Acknowledgment.—The authors wish to thank Mr. Thomas Parr, III, and Pfc. Vyto Adomaitis for their aid in carrying out some of the syntheses. Analyses were performed by various members of the Analytical Branch of the Chemical Corps Chemical and Radiological Laboratories, Army Chemical Center, Maryland.

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Optical Rotation of Peptides. VII. α- and γ-Dipeptides of Glutamic Acid and Alanine[1]

BY HOWARD SACHS AND ERWIN BRAND

RECEIVED MAY 15, 1953

Previous papers in this series[2] dealt with the synthesis and specific rotations of a number of alanine and lysine peptides. In this paper, the synthesis and specific rotations (in 0.5 *N* HCl) of eleven isomeric dipeptides containing glutamic acid (symbol: H·Glu·OH)[3] and alanine (H·Ala·OH)[3] are presented. Detailed data on the residue rotations[4] of glutamic acid and alanine residues in these peptides will be reported subsequently.

The dipeptides were prepared by coupling the appropriate N-carbobenzyloxyamino acid with amino acid benzyl esters according to the method of Boissonnas[5]; the resulting N-carbobenzyloxy dipeptide benzyl esters were reduced to the free peptides with palladium and hydrogen. Alanine peptides (H·Ala-Glu·OH) were obtained by coupling Z·Ala·OH with glutamic acid dibenzyl ester[6] (H·Glu·OBz). Glutamic acid α-peptides were
└OBz
synthesized from the carbobenzyloxy γ-benzyl ester[7] (Z·Glu·OH) and the γ-peptides from the
└OBz
carbobenzyloxy α-benzyl ester[6] (Z·Glu·OBz).

In the Van Slyke carboxyl nitrogen determination (ninhydrin),[8] γ-dipeptides of glutamic acid should yield 1 mole of COOH nitrogen (COOH, N), while the α-peptides should yield none. That this is the case for these two types of glutamic acid dipeptides, synthesized by the methods outlined above, can be seen from Table II (compounds 15–22).

In the Van Slyke amino nitrogen determination (nitrous acid), the α-peptides give correct analytical values (1 mole of amino N, *cf.* Table II, compounds 12–18). With the γ-peptides, both amino and peptide nitrogens react (2 moles of amino N, Table II, compounds 19–22). This observation constitutes an important distinction between α- and γ-peptides of glutamic acid. The underlying mechanism will be discussed elsewhere in connection with additional data.[9]

The purity of these peptides was further confirmed by chromatography; α- and γ-isomers are readily separable by this method (*cf.* Table II, column R_{Glu}).

It has been established[10] that the synthesis of dipeptides of glutamic acid *via* its carbobenzyloxy anhydride (Z·Glu·O),[11] yields mixtures of α- and γ-
└─┘
peptides. It was thought that the synthesis of pure γ-peptides could be accomplished *via* the γ-azide of carbobenzyloxyglutamic acid, (Z·Glu·OH)[10,12–14].
└N₃
However, we have found[15] that this procedure is not unequivocal, but leads to mixtures of α- and γ-peptides; from these mixtures, pure α- and γ-peptides can sometimes be obtained by fractional crystallization.[15]

In view of the difficulties encountered in the preparation of γ-peptides, it is essential that, in every case, homogeneity be established by all of the analytical procedures described above.

Experimental[16]

Starting Materials.—The syntheses and properties of some of the starting materials have been previously described: L- and D-alanine,[17] H·Ala·OBz (L) and (D) (ref. 17, compounds 5, 6), L- and D-glutamic acid,[6] H·Glu·OBz (L) and
└OBz
(D), and Z·Glu·OBz (L) (ref. 6, compounds 1, 2 and 5). Other starting materials used were: Z·Ala·OH (L) and (D),[11] Z·Glu·OH (L)[7] and H·Glu·OH (L)[7] (carboxyl nitrogen[8]
└OBz └OBz
content (ninhydrin, 100°, 7 min., *p*H 2.5): Calcd. for $C_{12}H_{15}O_4N$ (237.2): carboxyl N, 5.9. Found: carboxyl N, 5.9).

Carbobenzyloxy Dipeptide Benzyl Esters (Compounds 1–11).—The free COOH group of the carbobenzyloxyamino acids (Z·Ala·OH, Z·Glu·OH, Z·Glu·OBz) is converted into
└OBz
a tertiary amine salt. The tertiary amine salts, in turn, are converted with ethyl chlorocarbonate into the mixed an-

(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. Erwin Brand deceased July, 1953.

(2) Paper VI, E. Brand, B. F. Erlanger and H. Sachs, THIS JOURNAL, **74**, 1851 (1952).

(3) The following abbreviations and symbols are used (*cf.* E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946); ref. 2, Table I, footnote *a*; ref. 6. footnote 2): Z, carbobenzyloxy, $C_6H_5CH_2OCO$; Bz, $C_6H_5CH_2$; Ala, $NHCH(CH_3)CO$, C_3H_5ON; Glu, $NHCH(CH_2CH_2COOH)$-CO, $C_5H_7O_3N$; peptide linkage indicated by dash, –; configuration follows compound in parentheses, ( ). When the γ-carboxyl group of glutamic acid is substituted, the following symbol is used for the residue: Glu. *e.g.*, N-carbobenzyloxy-L-alanine benzyl ester,
└OH
Z·Ala·OBz (L); N-carbobenzyloxy-D-glutamic acid γ-benzyl ester, Z·Glu·OH (D); N-carbobenzyloxy-L-alanyl-D-glutamic acid dibenzyl
└OBz
ester, Z·Ala-Glu·OBz (LD); N-carbobenzyloxy-α-benzyl γ-L-glutamyl-
└OBz
D-alanine benzyl ester, Z·Glu·OBz (LD); γ-L-glutamyl-D-glutamic acid,
└Ala·OBz
H·Glu·OH (LD); α-L-glutamyl-L-alanine, H·Glu-Ala·OH (2L)
└Glu·OH

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(9) H. Sachs and E. Brand, unpublished work.

(10) *Cf.* discussion by W. J. LeQuesne and G. T. Young, *J. Chem. Soc.*, 1954 (1950).

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

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(15) H. Sachs and E. Brand, Am. Chem. Soc., Los Angeles Meeting, March, 1953, Abstracts p. 30c; H. Sachs and E. Brand, *Federation Proc.*, **12**, 262 (1953).

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

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

TABLE I

CARBOBENZYLOXY DIPEPTIDE BENZYL ESTERS; ANALYTICAL DATA AND SPECIFIC ROTATIONS IN GLACIAL ACETIC ACID

No.	Compound[a]	Molecular formula	Mol. wt.	M.p., °C. (cork)	Nitrogen, % Calcd.	Found	$[\alpha]^{23}D$ c, 2
			α-Dipeptide derivatives				
1	Z·Ala-Glu·OBz (LL) └OBz	$C_{30}H_{32}O_7N_2$	532.6	104–105	5.3	5.2	−16.6°
2	Z·Ala-Glu·OBz (LD) └OBz	$C_{30}H_{32}O_7N_2$	532.6	112–113	5.3	5.2	− 3.7
3	Z·Ala-Glu·OBz (DL) └OBz	$C_{30}H_{32}O_7N_2$	532.6	112–113	5.3	5.3	+ 3.8
4	Z·Glu-Ala·OBz (LL) └OBz	$C_{30}H_{32}O_7N_2$	532.6	102–104	5.3	5.3	−21.2
5	Z·Glu-Ala·OBz (LD) └OBz	$C_{30}H_{32}O_7N_2$	532.6	120–121	5.3	5.3	+ 2.6
6	Z·Glu-Glu·OBz (LL) └OBz └OBz	$C_{39}H_{40}O_9N_2$	680.7	104–105	4.1	4.1	−10.4
7	Z·Glu-Glu·OBz (LD) └OBz └OBz	$C_{39}H_{40}O_9N_2$	680.7	91–92	4.1	4.2	− 0.5
			γ-Dipeptide derivatives				
8	Z·Glu·OBz (LL) └Ala·OBz	$C_{30}H_{32}O_7N_2$	532.6	124–126	5.3	5.2	−16.2[c]
9	Z·Glu·OBz (LD) └Ala·OBz	$C_{30}H_{32}O_7N_2$	532.6	124–125	5.3	5.2	+14.7[b]
10	Z·Glu·OBz (LL) └Glu·OBz └OBz	$C_{39}H_{40}O_9N_2$	680.7	140.5–142	4.1	4.0	− 5.2
11	Z·Glu·OBz (LD) └Glu·OBz └OBz	$C_{39}H_{40}O_9N_2$	680.7	129.5–131	4.1	4.2	+ 3.3

[a] For an explanation of the symbols, *cf.* ref. 3. [b] At 25°. [c] At 26°.

TABLE II

DIPEPTIDES OF GLUTAMIC ACID AND ALANINE; ANALYTICAL DATA,[a] *R* VALUES AND SPECIFIC ROTATIONS IN 0.5 *N* HCl

No.	Compound[b]	Molecular formula	Mol. wt.	Nitrogen, % Calcd.	Found	Amino N,[c] % Calcd.	Found	Carboxyl N,[d] % Calcd.	Found	R_f[e]	R_{Glu}[f]	$[\alpha]^{24}D$ c, 1–2
						α-Peptides						
12	H·Ala-Glu·OH (LL)	$C_8H_{14}O_5N_2 \cdot H_2O$	236.2	11.8	11.8	5.9	5.9	0.0	0.0	0.3	1.1	− 9.3
13	H·Ala-Glu·OH (LD)	$C_8H_{14}O_5N_2$	218.2	12.8	12.9	6.4	6.4	.0	.0	.3	1.2	+32.4[g]
14	H·Ala-Glu·OH (DL)	$C_8H_{14}O_5N_2$	218.2	12.8	12.6	6.4	6.5	.0	.0	.3	1.1	−32.9[g]
15	H·Glu-Ala·OH (LL)	$C_8H_{14}O_5N_2$	218.2	12.8	12.7	6.4	6.6	.0	.0	.4	1.7	+ 7.3[h]
16	H·Glu-Ala·OH (LD)	$C_8H_{14}O_5N_2$	218.2	12.8	12.9	6.4	6.4	.0	.0	.4		+79.7
17	H·Glu-Glu·OH (LL)[i]	$C_{10}H_{16}O_7N_2$	276.2	10.1	10.0	5.1	5.3	.0	.0	.1	1.2	+18.2
18	H·Glu-Glu·OH (LD) └OH └OH	$C_{10}H_{16}O_7N_2$	276.2	10.1	10.2	5.1	5.0	.0	.0	.1		+56.4
						γ-Peptides						
19	H·Glu·OH (LL)[j] └Ala·OH	$C_8H_{14}O_5N_2$	218.2	12.8	12.8	6.4	12.3	6.4	6.4	0.4	1.1	−11.5
20	H·Glu·OH (LD) └Ala·OH	$C_8H_{14}O_5N_2$	218.2	12.8	13.0	6.4	12.0	6.3	6.4	.4	1.1	+63.1
21	H·Glu·OH (LL)[k] └Glu·OH	$C_{10}H_{16}O_7N_2$	276.2	10.1	10.1	5.1	10.1	5.1	5.0	.1	0.9	+ 3.8
22	H·Glu·OH (LD) └Glu·OH	$C_{10}H_{16}O_7N_2$	276.2	10.1	10.2	5.1	10.1	5.1	4.9	.1	0.9	+36.7[l]

[a] Compounds 12–14, 19–22 dried at 100° *in vacuo*, compounds 15, 17, 18 at 78°, compound 16 at 56°. [b] For an explanation of the symbols, *cf.* ref. 3. [c] Reaction time with nitrous acid was 3 minutes. [d] Reaction time with ninhydrin was 7 minutes at *p*H 2.5; *cf.* ref. 8. [e] After 40 hours; phenol–H_2O–NH_3 (ref. 18). [f] After 90 hours; butanol–acetic acid–H_2O (ref. 19). [g] At 23°. [h] At 27°. [i] Previously prepared (*cf.* ref. 11) with $[\alpha]^{18}D$ +19.9° (2.0% in H_2O + 1 equiv. HCl); we find $[\alpha]^{23}D$ +20.0° (0.9% in H_2O + 1 equiv. HCl). [j] Previously prepared (*cf.* ref. 13) with $[\alpha]^{18}D$ −22.1° (5.0% in H_2O); probably contained some α-isomer. We find $[\alpha]^{23}D$ −27.0° (5.3% in H_2O). [k] Previously prepared (*cf.* ref. 12) with $[\alpha]^{17}D$ +6.0° (1.1% in H_2O + 1 equiv. HCl); probably contained some α-isomer. [l] At 22°.

hydrides of ethylcarbonic acid. The peptide linkage is then formed by the action of amino acid esters on these anhydrides

$$\text{Z·Ala·ONHR}_3 \longrightarrow \text{Z·Ala·O·CO(OC}_2\text{H}_5) +$$

$$\text{H·Glu·OBz (└OBz)} \longrightarrow \text{Z·Ala-Glu·OBz (└OBz)} + CO_2 + C_2H_5OH$$

Tri-*n*-butylamine (2.4 ml., 0.01 mole) is added to 0.01 mole of carbobenzyloxyamino acid in 20 ml. of dioxane, cooled to 5–10°, and 0.95 ml. (0.01 mole) of ethyl chlorocarbonate added. After standing at this temperature for 30 minutes, 25 ml. of a cooled (10°) dioxane solution, containing 0.013 mole of amino acid benzyl ester hydrochloride and 0.013 mole of tri-*n*-butylamine, is added. The reaction mixture is kept in the ice-box overnight; then 100 ml. of ethyl acetate, followed by 150 ml. of 0.5 *N* HCl, is added. The ethyl acetate layer is washed successively with 0.5 *N* HCl, 5% $NaHCO_3$ and H_2O, and dried over Na_2SO_4. Upon removal of the solvent *in vacuo*, crystalline products are obtained, which are recrystallized first from ethyl acetate–

petroleum ether, and then from methanol–H_2O. The yield of pure compounds varies from 3.2 to 5.5 g. (60–80%, based on the carbobenzyloxyamino acid used).

Dipeptides (Compounds 12–22).—The carbobenzyloxy dipepetide benzyl esters are hydrogenated in the usual way,[2] using 80–90% acetic acid as solvent (a volume of 150 ml. per 0.015 mole of compound). Reduction is complete in approximately six hours. The peptides are recrystallized from H_2O (compounds 13, 14, 20), H_2O–ethanol (compounds 14, 15, 17, 18, 19, 21, 22), or 90% methanol and ether (compounds 12, 16). The yield of the individual pure peptides varies from 2.3 to 3.5 g. (70–85%).

Chromatography of Peptides.—Ascending, one dimensional, paper partition chromatography is employed using Whatman No. 1 paper and two solvent systems, (a) phenol–water–NH_3,[18] and (b) butanol–acetic acid–water (50:10: 40).[19] A wad of filter paper is attached to the top of the paper cylinder.[20] This makes it possible to develop the chromatograms for 44–96 hours.

All peptides traveled as single spots in both systems. The α- and γ-isomers are readily separable in solvent system (b) as indicated by the R_{Glu} values in Table II.

This work was aided by a contract between the Office of Naval Research, Department of the Navy, and Columbia University (NR 124-260).

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Benzyl Esters of Glutamic Acid[1]

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RECEIVED APRIL 28, 1953

Benzyl esters of glutamic acid (symbol, H·Glu·OH)[2] are useful intermediates in peptide synthesis. The preparation and properties of the L- and D-isomers of the α- and of the dibenzyl esters are presented in this paper.

Experimental[3]

The starting materials, L- and D-glutamic acid,[4] had specific rotations $[\alpha]^{25}D$ +31.6° (1.0% in 6 *N* HCl), and $[\alpha]^{28}D$ −31.3° (1.3% in 6 N HCl), respectively. All melting points are corrected.

┌OBz
1. **H·Glu·OBz·HCl** (L).—A suspension of 10 g. (0.068 mole) of L-glutamic acid in 150 ml. of benzyl alcohol is warmed to 55°, agitated with a magnetic stirrer while dry HCl is passed in for one hour, and the temperature permitted to rise. The mixture is transferred to a still, and 75 ml. of benzene added, which is distilled off with most of the H_2O at a bath temperature of about 40°. The mixture is now left *in vacuo* (approximately 10 mm.) for one hour at a bath temperature of 85°. Then, dry HCl is again passed in for one hour as described above. Unchanged glutamic acid hydrochloride (about 2 g.) is now filtered off, benzene added, and the process described previously is repeated. Dry HCl is passed in for a third time; after removal of about one-half of the benzyl alcohol *in vacuo* (steam-bath), the di-ester hydrochloride is precipitated with ether (5–7 volumes), and recrystallized from methanol–ether. The yield of pure compound is 15 g. (61%, not counting the recovered glutamic acid), m.p. 100–102°, $[\alpha]^{22}D$ +9.4° (1.5% in 0.1 *N* HCl).

Anal. Calcd. for $C_{19}H_{21}O_4N·HCl$ (363.8): N, 3.9; amino N, 3.9. Found: N, 3.9; amino[5] N, 3.9.

┌OBz
2. **H·Glu·OBz·HCl** (D).—This compound is obtained by the same procedure and in similar yield from D-glutamic acid as the L-isomer; m.p. 100–102°, $[\alpha]^{25}D$ −9.0° (2.0% in 0.1 *N* HCl).

Anal. Calcd. for $C_{19}H_{21}O_4N·HCl$ (363.8): N, 3.9; amino N, 3.9. Found: N, 3.9; amino[5] N, 4.0.

3. **H·Glu·OBz** (L).—Ten grams (0.027 mole) of compound 1, H·Glu·OBz·HCl (L), is dissolved in 100 ml. of
└OBz
glacial acetic acid. Ten ml. (0.12 mole) of constant boiling HI (sp. gr. 1.7) is added and the solution kept at 50° for 5.5 hours. The reaction mixture is taken down *in vacuo* and the resulting oil repeatedly (at least twice) treated with 50 ml. of benzene, which each time is distilled off *in vacuo*. The dark brown sirup is then taken up in 60 ml. of cold (−10°) 95% ethanol containing 7 ml. (0.029 mole) of tri-*n*-butylamine. Additional tri-*n*-butylamine (3–4 ml.) is added to bring the *p*H (moist *p*H paper) to approximately 7, whereupon the product begins to crystallize out. After storing in the ice-box overnight, the product is filtered off and washed copiously with absolute ethanol and ether to give 5.7 g. of crystalline material. The crystals are dissolved at room temperature in 11 ml. of water containing 0.034 mole of HCl, decolorized with charcoal, and an equal volume of absolute ethanol is added. Upon neutralization with tri-*n*-butylamine, crystallization takes place; the mixture is then cooled (0°) for several hours. The yield of pure compound is 4.3 g. (67%), m.p. 147–148°, $[\alpha]^{25}D$ +12.2° (2.9% in 0.1 *N* HCl).

Anal. Calcd. for $C_{12}H_{15}O_4N$ (237.2): N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0. Found: N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0.

4. **H·Glu·OBz** (D).—This is obtained from compound 2 by the same procedure and yield as the L-isomer; m.p. 147–148°, $[\alpha]^{25}D$ −11.9° (2.0% in 0.1 *N* HCl).

Anal. Calcd. for $C_{12}H_{15}O_4N$ (237.2): N, 5.9; amino N, 5.9; carboxyl nitrogen,[6] 0.0. Found: N, 5.9; amino N, 6.1; carboxyl nitrogen,[6] 0.0.

5. **Z·Glu·OBz** (L).—5.0 g. (0.021 mole) of H·Glu·OBz (L) (compound 3) is suspended in a cooled (0°), and vigorously stirred solution of 3.45 g. (0.025 mole) of K_2CO_3 in 20 ml. of water. When almost all of the ester has dissolved, 4.25 g. (0.025 mole) of carbobenzyloxy chloride is added in four portions over a period of 30 minutes, maintaining the *p*H at approximately 8 by addition of a 10% K_2CO_3 solution (total of 15–20 ml.); and stirring is continued for an additional 10 minutes. The reaction mixture is extracted twice with 30 ml. of ether, and acidified with 6 *N* HCl, yielding a heavy oil which solidifies on standing. The product is recrystallized from CCl_4 or ethanol–water; yield of pure compound[7] is 5.5–6.6 g. (70–85%), with m.p. 95–96°, $[\alpha]^{24}D$ −10.4° (1.7% in glacial acetic acid).

(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. Erwin Brand deceased July, 1953.

(2) For symbols and abbreviations, see the preceding paper by H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953). *E.g.*, D-glutamic acid-α-benzyl ester, H·Glu-OBz (D); L-glutamic acid-γ-benzyl ester, H·Glu·OH (L); L-glutamic acid dibenzyl ester hydrochloride, H.
└OBz
Glu·OBz·HCl (L); N-carbobenzyloxy-L-glutamic acid α-benzyl ester,
└OBz
Z·Glu·OBz (L).

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

(4) D-Glutamic acid was prepared by the enzymatic resolution of acetyl-DL-glutamic acid according to V. E. Price, J. B. Gilbert and J. P. Greenstein, *J. Biol. Chem.*, **179**, 1169 (1949). It was also obtained from DL-pyrrolidone carboxylic acid by alkaloid resolution (G. Hillmann and A. Elies, *Z. physiol. Chem.*, **283**, 31 (1948)); we are indebted to Dr. R. Dische for this material.

(5) The compound requires a reaction time of 10 minutes in the Van Slyke, manometric, amino N procedure.

(6) *Cf.* D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941); reaction time with ninhydrin was for seven minutes at *p*H 2.5.

(7) A mixture of Z·Glu·OBz and Z·Glu·OH was obtained as an oil by
└OBz
M. Bergmann, L. Zervas and L. Salzmann (*Ber.*, **66**, 1288 (1933)), by treating N-carbobenzyloxy-L-glutamic anhydride with benzyl alcohol at 100°. W. J. LeQuesne and G. T. Young (*J. Chem. Soc.*, 1954 (1950)) fractionated the mixture with Na_2CO_3 and obtained a solid, m.p. 78–81°, which they considered to be Z·Glu·OBz (L).