ing to the method of Witt.[5] The first indicator was recrystallized from alcohol, the second from water, and the third from mixed alcohol–acetone. Rough determinations of the melting points gave values in approximate agreement with those reported by Hammett and Deyrup.[3]

The source and purity of the sodium perchlorate and perchloric acid have been reported elsewhere.[6] The mixtures studied were prepared by the dilution of stock solutions of known concentration.

The colorimetric measurements were carried out with a Leitz photoelectric colorimeter: since the indicators had a yellow color in the basic form, and were colorless in the acidic form, a filter of blue tint was employed. A solution 6.0 formal in sodium perchlorate, in which the indicators were entirely converted to the basic form, was employed as a reference, and mixtures of perchloric acid and sodium perchlorate and the identical quantity of indicator were compared to this reference solution. All studies were made at room temperature (25 ± 2°).

Results

The results, calculated using the pK_a values given by Hammett[2] for the various indicators, are presented in the following table.

DETERMINATION OF H_0 FOR PERCHLORIC ACID–SODIUM PERCHLORATE MIXTURES OF CONSTANT IONIC STRENGTH 6.0 FORMAL

Perchloric acid, F	Sodium perchlorate, F	The quantity $-H_0$ determined with 2,4-Dichloro-6-nitro-aniline	*p*-Chloro-nitro-aniline	*o*-Nitro-aniline	$-H_0$, best value
6	0	2.91	2.93		2.92
5	1	2.66	2.63		2.64
4	2	2.37	2.32		2.34
3	3	(2.14)	2.01		2.0
2	4	(1.90)	1.72	1.73	1.72
1	5	(1.59)	1.31	1.39	1.35
0.5	5.5		0.98	1.02	1.00
0.3	5.7		0.72	0.76	0.74

Parentheses indicate determinations of $-H_0$ of lower accuracy resulting from the necessity of measuring a small difference when a large proportion of the indicator is in the basic form.

The results may be compared with those of Hammett and Deyrup[3] for pure perchloric acid 6.0 formal (about 8.4 molar) by interpolation from their data. The figure obtained, when corrected for a change in the zero-point of the H_0 scale[4] is −2.77, which is somewhat lower than the value of −2.92 reported above. The disagreement is not considered serious, since different lots of indicators, different instrumental techniques and reagents were employed.

The author wishes to thank Professor J. J. Beaver for the use of his photoelectric colorimeter, and Professor L. P. Hammett for discussions of the method.

(5) Witt, *Ber.*, **8**, 820 (1875).

(6) G. Harbottle and R. W. Dodson, THIS JOURNAL, **73**, 2442 (1951).

CHEMISTRY DEPARTMENT
COLUMBIA UNIVERSITY
NEW YORK, N. Y.

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On the Freezing Point Diagram of the Germanium–Manganese System

BY JAMES H. DOWNING AND DANIEL CUBICCIOTTI

In the present attempt to determine the freezing point diagram of the germanium–manganese system no suitable thermocouple protection tube was found for melts containing more than 32 atom per cent. manganese. Therefore, the results are fragmentary.

The freezing points were determined with a chromel–alumel thermocouple in an Alundum protection tube. An alundum crucible was used to hold the melt. The entire system was contained in a porcelain tube filled with helium to prevent attack by air. The manganese had a tendency to attack the Alundum protection tube. The attack by mixtures containing more than 32 atom per cent. manganese was too great to consider the results reliable.

Germanium metal, C.P., and manganese metal, 99.9%, were obtained from the A. D. Mackay Co., New York. The freezing point observed for the germanium, 957°, agreed well with the value of 959° given by Kelley.[1]

The results obtained are given in Fig. 1. The curve indicates the temperatures, for each composition, at which a solid, almost pure germanium, began to precipitate. Since the eutectic halt was observed at 5% manganese, the solid precipitating was almost pure germanium, containing less than 5% manganese at 720°.

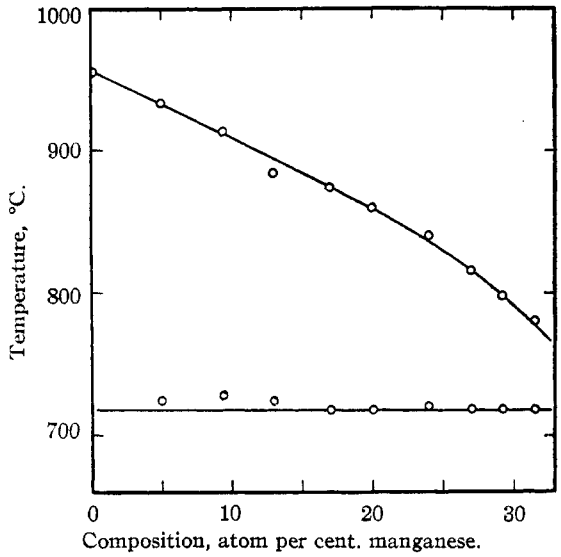

Fig. 1.—The freezing point diagram of the germanium–manganese system.

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(1) K. K. Kelley, Bur. Mines Bull. No. 393 (1936).

DEPARTMENT OF CHEMISTRY
ILLINOIS INSTITUTE OF TECHNOLOGY
CHICAGO 16, ILLINOIS

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Optical Rotation of Peptides. III. Lysine Dipeptides[1]

BY BERNARD F. ERLANGER AND ERWIN BRAND

The first two papers in this series dealt with glycine and alanine dipeptides[2] and tripeptides.[3] In

(1) This report is part of a dissertation submitted by Bernard F. Erlanger in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University. Presented in part before the Division of Biological Chemistry at the 118th Meeting of the A. C. S., Chicago, Ill., September, 1950.

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

(3) Brand, Erlanger, Sachs and Polatnick, *ibid.*, **73**, 3510 (1951).

TABLE I

CARBOBENZOXY LYSINE DIPEPTIDES AND DERIVATIVES

Number	Compound[a]	Molecular formula	Mol. wt.	M.p., °C. (cor.)	Nitrogen, % Calcd.	Found	Neut. equiv.[b] Found
		Carbobenzoxy dipeptide esters					
3	Z·Gly-Z·Lys·OMe (L)[c]	$C_{25}H_{31}O_7N_3$	485.5	97			
4	Z_2·Lys-Gly·OEt (L)[d]	$C_{26}H_{33}O_7N_3$	499.6	90			
5	Z_2·Lys-Gly·OBz (L)	$C_{31}H_{35}O_7N_3$	561.6	125	7.5	7.6	
6	Z·Ala-Z·Lys·OMe (L-L)	$C_{25}H_{33}O_7N_3$	499.6	90	8.4	8.3	
7	Z·Ala-Z·Lys·OMe (D-L)	$C_{26}H_{33}O_7N_3$	499.6	130	8.4	8.5	
7a	Z·Ala-Z·Lys·OMe (L-D)	$C_{26}H_{33}O_7N_3$	499.6	128	8.4	8.3	
8	Z_2·Lys-Ala·OEt (L-L)	$C_{27}H_{35}O_7N_3$	513.6	119	8.2	8.3	
9	Z_2·Lys-Ala·OBz (L-L)	$C_{32}H_{37}O_7N_3$	575.6	132	7.3	7.3	
10	Z_2·Lys-Ala·OEt (L-D)	$C_{27}H_{35}N_7O_3$	513.6	131.5.	8.2	8.2	
11	Z_2·Lys-Z·Lys·OMe (L-L)	$C_{37}H_{46}O_9N_4$	690.8	115–117	8.1	8.1	
12	Z_2·Lys-Z·Lys·OMe (L-D)	$C_{37}H_{46}O_9N_4$	690.8	134	8.1	8.2	
		Carboxybenzoxy dipeptide hydrazides					
13	Z·Gly-Z·Lys·$NHNH_2$ (L)[e]	$C_{24}H_{31}O_6N_5$	485.5	167	14.4	14.4	
14	Z·Ala-Z·Lys·$NHNH_2$ (L-L)	$C_{25}H_{33}O_6N_5$	499.6	165	14.0	14.2	
15	Z·Ala-Z·Lys·$NHNH_2$ (L-D)	$C_{25}H_{33}O_6N_5$	499.6	190–191	14.0	13.9	
16	Z_2·Lys-Z·Lys·$NHNH_2$ (L-L)	$C_{36}H_{46}O_8N_6$	690.8	187	12.2	12.1	
17	Z_2·Lys-Z·Lys·$NHNH_2$ (L-D)	$C_{36}H_{46}O_8N_6$	690.8	176	12.2	12.2	
		Carbobenzoxy dipeptides					
18	Z_2·Lys-Gly·OH (L)[f]	$C_{24}H_{29}O_7N_3$	471.5	160			472
19	Z·Gly-Z·Lys·OH (L)	$C_{24}H_{29}O_7N_3$	471.5	75	8.9	8.8	473
20	Z·Ala-Z·Lys·OH (L-L)	$C_{25}H_{31}O_7N_3$	485.5	134	8.7	8.6	490
21	Z·Ala-Z·Lys·OH (D-L)	$C_{25}H_{31}O_7N_3$	485.5	162	8.7	8.6	487
22	Z_2·Lys-Ala·OH (L-L)	$C_{25}H_{31}O_7N_3$	485.5	150	8.7	8.7	483
23	Z_2·Lys-Ala·OH (L-D)	$C_{25}H_{31}O_7N_3$	485.5	159	8.7	8.6	490
24	Z_2·Lys-Z·Lys·OH (L-L)	$C_{36}H_{44}O_9N_4$	676.8	145	8.3	8.3	677
25	Z_2·Lys-Z·Lys·OH (L-D)	$C_{36}H_{44}O_9N_4$	676.8	146	8.3	8.3	676

[a] The following abbreviations are used (*cf.* ref. 2, Table I, footnote *a*): Z: carbobenzoxy, $C_6H_5\cdot CH_2OCO$; 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.*, carbobenzoxy-D-alanyl-ε-carbobenzoxy-L-lysine methyl ester: Z·Ala-Z·Lys·OMe (D-L); L-lysyl-D-alanine hydrochloride: H·Lys-Ala·OH·HCl (L-D). [b] Neutralization equivalent, obtained by titration in alcohol (Ellenbogen and Brand, Am. Chem. Soc., Philadelphia Meeting, April 1950, Abstracts p. 56-C.) [c] Previously prepared[6] from carbobenzoxy-glycyl chloride with the same m.p. [d] Previously prepared (Bergmann, *et al., Z. physiol. Chem.*, **224**, 26 (1934)) from dicarbobenzoxy-L-lysyl chloride with the same m.p. [e] Previously prepared[6] with the same m.p. [f] Previously prepared (Bergmann, *et al., ibid.*, **224**, 26 (1934)) with m.p. 158–159°.

this paper the syntheses and specific rotations (in 0.5 *N* HCl) of eight lysine dipeptides are presented. More detailed data on their specific rotations and on the *residue rotations*[4] of alanine and lysine residues will be reported subsequently.

Experimental

Starting Materials.—The syntheses and properties of most of the starting materials have been described: L- and D-alanine[2]; α-carbobenzoxy hydrazides of glycine, of L- and D-alanine (ref. 2, Compounds 1–3) and of ε-carbobenzoxy-L-lysine[5]; methyl ester hydrochlorides of ε-carbobenzoxy-L- and -D-lysine[6]; benzyl ester hydrochlorides of glycine and of L- and D-alanine (ref. 2, Compounds 4–6).

1. ε-Carbobenzoxy-L-lysine Benzyl Ester Hydrochloride.—The specific rotation $[\alpha]^{26}D$ (2% in 6 *N* HCl) of the lysine used in these and other syntheses was +25.9° for the L-isomer and −24.9° for the D-isomer.[7]

ε-Carbobenzoxy-L-lysine carbamino anhydride is prepared by the method of Bergmann[6] from L-lysine hydrochloride *via* dicarbobenzoxylysine. After recrystallization from ethyl acetate–petroleum ether, the anhydride is converted into the benzyl ester hydrochloride, essentially as described for the corresponding alanine derivatives. From 25 g. (0.137 mole) of L-lysine HCl, 32 g. (58%) of recrystallized (hot water) ε-carbobenzoxy-L-lysine benzyl ester hydrochloride is obtained; m.p. 139°; $[\alpha]^{22}D$ −9.9° (0.5% in 0.1 *N* HCl).

Anal. Calcd. for $C_{21}H_{26}O_4N_2\cdot HCl$ (406.9): N, 6.9; NH_2-N, 3.5; HCl, 9.0. Found: N, 6.9; NH_2-N, 3.5; HCl, 8.9.

2. ε-Carbobenzoxy-D-Lysine Benzyl Ester Hydrochloride.—This compound is obtained from ε-carbobenzoxy-D-lysine carbamino anhydride by the same procedure and in the same yield as the corresponding L-derivative; m.p. 139°, $[\alpha]^{25}D$ +9.5° (0.5% in 0.1 *N* HCl).

Anal. Found: N, 7.0; NH_2-N, 3.5; HCl, 9.0.

Carbobenzoxy-dipeptide Esters (Compounds 3–12).—The solutions of the azides of carbobenzoxy-glycine and carbobenzoxy-alanine are prepared as described,[2] dicarbobenzoxy-L-lysine azide according to Bergmann.[5] The cold, ethereal azide solution is added in one portion to a cold, ethereal solution of an amino acid ester, prepared as previously described.[2] The mixture is then warmed to 25°. Snow-white crystals of the coupling products soon appear, aided by scratching. In some cases a small amount of heavy yellow oil accumulates on the bottom, from which the rest of the reaction mixture is decanted whenever convenient. After about 20 hours at room temperature, the reaction mixture is cooled to −5°, the crystals collected and washed with ether. The yield of pure, recrystallized (ethyl acetate–petroleum ether) dicarbobenzoxy-dipeptide esters is 50–60%, based on the hydrazide used.

The preparation of tricarbobenzoxy-L-lysyl-L-lysine

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

(5) Bergmann, Zervas and Greenstein, *Ber.*, **65**, 1692 (1932).

(6) Bergmann, Zervas and Ross, *J. Biol. Chem.*, **111**, 245 (1935). ε-Carbobenzoxy-L-lysine methyl ester hydrochloride is recrystallized from methanol–ether instead of from acetone. The synthesis of the D-form is identical (m.p. 117°).

(7) We are indebted to Dr. A. A. Albanese for the D-lysine·HCl, which was furnished him by the Electrochemical Division of the du Pont Company.

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.	Found	Amino N, % Calcd.	Found	HCl, % Calcd.	Found	Neut. equiv.[b] Calcd.	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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DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK 32, N. Y.

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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).