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[6] 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.[3]

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).

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK 32, N. Y. RECEIVED NOVEMBER 5, 1951

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[4]; 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)	$C_{26}H_{39}O_6N_5 \cdot 2HCl$	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)	$C_{26}H_{39}O_6N_5 \cdot HCl$	395.9	17.7	17.5	7.1	7.1	9.2	9.1	198	190	− 18.6
7	H.Ala-Lys-$(Ala)_3$-Ala.OH·HCl.H_2O (5L)	$C_{26}H_{54}O_8N_8 \cdot HCl \cdot H_2O$	485.0	17.3	17.1	5.8	5.8	7.5	7.3	243	244	−109.3[c]
8	H.Ala-Lys-$(Ala)_3$-Ala.OH·HCl (LDLLL)	$C_{26}H_{54}O_8N_8 \cdot HCl$	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-$(Ala)_3$-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*.

(4) **Z.Ala-Z.Lys-Ala-Ala.OBz** (LDLLL).—The method of preparation of this compound is the same as that of Compounds 1 (a) and 2. The azide solution is prepared from Z.Ala-Z.Lys.$NHNH_2$ (LD, ref. 3, Cmpd. 15) and added to a solution of the tripeptide benzyl ester, prepared from a 50% molar excess of its hydroiodide (ref. 5, Cmpd. 2). A white gelatinous precipitate forms almost immediately. After standing overnight at room temperature, the material is collected and recrystallized from absolute ethanol. Yield of pure product is 62%; m.p. 213–214°.

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

Peptides (Compounds 5–8).—The peptides are isolated as hydrochlorides which are more or less hygroscopic (Cmpds. 6–8 as monohydrochlorides; Cmpd. 5 as dihydrochloride).

Hydrogenolysis of 0.005 mole of a carbobenzoxy peptide benzyl ester is carried out in 100 cc. of 85% acetic acid, containing 0.005 mole of *N* HCl (0.01 mole *N* HCl in the case of Cmpd. 1), with palladium black as catalyst in a rapid stream of hydrogen, as previously described.[3,4] Concentration of the filtrates *in vacuo* results in oils which crystallize upon dissolving in a small amount of absolute methanol and adding absolute ether. The compounds are recrystallized from a minimum amount of water by the addition of absolute alcohol followed by ether. The pure peptide hydrochlorides are obtained in 70–80% yield. For analysis and rotation measurements they are dried over P_2O_5 at 100° in high vacuum; the data are in Table I.

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

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK 32, N. Y. RECEIVED DECEMBER 10, 1951

Chemistry of Technetium. II. Preparation of Technetium Metal[1]

BY J. W. COBBLE, C. M. NELSON, G. W. PARKER, W. T. SMITH, JR.,[2] AND G. E. BOYD

Recent large scale separations of technetium from fission product wastes at the Oak Ridge National Laboratory[3] have made chemical studies possible using readily weighable amounts.[4] The preparation of fractional gram quantities of pure metallic technetium was of interest since thus far only microgram amounts have been examined.[5,6] A tetraphenylarsonium perchlorate "process" precipitate containing coprecipitated pertechnetate was the starting material. This precipitate, which is quite stable toward common reagents, may be decomposed with difficulty by perchloric–sulfuric acid digestion. However, an electrolysis of its homogeneous solution in concentrated sulfuric acid afforded a convenient method.

About 0.2 g. of technetium together with 3–4 g. of tetraphenylarsonium perchlorate carrier in approximately one liter of sulfuric acid were electrolyzed for 24 hours with large platinum electrodes (C.D. = 10 ma./cm.^{2}, 2–3 volts). The black, technetium-containing solid which separated was filtered, dried, transferred to an all-glass distilling apparatus and then put into solution by gently warming with a mixture of five ml. each of concentrated nitric, perchloric and sulfuric acids. After the initially vigorous reaction subsided, the technetium was co-distilled with the perchloric acid and collected under dilute ammonium hydroxide. A variety of colors were noted in the acid distillate fractions, presumably owing to complexes similar to those postulated for manganese(VII)[7] and to those observed by us with rhenium(VII). The ammoniacal condensate was made slightly acid with HCl, bromine water was added and technetium sulfide (Tc_2S_7) precipitated with hydrogen sulfide. The coagulation of the slowly-forming, jet-black precipitate may be hastened by conducting operations on a water-bath at *ca.* 100°. The freshly precipitated sulfide dissolved readily in ammoniacal hydrogen peroxide and on evaporation to dryness NH_4TcO_4 and $(NH_4)_2SO_4$ were deposited. Technetium metal was prepared by hydrogen reduction of the latter mixture contained in a platinum boat. The initial reduction at low temperatures produced a black mass (TcO_2 ?) which prevented the loss of NH_4TcO_4 by sublimation. At the final higher temperature (500–600°) the $(NH_4)_2SO_4$ was volatilized leaving the technetium behind. Approximately 0.6 g. of spectrochemically pure metallic technetium has been isolated in the foregoing manner.

The metal, which shortly after reduction appeared as a silver-gray spongy mass, tarnished slowly in moist air. It did not dissolve in hot or cold, concentrated or dilute hydrochloric acid, nor was it attacked appreciably by alkaline hydrogen peroxide in agreement with Fried.[5] The metal did dissolve readily in nitric acid and aqua regia, and burned in oxygen to form Tc_2O_7.[4] Its atomic weight from previous chemical analyses on milligram quantities of the oxide was found to be 98.8 ± 0.1 which may be compared with the mass spectrometer value of 98.913.[8] Measurements on an X-ray diffraction pattern taken on the same preparation confirmed in detail the $\sin^2\theta$ and intensity values of Mooney.[9,10]

Whereas the specific activity of pure technetium is not large (*ca.* 20 μc./mg.) it may constitute a radiation hazard in some circumstances. Pure, dry compounds have been found to show about 10 "R"/hr./100 mg. at their surfaces owing to the low energy beta particles emitted (300 kev. maximum energy). These radiations, however, are completely absorbed by ordinary glass laboratory equipment (*i.e.*, beakers, desiccators, etc.). Whether or not technetium is, in addition, a radiological poison is not known. Accordingly, small quantities should be handled with the foregoing facts in mind.

(1) This work was performed for the Atomic Energy Commission at Oak Ridge National Laboratory.

(2) University of Tennessee and Consultant, Oak Ridge National Laboratory.

(3) Hot Laboratory Group, Chemistry Division, G. W. Parker, Leader (W. J. Martin, G. M. Hebert, G. E. Creek and P. M. Lantz).

(4) G. E. Boyd, J. W. Cobble, C. M. Nelson and W. T. Smith, Jr., THIS JOURNAL, **74**, 556 (1952).

(5) S. Fried, *ibid.*, **70**, 442 (1948).

(6) R. C. L. Mooney, *Phys. Rev.*, **72**, 1269 (1947).

(7) W. M. Latimer and J. H. Hildebrand, "Reference Book of Inorganic Chemistry," The Macmillan Company, New York, N. Y., 1943, p. 374.

(8) M. G. Inghram, D. C. Hess, Jr., and R. J. Hayden, *Phys. Rev.*, **72**, 1269 (1947).

(9) The authors are indebted to Mr. R. D. Ellison of the Chemistry Division, ORNL, for this X-ray identification.

(10) R. C. L. Mooney, *Acta Cryst.*, **1**, 161 (1948).

CHEMISTRY DIVISION
OAK RIDGE NATIONAL LABORATORY
OAK RIDGE, TENNESSEE AND
THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE RECEIVED DECEMBER 12, 1951

Separation of Keto-acids by Cellulose Columns[1]

BY FREDERICK D. DREW, LAWRENCE M. MARSHALL AND FELIX FRIEDBERG

Because of the instability of keto-acids the separation of these compounds as 2,4-dinitrophenylhydrazones becomes particularly useful. One such separation by column chromatography for biologically important keto-acids has been reported.[2] Cavallini, *et al.*,[3] suggested filter paper chromatography of 2,4-dinitrophenylhydrazones. In this report

(1) This investigation was supported in part by research grants from the National Cancer Institute and the United States Public Health Service, and from the Damon Runyan Memorial Fund.

(2) G. A. LePage, *Cancer Research*, **10**, 393 (1950).

(3) D. Cavallini, N. Frontali and G. Toschi, *Nature*, **163**, 568 (1949).