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

Dipeptides of β-Amino Acids. II. Derivatives of β-Phenyl-β-alanine

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In a previous paper[1] three dipeptides of β-alanine were described, but difficulties were reported in the attempt to prepare a corresponding peptide from the β-phenyl derivative of β-alanine. The work was continued because only two examples of dipeptides containing β-phenyl-β-alanine are recorded in the literature, and neither of these is of the type in which we were interested. In these two peptides, prepared by Abderhalden and Hasse,[2] the carboxyl group of β-phenyl-β-alanine is free, while the amino group is combined with another amino acid. In the following peptides which we have now synthesized, the position of the β-amino acid is reversed, leaving its amino group uncombined:

I $NH_2CH(C_6H_5)CH_2CONHCH_2COOH$
II $NH_2CH(C_6H_5)CH_2CONHCH(CH_2C_6H_5)COOH$

The peptides I and II were obtained by the Bergmann carbobenzoxy method,[3] through condensation of the acid chloride of carbobenzoxy-β-phenyl-β-alanine with the free ethyl ester of the second amino acid. Hydrolysis of the resulting esters and removal of the protecting group was accomplished as usual.

A third peptide, isomeric with II, was also prepared

III $NH_2CH(CH_2C_6H_5)CONHCH(C_6H_5)CH_2COOH$

In this case the carbobenzoxy method was found impracticable because of the instability of the necessary acid chloride. Attempts to prepare carbobenzoxy-β-phenyl-α-alanyl chloride by treatment of the carbobenzoxy acid with phosphorus pentachloride gave the N-carbonic acid anhydride of phenyl-α-alanine[4] in 45% yield. The formation of this anhydride might be explained as due to the rapid breakdown of the acid chloride in the following way

$$C_6H_5CH_2OCONHCH(CH_2C_6H_5)COCl \longrightarrow C_6H_5CH_2Cl + \underline{OCONHCH(CH_2C_6H_5)CO}$$

An analogous reaction was given by Bergmann and Zervas[5] to account for the decomposition of the acid chloride of carbobenzoxyglycine when heated at its melting point. It is to be noted, however, that the decomposition of carbobenzoxyphenylalanyl chloride occurred under surprisingly mild conditions, *i. e.*, on standing in ether solution at room temperature. A similar cleavage of the acid chloride of N-carbomethoxyphenylalanine was brought about[4] by heating at 60°.

The preparation of the peptide III by amination of a halogen acyl amino acid was considered unlikely to be successful because of the tendency of α-halogen hydrocinnamoyl compounds to become unsaturated during the treatment with ammonia.[6]

The peptide III was finally obtained by the azlactone method of Bergmann, Witte and Stern.[7] The azlactone of N-acetylphenyl-α-alanine was condensed with the ethyl ester of phenyl-β-alanine, and the resulting acetyl peptide ester was hydrolyzed to the free peptide III by aqueous hydrobromic acid. The stability of the —CO—NH— bond toward the latter reagent appeared to be as great in this peptide of a β-amino acid as in the peptides of the α-amino acids prepared by Bergmann.

Experimental Part

N-Carbobenzoxy-β-phenyl-β-alanine.[8]—To a solution of 9.9 g. (0.06 mole) of β-phenyl-β-alanine in 146 ml. of 1 *N* sodium hydroxide at 7° was added dropwise during stirring 0.06 mole of benzyl chlorocarbonate.[9] After the addition, which took forty-five minutes, stirring was continued for an hour, while the temperature of the mixture was allowed to rise to 15°. A heavy precipitate of the sodium salt of carbobenzoxy-β-phenyl-β-alanine was filtered, washed with water, then with ether, and suspended in 300 ml. of water for acidification with cold dilute hydrochloric acid. The gummy product, which solidified on standing, was recrystallized from dry carbon tetrachloride. A further quantity of the practically pure acid was obtained from acidification of the aqueous filtrate from the sodium salt, giving a total yield[10] of 5.4 g. (30%). The pure substance melted at 126–127.5°.[11]

(1) Dyer and Ballard, THIS JOURNAL, **59**, 1697 (1937).
(2) Abderhalden and Haase, *Fermentforsch.*, **12**, 313-328 (1931).
(3) Bergmann, Zervas, Fruton, Schneider and Schleich, *J. Biol. Chem.*, **109**, 337 (1935).
(4) Leuchs and Geiger, *Ber.*, **41**, 1724 (1908).
(5) Bergmann and Zervas, *ibid.*, **65**, 1195 (1932).
(6) Fischer, *ibid.*, **37**, 3069 (1904).
(7) Bergmann, Witte and Stern, *Ann.*, **449**, 300 (1926).
(8) This substance was prepared by Miss Elizabeth Ballard.
(9) Ref. 5, p. 1194.
(10) All percentage yields are given for material recrystallized once.
(11) All melting points are corrected.

Anal. Calcd. for $C_{17}H_{17}O_4N$: C, 68.20; H, 5.72; N, 4.68. Found[12]: C, 68.44; H, 6.00; N, 4.73.

N-Carbobenzoxy-β-phenyl-β-alanylglycine Ethyl Ester.—To a suspension of 0.015 mole of carbobenzoxy-β-phenyl-β-alanine in 100 ml. of anhydrous ether at 0° was added 0.015 mole of phosphorus pentachloride, and the mixture was then allowed to stand at room temperature for four hours while the reactants dissolved. A trace of impurity was filtered off and the ether evaporated under reduced pressure at 30°. The residue, a solid if all materials were absolutely anhydrous, was washed thoroughly with dry petroleum ether, and dissolved at once in anhydrous ethyl acetate. This solution was added gradually, during cooling, to a dry ethyl acetate solution[13] of glycine ethyl ester prepared from 0.06 mole of glycine ester hydrochloride. The mixture was allowed to stand for several hours, and the product was separated according to the procedure described by Bergmann and co-workers.[8] When recrystallized from a small volume of ethyl acetate (with hot filtration to remove a persistent amorphous impurity), the peptide ester separated in rosets of long silky needles; yield 53%. The melting point of the apparently pure peptide ester was not sharp, 123–133°, but was unchanged by repeated fractional crystallizations.

Anal. Calcd for $C_{21}H_{24}O_5N_2$: C, 65.60; H, 6.29; N, 7.29. Found: C, 65.81; H, 6.28; N, 7.23.

N-Carbobenzoxy-β-phenyl-β-alanyl-β-phenyl-α-alanine Ethyl Ester.—The preparation was similar to that described above, except that the phenylalanine ethyl ester from 0.032 mole of its hydrochloride was used with the acid chloride from 0.015 mole of carbobenzoxy-β-phenyl-β-alanine. The product crystallized from ethanol as needles, m. p. 142–144°; yield 58%.

Anal. Calcd. for $C_{28}H_{30}O_5N_2$: N, 5.90 Found: N, 5.76.

N-Carbobenzoxy-β-phenyl-β-alanylglycine.—A mixture of 1.5 g. of the corresponding ester and 40 ml. of *N* sodium hydroxide was shaken for four hours, diluted with 100 ml. of water, and filtered. Undissolved material was shaken again with dilute base for two hours. The combined aqueous alkaline solutions gave, when acidified, a gelatinous precipitate, which was dried and recrystallized from acetone. The pure acid melted at 190.5–191.5° with effervescence; yield 65%.

Anal. Calcd. for $C_{19}H_{20}O_5N_2$: N, 7.86. Found: N, 7.68.

N-Carbobenzoxy-β-phenyl-β-alanyl-β-phenyl-α-alanine.—A solution of 2.0 g. of the corresponding ester in 20 ml. of boiling ethanol was added to a boiling solution of 0.29 g. of potassium hydroxide in 15 ml. of ethanol, and the mixture was heated for two minutes on the steam-bath and then cooled rapidly. The gelatinous acid obtained on diluting and acidifying was crystallized from acetone; yield 90%; m. p. when pure 190–192° with decomposition.

Anal. Calcd. for $C_{26}H_{26}O_5N_2$: C, 69.95; H, 5.87; N, 6.28. Found: C, 69.62; H, 5.39; N, 6.11.

Reduction of Carbobenzoxy Peptides.—The carbobenzoxy group was removed by catalytic reduction.[14] The time needed was shortened by the use of slight pressure and continuous shaking.

β-Phenyl-β-alanylglycine.—Needles from water, decomposition point 245° (on rapid heating), yield 67%.

Anal. Hydrate: Calcd. for $C_{11}H_{14}O_3N_2 \cdot H_2O$: N, 11.66; H_2O, 7.50. Found: N, 11.62; H_2O, 7.34. Anhydrous material, dried in the oven at 125°: Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.48; H, 6.35; N, 12.61. Found: C, 59.25; H, 6.55; N, 12.37.

β-Phenyl-β-alanyl-β-phenyl-α-alanine.—Needles from 1:10 glacial acetic acid–water solution, m. p. 263–264° with decomposition, yield 60%.

Anal. Calcd. for $C_{18}H_{20}O_3N_2$: C, 69.25; H, 6.41; N, 8.97. Found: C, 68.87; H, 6.63; N, 8.86.

Treatment of Carbobenzoxy-β-phenyl-α-alanine with Phosphorus Pentachloride.[15]—To a solution of 1.3 g. (0.0043 mole) of carbobenzoxy-β-phenyl-α-alanine[16] in 18 ml. of ether at 0° was added 0.9 g. (0.0043 mole) of phosphorus pentachloride, and the mixture was kept at 0° for half an hour, then at room temperature for one and one-quarter hours. By this time a crystalline solid had separated (0.37 g.), which melted at 128°, was very soluble in dilute base, and contained no chlorine. It was identified by its properties and analysis as the N-carbonic acid anhydride of phenylalanine (4-benzyl-3,4-dihydro-2,5-oxazoledione), first described by Leuchs and Geiger.[4] Since the yield of the anhydride was 45%, and only negligible quantities of the desired acid chloride were present in the filtrate, this method of synthesis was discontinued. It is possible that the acid chloride could be obtained by a shorter reaction time. Bergmann and co-workers[17] prepared an acid chloride from 2 g. of carbobenzoxy-*l*-phenylalanine in fifteen minutes. Gulland, Partridge, and Randall[18] recently used the acid chloride of carbobenzoxy-*dl*-phenylalanine, but gave no preparative data.

N-Acetyl-β-phenyl-α-alanyl-β-phenyl-β-alanine Ethyl Ester.[15]—A solution of 1.2 g. (0.0063 mole) of the azlactone of N-acetyl-β-phenyl-α-alanine[7] in 8 ml. of anhydrous ether was added to a dry ether solution of the free ester of β-phenyl-β-alanine prepared from 1.87 g. (0.0082 mole) of its ester hydrochloride. The condensation product, which separated in a few minutes, was recrystallized from ethyl acetate; m. p. 195–196°; yield 46%. *Anal.* Calcd. for $C_{22}H_{26}O_4N_2$: C, 69.08; H, 6.86; N, 7.32. Found: C, 68.66; H, 6.87; N,[19] 7.12.

β-Phenyl-α-alanyl-β-phenyl-β-alanine.—The N-acetyl peptide ester described above was hydrolyzed by the method of Bergmann and co-workers.[7] In this case three and a half hours of boiling with hydrobromic acid were necessary to complete the reaction of 1.77 g. of the peptide ester. The hydrobromide of the free peptide, obtained in 67% yield, melted at 212–213° with decomposition

(12) Carbon and hydrogen analyses were done by Mr. Robert L. Peck of Yale University, and by the Arlington Laboratories, Chagrin Falls, Ohio.

(13) Unlike reactions described previously (ref. 1, p. 1698) this condensation cannot be effected in an aqueous alkaline medium.

(14) Ref. 1, p. 1698, and ref. 5, p. 1192.

(15) From the thesis of Elizabeth Stephey, presented to the Women's College of the University of Delaware in partial fulfillment of the requirements for the A.B. degree with distinction in chemistry, 1940.

(16) Ref. 5, p. 1198.

(17) Bergmann, Zervas, Rinke and Schleich, *Z. physiol. Chem.*, **224**, 37 (1934).

(18) Gulland, Partridge and Randall, *J. Chem. Soc.*, 419 (1940).

(19) Nitrogen in this compound could be determined by the Kjeldahl method only if the acid digestion was preceded by reduction with zinc dust and if the time of digestion was prolonged.

after recrystallization from glacial acetic acid. The hydrobromide was very hygroscopic.

Anal. Calcd. for $C_{18}H_{21}O_3N_2Br$: Br, 20.32. Found: Br, 19.75.

The free peptide was obtained from the hydrobromide by the usual silver carbonate–hydrogen sulfide method. After crystallization from a 1:2 methanol–water mixture, it melted at 232–233° with decomposition.

Anal. Calcd. for $C_{18}H_{20}O_3N_2$: C, 69.25; H, 6.41; N, 8.97. Found: C, 69.23; H, 6.41; N, 8.75.

β-Phenyl-β-alanine ethyl ester hydrochloride, prepared in quantitative yield by treatment of an absolute ethanol solution of the acid with hydrogen chloride,[20] separated from 1:4 absolute ethanol–ether solutions as silky needles, m. p. 137–138°.[21]

(20) Posner recorded the failure of this reaction with sulfuric acid as a catalyst [*Ber.*, **38**, 2321 (1905)].

(21) Houben and Pfankuch prepared this substance [*ibid.*, **59**, 1604 (1926)], but did not give the melting point.

Anal. Calcd. for $C_{11}H_{16}O_2NCl$: N, 6.10. Found: N, 6.02.

β-Phenyl-β-alanine Hydrobromide.—Crystals from glacial acetic acid, m. p. 182–183°.

Anal. Calcd. for $C_9H_{12}O_2NBr$: N, 5.69; Br, 32.48. Found: N, 5.65; Br,[22] 32.25.

Summary

Three dipeptides of β-phenyl-β-alanine are described. The synthesis of β-phenyl-β-alanylglycine and of β-phenyl-β-alanyl-β-phenyl-α-alanine was accomplished by the carbobenzoxy method, that of β-phenyl-α-alanyl-β-phenyl-β-alanine by the azlactone method. The instability of carbobenzoxy-β-phenyl-α-alanine has been noted.

(22) Analysis by Betty H. Whitenack.

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Porphyrin Studies. IV.[1] The Synthesis of α,β,γ,δ-Tetraphenylporphine

BY PAUL ROTHEMUND AND AMEL R. MENOTTI[2]

The subject of this report represents an application of the general reaction between pyrrole and aldehydes, reported some time ago.[3]

A systematic study of some of the many possibilities of this reaction is in progress in order to secure more information on the chemical properties of porphine and the substituted porphine ring system, especially in connection with the study of chlorophyll and photosynthesis.

In the preceding paper[1] a table was given summarizing some of the experimental results obtained. α,β,γ,δ-Tetraphenylporphine (Fig. 1), the synthesis of which is being reported here, was mentioned, and characterized by hydrochloric acid number 13.5, and 8.5, respectively.

When we condensed pyrrole and benzaldehyde, in pyridine solution, in sealed tubes at 220° for forty-eight hours, α,β,γ,δ-tetraphenylporphine $C_{44}H_{30}N_4$ was formed. It crystallized from the reaction mixture in beautiful lustrous deep-blue needles and had a hydrochloric acid number of 13.5.[4] If, however, pyrrole, benzaldehyde and pyridine were refluxed in methanol solution at atmospheric pressure for several days, two porphyrins were obtained. Their separation could be accomplished by fractionation of an ether solution with hydrochloric acid of different concentration, and yielded the above-mentioned α,β,γ,δ-tetraphenylporphine of hydrochloric acid number 13.5, and, in smaller amount, a porphyrin of hydrochloric acid number 8.5.

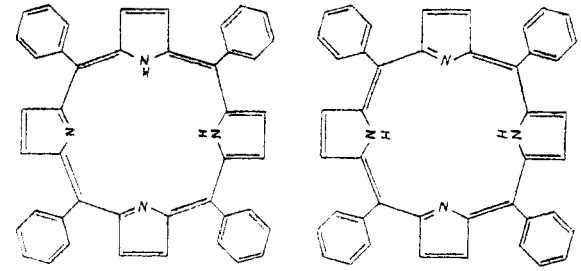

Fig. 1.—α,β,γ,δ-Tetraphenylporphine.

We are continuing the study of the porphyrin with the lower hydrochloric acid number and hope to report on our findings shortly. Figure 2 shows the great similarity of the absorption spectra of the two porphyrins and of their hydrochlorides. This property leads to the assumption that a close structural relationship exists between the two compounds, most likely isomerism in the porphine ring system as indicated in

(1) Paper III, THIS JOURNAL, **61**, 2912 (1939).

(2) From the dissertation submitted by Amel R. Menotti to the Faculty of the Graduate School of the Obio State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) Rothemund, THIS JOURNAL, **57**, 2010 (1935).

(4) Determination by the method of Willstätter in the improved form suggested by Fischer and Kirstahler, *Z. physiol. Chem.*, **198**, 47 (1931).