[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Optical Rotation of Peptides. VIII. Glutamic Acid Tripeptides[1]

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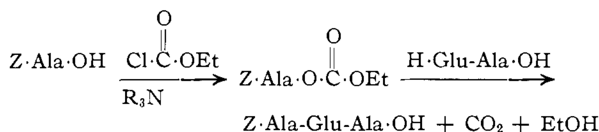
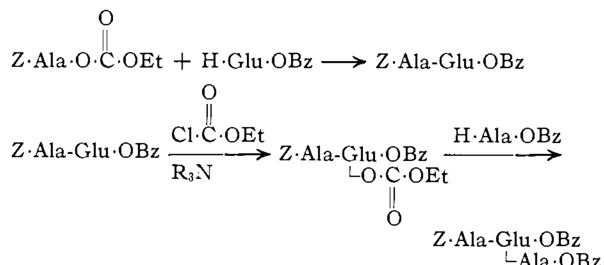
The synthesis and optical rotations of fifteen tripeptides of glutamic acid and alanine are reported. Evidence for the homogeneity and optical purity of these compounds is presented.

Previous papers in this series[3] dealt with the synthesis and specific rotations of a number of alanine, lysine and glutamic acid peptides. In this paper, the synthesis and specific rotation [in 0.5 *N* HCl] of fifteen isomeric tripeptides containing glutamic acid [symbol: H·Glu·OH][4] and alanine [H·Ala·OH][4] are presented. More detailed data on the residue rotations[5] of glutamic acid and alanine residues in these peptides, as well as their rotatory dispersion, will be reported subsequently.

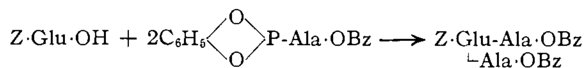
The tripeptides [compounds 21–24 H·Ala-Glu-Ala·OH] were prepared by coupling N-carbobenzyloxy-L- or -D-alanine with the appropriate disodium salt of α-glutamylalanine[3] by the procedure of Boissonnas[6]; the protecting carbobenzyloxy group was then removed with palladium and hydrogen.



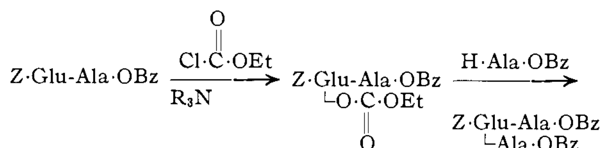
Compounds 25–29 were synthesized by the reaction of a carbobenzyloxy amino acid, [Z·Ala·OH (L) or (D) for compounds 25–28, Z·Glu·OBz[7] (L) for compound 29] with the sodium or potassium salt of L-α-benzyl glutamate[7] as above; the resulting N-carbobenzyloxy dipeptide benzyl esters containing a free γ-carboxyl group were subsequently coupled with the appropriate amino acid benzyl esters by the same method.[3,6]



Compounds 30 and 31 (H·Glu·Ala·OH └Ala·OH [LL L] and [LD D]) were prepared by treating N-carbobenzyloxy-L-glutamic acid with the *o*-phenylene phosphite amide of L- or D-alanine benzyl ester by the procedure of Anderson, *et al.*[8] The protecting groups were then removed in the usual manner.[3]



Compounds 32–35 were prepared by coupling pure [*cf.* Experimental part] Z·Glu-Ala·OBz (L-L) or (L-D) with the appropriate amino acid benzyl ester by the procedure of Boissonnas,[6] followed by removal of the protecting groups.



All of the carbobenzyloxy tripeptide derivatives were recrystallized (usually from at least two different solvents) to constant rotation, prior to removal of the protecting groups. The optical purity of the free peptides was further confirmed by hydrolysis to a mixture of the component unracemized amino acids, separable by means of an anion exchange resin. The chemical homogeneity of these compounds was demonstrated by means of paper partition chromatography and stepwise degradation by the phenylthiocarbamyl method of Edman.[9] The degradation experiment was designed to show that rearrangement[10] had not occurred during the coupling of Z·Glu-Ala·OBz └O·C(=O)·OEt with an amino acid benzyl ester. This was verified by

(1) From a dissertation to be submitted by Howard Sachs in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Deceased July 11, 1953.

(3) Paper VII, H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953).

(4) The following abbreviations and symbols are used (*cf.* E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946); ref. 3, footnote 3): Z: carbobenzyloxy, $C_6H_5CH_2OCO$; Bz: $C_6H_5CH_2$; Ala: NH·CH(CH_3)·CO, C_3H_5ON; Glu: CH·CH(CH_2CH_2COOH)·CO, $C_5H_7O_3N$; peptide linkage indicated by hyphen; configuration follows compound in parentheses. When the γ-carboxyl group of glutamic acid is substituted, the substituent in the γ-position is indicated below the line: Glu └. *E.g.:* N-carbobenzyloxy-L-alanyl-α-L-glutamyl-D-alanine: Z·Ala-Glu-Ala·OH (L-L-D); L-alanyl-α-D-glutamyl-L-alanine: H·Ala-Glu-Ala·OH (L-D-L); N-carbobenzyloxy-D-alanyl-α-benzyl-γ-L-glutamyl-L-alanine benzyl ester: Z·Ala-Glu·OBz └Ala·OBz (D-L-L); L-alanyl-γ-D-glutamyl-D-alanine: H·Ala-Glu·OH └Ala·OH (L-D-D); γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid: H·Glu·OH └Glu·OH └Glu·OH (L-L-L); D-glutamyl-α-D-alanine-γ-L-alanine: H·Glu-Ala·OH └Ala·OH [D-D L].

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

(6) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(7) H. Sachs and E. Brand, THIS JOURNAL, **75**, 4610 (1953).

(8) G. W. Anderson, J. Blodinger, R. W. Young and A. D. Welcher *ibid.*, **74**, 5304 (1952).

(9) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(10) Somewhat analogous rearrangements have been reported in the glutaric acid series; *cf.* J. Cason, *J. Org. Chem.*, **13**, 227 (1948); S. Ställberg-Stenhagen, THIS JOURNAL, **69**, 2568 (1947).

TABLE I

CARBOBENZYLOXY GLUTAMIC ACID TRIPEPTIDE DERIVATIVES; ANALYTICAL DATA AND SPECIFIC ROTATIONS IN GLACIAL ACETIC ACID[a]

No.	Compound[b]	Molecular formula	Mol. wt.	M.p., °C. (cor.)	Nitrogen, % Calcd.	Nitrogen, % Found	Neut. equiv.[c] Calcd.	Neut. equiv.[c] Found	$[\alpha]^{23}D$ (c, 2)
6	Z·Ala-Glu-Ala·OH (L-L-L)	$C_{19}H_{25}O_8N_3$	423.4	[d]	9.9	9.9	212	214	−31.6°[a]
7	Z·Ala-Glu-Ala·OH (L-L-D)	$C_{19}H_{25}O_8N_3$	423.4	157–159	9.9	10.1	212	215	−29.5[e]
8	Z·Ala-Glu-Ala·OH (D-L-L)	$C_{19}H_{25}O_8N_3$	423.4	210–212	9.9	10.1	212	215	−11.4[f]
9	Z·Ala-Glu-Ala·OH (D-L-D)	$C_{19}H_{25}O_8N_3 \cdot H_2O$[g]	441.4	118–120	9.5	9.3	221	216	− 4.5
10	Z·Ala-Glu·OBz (L-L-L) └Ala·OBz	$C_{33}H_{37}O_8N_3$	603.7	150–151	7.0	7.0			−28.8
11	Z·Ala-Glu·OBz (L-L-D) └Ala·OBz	$C_{33}H_{37}O_8N_3$	603.7	148–150	7.0	7.0			+ 4.0
12	Z·Ala-Glu·OBz (D-L-L) └Ala·OBz	$C_{33}H_{37}O_8N_3$	603.7	156–158	7.0	6.9			− 7.9[e]
13	Z·Ala-Glu·OBz (D-L-D) └Ala·OBz	$C_{33}H_{37}O_5N_3$	603.7	169–171	7.0	7.0			+19.7[e]
14	Z·Glu·OBz (L-L-L) └Glu·OBz └Glu·OBz └OBz	$C_{51}H_{53}O_{12}N_3$	900.0	147–149	4.7	4.7			− 5.3[h]
15	Z·Glu-Ala·OBz └Ala·OBz [L-L / L]	$C_{33}H_{37}O_8N_3$	603.7	200–201	7.0	7.0			−37.8[h]
16	Z·Glu-Ala·OBz └Ala·OBz [L-D / D]	$C_{33}H_{37}O_8N_3$	603.7	169–170	7.0	6.9			+23.3
17	Z·Glu-Ala·OBz └Ala·OBz [L-L / D]	$C_{33}H_{37}O_8N_3$	603.7	186–187	7.0	6.9			+ 3.3
18	Z·Glu-Ala·OBz └Ala·OBz [L-D / L]	$C_{33}H_{37}O_8N_3$	603.7	189–190	7.0	6.9			− 5.9
19	Z·Glu-Ala·OBz (L-L) └Gly-OBz	$C_{32}H_{34}O_8N_3$	588.7	182–183	7.1	7.1			−15.7
20	Z·Glu-Ala·OBz (L-D) └Gly·OBz	$C_{32}H_{34}O_8N_3$	588.7	182–183	7.1	7.0			+11.6

[a] Compound 6 in glacial acetic acid containing 0.3 ml. of H_2O per 3 ml. of solution. [b] For an explanation of symbols see ref. 4. [c] *Cf.* ref. 14. [d] Sinters at 110°, melts 130–140° with decomposition upon continued heating. [e] At 24°. [f] At 22°. [g] Calcd. for $C_{19}H_{25}O_8N_3 \cdot H_2O$ (441.4): C, 51.7; H, 6.1. Found: C, 54.0; H, 6.1. [h] At 28°.

TABLE II

TRIPEPTIDES OF GLUTAMIC ACID AND ALANINE; ANALYTICAL DATA, R_f VALUES AND SPECIFIC ROTATIONS IN 0.5 *N* HCl

No.	Compound[a]	Molecular formula	Mol. wt.	Nitrogen, % Calcd.	Nitrogen, % Found	Amino N, %[b] Calcd.	Amino N, %[b] Found	R_f[c]	R_f[d]	$[\alpha]^{24}D$ (c, 2)
21	H·Ala-Glu-Ala·OH (L-L-L)	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.5	4.8	4.7	0.34	0.24	−41.7°
22	H·Ala-Glu-Ala·OH (L-L-D)	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.6	4.8	4.6	.38	.27	+ 6.9
23	H·Ala-Glu-Ala·OH (D-L-L)	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.7	4.8	4.8	.37	.28	−63.8
24	H·Ala-Glu-Ala·OH (D-L-D)	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.3	4.8	4.8	.39	.29	−13.3
25	H·Ala-Glu-OH (L-L-L) └Ala·OH	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.8	4.8	4.7	.38	.21	−29.9[e]
26	H·Ala-Glu·OH (L-L-D) └Ala·OH	$C_{11}H_{19}O_6N$	289.3	14.5	14.4	4.8	4.6	.37	.22	+26.2[e]
27	H·Ala-Glu·OH (D-L-L) └Ala·OH	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.5	4.8	4.8		.19	−47.5
28	H·Ala-Glu·OH (D-L-D) └Ala·OH	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.2	4.8	4.7	.35	.22	+ 7.6[f]
29	H·Glu·OH[g] (L-L-L) └Glu·OH └Glu·OH	$C_{15}H_{23}O_{10}N_3$	405.4	10.4	10.4	3.5	8.9	.06	.09	− 7.2
30	H·Glu·Ala·OH (L-L-L) └Ala·OH	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.5	4.8	5.7	.41	.29	−15.4
31	H·Glu-Ala·OH └Ala·OH [L-D / D]	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.8	4.8	5.9	.38	.25	+92.2[e]
32	H·Glu-Ala·OH └Ala·OH [L-L / D]	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.3	4.8	5.9	.40	.33	+32.8[h]
33	H·Glu-Ala·OH └Ala·OH [L-D / L]	$C_{11}H_{19}O_6N_3$	289.3	14.5	14.3	4.8	6.1	.37	.28	+34.5[e]
34	H·Glu-Ala·OH (L-L) └Glu·OH	$C_{10}H_{16}O_6N_3$	274.3	15.3	15.2	5.1	5.7	.29	.17	+ 7.9[f]
35	H·Glu-Ala·OH (L-D) └Gly·OH	$C_{10}H_{16}O_6N_3$	274.3	15.3	15.2	5.1	7.2	.24	.17	+61.2[f]

[a] For an explanation of the symbols see ref. 4. [b] Reaction time with nitrous acid, 3 minutes; compounds 29–35, the γ-peptide N as well as the α-amino N have partially reacted. [c] After 24 hours, phenol–H_2O (ref. 17). [d] After 24 hours, butanol–acetic acid–H_2O (ref. 18). [e] At 25°. [f] At 26°. [g] Carboxyl nitrogen content (ninhydrin, 100°, 7 minutes, *p*H 4.7). Calcd. for $C_{15}H_{23}O_{10}N_3$ (405.4): carboxyl N, 3.5. Found: carboxyl N, 3.4. [h] At 23°.

conversion of H·Glu-Ala·OH (L-L) to the phenyl-
└Gly·OH
thiocarbamyl derivative, PTC·Glu-Ala·OH (L-L)
└Gly·OH
Subsequent cleavage of this compound yielded the corresponding thiohydantoin γ-peptide and alanine.

Experimental[11]

The synthesis and properties of some of the starting materials have been previously described: L- and D-alanine,[12]

(11) We are indebted for analytical work to T. Zelmenis (total and amino N).

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

H·Ala·OBz (L) and (D), and H·Gly·OBz (ref. 12, compounds 4–6), L- and D-glutamic acid, H·Glu·OBz (L), H·Glu·OBz (L) and (D) and Z·Glu·OBz (L) (ref. 7, compounds 1, 3, 4 and 5), Z·Glu·Ala·OBz (L-L) and (L-D) (ref. 3, compounds 4 and 5). Other starting materials used were: Z·Glu·OH (L),[13] Z·Glu (L)[13] and Z·Ala·OH (L) and (D).[13]

(1) **Z·Ala-Glu·OBz** (L-L).—This compound was prepared by coupling Z·Ala·OH (L) with the sodium salt of H·Glu·OBz (L)[7] by the mixed anhydride procedure.[3,6] The sodium salt was prepared by dissolving 3.6 g. (0.015 mole) of H·Glu·OBz (L) in 40 ml. of hot 40% dioxane, cooling rapidly to about 20° and then adding 1.6 g. (0.015 mole) of Na_2CO_3 with continued cooling to 0°. This was then added to 25 ml. of a cooled (5–10°) dioxane solution containing the mixed anhydride prepared from 2.9 g. (0.013 mole) of Z·Ala·OH (L), tri-*n*-butylamine (3.2 ml., 0.013 mole) and ethyl chlorocarbonate (1.2 ml., 0.013 mole). To the turbid reaction mixture was added 10–20 ml. of H_2O, whereupon a homogeneous solution was obtained, which was kept in the ice-box overnight. Most of the solvent was then removed *in vacuo* and the remaining solution (about 5 ml.) was taken up in 10 ml. of H_2O. The turbid aqueous solution was extracted clear with 20 ml. of ether, acidified with 6 *N* HCl and the resulting oil taken up in 40 ml. of ethyl acetate. The aqueous layer was extracted with two additional portions of 40 ml. of ethyl acetate; the combined ethyl acetate extracts were washed with 25 ml. of cold H_2O and dried over Na_2SO_4. After removal of the solvent *in vacuo*, a crystalline product was obtained by triturating the residue with ether. It was recrystallized from ethanol–H_2O and *n*-propyl alcohol–H_2O. The yield of pure compound was 2.3–2.9 g. (40–50%), m.p. 147–149°, $[\alpha]^{24}D$ −21.2° (1.6% in glacial acetic acid).

Anal. Calcd. for $C_{23}H_{26}O_7N_2$ (442.5): N, 6.3; neut. equiv., 443. Found: N, 6.1; neut. equiv.,[14] 449.

(2) **Z·Ala-Glu·OBz** (D-L).—This compound was obtained by the same procedure and in similar yield from Z·Ala·OH (D) and H·Glu·OBz (L) as described for the (L-L) isomer; m.p. 132–135°, $[\alpha]^{24}D$ +4.8° (1.7% in glacial acetic acid).

Anal. Calcd. for $C_{23}H_{26}O_7N_2$ (442.5): N, 6.3; neut. equiv., 443. Found: N, 6.4; neut. equiv.,[14] 446.

(3) **Z·Glu·OBz** (L-L).—This was prepared in a manner **Glu·OBz** analogous to the one described for compounds 1 and 2. The mixed anhydride of Z·Glu·OBz (L) and ethylcarbonic acid was prepared as previously described[3] from 4.8 g. (0.013 mole) of Z·Glu·OBz (L), 3.2 ml. (0.013 mole) of tri-*n*-butylamine and 1.2 ml. (0.013 mole) of ethyl chlorocarbonate, all in 25 ml. of dioxane (5–10°). H·Glu·OBz (L) (4.5 g., 0.019 mole) was suspended in 30 ml. of cold (0°) H_2O containing 2.64 g. (0.019 mole) of K_2CO_3, and the mixture stirred vigorously until most of the ester had dissolved. This was then added to the above dioxane solution of mixed anhydride plus an additional 15–20 ml. of dioxane and 10 ml. of H_2O to give a clear supernatant and some undissolved L-α-benzyl glutamate. Stirring was continued for one hour at 0° and the reaction mixture then stored in the ice-box overnight. The product was isolated as described above and recrystallized from ethanol–H_2O. The yield of pure compound was 2.3–3.1 g. (30–40%), m.p. 148–151°, $[\alpha]^{24}D$ −6.5° (1.4% in glacial acetic acid).

Anal. Calcd. for $C_{32}H_{34}O_9N_2$ (590.6): N, 4.7; neut. equiv., 591. Found: N, 4.8; neut. equiv.,[14] 594.

(4) **Z·Glu-Ala·OBz** (L-L).—This was prepared by treating N-carbobenzyloxy-L-glutamic anhydride[13] with H·Ala·OBz (L) as described by LeQuesne and Young.[15] It was recrystallized from ethanol–H_2O; yield 35–45%, m.p. 153–154°, $[\alpha]^{25}D$ −25.8° (1.1% in glacial acetic acid).

Anal. Calcd. for $C_{23}H_{26}O_7N_2$ (442.5): N, 6.3; neut. equiv., 442. Found: N, 6.3; neut. equiv.,[14] 437.

(5) **Z Glu-Ala·OBz** (L-D).—This compound was obtained in the same manner and in similar yield as described for the (L-L)-isomer, m.p. 148–151°, $[\alpha]^{23}D$ +8.8° (1.25% in glacial acetic acid).

Anal. Calcd. for $C_{23}H_{26}O_7N_2$ (442.5): N, 6.3; neut. equiv., 442. Found: N, 6.4; neut. equiv., 439.

The homogeneity of compound 5 was demonstrated by catalytic reduction and analysis of the free peptide in solution without isolation or further purification.

Compound 5, 0.24 g. (0.54 mmole), was hydrogenated in 90% acetic acid and after removal of all of the solvent *in vacuo*, the residue was taken up in 0.5 *N* HCl and brought to a volume of 10 cc.; aliquots were then analyzed for total, amino and carboxyl nitrogen and optical rotation; from these analytical figures the concentration of peptide was calculated and the specific rotation determined.

Anal. Calcd. for $C_8H_{14}O_5N_2$ (218.2): mg. N/cc., 1.5; mg. amino N/cc., 0.75; mg. carboxyl N/cc., 0. Found: mg. N/cc., 1.4; mg. amino N/cc., 0.73; mg. carboxyl N/cc., 0; $[\alpha]^{23}D$ +81.2° (1.2% in 0.5 *N* HCl).

The authentic α-peptide had $[\alpha]^{24}D$ +79.7° (1.2% in 0.5 *N* HCl). Paper chromatography (butanol–acetic acid–H_2O) of an aliquot of the above solution gave a single spot with R_{Glu} corresponding to authentic H·Glu-Ala·OH (L-D).[3]

Carbobenzyloxy Tripeptides (Compounds 6–9).—Z·Glu-Ala·OBz[3] (L-L) or (L-D) (7.1 g., 0.013 mole) was dissolved in 150 ml. of 95% methanol and hydrogenated in the usual way[3]; about 20–25 ml. of H_2O was added in small portions during the first three hours. After the reduction was complete (about six hours), the catalyst was filtered off and the solution taken down *in vacuo* to a sirup which was twice treated with 25 ml. of H_2O which each time was distilled off *in vacuo*. The final sirup was then taken up in 13 ml. of cold (0°) 2 *N* NaOH. This cooled solution of dipeptide disodium salt was added in one portion to 25 ml. of a cooled (5–10°) dioxane solution containing the mixed anhydride of ethylcarbonic acid and Z·Ala·OH (L) or (D) (prepared as previously described[3] from Z·Ala·OH (3.0 g., 0.013 mole), tri-*n*-butylamine (3.2 ml., 0.013 mole), and ethyl chlorocarbonate (1.2 ml., 0.013 mole)). More dioxane or H_2O may be added accordingly in order to give a homogeneous mixture. This was kept in the ice-box overnight, and the products isolated as described above (compound 1). These were recrystallized from acetone–petroleum ether, *n*-propyl alcohol–petroleum ether or ethyl acetate–ether. The yield of pure compounds varied from 2.8–3.3 g. (50–60%).

Carbobenzyloxy Tripeptide Benzyl Esters (Compounds 10–14).—These compounds were obtained by coupling compound 1, 2 or 3 with the appropriate amino acid benzyl ester by the mixed anhydride procedure as described for carbobenzyloxy dipeptide benzyl esters.[3] They were recrystallized from ethanol–H_2O, methanol–H_2O or ethyl acetate. The yield of pure compounds was 60–80% based on the carbobenzyloxy dipeptide used.

Carbobenzyloxy Tripeptide Benzyl Esters (Compounds 15, 16).—H·Ala·OBz·HCl (L) or (D) (2.2 g., 0.01 mole) was suspended in 20 ml. of dry ether, cooled (0°), and 2.8 ml. (0.02 mole) of triethylamine added. *o*-Phenylene chlorophosphite,[16] 1.8 g. (0.01 mole) in 20 ml. of ether, was added rapidly with cooling and the reaction mixture was then agitated with a magnetic stirrer for 30 minutes at room temperature. The triethylamine hydrochloride was filtered off and the ether removed *in vacuo*. To the colorless sirup was added 0.84 g. (0.003 mole) of N-carbobenzyloxy-L-glutamic acid partially dissolved in 25 ml. of dry toluene. The mixture was brought to boiling; at this point complete solution occurred and refluxing was continued for one hour. Then, about one-half of the solvent was removed *in vacuo* leaving behind a gelatinous product, which was filtered off and washed with cold ethyl acetate. This was recrystallized from methanol or *n*-propyl alcohol; yield of pure compounds was 0.9–1.0 g. (50–55% based on the amount of N-carbobenzyloxy-L-glutamic acid used). Compound 15 was also prepared by treating Z·Glu-Ala·OBz (L-L) (mixed carbonic acid anhydride) with H·Ala·OBz (L) as described below for compounds 17–20.

Carbobenzyloxy Tripeptide Benzyl Esters (Compounds 17–20).—These compounds were obtained by coupling compounds 4 or 5 with the appropriate amino acid benzyl ester by the mixed anhydride procedure[6] as described for carbobenzyloxy dipeptide benzyl esters.[3] They were recrystallized from ethanol, methanol or *n*-propyl alcohol. The yield of pure compounds was 60–80% based on the carbobenzyloxy dipeptide used.

(13) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(14) Obtained by titration in alcohol, *cf.* E. Brand, B. F. Erlanger and H. Sachs, THIS JOURNAL, **74**, 1851 (1952).

(15) W. J. LeQuesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950).

(16) We are indebted to Dr. Young of the American Cyanamid Company for a gift of this reagent.

TABLE III

SPECIFIC ROTATIONS OF PEPTIDE HYDROLYSATES

No.	Compound hydrolyzed	$[\alpha]^{25}_D$ of hydrolysate Calcd.[a]	Found	Amino acids separated by anion exchanger	$[\alpha]^{25}_D$ of amino acids Calcd.	Found
	H·Glu·OH (L) control		+29.7°[b]			
	H·Ala·OH (D) control		−13.1[c]			
32	H·Glu-Ala·OH [L-L] └Ala·OH [D]	+29.7°	+28.9[d]	H·Ala·OH (D-L) H·Glu·OH (L)	0.0°	0.0°
29	H·Glu·OH (L-L-L) └Glu·OH └Glu·OH	+29.7	+29.6[f]			
31	H·Glu-Ala·OH [L-D] └Ala·OH [D]			H·Ala·OH (D) H·Glu·OH (L)	−13.1 +29.7	−13.6[g] +31.1[h]

[a] Calculated on the basis of the values obtained for the L-glutamic acid and D-alanine controls. [b] 1.4% in 6 N HCl. [c] 3.9% in 2 N HCl. [d] 1.8% in 2 N HCl. [e] 2.0% in N HCl. [f] 3.5% in 6 N HCl. [g] 1.9% in N HCl. [h] 0.8% in 6 N HCl.

Tripeptides (Compounds 21–35).—The carbobenzyloxy tripeptides (compounds 6–9) were hydrogenated in the usual way[3] using 70–80% methanol as solvent (a volume of 50 ml. per 0.005 mole of compound, except for compound 6 which required 150 ml. of 50% methanol per 0.006 mole).

The carbobenzyloxy tripeptide benzyl esters (compounds 10, 11) were hydrogenated in 95% methanol (75 ml. per 0.005 mole); for compounds 12–20, 95% acetic acid was used (100 ml. per 0.005 mole of compounds). During the first two hours 15 ml. of H_2O was added; approximately six hours were required for complete reduction. The peptides were recrystallized from H_2O–ethanol (compounds 23–25, 27, 34 and 35) or H_2O–ethanol–ether (compounds 21, 22, 26, 28–33). The yield of pure peptides varied from 1.0–1.2 g. (70–85%).

Chromatography of Peptides.—Ascending, one dimensional, paper partition chromatography was employed using Whatman No. 1 paper and two systems, (a) phenol–H_2O, containing 8-hydroxyquinoline,[17] and (b) butanol–acetic acid–H_2O (50:10:40).[18] All peptides traveled as single spots in both systems. R_f values are given in Table II.

Hydrolysis of Peptides.—H·Glu-Ala·OH [L-D / D] └Ala·OH (compound 31, 0.1065 g., (0.37 mmole)), H·Glu-Ala·OH [L-L / D] └Ala·OH (compound 32, 0.1039 g. (0.36 mmole)), and H·Glu·OH └Glu·OH └Glu·OH (L-L-L) (compound 29, 0.16 mmole)) were each dissolved in 1.5 ml. of 6 N HCl and the solution was refluxed for 20 hours. Controls of L-glutamic acid and D-alanine were treated in a similar manner. The last four solutions were then brought to a known volume and the specific rotation determined (*cf.* Table III). The hydrolysates of compounds 31 and 32 were diluted to 100 cc. with H_2O and then passed through 1R-4B anion exchange columns. The glutamic acid and HCl were picked up by the column while the alanine passed through. The L-glutamic acid was subsequently eluted with dilute HCl. The effluents were taken to dryness *in vacuo*, and the residues were then taken up in a known volume of standard acid (see below) and aliquots taken for carboxyl nitrogen analysis, optical rotation and paper chromatography. Ascending, one-dimensional, paper partition chromatography using Whatman No. 1 paper and phenol–H_2O containing 8-hydroxyquinoline[18] gave single spots for each fraction, corresponding to either glutamic acid or alanine. The concentration of amino acid in each effluent fraction was calculated from the carboxyl nitrogen values. The recoveries of the amino acids varied from 90–95%. Their specific rotations are presented in Table III.

Stepwise Degradation.—H·Glu-Ala·OH └Gly·OH (L-L) (compound 34, 0.041 g. (0.15 mmole)) in 3 ml. of pyridine–H_2O (1:1) was treated with 0.1 g. (0.74 mmole) of phenyl isothiocyanate according to Edman.[9] The reaction mixture was then washed four times with benzene, cooled and brought to pH 3 with N HCl. The precipitate was taken up in ethyl acetate and this was washed with H_2O, dried over Na_2SO_4 and then taken to dryness *in vacuo*. Two ml. of purified nitromethane saturated with HCl was added and after 15–30 minutes at 40° the crystalline material was centrifuged, washed with ether and taken up in 0.5 ml. of H_2O. Paper chromatography of an aliquot of this solution (phenol–H_2O, 8-hydroxyquinoline)[17] gave a single spot with R_f 0.52, corresponding to authentic L-alanine hydrochloride (R_f 0.38 for glycine hydrochloride run simultaneously). Alkaline hydrolysis[9] of the thiohydantoin fraction and paper chromatography (butanol–acetic acid–H_2O) of the hydrolysate gave two spots corresponding to glutamic acid and glycine.

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