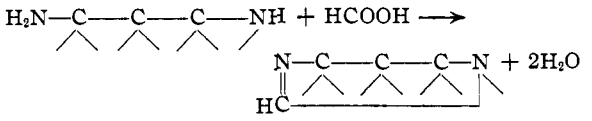
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE WOMEN'S COLLEGE OF THE UNIVERSITY OF DELAWARE]

Dipeptides of Beta Amino Acids

BY ELIZABETH DYER AND ELIZABETH BALLARD

This work originated in a study of the possibility of pyrimidine synthesis by ring closure of diamino compounds with formic acid. Since the method has been used frequently for the preparation of condensed benzene pyramidines, the quinazolines,[1] it would be of interest to investigate its applicability to simple pyrimidines. Such a synthesis would be represented by the scheme



The necessary 1,3-arrangement of amino groups is present in dipeptides in which the free amino group is in the β-position. Consequently, the first objective of this investigation was to synthesize the following dipeptides

$NH_2CH_2CH_2CONHCH_2COOH$	I
$NH_2CH_2CH_2CONHCH(COOH)CH_2C_6H_5$	II
$NH_2CH_2CH_2CONHCH(C_6H_5)CH_2COOH$	III
$NH_2CH(C_6H_5)CH_2CONHCH_2COOH$	IV

The compound (IV) is an open-chain analog of the *o*-aminohippuric acid used, together with formic acid, by Späth[2] to prepare the condensed benzene pyrimidine which is an oxidation product of the alkaloid vasicine.

The first method investigated for the synthesis of these peptides was the preparation of β-halogen acyl amino acids, which on amination would be expected to give the corresponding beta amino peptides.[3] Accordingly, three new β-halogen acyl amino acids were prepared, the β-chloropropionyl derivatives of glycine (V), of β-phenyl-α-alanine (VI), and of β-phenyl-β-alanine (VII). However, amination of (V) or its ester yielded the ammonium salt or amide of β-chloropropionylglycine, or a non-crystallizing oil which was not the desired peptide. Treatment of (VI) and (VII) with ammonia was also unsuccessful. Efforts to obtain a halogen acyl derivative corresponding to (IV) by treating the acid chloride of β-bromohydrocinnamic acid with glycine yielded cinnamoylglycine as the sole product. Furthermore, ammonia could not be added to the double bond of cinnamoylglycine.

However, a second method of peptide synthesis, the Bergmann[4] carbobenzoxy procedure, was entirely successful for the preparation of the peptides (I), (II) and (III). The peptide (IV) could not be obtained by this series of reactions. The cause of the failure in this case is being investigated. It may possibly be due to an increased lability of the β-amino group, caused by the proximity of the phenyl group.

The peptides (I), (II) and (III) were boiled with formic acid to test the possibility of ring closure. These substances were found, however, to be unreactive, and to possess a stability remarkable for compounds containing β-amino groups, since no pyrimidines were formed and the peptides were largely recovered unchanged. A simpler compound possessing the proper 1,3-arrangement of amino groups, diaminoacetone, was treated with formic acid in a similar way, but no pyrimidine was isolated.

Grateful acknowledgment is made to Professor Treat B. Johnson of Yale University for suggesting this problem; to the American Association for the Advancement of Science for a grant in support of the research; to the Department of Chemistry of Yale University for the use of the laboratory during the summer of 1935; and to the du Pont Experimental Station for the use of its library.

Experimental Part

Preparation of β-Chloropropionylamino Acids.—One equivalent of β-chloropropionyl chloride, prepared by the Abderhalden method[3] (p. 521), was added dropwise to a cold solution containing one equivalent of the amino acid dissolved in 2.2 to 2.5 equivalents of normal sodium hydroxide. The mixture was kept at 0 to −3° and stirred continuously until the odor of the acid chloride had disappeared (about one hour for 0.05 mole). The product, in the case of the β-chloropropionyl derivatives of phenylalanine and β-phenylalanine, was precipitated by the slow addition of an excess of cold dilute hydrochloric acid. Since β-chloropropionylglycine is soluble in water, it was separated by evaporation of the aqueous acid solution to dryness *in vacuo* at 40°, and extraction of the dry powdered residue with ethyl acetate in a Soxhlet extractor.

β-Chloropropionylglycine.[5]—Rhombic plates from ethyl acetate, m. p. 133–135°,[6] yield[7] 70%.

(1) Gabriel and Colman, *Ber.*, **37**, 3645 (1904).

(2) Späth and Kuffner, *ibid.*, **67**, 1495 (1934).

(3) Abderhalden, Ryndin and Schwab, *Fermentforschung*, **11**, 515 (1930).

(4) Bergmann and Zervas, *Ber.*, **65B**, 1192 (1932).

(5) Acknowledgment is made to Mary D. Caulk for the preparation and study of this substance.

(6) All melting points are corrected.

(7) The yields in all cases are calculated on the recrystallized products.

Anal. Calcd. for $C_5H_8O_3NCl$: C, 36.24; H, 4.87; N, 8.45. Found:[8] C, 36.50; H, 5.10; N, 8.48, 8.61.

β-Chloropropionyl-β-phenyl-α-alanine.[9]—The immediate reaction product was a gummy precipitate, which crystallized after being dried *in vacuo* and treated with cold ether; hexagonal plates from water, m. p. 123–125°, yield 44%.

Anal. Calcd. for $C_{12}H_{14}O_3NCl$: C, 56.46; H, 5.52; N, 5.48. Found: C, 56.60; H, 5.56; N, 5.52, 5.43.

β-Chloropropionyl-β-phenyl-β-alanine.[10,11]—Needles from water, m. p. 162.5–163°, yield 27%.

Anal. Calcd. for $C_{12}H_{14}O_3NCl$: N, 5.48. Found: N, 5.35, 5.43.

Ethyl Ester of β-Chloropropionylglycine.—This was prepared in a similar way by adding β-chloropropionyl chloride to a cold, stirred solution of glycine ester hydrochloride in a water–chloroform mixture, using sodium carbonate solution instead of sodium hydroxide. The product was precipitated from the dried, concentrated chloroform layer by petroleum ether as narrow plates, m. p. 71–72.5°, yield 74%.

Anal. Calcd. for $C_7H_{12}O_3NCl$: N, 7.23. Found: N, 7.25, 7.35.

Amide of β-Chloropropionylglycine.—Thin plates from alcohol, m. p. 174–175°.

Anal. Calcd. for $C_5H_9O_2N_2Cl$: N, 17.02. Found: N, 16.83, 16.82.

Condensation of β-Bromohydrocinnamoyl Chloride with Glycine.—β-Bromohydrocinnamic acid[12] was treated with thionyl chloride at 45°, and the resultant acid chloride, after removal of excess thionyl chloride, was added to glycine in alkaline solution under the same conditions as were used for the preparation of β-chloropropionylglycine. After acidification the chief product was, however, cinnamoylglycine, with small amounts of cinnamic anhydride.

Amination of Chloropropionylamino Acids.—Treatment of β-chloropropionylglycine with cold aqueous ammonia resulted in the formation of ammonium chloride and a non-crystalline oil from which neither the peptide nor pure solid derivatives of any kind could be obtained. When β-chloropropionylglycine ester was treated with alcoholic ammonia, the ammonium salt or the amide of β-chloropropionylglycine was obtained. The reaction of the β-chloropropionyl derivatives of β-phenyl-α-alanine and of β-phenyl-β-alanine with ammonia yielded mixtures of solid products, none of which gave the desired peptides. Furthermore, cinnamoylglycine could not be made to add ammonia to give a β-amino peptide.

Preparation of Carbobenzoxy-β-alanyl Amino Acids.—The carbobenzoxy-β-alanyl chloride needed for these condensations was prepared by treating carbobenzoxy-β-alanine, obtained by the method of Sifferd and du Vigneaud,[13] with thionyl chloride at 50°. The excess thionyl chloride and gaseous products were separated from the acid chloride by evaporation at 45° under 20 mm. pressure. The residue was treated with ether, and the solution of the acid chloride filtered from a precipitate composed largely of β-alanine hydrochloride (formed in 15–20% yield). Although the formation of this by-product can be avoided by using phosphorus pentachloride[13] (p. 758), the method was not used by us because of the lack of a source of very high vacuum to remove phosphorus-containing by-products.

The freshly prepared ether solution containing 0.05 mole of the acid chloride was added dropwise during stirring to a solution of 0.05 mole of the amino acid in an excess of cold normal sodium hydroxide kept at 0°. After about an hour the aqueous layer was extracted with ether to remove by-products, and then acidified. The product separated as an oil which became granular on stirring in the cold.

Carbobenzoxy-β-alanylglycine.—Microscopic needles from ethyl acetate, m. p. 145–146°, yield 44% (calculated from the carbobenzoxy-β-alanine used).

Anal. Calcd. for $C_{13}H_{16}O_5N_2$: N, 10.00. Found: N, 10.01, 10.18.

Carbobenzoxy-β-alanyl-β-phenyl-α-alanine.—Long narrow plates from water, or from 1:2 alcohol–water solutions, m. p. 144.5–145°, yield 40%.

Anal. Calcd. for $C_{20}H_{22}O_5N_2$: N, 7.57. Found: N, 7.69, 7.76.

Carbobenzoxy-β-alanyl-β-phenyl-β-alanine.—Balls of fine needles from ethyl acetate, m. p. 151.5–153°, yield 28%.

Anal. Calcd. for $C_{20}H_{22}O_5N_2$: N, 7.57. Found: N, 7.71, 7.75.

Preparation of β-Alanyl Amino Acids.—The application of the Bergmann method[4] to the reduction of these carbobenzoxy derivatives is illustrated by the following example. A rapid stream of pure hydrogen was passed into a solution containing 4 g. of carbobenzoxy-β-alanylglycine, 60 cc. of methyl alcohol, 15 cc. of water, 5 cc. of glacial acetic acid, and 0.2 g. of colloidal palladium. After about fourteen hours 0.1 g. of fresh catalyst was added, and hydrogenation was continued until no more carbon dioxide was evolved (thirty-eight hours longer). The filtrate from the palladium was evaporated to dryness, the residue dissolved in the minimum amount of boiling water, and the product crystallized by the addition of alcohol.

β-Alanylglycine.—Rosets of fine needles from 80% alcohol, m. p. 230° with effervescence, yield 98%.

Anal. Calcd. for $C_5H_{10}O_3N_2$: C, 41.07; H, 6.90; N, 19.18. Found: C, 40.87; H, 6.79; N, 19.17.

The hydrochloride separates with solvent of crystallization when precipitated from concentrated aqueous solution by acetone, or when recrystallized from absolute alcohol

Anal. Calcd. for $C_5H_{11}O_3N_2Cl$: N, 15.35. Found: N, 15.71.

β-Alanyl-β-phenyl-α-alanine.—Irregular plates from 60% alcohol, m. p. 264–265° with decomposition, yield 80%.

(8) Carbon and hydrogen analyses were done by Mrs. G. M. Wellwood, Harvard University.

(9) The phenylalanine was prepared according to "Organic Syntheses," Vol. XIV, p. 80.

(10) Described by Martha B. Mason in a thesis presented to the Women's College of the University of Delaware in partial fulfilment of the requirements for the A.B. degree with distinction in chemistry, 1936.

(11) The β-phenyl-β-alanine was prepared from hydrobenzamide, Johnson and Livak, THIS JOURNAL, **58**, 301 (1936).

(12) Prepared according to Anschütz and Kinnicutt, *Ber.*, **11**, 1221 (1878).

(13) Sifferd and du Vigneaud, *J. Biol. Chem.*, **108**, 757 (1935)

Anal. Calcd. for $C_{13}H_{16}O_3N_2$: C, 60.98; H, 6.82; N, 11.86. Found: C, 61.08; H, 6.97; N, 11.98, 12.11.

Hydrochloride, precipitated from ethanol solution by dry acetone, m. p. 205.5–207°.

Anal. Calcd. for $C_{12}H_{17}O_3N_2Cl$: N, 10.28. Found: N, 10.15, 10.41.

β-Alanyl-β-phenyl-β-alanine.—Microscopic narrow prisms from 60% alcohol, m. p. 235–236° with effervescence, yield 75%.

Anal. Calcd. for $C_{12}H_{16}O_3N_2$: C, 60.98; H, 6.82; N, 11.86. Found: C, 60.87; H, 6.95; N, 11.83, 11.87.

Hydrochloride, obtained as crystalline material by repeated precipitation from a concentrated methyl alcohol solution by dry acetone, m. p. 180–182°.

Anal. Calcd. for $C_{12}H_{17}O_3N_2Cl$: N, 10.28. Found: N, 10.36.

Attempts at Ring Closure with the Dipeptides and with Diaminoacetone.—When a sample of each of the peptides described above was dissolved in 98.6% formic acid and the solution boiled for thirty to ninety minutes, the peptide was recovered in 60 to 80% yield on evaporation of the solvent. Similarly, when a mixture of the sulfate of diaminoacetone[14] and dry sodium formate was heated under varying conditions with 95% formic acid, no pyrimidine was isolated.[15] In this case, however, there was evidence of considerable change, the nature of which has not been determined.

Summary

1. The synthesis of the peptides β-alanylglycine, β-alanyl-β-phenyl-α-alanine, and β-alanyl-β-phenyl-β-alanine has been accomplished through the carbobenzoxy derivatives, but not by the amination of the corresponding β-halogen acyl amino acids.

2. These peptides do not combine with formic acid to give pyrimidines.

(14) Obtained from diaminoacetone hydrochloride, prepared according to Koessler and Hanke, THIS JOURNAL, **40**, 1718 (1918); and Adams, Chiles and Rassweiler, "Organic Syntheses," Coll. Vol. I, p. 9.

(15) From the thesis of Kathleen Spencer, presented to the Women's College of the University of Delaware in partial fulfilment of the requirements for the A.B. degree with distinction in chemistry, 1937.

NEWARK, DELAWARE RECEIVED JULY 17, 1937

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BROWN UNIVERSITY]

Properties of Electrolytic Solutions. XX. Freezing Points of Solutions of Electrolytes in Benzene[1]

BY DAVID A. ROTHROCK, JR.,[2] AND CHARLES A. KRAUS

I. Introduction

In previous papers of this series[3,4] an apparatus has been described for the determination of freezing points of dilute benzene solutions and data have been presented for a few quaternary ammonium salts in the concentration range 0.001 to 0.025 *N*. Geddes[5] and Kraus have shown that ammonium salts may be divided into three general classes according to their polarization–concentration curves. The purpose of the present investigation was to determine the freezing-point curves of a typical salt from each of these groups. Several improvements were made on the freezing-point apparatus as described by Batson and Kraus.[4] The accuracy of the results was determined by numerous measurements of the freezing-point curve for triphenylmethane, a normal solute. When the reproducibility of results with the improved apparatus had been established, the freezing-point curves were determined for solutions of tri-*n*-butylammonium picrate, tri-*n*-butylammonium iodide, and tetra-*n*-butylammonium perchlorate.

(1) This paper is an abstract of a portion of a thesis presented by David A. Rothrock, Jr., in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1936.

(2) Metcalf Fellow in Chemistry at Brown University.

(3) Kraus and Vingee, THIS JOURNAL, **56**, 511 (1934).

(4) Batson and Kraus, *ibid.*, **56**, 2017 (1934).

(5) Geddes and Kraus, *Trans. Faraday Soc.*, **32**, 583 (1936).

II. Materials, Apparatus and Procedure

Materials: Benzene.—Thiophene-free benzene was purified by the method described by Batson and Kraus.[4] Fractional crystallization was found to be superior to distillation as a method of purification. The pure product was stored over sodium–lead alloy (NaPb) in an all-glass still.

Triphenylmethane.—After four recrystallizations from ethyl alcohol, the product had a constant melting point of 92.5°.

Salts.—The tri-*n*-butylammonium picrate was prepared by Professor Fuoss by the method described by Kraus and Fuoss;[6] m. p. 106°. Tri-*n*-butylammonium iodide was prepared by J. A. Geddes by the addition of hydriodic acid to an ether solution of tributylamine. The crude compound was recrystallized from ethyl acetate; m. p. 102°. The tetra-*n*-butylammonium perchlorate was prepared in Rogers Laboratory by the method described by Luder, P. B. Kraus, C. A. Kraus and Fuoss;[7] m. p. 207°.

(6) Kraus and Fuoss, THIS JOURNAL, **55**, 21 (1933).

(7) Luder, Kraus, Kraus and Fuoss, *ibid.*, **58**, 255 (1936).