bath. A solution of 5.55 g. (0.24 mole) of sodium in 260 cc. of absolute alcohol was added slowly, with vigorous stirring, over a period of two hours. Precipitation of the sodium salt of IV and probably some sodium ethylate occurred to form a very thick slurry. This was stirred overnight, then filtered. The salt was washed twice with 125-cc. portions of ether, transferred to a separatory funnel, covered with 200 cc. of ether and acidified with 100 cc. of 10% hydrochloric acid. Vigorous shaking was continued until all the solid had disappeared. The ether layer was separated and the aqueous phase extracted with 100 cc. of ether. The ether solutions were combined and washed with two 200-cc. portions of water. Drying and concentration left 57.9 g. (91.6%) of crystalline IV. The identity of this material and that obtained by alkylation of ethyl nitroacetate was established by a mixed melting point.

DL-**Tryptophan.**—The reduction of 57.9 g. (0.221 mole) of IV was carried out in an Aminco rocking hydrogenator using 150 cc. of absolute alcohol and 6 g. of Raney nickel catalyst at 100° for one hour. The bomb was heated as rapidly as possible since we have found that a rapid reduction is necessary to avoid undesirable by-products and low yields. The catalyst was removed by filtration and to the filtrate was added 60 g. of 20% sodium hydroxide. The solution was allowed to stand overnight at room temperature. The *p*H was then adjusted to 5.9 with glacial acetic acid and crystalline material separated. After the mixture had stood in the ice-box overnight, the tryptophan was filtered and washed with water, alcohol and ether. The product was dried *in vacuo*. There was thus obtained 39.3 g. (87.1%) of white, crystalline *dl*-tryptophan; m. p. 265° (uncor., dec.).

Ethyl α-Carbethoxy-β-(3-indole)-propionate (VI).—Ethyl α-nitro-α-carbethoxy-β-(3-indole)-propionate (III), 16.72 g. (0.05 mole), in 50 cc. of absolute alcohol was reduced catalytically at 180 atm. and 100° in the presence of approximately 3 g. of Raney nickel. The observed hydrogen absorption was approximately 3.8 moles per mole of III. The oil which remained after filtration and concentration crystallized readily, representing a nearly quantitative yield of VI. An analytical sample was prepared by recrystallizing several times from 75% alcohol, m. p. 62.0–62.5° (uncor.).[5]

Anal. Calcd. for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.74; H, 6.52; N, 5.06.

Ethyl α-Hydroxyamino-α-carbethoxy-β-(3-indole)-propionate (VII).—To 3.34 g. (0.01 mole) of III in 25 cc. of glacial acetic acid was added 0.5 cc. of water and then 5 g. of zinc dust in small portions. The temperature was held below 45° during the addition. After forty minutes, the zinc and zinc acetate were removed by centrifugation and washed with glacial acetic acid. Concentration at reduced pressure was followed by partitioning of the crude between water and ether. The ether was washed with 5% sodium hydroxide, then with water. Drying and concentration left 1.80 g. (56%) of crystalline material. After several recrystallizations from alcohol the hydroxylamino ester melted at 131–132° (uncor.).

Anal. Calcd. for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.75. Found: C, 60.16, 59.75; H, 6.41, 6.09; N, 9.00, 9.08.

Acknowledgments.—The authors wish to thank Mr. Jack Richmond for assistance with a portion of the laboratory work, and Mr. Harold Emerson and his associates for some of the microanalyses.

Summary

1. A new synthesis of *dl*-tryptophan employing ethyl nitromalonate and gramine is reported.

2. An improved method for preparing and stabilizing esters of nitromalonic acid is described.

KALAMAZOO, MICHIGAN RECEIVED APRIL 27, 1949

[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH SCHOOL OF MEDICINE]

Some Peptides and Peptide Derivatives Containing Leucine and Alanine

BY W. J. POLGLASE AND EMIL L. SMITH

In order to investigate further the specificity of the enzyme, leucine aminopeptidase,[1] a number of new leucine compounds were required. For a study of the stereochemical specificity[2] of this enzyme, dipeptides containing L-leucine in combination with L- and D-alanine and β-alanine were prepared. We wish to report the synthesis of D-alanyl-L-leucinamide acetate, L-alanyl-L-leucinamide acetate, L-leucyl-L-alaninamide acetate, L-leucyl-D-alaninamide acetate and β-alanyl-L-leucinamide hydrochloride. The preparation of L-alanyl-L-leucine is given in detail since this compound has not been previously synthesized by the carbobenzoxy method. The preparation of L-leucyl-L-alanine and L-leucyl-D-alanine by the carbobenzoxy method has already been described by Bergmann and co-workers.[3] Some additional data on intermediate products in the synthesis of these two peptides have now been obtained and a synthesis from racemic alanine was accomplished.

It has been the practice of most workers when preparing a dipeptide containing a D-amino acid first to resolve a racemic mixture of the amino acid by the use of an optically active base. In the preparation of a dipeptide by the carbobenzoxy method it is often possible to obtain one or two crystalline intermediate compounds as well as the final crystalline dipeptide. Thus, if a carbobenzoxy-L-amino acid is coupled to a DL-amino acid ester there may be as many as three synthetic steps at which to effect separation of the resulting diastereoisomers. We have found this method particularly useful in the preparation of L-leucyl-D-alanine and L-leucyl-L-alanine. Thus, carbobenzoxy-L-leucine was coupled through the azide with the methyl ester of DL-alanine. Carbobenzoxy-L-leucyl-D-alanine methyl ester was readily crystallized from the mixture. The diastereoisomeric compound could not be crystallized from

(1) K. Linderstrøm-Lang, *Z. physiol. Chem.*, **182**, 151 (1929); E. L. Smith and M. Bergmann, *J. Biol. Chem.*, **153**, 627 (1944).

(2) E. L. Smith and W. J. Polglase, *Federation Proc.*, **8**, 252 (1949), and to be published.

(3) M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider and H. Schleich, *J. Biol. Chem.*, **109**, 325 (1935).

the mother liquor but after saponification with alkali, the resulting carbobenzoxy-L-leucyl-L-alanine was crystallized. Reduction by hydrogenation of the carbobenzoxy dipeptides gave the pure dipeptides in both instances. This procedure for the preparation of dipeptides containing a D-amino acid residue is being investigated further with other amino acids.

There appear to be few instances of the preparation of peptides by such methods. However, Behrens, Doherty and Bergmann[4] separated the products resulting from the reduction of acetyldehydrophenylalanyl-L-leucine; they obtained by differential crystallization acetyl-L-phenylalanyl-L-leucine and acetyl-D-phenylalanyl-L-leucine. Recently, Cook, Cox and Farmer[5] coupled *l*-α-bromisovaleryl chloride and DL-N-methylvaline; these products were converted to the hydroxy compounds and then to the lactones. The diastereoisomeric lactones were then separated chromatographically.

The D-alanine used in this work was prepared as described by Fischer.[6] The carbobenzoxy-D-alanine was obtained by an enzymatic resolution of carbobenzoxy-DL-alanine.[7] In the presence of aniline and cysteine-papain, the carbobenzoxy-L-alaninanilide is synthesized and crystallizes from solution; the carbobenzoxy-D-alanine is readily recovered from the mother liquor.[8] The procedure has successfully been used for the resolution of the DL mixtures of derivatives of several amino acids.[9]

Experimental

Preparation of Amino Acid Esters.—The amino acid was dissolved in ten times its weight of anhydrous methanol saturated with anhydrous hydrogen chloride at 0° and allowed to stand at 0° overnight. The reaction mixture was then concentrated to a sirup under reduced pressure and the concentration repeated several times after the addition of anhydrous methanol. The amino acid ester hydrochloride generally crystallized and was suspended in ether and collected by filtration. A solution of the free ester in the desired organic solvent was then prepared as described by Fischer.[10]

Coupling of Amino Acids.—Following the well-known method of Bergmann and Zervas,[7] the amino acid (or amino acid ester) required as the first moiety of the desired peptide was acylated with a 10% excess of carbobenzoxy chloride in the presence of two equivalents of alkali. The resulting compound was converted to the chloride (or azide) and allowed to react with the ester of the amino acid required as the second moiety. The carbobenzoxy dipeptide ester thus obtained was then saponified to the carbobenzoxy dipeptide or ammonolyzed to the carbobenzoxy dipeptide amide. Hydrogenation in the usual manner[7] of a methanolic solution of the carbobenzoxy compound in the presence of acetic acid and water and with palladium as catalyst gave the desired dipeptide or dipeptide amide acetate.

Carbobenzoxy-L-leucyl-D-alanine Methyl Ester.—This was prepared from D-alanine methyl ester hydrochloride and carbobenzoxy-L-leucine azide as described by Bergmann and co-workers,[3] m. p. 129–130°, which is the same as that previously given.[3] The optical rotation has not been reported; $[\alpha]^{27}D$ −1° (*c* 4, ethanol). The same product was obtained from carbobenzoxy-L-leucine azide and racemic alanine methyl ester hydrochloride. An ether solution of carbobenzoxy-L-leucine azide prepared from 15.6 g. of carbobenzoxy-L-leucine hydrazide[3] was added to an ether solution of DL-alanine methyl ester obtained from 5.0 g. of the hydrochloride. The mixture was allowed to stand at room temperature overnight in the hood. The ether solution was then washed with water, *N* hydrochloric acid, saturated bicarbonate and dried over sodium sulfate. The filtered solution was concentrated under reduced pressure to about 70 cc. whereupon the carbobenzoxy-L-leucyl-D-alanine methyl ester crystallized; yield 2.95 g.; m. p. 126–129°. This compound was recrystallized from ethyl acetate–petroleum ether; m. p. 129–130°, unchanged upon admixture with a specimen prepared with pure D-alanine; $[\alpha]^{21}D$ −1° (*c* 4, ethanol).

L-Leucyl-D-alanine.—This was prepared as previously described[3] by saponification of 2.02 g. of carbobenzoxy-L-leucyl-D-alanine methyl ester and reduction of the sirupy carbobenzoxy dipeptide; yield 0.9 g. The peptide was recrystallized from methanol–ethyl acetate; yield 0.7 g.; $[\alpha]^{27}D$ +80° (*c* 1, water). Bergmann, *et al.*,[3] give $[\alpha]^{22}D$ +76.0 (*c* 2.5, water).

Anal. Calcd. for $C_9H_{18}O_3N_2$: N, 13.86. Found: N, 13.72.

Carbobenzoxy-L-leucyl-D-alaninamide.—Carbobenzoxy-L-leucyl-D-alanine methyl ester, 2.20 g., was dissolved in 30 cc. of methanol previously saturated at 0° with ammonia. After forty hours in a pressure bottle at room temperature, the solution was concentrated under reduced pressure and the amide crystallized. The product was recrystallized from ethanol–ether; needles, yield 1.65 g.; m. p. 181–182°; $[\alpha]^{22}D$ −6° (*c* 1, ethanol).

Anal. Calcd. for $C_{17}H_{25}O_4N_3$: N, 12.5. Found: N, 12.2.

L-Leucyl-D-alaninamide Acetate.—This was obtained by hydrogenation over palladium of 1 g. of the carbobenzoxy derivative in 20 cc. of methanol containing 1 cc. of acetic acid and 1 cc. of water. The reduction was complete in two hours, the catalyst was removed by filtration and the filtrate concentrated under reduced pressure. The product crystallized after repeated concentration with anhydrous methanol and was recrystallized from methanol–ethyl acetate: needles, yield 0.72 g.; m. p. 250–255° (dec.); $[\alpha]^{22}D$ +61° (*c* 1, water).

Anal. Calcd. for $C_{11}H_{23}O_4N_3$: N, 16.1. Found: N, 16.0.

Carbobenzoxy-L-leucyl-L-alanine Methyl Ester.—The synthesis of this compound has been described[3] (m. p. 92–93°) but the optical rotation was not reported. We observed m. p. 95–96°; $[\alpha]^{22}D$ −38° (*c* 1, ethanol).

Carbobenzoxy-L-leucyl-L-alanine.—This compound was previously described as a sirup. It was prepared as described[3] by saponification of the methyl ester. The compound crystallized at room temperature in large dodecahedra from a mixture of ethyl acetate, ethyl ether and petroleum ether (boiling range 65–110°): yield quantitative; m. p. 152–153°, $[\alpha]^{27}D$ −25° (*c* 1, ethanol).

Anal. Calcd. for $C_{17}H_{24}O_5N_2$: C, 60.7; H, 7.2; N, 8.33. Found: C, 60.4; H, 7.2; N, 8.44.

This compound was also obtained from the mother liquor of the carbobenzoxy-L-leucyl-D-alanine methyl ester resulting from coupling carbobenzoxy-L-leucine azide with racemic alanine ester. The mother liquor was concentrated to a sirup, dissolved in 20 cc. of acetone and saponi-

(4) O. K. Behrens, D. G. Doherty and M. Bergmann, *J. Biol. Chem.*, **136**, 61 (1940).

(5) A. H. Cook, S. F. Cox and T. H. Farmer, *Nature*, **162**, 61 (1948).

(6) E. Fischer, *Ber.*, **34**, 245 (1899).

(7) M. Bergmann and L. Zervas, *ibid.*, **65**, 1192 (1932).

(8) We are grateful to Dr. William H. Stein of the Rockefeller Institute for Medical Research for the preparation of carbobenzoxy-D-alanine and for his courtesy in permitting us to publish his method of preparation of this compound.

(9) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937); J. S. Fruton, G. W. Irving, Jr., and M. Bergmann, *ibid.*, **133**, 703 (1940); C. A. Dekker and J. S. Fruton, *ibid.*, **173**, 471 (1948); H. T. Hanson and E. L. Smith, *ibid.*, **179**, 815 (1949).

(10) E. Fischer, *Ber.*, **34**, 433 (1907).

fied with 11 cc. of *N* sodium hydroxide. After forty-five minutes at room temperature the solution was acidified and the acetone removed under reduced pressure. The resulting sirup was extracted into ethyl acetate and this solution extracted with 25 cc. of a saturated solution of sodium bicarbonate. The sodium bicarbonate solution was acidified and extracted with ethyl acetate and this extract dried with sodium sulfate. The ethyl acetate solution was concentrated to a sirup and the product crystallized from ethyl acetate, ethyl ether and petroleum ether (boiling range 65–110°); dodecahedra, yield 0.7 g., m. p. 152–153°, unchanged on admixture with a specimen prepared with L-alanine; $[\alpha]^{25}D$ −25° (*c* 1, ethanol).

L-Leucyl-L-alanine.—This was prepared as described by Bergmann and co-workers,[3] except that the above crystalline compound was the starting material. Three grams of carbobenzoxy-L-leucyl-L-alanine yielded 1.75 g. of the dipeptide. The compound was recrystallized from methanol–ethyl acetate. Fischer (see[3]) recrystallized this dipeptide by dissolving it in a large volume of hot absolute ethanol and collecting successive crops of crystals. We found that the methanol–ethyl acetate mixture gave higher yields of material having the required optical rotation. The dipeptide was dried *in vacuo* at 50°; $[\alpha]^{25}D$ + 22.9° (*c* 5, methanol) identical with that previously found.

Carbobenzoxy-L-leucyl-L-alaninamide.—This was prepared from 2.04 g. of the ester and methanol previously saturated with ammonia at 0°. After forty hours at room temperature in a pressure bottle, the solution was concentrated under reduced pressure to a crystalline mass. This was suspended in ether and filtered; needles, yield 1.76 g. The compound was recrystallized from ethanol–water; m. p. 189°, $[\alpha]^{27}D$ − 26° (*c* 0.6, ethanol).

Anal. Calcd. for $C_{17}H_{25}O_4N_3$: C, 60.9; H, 7.5; N, 12.5. Found: C, 60.9; H, 7.3; N, 12.6.

L-Leucyl-L-alaninamide Acetate.—This was prepared by hydrogenation of 1.03 g. of carbobenzoxy-L-leucyl-L-alaninamide. The reduction was complete in ninety minutes; needles, yield 0.65 g.; m. p. 250–255° (dec.); $[\alpha]^{21}D$ + 4° (*c* 1, water). The compound was dried *in vacuo* at 50°.

Anal. Calcd. for $C_{11}H_{23}O_4N_3$: C, 50.6; H, 8.9; N, 16.1. Found: C, 51.2; H, 9.0; N, 15.9.

Enzymatic Preparation of Carbobenzoxy-D-alanine.[8]—Commercial papain, 10.2 g., was stirred for thirty minutes with 255 cc. of water and filtered. Two hundred cc. of the filtrate was added to a mixture of 112 g. of carbobenzoxy-DL-alanine,[7] 135 cc. of 2 *N* sodium hydroxide, 46 cc. of aniline, 3.0 g. of cysteine and 200 cc. of citrate buffer, *p*H 5.0. The volume of the mixture was adjusted to 1,000 cc. by the addition of water and then incubated for seven days at 40°. The mixture was cooled to 0°, filtered and the precipitate washed twice with 250-cc. quantities of water. The filtrate was boiled and filtered and this filtrate was acidified with 100 cc. of 20% hydrochloric acid. The acidic solution was extracted with ether and the ether solution was extracted with saturated sodium bicarbonate solution. This solution was acidified and extracted with ether, the ether solution was washed with water and dried over sodium sulfate. The solution was then concentrated under reduced pressure to a sirup which crystallized on standing *in vacuo*. The crystalline residue was tritrated at 0° with 200 cc. of 2 *N* hydrochloric acid, filtered and the product dried in air; yield 49.5 g. For recrystallization, the product was dissolved in 200 cc. of ethyl ether and 600 cc. of light petroleum ether was added slowly, in portions. The carbobenzoxy-D-alanine was collected by filtration; m. p. 84–86°, $[\alpha]^{28}D$ + 14.0° (*c* 9, acetic acid). The constants reported by Bergmann and Zervas for carbobenzoxy-L-alanine[7] are: m. p. 84°; $[\alpha]^{17}D$ −14.3° (*c* 9, acetic acid). Hunt and du Vigneaud[11] give $[\alpha]^{27}D$ −13.9° and + 14.0 for the L and D derivatives.

Carbobenzoxy-D-alanyl-L-leucine Methyl Ester.—Carbobenzoxy-D-alanine, 5.0 g., was dissolved in 20 cc. of anhydrous ethyl ether and shaken at 0° with 5.0 g. of phosphorus pentachloride until all but a trace of the latter had dissolved. Light petroleum ether, previously cooled to −50° (*cf.* 11) was added and the oil which precipitated was washed three times with petroleum ether. The oil was then dissolved in 30 cc. of chloroform, previously cooled to −50°. This solution was added to a solution of L-leucine methyl ester (from 8.2 g. of the hydrochloride) in 30 cc. of chloroform. After thirty minutes at 0° and one hour at room temperature, the chloroform solution was washed with water, saturated sodium bicarbonate and *N* hydrochloric acid. The chloroform layer was dried over sodium sulfate and concentrated to a sirup which crystallized upon addition of petroleum ether; yield 5.7 g. The product was recrystallized from ethyl ether–petroleum ether (boiling range 30–60°); m. p. 72–73°; $[\alpha]^{27}D$ −9° (*c* 1, ethanol).

Anal. Calcd. for $C_{18}H_{26}O_5N_2$: N, 8.0. Found: N, 7.9.

Carbobenzoxy-D-alanyl-L-leucinamide.—This was prepared at room temperature by ammonolysis for forty hours with saturated methanol–ammonia of 3.75 g. of the corresponding ester. The amide crystallized upon concentration of the reaction mixture under reduced pressure; needles, yield 2.84 g. The product was recrystallized from ethanol–water; m. p. 187–188°; $[\alpha]^{30}D$ − 6° (*c* 1, ethanol).

Anal. Calcd. for $C_{17}H_{25}O_4N_3$: N, 12.5. Found: N, 12.5.

D-Alanyl-L-leucinamide Acetate.—This was obtained by hydrogenation of 2.25 g. of carbobenzoxy-D-alanyl-L-leucinamide. It crystallized in needles after repeated concentration of the filtered reaction mixture with ethanol; yield 1.61 g. The compound melted at 147°, immediately re-solidified and melted with decomposition at 249–250°; $[\alpha]^{26}D$ − 35° (*c* 1.5, water). The compound was dried *in vacuo* at 25°.

Anal. Calcd. for $C_{11}H_{23}O_4N_3$: C, 50.6; H, 8.9; N, 16.1. Found: C, 50.6; H, 8.9; N, 16.1.

Carbobenzoxy-L-alanyl-L-leucinamide.—Carbobenzoxy-L-alanine, 11.1 g., was converted to the acid chloride and dissolved in chloroform (75 cc.) as described above in the preparation of carbobenzoxy-D-alanyl-L-leucine methyl ester. The chloroform solution was added to a chloroform solution of leucine methyl ester obtained from 9.1 g. of the hydrochloride. After about five minutes, potassium bicarbonate, 5 g. in water, was added and the mixture shaken at frequent intervals. After one hour, the chloroform layer was washed with dilute hydrochloric acid and with water, dried over sodium sulfate and concentrated under reduced pressure to a sirup. The entire product was converted to the amide with saturated methanol–ammonia. After forty hours the solution was concentrated under reduced pressure and the amide crystallized: needles, yield 4.3 g.; m. p. 185–187°. The product was recrystallized from ethanol–water, m. p. 188–189°; $[\alpha]^{30}D$ − 41° (*c* 1, ethanol).

Anal. Calcd. for $C_{17}H_{25}O_4N_3$: N, 12.5. Found: N, 12.6.

L-Alanyl-L-leucinamide Acetate.—This was prepared by hydrogenation of 3.00 g. of carbobenzoxy-L-alanyl-L-leucinamide; needles, yield 2.32 g. The compound was dried at 25° *in vacuo*. It softened at 158° and gradually darkened on further heating, melting at 250°; $[\alpha]^{28}D$ −9° (*c* 2, water).

Anal. Calcd. for $C_{11}H_{23}O_4N_3$: C, 50.6; H, 8.9; N, 16.1. Found: C, 50.9; H, 9.2; N, 16.0.

L-Alanyl-L-leucine.—This compound has been prepared by Fischer[12] but its synthesis by the carbobenzoxy method has not been reported. Carbobenzoxy-L-alanine, 22.3 g., was coupled with leucine methyl ester (from 20 g. of the hydrochloride) as described above in the preparation of carbobenzoxy-L-alanyl-L-leucinamide. The resulting sirupy carbobenzoxy-L-alanyl-L-leucine methyl ester was saponified in 150 cc. of acetone and 45 cc. of *N* sodium

(11) M. Hunt and V. du Vigneaud, *J. Biol. Chem.*, **124**, 699 (1938).

(12) E. Fischer, *Ber.*, **40**, 1754 (1907).

hydroxide. After forty-five minutes at room temperature, the solution was neutralized with *N* hydrochloric acid and the acetone removed by distillation under reduced pressure. The water insoluble sirup was taken into ethyl acetate, and the solution dried and concentrated. The resulting sirup was hydrogenated in 60 cc. of methanol containing 4 cc. of acetic acid and 4 cc. of water with palladium catalyst. The reduction was complete after forty-eight hours. The catalyst was removed by filtration, the filtrate concentrated under reduced pressure to a sirup from which the acetic acid and water were removed by repeated concentration with anhydrous methanol. The product was crystallized from methanol–ether and dried at 75° *in vacuo:* yield 2.60 g.; $[\alpha]^{21}D$ − 17.0° (*c* 5, water). Fischer[12] found $[\alpha]^{20}D$ − 17.2 and − 16.8°.

Carbobenzoxy-β-alanyl-L-leucinamide.—Carbobenzoxy-β-alanine,[13] 6.65 g., in anhydrous ethyl ether, 25 cc., was converted to the acid chloride as previously described[13] and was added to an ether solution of L-leucine methyl ester prepared from 5.9 g. of the hydrochloride. After a few minutes at 0°, 3.5 g. of potassium bicarbonate in water was added and the mixture shaken frequently for an hour during which time the mixture was allowed to come to room temperature. The ether layer was separated and washed with dilute hydrochloric acid and with water. The solution was dried over sodium sulfate and then concentrated under reduced pressure to a sirup. The sirupy product was converted to the amide as described above; needles, yield 5.70 g., m. p. 172–173°. The amide was recrystallized from ethanol–water, m. p. 174–175°.

Anal. Calcd. for $C_{17}H_{25}O_4N_3$: C, 60.9; H, 7.5; N, 12.5. Found: C, 61.0; H, 7.2; N, 12.5.

(13) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

β-Alanyl-L-leucinamide Hydrochloride.—This was prepared by hydrogenation of 2.50 g. of carbobenzoxy-β-alanyl-L-leucinamide. The reduction was complete in three hours and the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to a sirup and this procedure repeated several times after the addition of successive small portions of ethanol. Since the product failed to crystallize as the acetate, the sirup was dissolved in 30 cc. of ethanol and 1 cc. of concentrated hydrochloric acid was added. The compound crystallized as the hydrochloride upon the addition of ether as plates; yield after drying *in vacuo* over sulfuric acid at 25°, 1.55 g.; m. p. 120° with evolution of gas; hygroscopic, $[\alpha]^{26}D$ − 17° (*c* 2, water).

Anal. Calcd. for $C_9H_{20}O_2N_3Cl$: C, 45.5; H, 8.5; N, 17.7. Found: C, 45.3; H, 8.6; N, 17.6.

Acknowledgment.—This investigation was aided by grants from the United States Public Health Service.

Summary

1. The preparation and properties of some dipeptides and dipeptide derivatives containing L-leucine, D- and L-α-alanine and β-alanine are described.

2. L-Leucyl-D-alanine and L-leucyl-L-alanine have been synthesized from L-leucine and racemic alanine.

3. The enzymatic preparation of carbobenzoxy-D-alanine from carbobenzoxy-DL-alanine is described.

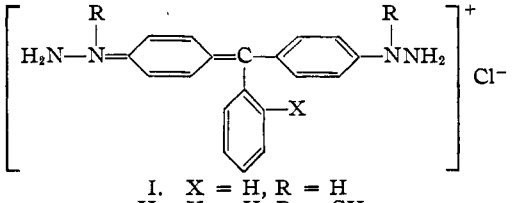
SALT LAKE CITY, UTAH RECEIVED APRIL 30, 1949

[CONTRIBUTION FROM THE BALLISTIC RESEARCH LABORATORIES

Triphenylmethane Dyes Containing the Hydrazine Group and Their Condensation Products with Aldehydes

BY LESTER P. KUHN AND LOUIS DEANGELIS

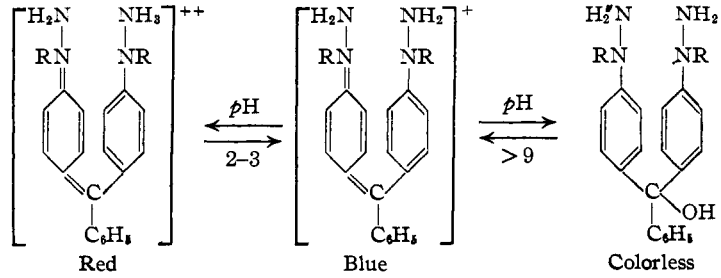
In order to obtain an improved reagent for the determination of aldehydes we sought to prepare a



I. X = H, R = H
II. X = H, R = CH_3
III. X = SO_3^-, R = CH_3

dye which contains a functional group capable of reacting readily with aldehydes and which as a result of its reaction would change color. Schwarzenbach[1] prepared N,N′ substituted phenylhydrazine sulfonphthalein dyes and showed that they are deeply colored stable compounds quite similar in properties to the aniline sulfonphthaleins. More recently *p,p′*-dihydrazinotriphenylmethyl chloride, I, and its N-methyl derivative, II, have been prepared.[2] It was shown that these dyes react readily with aldehydes, but not with ketones, and that the color of the dye solution changes from red to blue or to green. In the present work the preparation of I and II has been repeated, and the new dye III has been prepared. An attempt has been made to further evaluate these compounds as reagents for the determination of aldehydes and also to explain the color changes which are observed.

The dyes I, II and III can exist in several different states depending upon the *p*H of the solution in the manner shown here.



(1) Schwarzenbach, *Helv. Chim. Acta*, **20**, 498 (1937).

(2) Ciusa and Ottolino, *Gazz. chim. ital.*, **75**, 171 (1945); *C. A.*, **41**, 4137 (1947).