substances giving colored spots with ninhydrin were observed. Since 1 μg. or less of an amino acid could have been detected by this method, it would appear that no more than 0.2% (theoretical yield) of any amino acid was present.

Alkaline Fusion of Vitamin B_{12}.—Ten milligrams of vitamin B_{12} was ground in a mortar with 100 mg. of sodium hydroxide. The powdered mixture was placed in a tube, moistened with water, and the tube was heated slowly to 250°. The aqueous distillate gave a negative test with Ehrlich reagent. The bottom of the tube containing the alkali melt was then gently heated with a free flame. A small amount of distillate collected on the cool upper walls of the tube. The distillate was washed from the tube with a few drops of methanol and the solution treated with a drop of Ehrlich reagent (*p*-dimethylaminobenzaldehyde in ethanol) and a drop of concentrated hydrochloric acid. A deep red color developed immediately.

In another experiment, the fusion was carried out at reduced pressure and the distillate was collected in a dry-ice trap. The distillate gave a strong positive test with Ehrlich reagent, and when added dropwise to a 4% aqueous solution of mercuric chloride, precipitation took place immediately.

Acknowledgment.—The authors are indebted to Miss Janice Mayfield and Mr. John Valiant for technical assistance, to Mr. R. N. Boos and his associates for the microanalyses, and to Miss Muriel Caswell, Mr. John Messerly, and their colleagues for the microbiological assays. They wish to thank Mr. F. A. Bacher, Dr. J. B. Conn, Dr. Charles Rosenblum, and Dr. N. R. Trenner for their coöperation on some of the physical measurements reported here.

Summary

The vitamin B_{12} molecule possesses one cobalt and one phosphorus atom. An ebullioscopic molecular weight determination gave a value of 1490 ± 150. Its composition is typified by $C_{61-64}H_{86-92}N_{14}O_{13}PCo$, with $C_{62}H_{86-90}N_{14}O_{13}PCo$ and $C_{63}H_{88-92}N_{14}O_{13}PCo$ agreeing very well with the analytical data.

Vitamin B_{12} is levorotatory, and shows absorption maxima at 2780, 3610 and 5500 Å. which do not shift markedly with a change in *p*H.

Hydrolysis of vitamin B_{12} does not liberate α-amino acids; thus, the molecule is not a peptide. Alkali fusion of vitamin B_{12} forms products which react with *p*-dimethylaminobenzaldehyde, characteristic of certain cyclic five-membered nitrogen-containing compounds including pyrroles.

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[CONTRIBUTION FROM THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY, DEPARTMENT OF CHEMISTRY]

A New Synthetic Route to Peptides

BY JOHN C. SHEEHAN AND VICTOR S. FRANK[1]

Knowledge of protein structure gained through the synthesis and study of relatively simple peptides has contributed enormously to a better understanding of the chemical nature of toxins, antibodies, hormones, enzymes and viruses. With the recent discoveries of the peptide nature of certain antibiotic substances, including gramicidin,[2] tyrocidin[3] and diplococcin,[4] there is renewed interest in practical methods for the synthesis of peptides.

Excellent reviews of the early work on the chemistry of the peptides may be found in the collected papers of Fischer[5] and of Abderhalden.[6] Bergmann has edited a volume[7] in which the field was reviewed to 1923, and Greenstein has written a more recent survey[8] of peptide syntheses.

Most of the methods for peptide synthesis involve the protection of the amino group while the carboxyl function is converted to an acid chloride, anhydride, azide or ester for coupling with a second amino acid or peptide. Removal of the masking group completes the synthesis. The procedures of Fischer include the use of diketopiperazines,[9] α-haloacyl halides,[10] amino acid chloride hydrochlorides[11] and esters of peptides.[12] N-Carboxyamino acid anhydrides[13] were also employed. Bergmann's azlactone methods[14] permitted the synthesis of peptides containing tyrosine, arginine, histidine, glutamic acid and other amino acids more complex than Fischer was able to use. Blocking groups which could not be removed without destruction of the peptide include the benzoyl,[15] carbomethoxy[16] and carboethoxy.[10]

The introduction, in 1932, by Bergmann and Zervas[17] of the use of the carbobenzoxy group (removable by hydrogenolysis) in peptide synthesis made practical the preparation of a wide variety of peptides. Bergmann's procedure has been used very extensively by other workers, including Dunn, Fruton, Greenstein, Harington, du Vig-

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(2) Hotchkiss and Dubos, *J. Biol. Chem.*, **132**, 793 (1940).

(3) Hotchkiss, *ibid.*, **141**, 171 (1941); Cristensen, Edwards and Piersma, *ibid.*, **141**, 187 (1941).

(4) Oxford, *Biochem. J.*, **38**, 178 (1944).

(5) Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vol. I, Springer, Berlin, 1909.

(6) Abderhalden, "Neuere Ergebnisse auf dem Gebiete der Speziellen Eiweisschemie," Fischer, Jena, 1909.

(7) Bergmann, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vol. II, Springer, Berlin, 1923.

(8) Schmidt, "The Chemistry of Amino Acids and Proteins," C. C. Thomas, Springfield, Ill., 1945, pp. 252–333.

(9) Fischer and Fourneau, *Ber.*, **34**, 2868 (1901).

(10) Fischer and Otto, *ibid.*, **36**, 2106, 2982 (1903).

(11) Fischer, *ibid.*, **38**, 2914 (1905).

(12) Fischer, *ibid.*, **39**, 453, 3893 (1906).

(13) Sigmund and Wessely, *Z. physiol. Chem.*, **157**, 91 (1926).

(14) Bergmann, Stern and Witte, *Ann.*, **449**, 277 (1926).

(15) Curtius, *J. prakt. Chem.*, **26**, 175 (1882).

(16) Fischer, *Ber.*, **41**, 2860 (1908).

(17) Bergmann and Zervas, *ibid.*, **65**, 1192 (1932).

neaud and their associates. However, these investigators have experienced some practical difficulties which limit the usefulness of the method. Sulfur-containing peptides, for example, cannot be treated successfully in the usual way. Bergmann found that the carbobenzoxy group in such compounds could be removed by catalytic hydrogenolysis only with very large excess of palladium catalyst, and in poor yield. Alternate procedures for removing the blocking group under rather drastic conditions have been developed by du Vigneaud[18] and by Harington.[19] In the preparation of α-glutamylcysteylglycine (glutathione), Harington[19] and Mead used phosphonium iodide, and du Vigneaud and Miller[20] employed sodium and liquid ammonia to remove the carbobenzoxy group.

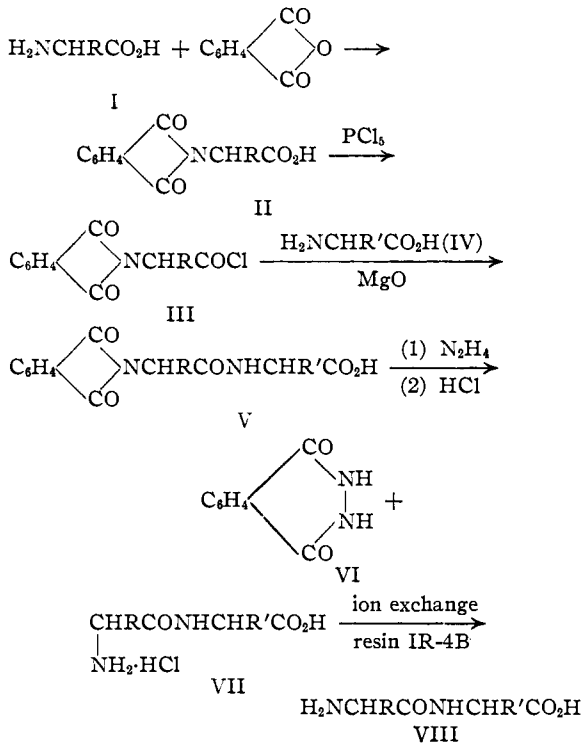
Another serious disadvantage of the Bergmann method is the strong tendency for carbobenzoxyamino acyl chlorides to decompose under mild conditions to the corresponding N-carboxy (Leuchs') anhydrides. In addition, many of the acid chlorides have been obtained only as oils. Carbobenzoxy amino acid derivatives are frequently difficult to crystallize.

Because of the marked instability of the chlorides, most workers have employed the corresponding acid azides. These are generally not isolated, but are prepared from the esters (by treatment with hydrazine followed by nitrous acid) and are used immediately to avoid losses due to rearrangement. A recent example of a peptide synthesis by this route is the preparation of L-leucylglycylglycine. The removal of the blocking group in this typical case required from one to six days for complete hydrogenolysis.[21]

Although the carbobenzoxy procedure has been the method of choice for fifteen years and has led to outstanding advances, it has become apparent that a new protecting scheme is desirable and perhaps even necessary for the synthesis of polypeptides of recent interest.

The phthalyl group has been utilized in a variety of cases as a masking group in the preparation of amines and of amino acids. Earlier methods for its removal involved prolonged heating with strong mineral acids, often preceded by hydrolysis with alkali. In 1926, Ing and Manske[22] reported an improved method for cleaving N-alkyl phthalimides by treatment with alcoholic hydrazine hydrate followed by hydrochloric acid. The final products were phthalhydrazide and the hydrochloride of the desired amine. Radenhausen[23] apparently was the first to observe this cleavage, although he did not identify both of the products. When phthalylglycine ester was allowed to react with one equivalent of hydrazine hydrate in alcohol, phthalhydrazide was obtained instead of the desired phthalimidoacetyl hydrazide. The solid nitrogenous by-product presumed by Radenhausen to be glycine ester was probably 2,5-diketopiperazine.

We have developed a new route for the synthesis of peptides,[24] utilizing the phthalyl protecting group and the Ing and Manske procedure for its removal. The method may be formulated as



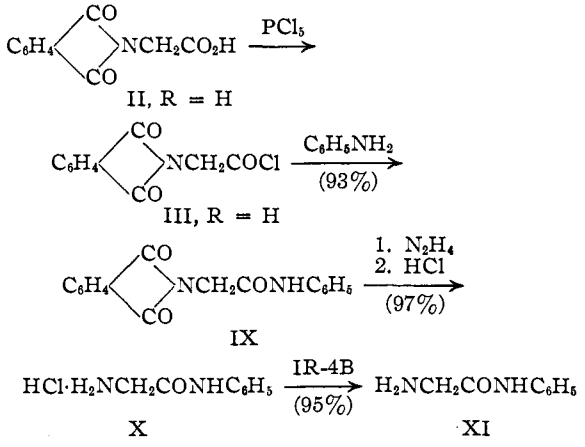
Barber and Wragg[25] made the interesting observation that the product of the reaction of an N-substituted phthalimide with hydrazine hydrate is an amine salt of phthalhydrazide. They did not obtain an intermediate substance of the type postulated by Ing and Manske.[22] Boiling with hydrochloric acid according to the usual procedure was unnecessary, since it was found possible to separate the desired amine from the salt by milder methods, including thermal dissociation, solvent extraction or basification. Mosher[26] had previously noted that the usual treatment with hydrochloric acid was unnecessarily strenuous.

It was found in this work that the action of hydrazine upon phthalylglycine anilide led to the direct formation of phthalhydrazide and glycine anilide (X); it was also found that glycine anilide

(18) Loring and du Vigneaud, *J. Biol. Chem.*, **111**, 385 (1935).
(19) Harington and Mead, *Biochem. J.*, **29**, 1604 (1935).
(20) du Vigneaud and Miller, *J. Biol. Chem.*, **116**, 469 (1936).
(21) Dunn, *et al.*, *J. Org. Chem.*, **12**, 490 (1947).
(22) Ing and Manske, *J. Chem. Soc.*, 2348 (1926).
(23) Radenhausen, *J. prakt. Chem.*, [2] **52**, 446 (1895).
(24) Since the preparation of this work for publication, a note (Kidd and King, *Nature*, **162**, 776 (1948)), has appeared which states, without giving experimental details, that phthalylglutamic acid anhydride was condensed with various amines, including amino acids, and that hydrazine was employed for removal of the phthalyl group from the product.
(25) Barber and Wragg, *Nature*, **158**, 514 (1946).
(26) Mosher, THIS JOURNAL, **68**, 1565 (1946).

was more readily obtained in high yield as the hydrochloride. Separation from phthalhydrazide was effected by mild digestion with dilute hydrochloric acid.

The preparation of glycine anilide was undertaken as a model peptide synthesis, in order to test the applicability of the hydrazine method of cleavage. For this purpose, phthalylglycine[27] (II, R = H) was converted *via* the acid chloride[28] (III, R = H) to the protected anilide (IX),[29] which was treated with hydrazine and then extracted with dilute hydrochloric acid. The hydrochloride (X) was formed in excellent yield. This is apparently the first instance in which the Ing and Manske procedure has been applied to a phthalimide containing an amide function.[24]



The free amine (XI) was best liberated from the hydrochloride by means of the ion-exchange resin IR-4B, which acts as an acid adsorber. Glycine anilide was obtained as the known dihydrate[30] in 95% yield by this procedure. This series of reactions constitutes a new synthesis for the anilide in 70% over-all yield from glycine. The method of Dubsky and Granacher[30] from chloroacetanilide was reported to give the product in 55% yield.

The transformation of glycine into glycylglycine by this route was accomplished as indicated (I–VIII). The intermediate phthalyl dipeptide (V, R and R′ = H) had previously been obtained from phthalic anhydride and glycylglycine for use in enzymatic studies.[31] In this investigation the phthalyl derivative was synthesized by a Schotten–Baumann type of acylation of glycine, using phthalylglycyl chloride[28] and magnesium oxide. The removal of the protecting group was effected by means of alcoholic hydrazine hydrate, and the desired peptide hydrochloride (VII, R and R′ = H) was obtained in high yield. The insensitivity of the peptide bond to the reagent under the conditions employed was demonstrated by allowing two moles to react with one of the pththalyl compound. In this case, as in the case of reactions carried out with equimolar quantities, glycylglycine hydrochloride was isolated in excellent yield. The free dipeptide was converted to the acetyl derivative[32] for identification. This five-step route resulted in a 60% yield based on glycine, compared with about 40% by Fischer's (five-step) method[9] *via* diketopiperazine. The preparation of glycylglycine by the carbobenzoxy method has not been reported. However, two of the four steps necessary have been carried out. Carbobenzoxyglycyl chloride has been obtained in 55% over-all yield from glycine, as an unstable low-melting compound.[17]

It was found that the use of sodium hydroxide in the aceylation reaction led to the formation of a product other than the desired phthalyl derivative. When phthalylglycyl chloride was added to a cold solution of glycine in aqueous sodium hydroxide, none of the compound (V, R and R′ = H) could be isolated from the reaction mixture. Reese[33] has reported that strong bases will open the phthalimide ring of phthalylglycine to give the salt of the corresponding phthalamic acid. Apparently the product obtained in this work was the analogous phthalamic acid derived from phthalylglycylglycine. This substance was converted to the desired compound in fair yield by heating it above its melting point for a short time.

The procedure developed for the synthesis of glycylglycine has been extended to the preparation of glycyl-DL-phenylalanine, glycyl-L(+)-cysteine, and DL-phenylalanylglycylglycine. The intermediate phthalyl derivative was not purified in the synthesis of the sulfur-containing peptide, but was treated with hydrazine to remove the blocking group, followed by sodium hydrosulfite in order to reduce the product completely to the sulfhydryl compound. The optically active dipeptide was isolated as the stable hydrochloride in 60% over-all yield. This dipeptide has been prepared recently by Cavallito[34] in unstated yield, using the carbobenzoxy procedure and the sodium–liquid ammonia technique for removing the protecting group. The corresponding cysteine peptide was prepared by Greenstein[35] in approximately 20% yield, employing the same process followed by oxidation of glycylcysteine which was not isolated.

Glycyl-DL-phenylalanine[36] was synthesized in the manner described for glycylglycine in 61% yield from DL-phenylalanine. The tripeptide[37] DL-phenylalanylglycylglycine, was prepared in a similar fashion. The over-all yield was 53% based on DL-phenylalanine.

(27) Drechsel, *J. prakt. Chem.*, [2] **27**, 418 (1883).
(28) Gabriel, *Ber.*, **40**, 2648 (1907).
(29) Scheiber, *ibid.*, **46**, 1103 (1913).
(30) Dubsky and Granacher, *ibid.*, **50**, 1703 (1917).
(31) Utzino, *J. Biochem.* (*Tokyo*), **9**, 453 (1928).
(32) Fischer and Otto, *Ber.*, **36**, 2115 (1903).
(33) Reese, *Ann.*, **242**, 1 (1881).
(34) Cavallito, *J. Biol. Chem.*, **164**, 30 (1946).
(35) Greenstein, *ibid.*, **128**, 241 (1939).
(36) Abderhalden and Schweitzer, *Fermentforschung*, **10**, 341 (1929).
(37) Fischer, *Ber.*, **37**, 3062 (1904).

The advantages of the phthalyl protecting group over the carbobenzoxy group in peptide synthesis are apparent in a step-by-step comparison of the two procedures. Phthalic anhydride is commercially available in pure crystalline form, while benzyl chloroformate[17] must be prepared from phosgene and is a liquid which cannot be purified. The recorded yields[27,38,39] of phthalyl amino acids (75–95%) are equal to or higher than those reported[17,40,41,18,19,20] for carbobenzoxy amino acids (70–90%). The phthalyl compounds are readily crystallized substances which have been recommended as derivatives for identification, but the carbobenzoxy amino acids are frequently difficult to obtain in crystallized form.

Carbobenzoxyamino acid chlorides are unstable (decomposing to the N-carboxyamino acid anhydrides), and several individuals in the series have been prepared only as oils (70–80%). However, phthalylamino acyl chlorides are easily obtained in 80–90% yields as stable, crystalline compounds. Carbobenzoxyamino acid azides (which are employed to circumvent the use of the chlorides) are prepared in three additional steps from the acids and are also subject to decomposition. Prolonged hydrogenation is frequently needed for removal of the carbobenzoxy groups,[21] but phthalyl groups are rapidly cleaved with hydrazine. Sulfur-containing compounds present no special difficulty in the removal of the phthalyl group with hydrazine, as they do in removal of the carbobenzoxy group by hydrogenation. No racemization is encountered with the use of the Bergmann procedure. The preparation of optically active glycyl L-(+)-cysteine by the phthalyl route offered no unusual difficulty. The conversion of optically active amino acids to the corresponding phthalyl derivatives without racemization has been reported.[42,30] It therefore seems likely that the phthalyl synthesis can be extended to cases in which an optically active amino acid derivative is the acylating agent, and preliminary experiments indicate this to be so. Over-all yields of dipeptides obtained by the carbobenzoxy procedure have ranged from 20–50%, while the three dipeptides prepared in this work were obtained in 60% yield.

We wish to express our appreciation to Swift and Company for the support of a fellowship for one of us (V. S. F.).

Experimental[43]

Phthalylglycyl Chloride (III, R = H).—The following procedure is a modification of that of Gabriel.[28] A suspension of 20.5 g. (0.1 mole) of phthalylglycine[27] and 20.8 g. (0.1 mole) of phosphorus pentachloride in 200 ml. of benzene was heated in a water-bath at 60°. The mixture was shaken occasionally until a clear solution was obtained (about thirty minutes). After heating for a total of two hours, the solution was concentrated under reduced pressure and the dry residue was crystallized from benzene and petroleum ether (30–60°). The yield was 18.1 g. (81%), m. p. 83–85° (Gabriel[28] reported 84–85°).

Glycine Anilide (XI).—A suspension of 2.80 g. (0.01 mole) of phthalylglycine anilide[29] in 100 ml. of alcohol containing 10 ml. of 1 *N* alcoholic hydrazine hydrate (0.01 mole) was heated under reflux for one hour. After concentration under reduced pressure, the dry residue was warmed to 50° with 50 ml. of approximately 2 *N* hydrochloric acid for five minutes, and allowed to cool to room temperature during thirty minutes. Phthalylhydrazide was removed by filtration, and evaporation of the solvent followed by recrystallization from alcohol yielded 1.96 g. of crude hydrochloride. A second recrystallization from alcohol gave 1.82 g. (97.3%) of pure glycine anilide hydrochloride, m. p. 192–195°.

Calcd. for $C_8H_{11}ON_2Cl$: neut. equiv., 186.6. Found: neut. equiv., 186.2.

A solution of 0.933 g. (0.005 mole) of the hydrochloride in approximately 100 ml. of water was passed slowly through a 12 × 250 mm. column packed with Amberlite IR-4B acid adsorbing resin.[44] The halogen-free effluent was concentrated under reduced pressure to a volume of about 20 ml., and the crystalline hydrate of glycine anilide was obtained in 93% yield (0.780 g.); m. p. 60–62° with softening from 55° (reported[30] m. p. 62°).

Glycine anilide was also obtained from the phthalyl derivative (0.24 g., 0.86 millimole) and hydrazine hydrate (0.86 ml. of 1 *M* alcoholic solution) by boiling in 50 ml. of alcohol for one hour followed by extraction with water. Evaporation of the alcohol and water afforded 0.098 g. (61.5%) of the anilide (XI), m. p. 60.5–62.0°. A mixture with the product prepared from the hydrochloride showed no depression in melting point.

Phthalylglycylglycine (V, R and R′ = H).—A solution of 4.47 g. (0.02 mole) of phthalylglycyl chloride in 25 ml. of dioxane was added dropwise during thirty minutes to a stirred, cooled (5°) suspension of 1.50 g. (0.02 mole) of glycine and 1.21 g. (0.03 mole) of magnesium oxide in 75 ml. of water. After stirring for an additional ten minutes at room temperature, the mixture was acidified with hydrochloric acid. A portion of the product precipitated and was removed by filtration; the remainder was obtained by evaporation of the filtrate under reduced pressure. The crude material (5.01 g.) was recrystallized from alcohol, giving a total yield of 89.5% in two crops: 4.32 g., m. p. 229–231°, and 0.63 g., m. p. 228–230° (reported[31] for phthalylglycylglycine, prepared from glycylglycine and phthalic anhydride, m. p. 232°).

Glycylglycine from Phthalylglycylglycine.—A suspension containing 2.62 g. (0.01 mole) of phthalylglycylglycine and 10 ml. of 1 *M* alcoholic hydrazine hydrate in 30 ml. of alcohol was heated under reflux for one hour. After evaporation, the dry residue was warmed to 50° for ten minutes with 25 ml. of approximately 2 *N* hydrochloric acid, and allowed to cool to room temperature during thirty minutes. After removing the phthalhydrazide by filtration, glycylglycine hydrochloride monohydrate[9] was obtained in nearly quantitative yield by concentrating the filtrate under reduced pressure. Recrystallization from alcohol afforded 1.73 g. (92.8%)[45] of material, which was dissolved in 100 ml. of water and converted to the free dipeptide by means of Amberlite IR-4B acid absorbing resin. The halogen-free effluent was concentrated to a volume of 20 ml., and, by adding alcohol to the hot solution, 1.07 g. (87%) of glycylglycine was obtained as colorless plates, which decomposed without melting at 215–222°. This weight represents an over-all yield of 72.6% from phthalylglycyl chloride. The N-acetyl derivative was prepared in good yield; m. p. 186–187°. A mixture with a sample

(38) Gabriel, *Ber.*, **40**, 634 (1907).

(39) Billman and Harting, THIS JOURNAL, **70**, 1437 (1948).

(40) Bergmann, Zervas and Salzmann, *Ber.*, **66**, 1288 (1933).

(41) Bergmann, Zervas and Ross, *J. Biol. Chem.*, **111**, 245 (1935).

(42) Fling, Minard and Fox, THIS JOURNAL, **69**, 2466 (1947); Reese, *Ann.*, **242**, 9 (1887).

(43) All melting points are corrected.

(44) This material was obtained from the Rohm and Haas Company, Philadelphia, Pennsylvania.

(45) A similar experiment in which the amount of hydrazine solution was doubled also gave a nearly theoretical yield.

prepared by acetylation of authentic glycylglycine[12] showed no depression in melting point (reported[46] for N-acetylglycylglycylglycine, m. p. 187–189°).

Acylation of Glycine with Phthalylglycyl Chloride and Sodium Hydroxide.—A solution of 11.2 g. (0.005 mole) of phthalylglycyl chloride in 50 ml. of dioxane was added dropwise to a stirred solution of 3.75 g. (0.05 mole) of glycine and 6.0 g. (0.15 mole) of sodium hydroxide in 50 ml. of water. The acid chloride (III, R = H) was added in thirty minutes to the ice-cold solution, after which stirring was continued for ten minutes at room temperature. The solution was acidified with hydrochloric acid and evaporated under reduced pressure. Repeated crystallizations of the crude product from alcohol and from isoamyl alcohol gave colorless needles, m. p. 189–192°, not identical with phthalylglycylglycine (m. p. 232°, mixed m. p. 141–158°) nor with phthalylglycine (m. p. 192°, mixed m. p. 145–160°). The crude product was combined with the solid residues obtained by evaporation of the mother liquors, and the combined residues were heated in an oil-bath at 190–195° for forty-five minutes. The product was recrystallized from alcohol, giving 9.6 g., m. p. 212–218°. A second recrystallization from alcohol yielded 8.35 g. (63.7%), m. p. 229–231°, undepressed when mixed with an authentic sample of phthalylglycylglycine.

Phthalylglycyl-DL-phenylalanine (V, R = H, R′ = $CH_2C_6H_5$).—The acid chloride (III, R = H) (4.48 g., 0.02 mole) was dissolved in 25 ml. of dioxane, and the solution was added dropwise in thirty minutes to an ice-cold, stirred suspension of 3.30 g. (0.02 mole) of DL-phenylalanine and 1.21 g. (0.03 mole) of magnesium oxide in 50 ml. of water. The solution was stirred for ten minutes at room temperature, and subsequent acidification, concentration and recrystallization from dioxane gave two crops of colorless needles; 4.11 g., m. p. 195–198°, and 1.31 g., m. p. 189–195° (total yield 77.0%). A sample was recrystallized twice from isoamyl alcohol for analysis, m. p. 197.0–198.5°.

Anal. Calcd. for $C_{19}H_{16}O_5N_2$: C, 64.76; H, 4.58; N, 7.95; neut. equiv., 352. Found: C, 64.49; H, 4.91; N, 7.77; neut. equiv., 348, 349.

Glycyl-DL-phenylalanine (V, R = H, R′ = $CH_2C_6H_5$).—The protecting group was removed from the phthalyl derivative in the manner described for phthalylglycylglycine. From 3.52 g. (0.01 mole) of phthalylglycyl-DL-phenylalanine there was obtained 1.58 g. (97.5%) of phthalhydrazide and 2.32 g. (90%) of the dipeptide hydrochloride. After recrystallization from alcohol the yield was 2.19 g. (84.8%).

Calcd. for $C_{11}H_{15}O_3N_2Cl$: neut. equiv., 258. Found: neut. equiv., 254, 255.

The free dipeptide was prepared from the hydrochloride (1.29 g., 5 millimoles) by means of the ion exchange resin IR-4B. The halogen-free product was recrystallized twice from aqueous alcohol, giving 0.97 g. (88.2%), m. p. 254–258° (dec.). Abderhalden, *et al.*,[36] reported m. p. 260° (dec.).

Glycyl-L(+)-cysteine Hydrochloride (V, R = H, R′ = CH_2SH).—A solution of 2.24 g. (0.01 mole) of the acid chloride (III, R = H) in 50 ml. of dioxane was added dropwise to a stirred, cold (5°) mixture containing 1.59 g. (0.01 mole) of L(+)-cysteine hydrochloride, 0.91 g. (0.0225 mole) of magnesium oxide and 100 ml. of water. By acidification and concentration under reduced pressure, 4.57 g. of colorless material was obtained. This was dissolved in 200 ml. of alcohol containing 10 ml. of 1 *M* alcoholic hydrazine hydrate (0.01 mole) and the solution was heated under reflux for one hour. The solvent was evaporated and the residue was treated with 20 ml. of 1.25 *N* hydrochloric acid for fifteen minutes at room temperature. The resulting suspension was filtered, and the residue was washed with 50 ml. of water. From the concentrated filtrate and washings there was obtained 0.73 g. of crystalline material, m. p. 195–196°. The mother liquors were concentrated to dryness and the residue was recrystallized from glacial acetic acid, giving 1.10 g., m. p. 195–196°. The crude dipeptide hydrochloride (1.83 g.) was dissolved in 30 ml. of ice-water, and 5% sodium bicarbonate solution was added to *p*H 7.5–8.0. Two grams of sodium hydrosulfite dihydrate was added in 20 ml. of water, with stirring. The filtered solution was concentrated in an atmosphere of carbon dioxide, and the residue was recrystallized from approximately 1 *M* alcoholic hydrogen chloride, yielding 1.58 g. of the hydrochloride (73.5% from cysteine hydrochloride); m. p. 91–96° (dec.), $[\alpha]^{25}D$ +2.4 (reported[32] $[\alpha]^{25}D$ +2.5).

Calcd. for $C_5H_{11}O_3N_2SCl$: neut. equiv., 215. Found: neut. equiv., 211.

Phthalyl-DL-phenylalanine (II, R = $CH_2C_6H_5$).—A mixture of 2.96 g. (0.02 mole) of phthalic anhydride and 3.30 g. (0.02 mole) of DL-phenylalanine was heated in an oil-bath at 170–180° for thirty minutes, cooled, and recrystallized from water. The product was obtained as colorless needles in 94.5% yield (5.57 g.), m. p. 177.5–179.0°. (Billman and Harting[39] report m. p. 174–175° (uncor.) for their product which they obtained in 79% yield.)

Phthalyl-DL-phenylalanyl Chloride (III, R = $CH_2C_6H_5$).—Phthalyl-DL-phenylalanine (2.36 g., 8 millimoles) and phosphorus pentachloride (1.67 g., 8 millimoles) were suspended in 80 ml. of dioxane. After heating on a steam-bath for five minutes a clear solution resulted, which was heated for an additional fifteen minutes and concentrated to dryness under reduced pressure. The residue was recrystallized from benzene, giving 2.12 g. (84%) of colorless needles, m. p. 121–124° (uncor.). The analytical sample was recrystallized from benzene–petroleum ether to a constant m. p. of 124–126°.

Anal. Calcd. for $C_{17}H_{12}O_3NCl$: Cl, 11.31. Found: Cl, 10.92.

Phthalyl-DL-phenylalanine Anilide.—A solution of 0.314 g. (1 millimole) of phthalyl-DL-phenylalanyl chloride in 10 ml. of dioxane was added dropwise to a suspension of 0.47 g. (5 millimoles) of aniline in 25 ml. of ice-water with agitation. The mixture was acidified and concentrated under reduced pressure, yielding 0.358 g. of yellowish needles, m. p. 212.5–213.8°. Recrystallization from alcohol gave 0.349 g. (94.3%) of colorless needles, m. p. 213.0–213.8°. A sample was recrystallized from isoamyl alcohol for analysis; m. p. 213.0–213.8°.

Anal. Calcd. for $C_{23}H_{18}O_3N_2$: C, 74.58; H, 4.90; N, 7.57. Found: C, 74.66; H, 5.01; N, 7.45.

Phthalyl-DL-phenylalanylglycylglycine.—Phthalyl-DL-phenylalanyl chloride (2.78 g., 8.85 millimoles) in 20 ml. of dioxane was added dropwise in thirty minutes to an ice-cold, agitated suspension of 1.5 g. (8.85 milimoles) of glycylglycine hydrochloride and 0.715 g. (17.7 millimoles) of magnesium oxide in 75 ml. of water. After stirring for ten minutes at room temperature, the mixture was cooled in an ice-bath and acidified with concentrated hydrochloric acid. The product was obtained as a crystallizable oil in two crops totalling 2.13 g. (68%), m. p. 241–245° (dec.). A third crop was obtained from the mother liquors by evaporation under reduced pressure; 0.27 g. (8.6%), m. p. 241–244° (dec.). The combined product was recrystallized from isoamyl alcohol, yielding 2.27 g. (72.5%), m. p. 243–245° (dec.). A portion was recrystallized twice to constant melting point from isoamyl alcohol, giving colorless needles, m. p. 244.0–245.5° (dec.).

Anal. Calcd. for $C_{21}H_{19}O_6N_3$: C, 61.57; H, 4.68; N, 10.27. Found: C, 61.32; H, 4.89; N, 10.09.

DL-Phenylalanylglycylglycine.—A mixture of 2.05 g. (5 millimoles) of the phthalyl tripeptide, 5 ml. of 1 *M* alcoholic hydrazine hydrate (5 millimoles) and 500 ml. of alcohol was heated under reflux for two hours. The solvent was removed under reduced pressure, and the residue was digested with 50 ml. of 2 *N* hydrochloric acid for five minutes at 40°. Phthalhydrazide (0.788 g., 97.5%) was removed by filtration. The filtrate was concentrated under reduced pressure and the product was recrystallized

(46) Fischer and Otto, *Ber.*, **36**, 2115 (1903).

from alcohol. The hydrochloride of the tripeptide was obtained in 94.2% yield (1.49 g.). A sample was recrystallized from alcohol, forming clusters of colorless needles.

Calcd. for $C_{13}H_{18}O_4N_3Cl$: neut. equiv., 316. Found: neut. equiv., 312.

A solution of 316 mg. (1 millimole) of the hydrochloride in approximately 200 ml. of water was passed slowly through a 12 × 250 mm. column packed with Amberlite IR-4B resin. From the chloride-free effluent 260 mg. (96.8%) of the tripeptide was recovered by evaporation; m. p., 221–223° (dec.) (Sigmund and Wessely[13] reported m. p. 225–230° (dec.)).

Summary

A new method for the synthesis of peptides has been devised in which the amino group of an amino acid is protected by formation of the phthalyl derivative. This is converted to the corresponding phthalimidoacyl chloride which is used to acylate a second amino acid or peptide. Removal of the protecting group from the resulting phthalyl peptide is effected by treatment with hydrazine hydrate under conditions which do not affect the peptide linkage.

Procedures are given for the synthesis in good yield of glycine anilide (70%), glycylglycine (60%), glycyl-DL-phenylalanine (61%), glycyl-L(+)-cysteine hydrochloride (60%) and DL-phenylalanylglycylglycine (53%).

The advantages of the phthalyl protecting group in peptide syntheses are discussed.

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NOTES

The Isoxazolines and Isoxazoles from Benzal-*p*-bromoacetophenone and *p*-Bromobenzalacetophenone

BY A. H. BLATT

In a study of the configuration and rearrangement of the oximes of α,β-unsaturated ketones published in THIS JOURNAL,[1] some attention was given to the formation of the isoxazoline (III) from benzal-*p*-bromoacetophenone (I) and its oximes (II), and to the formation of the isoxazole (VIII) from the dibromide of benzal-*p*-bromoacetophenone (IV) and its oxime (VII). The reactions involved are shown in diagram I.

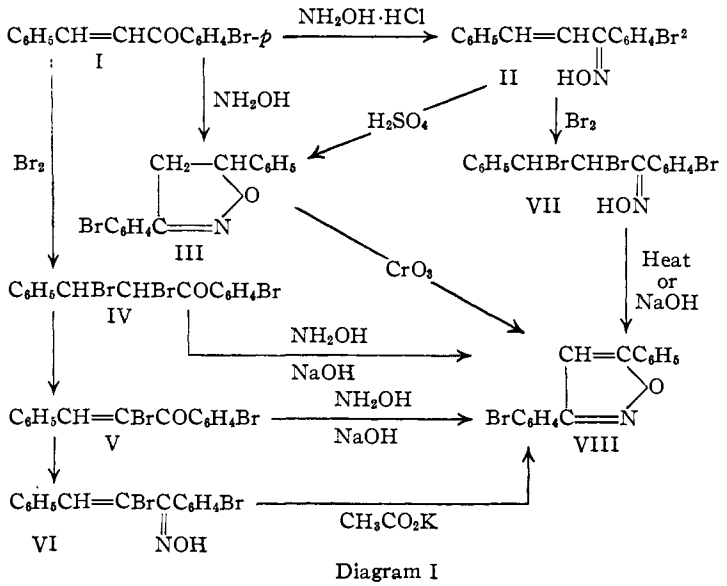

Diagram I

(1) Blatt, THIS JOURNAL, **53**, 1133 (1931); Blatt and Stone, *ibid.*, **53**, 4134 (1931).

(2) Only one of the pair of isomeric oximes is shown

At the time it was pointed out that the obvious and attractive mechanism for the formation of an isoxazoline from an α,β-unsaturated ketone and hydroxylamine was by a simple 1,4-addition of the reagent to the conjugated system followed by elimination of a molecule of water and the shift of a hydrogen atom from nitrogen to carbon. This mechanism could not be accepted, however, because it led to a structure for the isoxazoline that was contradicted by the chemical evidence. The 1,4-addition of hydroxylamine to benzal-*p*-bromoacetophenone would furnish the isoxazoline (X) below, in which the nitrogen atom is attached to what was the β-carbon atom of the conjugated system in the unsaturated ketone. The isoxazoline obtained, however, is III, above, with the nitrogen atom attached to what was the carbonyl carbon atom in the unsaturated ketone. This structure is established for the isoxazoline (III) by its formation from the oxime (II), and by its conversion to the isoxazole (VIII), whose structure in turn is established by its formation from the oximes (VI) and (VII).

It is necessary for us to reopen this old work because of the appearance of a set of papers by Barnes and his co-workers[3] which deals *inter alia* with the formation and structures of the isoxazolines and isoxazoles obtained from a number of

(3) (a) Barnes, Pierce and Cochrane, THIS JOURNAL, **62**, 1084 (1940); (b) Barnes and Cochrane, *ibid.*, **64**, 2262 (1942); (c) Barnes and Brandon, *ibid.*, **65**, 1070 (1943); (d) Barnes and Dodson, *ibid.*, **65**, 1585 (1943); (e) Barnes and Dodson, *ibid.*, **67**, 132 (1945); (f) Barnes and Spriggs, *ibid.*, **67**, 134 (1945); (g) Barnes and Snead, *ibid.*, **67**, 138 (1945); (h) Barnes, Pinkney and DaCosta, *ibid.*, **69**, 3129 (1947); (i) Barnes and Read, *ibid.*, **69**, 3132 (1947); (j) Barnes, Goodwin and Cotten, *ibid.*, **69**, 3135 (1947).