yl-5-thienylacetamide and the corresponding acid was obtained by hydrolysis.

Experimental

Attempted Transmetalation of 3-Methylthiophene.—A mixture of 23 g. (1 gram atom) of sodium sand, 250 ml. of anhydrous ether and 98 g. (1 mole) of 3-methylthiophene was refluxed and 68.5 g. (0.5 mole) of *n*-butyl bromide in 100 ml. of anhydrous ether was added dropwise with mechanical stirring. After the addition was completed the mixture was refluxed for an additional 2 hours and was carbonated with pieces of Dry Ice. Water was cautiously added, the alkaline aqueous layer was separated and acidified with concentrated hydrochloric acid whereupon 15 g. (21%) of 4-methyl-2-thiophenecarboxylic acid, m.p. 119–120°, was obtained.

There was also obtained from the ethereal layer 46% of 3-methylthiophene and 60% of a liquid, b.p. 198–202°. Using ethyl bromide as the alkyl halide there was obtained 10% of 4-methyl-2-thiophenecarboxylic acid, 60% of 3-methylthiophene and 40% of a liquid, b.p. 164–166°, 46–48° (13 mm.). For some unaccountable reason, when bromobenzene was used only a high recovery of 3-methylthiophene was observed.

2-Ethyl-4-methyl-5-acetylthiophene.—The organic liquid, b.p. 46–48° (13 mm.) obtained from the attempted transmetalation of 3-methylthiophene with ethyl bromide, was acetylated with acetic anhydride and stannic chloride by the method of Johnson and May.[4] From 16 g. (0.13 mole) there was obtained 15 g. (70%) of a colorless liquid, b.p. 125–126° (15 mm.). A semicarbazone was prepared in the usual manner and crystallized from alcohol, m.p. 185–186°.

Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.33; H, 6.66; N, 18.67. Found: C, 53.55; H, 6.60; N, 18.78.

The molecular formula, $C_{10}H_{15}N_3OS$, was calculated for the semicarbazone of a methylethylacetylthiophene. The semicarbazone of 5-ethyl-4-methyl-2-acetylthiophene is a known compound and has a m.p. of 228–229°. The ketone obtained is believed to be 2-ethyl-4-methyl-5-acetylthiophene.

2-Ethyl-4-methyl-5-thenylacetamide.—2-Ethyl-4-methyl-5-acetylthiophene was converted to 2-ethyl-4-methyl-5-thienylacetamide, m.p. 98–99°, in a yield of 35% by the Willgerodt reaction.[1]

Anal. Calcd. for $C_9H_{13}NOS$: N, 7.65. Found: N, 7.77.

Hydrolysis of this amide gave an oil which readily formed a solid *p*-bromophenacyl ester, m.p. 95–97°.

Anal. Calcd. for $C_{17}H_{17}BrO_3S$: C, 53.54; H, 4.46. Found: C, 53.82; H, 4.31.

(4) J. R. Johnson and C. E. May, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 8.

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Optical Rotation of Peptides. V. Alanine Tetra-, Penta- and Hexapeptides[1]

BY ERWIN BRAND, BERNARD F. ERLANGER AND HOWARD SACHS

Previous papers in this series dealt with the synthesis and specific rotation of alanine dipeptides[2] and tripeptides.[3] In this paper the syntheses and specific rotations (in 0.5 *N* HCl) of four isomeric alanine tetrapeptides,[4] pentaalanine (5L) and hexaalanine (6L) are presented. More detailed data on their specific rotations and on the *residue rotations*[5] of alanine residues will be reported subsequently.

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

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

(3) E. Brand, B. F. Erlanger, H. Sachs and J. Polatnick, *ibid.*, **73**, 3510 (1951).

(4) For abbreviations see Table I, footnote *a*.

(5) E. Brand and B. F. Erlanger, THIS JOURNAL, **72**, 3314 (1950).

Experimental

The synthesis and properties of most of the starting materials have been previously described: L- and D-alanine[2]; L- and D-alanine benzyl esters (ref. 2, Cmpds. 5, 6); carbobenzoxy-D-alanine hydrazide (ref. 2, Cmpd. 3); carbobenzoxydialanine hydrazide (Z.Ala-Ala.$NHNH_2$(2L), ref. 2, Cmpd. 17); and three isomeric carbobenzoxytrialanine hydrazides (ref. 3, Cmpds. 14–16).

The benzyl ester hydroiodides of di- and trialanine were used as intermediates in the synthesis of some of the higher peptides. These benzyl esters were prepared from their corresponding carbobenzoxy derivatives by reduction with phosphonium iodide, which removes the N-carbobenzoxy group more rapidly than the benzyl ester group.[6]

(1) **H.Ala-Ala.OBz.HI** (2L) ($C_{13}H_{18}O_3N_2$.HI, mol. wt. 378.2).—0.15 mole of Z.Ala-Ala.OBz (2L) (ref. 2, Cmpd. 13) is dissolved in 65 cc. of glacial acetic acid and warmed to 35–40°. Ph_4I (0.45 mole) is added and hydrogen passed through the solution for about two hours, when CO_2 evolution stops. The solution is evaporated *in vacuo*. Water is added and then distilled off *in vacuo*, in order to remove acetic acid; this treatment is repeated several times. The solution is finally distilled down to an oil, which is taken up in about 15 cc. of water and extracted with several portions of ether (caution—lachrymator!). The amount of H.Ala-Ala.OBz.HI (2L) present in this aqueous solution is determined by Van Slyke amino nitrogen determination. From this solution the free dipeptide benzyl ester is prepared in the usual fashion[2,3] for use in further synthesis.

Since neither the hydroiodide, the hydrochloride, nor the free dialanine benzyl ester is obtained in crystalline form, Compound 1 is identified by converting it into derivatives. Reaction of the free dialanine benzyl ester in ethyl acetate with an ethereal solution of carbobenzoxy-L-alanine azide yields a compound with the same melting point, mixed melting point (201°) and analysis as Z.Ala-Ala-Ala.OBz (3L), previously prepared by a different procedure (ref. 3, Cmpd. 9). Compound 1 is further identified by converting it into Z.Ala-$(Ala)_3$-Ala.OBz (5L) (*cf.* below, Cmpd. 7).

(2) **H.Ala-Ala-Ala.OBz.HI** (3L) ($C_{16}H_{23}O_4N_3$.HI, mol. wt. 449.3).—This compound is prepared from Z.Ala-Ala-Ala.OBz(3L)(ref. 3, Cmpd. 9) as described above for Compound 1. Since neither the hydroiodide nor the free trialanine benzyl ester is obtained in crystalline form, Compound 2 is identified by converting it into derivatives (*cf.* below, Cmpds. 6, 7 and 8).

Carbobenzoxytetraalanine Benzyl Esters (Compounds 3–6).—Compounds 3–5 are prepared by coupling the azide of a carbobenzoxytrialanine hydrazide[3] with free alanine benzyl ester essentially as described in detail for the synthesis of carbobenzoxydipeptide and -tripeptide esters.[2,3]

(3) **Z.Ala-$(Ala)_2$-Ala.OBz** (4L).—For the preparation of this compound, 0.1 mole of Z.Ala-Ala-Ala.$NHNH_2$ (3L) (ref. 3, Cmpd. 14) is dissolved in 150 cc. of glacial acetic acid, 12.5 cc. of 5 *N* HCl and 100 cc. of water. The solution is cooled to −5°, sodium nitrite (0.11 mole) added, and allowed to stand in an ice–salt-bath for three minutes, after which an additional 300 cc. of ice-cold water and 250 cc. of ice-cold ethyl acetate are added. The azide is extracted and washed with water, 3% aqueous $NaHCO_3$ and water (all ice-cold), dried over sodium sulfate and added in one portion to a cold, dry, ethereal solution of L-alanine benzyl ester[2] (previously prepared from 0.15 mole of its hydrochloride). After standing at room temperature for about 20 hours, the carbobenzoxytetraalanine ester is isolated and recrystallized from dioxane.

(4) **Z.Ala-$(Ala)_2$-Ala.OBz** (LDLL). (5) **Z.Ala-$(Ala)_2$-Ala.OBz** (LLDL).—For the preparation of these compounds, the carbobenzoxytrialanine azides are prepared from their respective carbobenzoxytrialanine hydrazides (ref. 3, Cmpds. 15, 16) by following exactly the procedure described for the preparation of carbobenzoxydialanine azides,[3] except that the carbobenzoxytrialanine azides are extracted with ethyl acetate instead of with ether–ethyl acetate. The azide solutions are then added in one portion to a 50% molar excess of free alanine benzyl ester in ether. After 20 hours the carbobenzoxytetraalanine benzyl esters are collected and recrystallized from 95% ethanol.

(6) **Z.Ala-$(Ala)_2$-Ala.OBz** (DLLL).—This compound is prepared by coupling 0.1 mole of carbobenzoxy-D-alanine azide[2] with 0.15 mole of H.Ala-Ala-Ala.OBz (3L) in ethyl

(6) C. R. Harington and T. H. Mead, *Biochem. J.*, **30**, 1599 (1936).

TABLE I

TETRA-, PENTA- AND HEXAALANINE DERIVATIVES

No.	Compound[a]	Molecular formula	Mol. wt.	M.p., °C. (cor.)	N, % Calcd.	N, % Found
3	Z.Ala-(Ala)$_2$-Ala.OBz (4L)	$C_{27}H_{34}O_7N_4$	526.6	246	10.6	10.7
4	Z.Ala-(Ala)$_2$-Ala.OBz (L-D-L-L)	$C_{27}H_{34}O_7N_4$	526.6	181.5	10.6	10.5
5	Z.Ala-(Ala)$_2$-Ala.OBz (L-L-D-L)	$C_{27}H_{34}O_7N_4$	526.6	213–214	10.6	10.6
6	Z.Ala-(Ala)$_2$-Ala.OBz (D-L-L-L)	$C_{27}H_{34}O_7O_4$	526.6	191–192	10.6	10.9
7	Z.Ala-(Ala)$_3$-Ala.OBz (5L)	$C_{30}H_{39}O_8N_5$	597.7	254	11.7	11.7
8	Z.Ala-(Ala)$_4$-Ala.OBz (6L)	$C_{33}H_{44}O_9N_6$	668.7	Decomp. >260	12.6	12.7

[a] The following abbreviations are used (*cf.* ref. 2, 3, Table I, footnote *a*): Z, carbobenzoxy, $C_6H_5CH_2OCO$; Ala, NH-(CHCH$_3$)CO; peptide linkage indicated by hyphen; Bz, $C_6H_5CH_2$; configuration follows compound in parentheses. *E.g.*, carbobenzoxy-L-alanyl-L-alanyl-L-alanyl-L-alanyl-L-alanyl-L-alanine benzyl ester: Z.Ala-(Ala)$_4$-Ala.OBz (6L); L-alanyl-D-alanyl-L-alanyl-L-alanine: H.Ala-(Ala)$_2$-Ala.OH (L-D-L-L).

TABLE II

TETRA-, PENTA- AND HEXAALANINE
ANALYTICAL DATA AND SPECIFIC ROTATION IN 0.5 *N* HCl

No.	Compound[a]	Molecular formula	Mol. wt.	Nitrogen, % Calcd.	Nitrogen, % Found	Amino N, % Calcd.	Amino N, % Found	$[\alpha]^{25}D$ (c = 2)
9	H.Ala-(Ala)$_3$-Ala.OH.H$_2$O (4L)	$C_{12}H_{22}O_5N_4.H_2O$	320.3	17.5	17.6	4.4	4.5	−131.0
10	H.Ala-(Ala)$_3$-Ala.OH (L-D-L-L)[b]	$C_{12}H_{22}O_5N_4$	302.3	18.5	18.5	4.6	4.5	− 14.2[c]
11	H.Ala-(Ala)$_3$-Ala.OH (L-L-D-L)	$C_{12}H_{22}O_5N_4$	302.3	18.5	18.6	4.6	4.5	− 5.0
12	H.Ala-(Ala)$_3$-Ala.OH (D-L-L-L)	$C_{12}H_{22}O_5N_4$	302.3	18.5	18.3	4.6	4.5	−145.1[d]
13	H.Ala-(Ala)$_3$-Ala.OH (5L)	$C_{15}H_{27}O_6N_5$	373.4	18.8	18.8	3.8	3.8	−149.7[e]
14	H.Ala-(Ala)$_4$-Ala.OH (6L)	$C_{18}H_{32}O_7N_6$	444.5	18.9	18.7	3.2	3.1	−156.6[f]

[a] See Table I, footnote (*a*). [b] Neutralization equivalent = 303, obtained by titration in alcohol (Ellenbogen and Brand, Am. Chem. Soc., Philadelphia Meeting, April, 1950, Abstracts p.56C); the other peptides are too insoluble for this determination. [c] At 21°. [d] At 22°. [e] At 23°. [f] At 23°, c = 0.9

acetate (for the preparation of the free ester, *cf.* above under Compounds 1 and 2). The compound is isolated after 20 hours at room temperature and recrystallized from 95% ethanol.

The yield of pure carbobenzoxytetraalanine benzyl esters is 65–75%, based on the hydrazide used.

(7) **Z.Ala-(Ala)$_3$-Ala.OBz (5L).**—The carbobenzoxypentaalanine benzyl ester is prepared in two ways: (a) by coupling 0.1 mole of carbobenzoxydialanine azide (prepared[7] from Cmpd. 17, ref. 2) with 0.15 mole of trialanine benzyl ester in ethyl acetate (for the preparation of the free ester, *cf.* above Cmpds. 1 and 2); (b) by coupling 0.1 mole of carbobenzoxytrialanine azide (prepared as described above under Cmpd. 3) with 0.15 mole of dialanine benzyl ester (for the preparation of the free ester, *cf.* above under Cmpd. 1).

The carbobenzoxypentaalanine benzyl ester obtained by both procedures is insoluble in most organic solvents. It is purified by extracting with 95% boiling ethanol for 30 minutes, with a 75% yield of the pure product.

(8) **Z.Ala-(Ala)$_4$-Ala.OBz (6L).**—The carbobenzoxyhexaalanine benzyl ester is prepared by coupling 0.1 mole of carbobenzoxytrialanine azide (prepared as described above under Cmpd. 3) with 0.15 mole of trialanine benzyl ester (for the preparation of the free ester, *cf.* above under Cmpds. 1 and 2).

Compound 8 is insoluble in most organic solvents. It is purified by extracting with 95% boiling ethanol for 30 minutes, with a 75% yield of the pure product.

Peptides—Isomeric Tetraalanines (Compounds 9–12).—The carbobenzoxytetraalanine benzyl esters (Cmpds. 3–6) are hydrogenated in the usual way,[3] using 80% acetic acid as solvent. A volume of 180 cc. is used for 0.008 mole of Compound 3 and 150 cc. for 0.015 mole each of Compounds 4–6. The peptides are recrystallized from water–alcohol and dried in high vacuum at 56°. The yield of pure peptides is about 85%.

Tetraalanine (4L) (Cmpd. 9) is the only peptide obtained with one molecule of water. A previous preparation[8] from an α-halogen acid halide contained one molecule of water and gave $[\alpha]^{22}D$ −122.5° (2.2% in 2 *N* HCl).

(7) As described previously[4] under "Carbobenzoxy Tripeptide Esters," except that ethyl acetate is used for extraction instead of ether–ethyl acetate.

(8) E. Abderhalden and W. Gohdes, *Fermentforschung*, **13**, 52 (1931).

(13) **H.Ala-(Ala)$_3$-Ala.OH (5L).**—For the preparation of pentaalanine the corresponding carbobenzoxy benzyl ester is reduced in dimethyl formamide in the following way: 0.0045 mole of the ester (Cmpd. 7) is dissolved in 225 cc. of dimethyl formamide at 105°. The solution is cooled quickly to 45°, resulting in a fine suspension. About 1 g. of palladium black suspended in about 10 cc. of dimethyl formamide is added quickly and followed immediately by a rapid stream of hydrogen. After about 30 minutes, when the solution has cooled to about 25°, 2.5 cc. of 2 *N* HCl is added. After another 15 minutes, 50 cc. of water is added gradually over a 30-minute period to keep the reduced peptide in solution—care being taken not to precipitate unreduced material. After a total of about two hours, CO_2 evolution ceases and the solution is clear. Hydrogenation is continued for another two hours. Palladium black is filtered off and washed with 50 cc. of 0.1 *N* HCl. The peptide is precipitated by adding 2 cc. of pyridine to the combined filtrate and washings. After standing overnight in the ice-box, the peptide is filtered off and purified by dissolving in the calculated quantity of 0.25 *N* HCl, precipitating with pyridine, and washing thoroughly with alcohol and ether. For analysis it is dried in high vacuum at 100°. The yield of the pure peptide is 85%.

It was previously prepared[8] with $[\alpha]^{18}D$ −136.4° (1.9% in 2 *N* HCl), containing one molecule of water which could not be removed in high vacuum at elevated temperature.

(14) **H.Ala-(Ala)$_4$-Ala.OH (6L).**—For the preparation of hexaalanine the corresponding carbobenzoxy benzyl ester is reduced in phenol in the following way: 0.0043 mole of the ester (Cmpd. 8) is dissolved in 175 cc. of phenol (saturated with water) by warming to 90°. The solution is then cooled rapidly to 40°. About 1 g. of palladium black suspended in about 10 cc. of phenol is added quickly to the slightly turbid solution, followed immediately by a rapid stream of hydrogen. After 20 minutes 2.5 cc. of 2 *N* HCl and 30 cc. of 95% ethanol are added. After hydrogenation has proceeded for one hour, 30 cc. of 50% ethanol is added to reduce the turbidity, and another 30 cc. after a second hour. At this point CO_2 evolution is negligible, and the solution becomes clear. Hydrogenation is continued for three more hours. Palladium black is then filtered off and washed with 50 cc. of a 1:1 (v./v.) mixture of 95% ethanol and 0.5 *N* HCl. An excess of pyridine is added to the combined filtrate and washings, followed by 250 cc. of 95% ethanol. After standing overnight in the ice-box, the gelatinous precipitate of the peptide is filtered off and washed

with alcohol and ether. The peptide is purified by dissolving in 250 cc. of water containing a 50% molar excess of HCl, filtering, adding 150 cc. of absolute ethanol to the clear solution, and precipitating with pyridine. The precipitate is centrifuged off and washed repeatedly, first with absolute alcohol and then with ether. For analysis the material is dried *in vacuo* at 100°. The yield of pure peptide is about 78%. It was previously prepared[8] containing one molecule of water which could not be removed in high vacuum at elevated temperature; since the preparation was insoluble, no rotation was reported.[8]

This work was aided by a contract between the Office of Naval Research Department of the Navy and Columbia University (NR 122-260).

CORRECTION.—In Paper IV. Lysine Tripeptides, by E. Brand, *et al.*, THIS JOURNAL, **73**, 4026 (1951), Compound 5 in Table I should read Z.Ala-Z.Lys-Ala.OBz (L-L-D), and Compound 14 in Table II should read H.Ala-Lys-Ala.OH.HCl (L-L-D).

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Optical Rotation of Peptides. VI. Tetra- and Pentapeptides Containing Alanine and Lysine[1]

BY ERWIN BRAND, BERNARD F. ERLANGER AND HOWARD SACHS

Previous papers in this series dealt with the synthesis and specific rotation of a number of alanine and lysine peptides.[2-5] In this paper the syntheses and specific rotations (in 0.5 *N* HCl) of two isomeric tetrapeptides and two isomeric pentapeptides containing lysine are presented. More detailed data on their specific rotations and on the *residue rotations*[6] of lysine and alanine residues in these peptides will be reported subsequently, as well as the action of certain proteolytic enzymes on these peptides.

Experimental[7]

The synthesis and properties of the starting materials have been previously described[8]: L-alanine[2]; L- and D-lysine[3]; Z.Ala-Z.Lys.$NHNH_2$ (2L and LD, ref. 3, Compds. 14, 15); Z.Ala-Z.Lys-Ala.$NHNH_2$ (3L, ref. 4, Compd. 10); benzyl ester hydroiodides of di- and tri-L-alanine (ref. 5, Cmpds. 1, 2).

Carbobenzoxytetrapeptide Benzyl Esters

(1) **Z.Ala-Z.Lys-Ala-Ala.OBz (4L).**—This compound is prepared by two different methods. (a) From a carbobenzoxy dipeptide azide and a dipeptide benzyl ester: 0.01 mole of Z.Ala-Z.Lys.$NHNH_2$ (2L, ref. 3, Cmpd. 14) is converted into the azide as described previously (ref. 4 under "Carbobenzoxy Tripeptide Esters") and added to an ethyl acetate solution of H.Ala-Ala.OBz (2L), prepared from 0.015 mole of the hydroiodide (ref. 5, Cmpd. 1). A white gelatinous precipitate forms almost immediately. The mixture is allowed to stand overnight at room temperature, the carbobenzoxytetrapeptide benzyl ester collected and recrystallized from absolute ethanol. The yield of pure product is 45%; m.p. 198° (all m.p. cor.).

Anal. Calcd. for $C_{38}H_{47}O_9N_5$ (717.8): N, 9.8. Found: N, 9.8.

(b) From a carbobenzoxy tripeptide azide and L-alanine benzyl ester: 0.006 mole of Z.Ala-Z.Lys-Ala.$NHNH_2$ (3L) (ref. 4, Cmpd. 10) is dissolved in 20 cc. of glacial acetic acid, 2 cc. of 5 *N* HCl and 120 cc. of water. The solution is cooled to −4°, and 0.006 mole of sodium nitrite dissolved in a small amount of water is added in one portion. The precipitated azide is extracted with ice-cold ethyl acetate, washed with ice-cold water and bicarbonate solution. If precipitation occurs in the ethyl acetate layer at this point, a small amount (10 cc.) of glacial acetic acid is added to it after removal of the aqueous bicarbonate layer. The clear ethyl acetate solution is then washed with ice-cold water, dried over sodium sulfate and added to L-alanine benzyl ester in ethyl acetate, prepared from 0.009 mole of the hydrochloride (ref. 2, Cmpd. 5). After standing overnight at room temperature, the carbobenzoxytetrapeptide benzyl ester is collected and recrystallized from absolute ethanol. Yield of pure product is 55%; m.p. 197°.

Anal. Calcd. for $C_{38}H_{47}O_9N_5$ (717.8): N, 9.8. Found: N, 9.8.

(2) **Z.Ala-Z.Lys-Ala-Ala.OBz (LDLL).**—The preparation of this compound is the same as that of Compound 1 (a), except that the isomeric (L-D) carbobenzoxy dipeptide hydrazide (ref. 3, Cmpd. 15) is used as one of the starting materials. The product is recrystallized from ethyl acetate. Yield of pure product is 45%; m.p. 166–167°.

TABLE I

TETRA- AND PENTAPEPTIDES CONTAINING ALANINE AND LYSINE ANALYTICAL DATA AND SPECIFIC ROTATION IN 0.5 *N* HCl BASIS: FREE PEPTIDE

No.	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)
5	H.Ala-Lys-Ala-Ala.OH·2HCl (4L)	$C_{15}H_{29}O_5N_5 \cdot 2HCl$	432.4	16.2	16.0	6.5	6.5	16.9	16.6	144	147	− 78.0
6	H.Ala-Lys-Ala-Ala.OH·HCl (LDLL)	$C_{15}H_{29}O_5N_5 \cdot HCl$	395.9	17.7	17.5	7.1	7.1	9.2	9.1	198	190	− 18.6
7	H.Ala-Lys-(Ala)$_2$-Ala.OH·HCl.H_2O (5L)	$C_{18}H_{34}O_6N_6 \cdot HCl \cdot H_2O$	485.0	17.3	17.1	5.8	5.8	7.5	7.3	243	244	−109.3[c]
8	H.Ala-Lys-(Ala)$_2$-Ala.OH·HCl (LDLLL)	$C_{18}H_{34}O_6N_6 \cdot HCl$	467.0	18.0	18.0	6.0	5.9	7.8	7.7	234	232	− 62.0[d]

[a] The following abbreviations are used (*cf.* refs. 2, 3 and 4, Table I, footnote *a*): Z, carbobenzoxy, $C_6H_5 \cdot CH_2OCO$; Ala, $NH(CHCH_3)CO$; Lys, $NH(CHC_4H_8NH_2)CO$; peptide linkage indicated by hyphen; Bz, $C_6H_5CH_2$; configuration follows compound in parentheses, *e.g.*, carbobenzoxy-L-alanyl-ε-carbobenzoxy-D-lysine hydrazide: Z.Ala-Z.Lys.$NHNH_2$ (L-D); carbobenzoxy-L-alanyl-ε-carbobenzoxy-L-lysyl-L-alanyl-L-alanine benzyl ester: Z.Ala-Z.Lys-Ala-Ala. OBz (4L); L-alanyl-D-lysyl-L-alanyl-L-alanyl-L-alanine monohydrochloride: H.Ala-Lys-(Ala)$_2$-Ala.OH.HCl (LDLLL). [b] Obtained by titration in alcohol (Ellenbogen and Brand, Am. Chem. Soc., Philadelphia Meeting, April, 1950, Abstracts, p. 56C). [c] At 23°. [d] At 27°.

Anal. Calcd. for $C_{38}H_{47}O_9N_5$ (717.8): N, 9.8. Found: N, 9.6.

Carbobenzoxypentapeptide Benzyl Esters

(3) **Z.Ala-Z.Lys-Ala-Ala.OBz (5L).**—The method of preparation of this compound is the same as that of Compound 1 (b). The carbobenzoxy tripeptide azide is coupled with the dipeptide benzyl ester, H.Ala-Ala.OBz (2L), prepared from a 50% molar excess of the hydroiodide (ref. 5, Cmpd. 1). Precipitation starts about 15 minutes after adding the azide solution to that of the dipeptide benzyl ester. After standing overnight at room temperature, the material is collected and recrystallized from glacial acetic acid–water. Yield of pure product is about 65%; m.p. 238–239°.

Anal. Calcd. for $C_{41}H_{52}O_{10}N_6$ (788.9): N, 10.7. Found: N, 10.5.

(1) Presented in part before the Division of Biological Chemistry at the 75th Anniversary Meeting of the A. C. S., New York, N. Y., September, 1951.

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

(3) B. F. Erlanger and E. Brand, *ibid.*, **73**, 4025 (1951).

(4) E. Brand B. F. Erlanger, J. Polatnick, H. Sachs and D. Kirschenbaum, *ibid.*, **73**, 4026 (1951).

(5) E. Brand, B. F. Erlanger and H. Sachs, *ibid.*, **74**, 1849 (1952).

(6) E. Brand and B. F. Erlanger, *ibid.*, **72**, 3314 (1950).

(7) We are indebted for analytical work to T. Zelmenis (total N) and to D. Kirschenbaum (amino N, HCl and neut. equiv.).

(8) For abbreviations see Table I, Footnote *a*.