysine (ref. 3, Cmpds. 1,2), various carbobenzoxy dipeptide hydrazides (ref. 3, Cmpds. 13–17).

Carbobenzoxy Tripeptide Esters (Compounds 1–9).—The coupling of the azides of carbobenzoxy dipeptide hydrazides with the free amino acid benzyl esters is carried out as described in detail previously.[2,3] However, the preparation of the azide solution differs for the synthesis of Compound 1 (containing glycine), Compounds 2–5 (containing alanine) and Compounds 6–9 (containing only lysine).

(1) Presented in part before the Division of Biological Chemistry at the 119th Meeting of the A.C.S., Boston, Mass., April, 1951.

(2) Erlanger and Brand, THIS JOURNAL, **73**, 3508 (1951).

(3) Erlanger and Brand, *ibid.*, **73**, 3508 (1951).

(4) Brand and Erlanger, *ibid.*, **72**, 3314 (1950).

TABLE I

CARBOBENZOXY LYSINE TRIPEPTIDE DERIVATIVES

No.	Compound[a]	Molecular formula	Mol. wt.	M.p., °C. (cor.)	Nitrogen, % Calcd.	Found
	Carbobenzoxy tripeptide esters					
1	Z·Gly-Z·Lys-Gly·OBz (L)	$C_{33}H_{38}O_8N_4$	618.7	120–122	9.1	9.2
2	Z·Ala-Z·Lys-Ala·OEt (3L)	$C_{30}H_{40}O_8N_4$	584.7	191–192	9.6	9.7
3	Z·Ala-Z·Lys-Ala·OBz (3L)	$C_{35}H_{42}O_8N_4$	646.7	183–184	8.7	8.7
4	Z·Ala-Z·Lys-Ala·OBz (L-D-L)	$C_{35}H_{42}O_8N_4$	646.7	160–161	8.7	8.8
5	Z·Ala-Z·Lys-Ala·OBz (L-D-D)	$C_{35}H_{42}O_8N_4$	646.7	173–174	8.7	8.8
6	Z_2·Lys-Z·Lys-Z·Lys·OMe (3L)	$C_{51}H_{64}O_{12}N_6$	953.1	142–145	8.8	8.8
7	Z_2·Lys-Z·Lys-Z·Lys·OBz (3L)	$C_{57}H_{68}O_{12}N_6$	1029.2	153–154	8.2	8.3
8	Z_2·Lys-Z·Lys-Z·Lys·OBz (L-D-L)	$C_{57}H_{68}O_{12}N_6$	1029.2	141–142	8.2	8.3
9	Z_2·Lys-Z·Lys-Z·Lys·OBz (L-D-D)	$C_{57}H_{68}O_{12}N_6$	1029.2	151–152	8.2	8.3
	Carbobenzoxy tripeptide hydrazide					
10	Z·Ala-Z·Lys-Ala·$NHNH_2$ (3L)	$C_{28}H_{38}O_7N_6$	570.6	208	14.7	14.6

[a] The following abbreviations are used (*cf.* ref. 2, 3, Table I, footnote *a*): Z: carbobenzoxy, C_6H_5·CH_2OCO; Gly: $NH(CH_2)CO$; Ala: $NH(CHCH_3)CO$; Lys: $NH(CHC_4H_8NH_2)CO$; peptide linkage indicated by hyphen; Me: CH_3; Et: C_2H_5; Bz: $C_6H_5CH_2$; configuration follows compound in parentheses. *E.g.*, α,ε-dicarbobenzoxy-L-lysyl-ε-carbobenzoxy-D-lysyl-ε-carbobenzoxy-L-lysine benzyl ester: Z_2·Lys-Z·Lys-Z·Lys·OBz (L-D-L); L-alanyl-D-lysyl-D-alanine monohydrochloride: H·Ala-Lys-Ala·OH·HCl (L-D-D).

TABLE II

LYSINE TRIPEPTIDES: ANALYTICAL DATA AND SPECIFIC ROTATION IN 0.5 *N* HCl (BASIS: FREE PEPTIDES)

No.	Compound[a]	Molecular formula	Mol. wt.	Nitrogen, % Calcd.	Found	Amino N, % Calcd.	Found	HCl, % Calcd.	Found	Neut. equiv.[b] Calcd.	Found	$[\alpha]^{25}D$ (c = 2)
11	H·Gly-Lys-Gly·OH·HCl (L)	$C_{10}H_{20}O_4N_4$·HCl	296.8	18.9	18.6	..	..	12.3	12.3	148	148	−32.1
12	H·Ala-Lys-Ala·OH·HCl (3L)	$C_{12}H_{24}O_4N_4$·HCl	324.8	17.3	17.2	8.6	8.6	11.2	11.4	162	163	−42.5
13	H·Ala-Lys-Ala·OH·HCl (L-D-L)	$C_{12}H_{24}O_4N_4$·HCl	324.8	17.3	17.1	8.6	8.3	11.2	11.1	162	161	+14.2[c]
14	H·Ala-Lys-Ala·OH·HCl (L-D-D)	$C_{12}H_{24}O_4N_4$·HCl	324.8	17.3	17.1	8.6	8.5	11.2	11.0	162	159	+12.4[d]
15	H·Lys-Lys-Lys·OH·3HCl (3L)	$C_{18}H_{38}O_4N_6$·3HCl	511.9	16.4	16.2	10.9	10.9	21.4	21.4	128	133	− 2.2[d]
16	H·Lys-Lys-Lys·OH·3HCl (L-D-L)	$C_{18}H_{38}O_4N_6$·3HCl	511.9	16.4	16.3	10.9	11.2	21.4	21.2	128	129	+27.7[e]
17	H·Lys-Lys-Lys·OH·3HCl (L-D-D)	$C_{18}H_{38}O_4N_6$·3HCl	511.9	16.4	16.5	10.9	10.9	21.4	21.2	128	130	+34.9[e]

[a] See Table I, footnote *a*. [b] Neutralization equivalent, obtained by titration in alcohol (Ellenbogen and Brand, Am. Chem. Soc., Philadelphia Meeting, April, 1950, Abstracts p. 56-C). [c] At 19°. [d] At 24°. [e] At 22°.

In the case of Compound 1, 0.025 mole of Z·Gly-Z·Lys·$NHNH_2$ (L, ref. 3, Cmpd. 13) is dissolved in a mixture of 40 cc. of glacial acetic acid, 24 cc. of 5 *N* HCl and 220 cc. of water, treated with 0.028 mole of sodium nitrite and taken up in 250 cc. of cold ether. Following the usual procedure, the azide solution is added in one portion to a cold, dry, ethereal solution of glycine benzyl ester (previously prepared from 0.03 mole of its hydrochloride).

In the case of Compounds 2–5, 0.015 mole of Z·Ala-Z·Lys·$NHNH_2$ (L-L or L-D, ref. 3, Compds. 14, 15) is dissolved in a mixture of 65 cc. of glacial acetic acid, 15 cc. of 5 *N* HCl and 100 cc. of water, treated with 0.018 mole of sodium nitrite, followed by an additional 150 cc. of ice-cold water. The azide is then extracted with 200 cc. of cold ethyl acetate, washed and dried in the usual way, and added in one portion to a cold, dry, ethereal solution of alanine benzyl (or ethyl) ester (previously prepared from 0.024 mole of its hydrochloride).

In the case of Compounds 6–9, 0.06 mole of Z_2.Lys-Z.Lys.$NHNH_2$ (L-L or L-D, ref. 3, Cmpds. 16, 17) is dissolved in a mixture of 70 cc. of glacial acetic acid and 50 cc. of water, treated with 0.075 mole of sodium nitrite, followed by an additional 200 cc. of ice-cold water. The azide is then extracted with 200 cc. of cold ethyl acetate, washed and dried in the usual way, and added in one portion to a cold, dry solution (1:1 ethyl acetate–ether) of ε-carbobenzoxy-lysine benzyl (or methyl) ester (previously prepared from 0.1 mole of its hydrochloride).

In all cases precipitation of the coupling products starts within 30 minutes. After standing for about 20 hours at room temperature, the reaction mixture is cooled to about −10°, the material collected and washed with ether. Compound 1 is recrystallized from ethyl acetate–petroleum ether, Compounds 3–5 from 85% methanol, and Compounds 2, 6–9 from 95% ethanol. The yield of pure recrystallized carbobenzoxy tripeptide esters is 70–80% based on the hydrazide use.

Carbobenzoxy Tripeptide Hydrazide (Compound 10).—Z·Ala-Z·Lys-Ala·$NHNH_2$ (3L) is prepared in the usual manner[2] from Compound 2, except that refluxing with hydrazine hydrate in alcohol is carried out for one and a half hours instead of one hour. The yield of pure recrystallized (95% ethanol) product is 75% based on the ester used.

Tripeptides (Compounds 11–17).—The tripeptides are isolated as hydrochlorides, which are all more or less hygroscopic.

Hydrogenolysis of 0.005 mole of a carbobenzoxy tripeptide benzyl ester is carried out in 100 cc. of 85% acetic acid, containing the amount of *N* HCl required for the epsilon amino groups, with palladium black as catalyst in a rapid stream of hydrogen. After cessation of CO_2 evolution, hydrogenolysis is continued for another two hours. Concentration of the filtrates *in vacuo* (bath temperature at 40°) results in oils which are dried over P_2O_5 in high vacuum. The glass-like solids (though sometimes crystals appear at this stage) crystallize from warm 95% methanol upon the addition of absolute ethanol. Recrystallization from the same solvents results in pure peptide hydrochlorides in 70–80% yield. For analysis and rotation measurements, the tripeptide hydrochlorides are dried at 56° in high vacuum.

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The Magnetic Susceptibility of $Co^{+++}aq.$

BY HAROLD L. FRIEDMAN, JOHN P. HUNT, ROBERT A. PLANE AND HENRY TAUBE

Co(III) in solid K_3CoF_6 has a magnetic moment corresponding to 4 unpaired electrons[1] while $Co(NH_3)_3F_3$, $Co(NH_3)_6Cl_3$ and $K_3Co(CN)_6$ are dia-

(1) Cartledge quoted (p. 109) in "The Nature of the Chemical Bond," Pauling, Cornell University Press, Ithaca, N. Y., 1939.