nitrile in chloroform (dried by distillation; alcohol not removed) for 1.5 hours. There was heat evolution. Concentration gave 17.6 g. of solid which became pink on exposure to air. The product was stirred with water containing enough ammonium hydroxide to make the water phase basic. This was filtered and washed with acetone to remove the now yellow color. The product was dissolved in hydrochloric acid and reprecipitated with ammonium hydroxide, m.p. 234–236°.

Anal. Calcd. for C_8H_9NO: C, 64.86; H, 8.17; N, 12.61. Found: C, 64.75; H, 8.17; N(K), 12.41; N(AP), 6.26 (sharp end-point).

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The Application of *p*-Nitrobenzyl Chloroformate to Peptide Synthesis

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The preparation of crystalline carbo-*p*-nitrobenzyloxy derivatives of several amino acids, through the use of the reagent, *p*-nitrobenzyl chloroformate is described. These crystalline derivatives include those of the amino acids for which crystalline carbobenzoxy derivatives have not yet been reported. The application of this reagent to peptide synthesis through preparation of glycyl-L-leucine and L-leucyl-L-leucine is reported.

Since the introduction of benzyl chloroformate (carbobenzoxy chloride) by Bergmann and Zervas[1] as a reagent for peptide synthesis, a large number of peptides have been prepared by methods which have made use of this reagent. The wide applicability of the carbobenzoxy method is now well established.[2] One disadvantage of the carbobenzoxy method arises from the benzyl chloroformate. The sirupy reagent, which is generally stored in toluene solution in the cold, gradually decomposes over a period of a few months and as a result there is some uncertainty as to the amount of active reagent present at any one time. A second disadvantage of the method arises from the difficulty experienced in crystallizing certain derivatives. The carbobenzoxy derivatives of DL- or L-hydroxyproline, DL- or L-proline, L-leucine and DL- or L-isoleucine have not been crystallized as yet. In attempts to surmount these difficulties recent modifications of the method have been introduced. These include the use of the carboallyloxy group[3] and of the *p*-bromobenzyloxy group[4] in peptide synthesis.

We have investigated the use of *p*-nitrobenzyl chloroformate in the preparation of carbo-*p*-nitrobenzyloxy derivatives of amino acids and peptides. It contains a nitro group which makes possible the detection of the derivatives in very small amounts by measurement of the ultraviolet absorption at about 265 mμ. The reagent[5] has the further advantage of being a stable, low melting crystalline solid. One preparation of the reagent was stored in a desiccator over phosphorus pentoxide in the cold for over a year, during which time numerous samples were withdrawn. No change in the melting point or other evidence of deterioration of the reagent was detected. The presence in the reagent of a nitro group would be expected to enhance the ease of crystallization of the derivatives prepared from this reagent. This was indeed the case and all derivatives prepared so far, including the carbo-*p*-nitrobenzyloxy derivatives of L-proline, hydroxy-L-proline, L-leucine and DL- and L-isoleucine which yield oils as the carbobenzoxy derivatives, have been obtained in crystalline form with no difficulty. The carbo-*p*-nitrobenzyloxy group was readily removed by hydrogenolysis.

Thiele and Dent[5] reported that phosgene does not react with *p*-nitrobenzyl alcohol in the cold and consequently prepared *p*-nitrobenzyl chloroformate in a sealed tube at 60–65°, using chloroform as a solvent. However, it was found that by using dioxane as a solvent and by permitting the reaction to proceed overnight, the reagent could be prepared in 95% yield at room temperature.

The carbo-*p*-nitrobenzyloxy derivatives of the amino acids were prepared in the usual manner.[1] In a preliminary study using glycine as a model it was found that a ratio of 1.25 moles of *p*-nitrobenzyl chloroformate to one of the amino acid gave the best yield. This ratio was used, therefore, in the preparation of the remainder of the derivatives described below.

It was of interest to discover if the carbo-*p*-nitrobenzyloxy group could be used in peptide synthesis with the same ease as the carbobenzoxy group. With this view in mind, glycyl-L-leucine and L-leucyl-L-leucine were prepared in good yield using the carbo-*p*-nitrobenzyloxy group to mask the amino group. These peptides were prepared through the reaction of the acid chlorides of carbo-*p*-nitrobenzyloxyglycine and carbo-*p*-nitrobenzyloxy-L-leucine, respectively, with L-leucine methyl ester. The resulting esters were saponified and the carbo-*p*-nitrobenzyloxy group was removed by hydrogenolysis. No racemization was observed and all the intermediate carbo-*p*-nitrobenzyloxy derivatives were readily obtained in crystalline form. These results demonstrate the applicability of this reagent to peptide synthesis.

It should be pointed out that although introduction of the nitro group into the reagent gives rise to certain favorable properties for peptide synthesis it also elicits certain disadvantages that are not present in the original carbobenzoxy chloride reagent. The presence of the nitro group precludes the use of the Kjeldahl method for nitrogen determination. In addition the presumed formation of *p*-toluidine

(1) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(2) J. Fruton, "Advances in Protein Chemistry," Vol. 5, ed. by M. Anson, J. Edsall and K. Bailey, Academic Press, Inc., New York, N. Y., 1949, p. 1.

(3) C. M. Stevens and R. Watanabe, THIS JOURNAL, **72**, 725 (1950).

(4) D. M. Channing, F. B. Turner and G. T. Young, *Nature*, **167**, 487 (1951).

(5) First reported by J. Thiele and F. Dent, *Ann.*, **302**, 258 (1898).

on reduction instead of the volatile and inert toluene may in some cases lead to difficulties, although in investigations performed so far, the *p*-toluidine has been readily removed as the slightly volatile acetate.

In the course of the preparation of carbo-*p*-nitrobenzyloxy derivatives of amino acids, especially when a large excess of the reagent was used, it was found that a crystalline by-product formed in the reaction mixture. This by-product was found to be di-(*p*-nitrobenzyl) carbonate, and was probably formed by coupling of the reagent with *p*-nitrobenzyl alcohol. The latter may have arisen from the hydrolysis of sodium *p*-nitrobenzyl carbonate resulting from the interaction of the reagent with alkali. Since the di-(*p*-nitrobenzyl) carbonate was insoluble in the alkaline reaction mixture, it was readily removed by filtration and did not interfere in the isolation of any of the derivatives prepared so far. Wolf and Seligman[6] have reported the presence of dinaphthyl carbonate as a by-product in the preparation of carbonaphthoxy derivatives of amino acids. When *p*-nitrobenzyl chloroformate was treated with alkali under the same conditions as those used in the preparation of the derivatives, di-(*p*-nitrobenzyl) carbonate was the chief product.

Experimental[7]

p-Nitrobenzyl Chloroformate.—Phosgene was bubbled into 180 ml. of cold, purified[8] dioxane until 174 g. had been absorbed. The stream of phosgene was passed successively through a dry trap, the dioxane, a second dry trap, a trap of cold toluene and finally a gas trap.[9] *p*-Nitrobenzyl alcohol (60 g., 0.39 mole) was dissolved in 75 ml. of dioxane with slight warming and this solution was added to the phosgene solution. The flask was stoppered with a cork and the reaction was allowed to proceed at room temperature overnight. The excess phosgene, the hydrogen chloride formed and the dioxane were distilled *in vacuo* (water-pump) with warming (the temperature of the bath was kept below 50°). Fresh dioxane was added and removed by vacuum distillation. This operation was repeated several times in order to remove the last traces of phosgene. The whole operation was carried out cautiously in a well-ventilated hood. The oily residue was dissolved in 120 ml. of toluene and the solution was cooled to about 0°. Petroleum ether (30–60°) was added to opalescence (about 150 ml.) and crystallization was induced by scratching the walls of the vessel. An additional 400 ml. of petroleum ether was added and the mixture was cooled to −50°. The crop of crystals was collected, washed with toluene–petroleum ether, petroleum ether and then dried *in vacuo* over phosphorus pentoxide; weight 80 g. (95%), m.p. 33.5–34° (Thiele and Dent reported 32° as the melting point[5]).

Di-(*p*-nitrobenzyl) Carbonate.—*p*-Nitrobenzyl alcohol (0.38 g.), *p*-nitrobenzyl chloroformate (0.54 g.) and 1 ml. of pyridine were heated on the steam-bath under reflux for one hour. To the cooled reaction mixture was added 10 ml. of 5% sodium bicarbonate and the mixture was cooled in an ice-bath. The crystalline product was collected, washed with bicarbonate solution, water and ethanol and dried; yield 0.43 g. (52%), m.p. 90–155°. When the product was recrystallized from amyl acetate, it melted at 171.5–172°. A sample was dried at 100° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{15}H_{12}O_7N_2$: C, 54.23; H, 3.64; N, 8.43. Found: C, 54.19; H, 3.67; N, 8.24.

Ethyl-*p*-nitrobenzyl Carbonate.—*p*-Nitrobenzyl chloroformate (0.6 g.) was dissolved in 6 ml. of absolute ethanol and the mixture allowed to stand at room temperature for 30 minutes. The mass of crystals which had formed was collected, washed with ethanol and dried; yield 0.52 g., m.p. 85–92°. After the product had been recrystallized from ethanol, the ester melted at 92–92.5°. A sample was dried at 78° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.53; H, 4.83; N, 6.08.

Preparation of Derivatives.—All of the derivatives described below were prepared in the same manner as that described for the glycine derivative unless otherwise noted.

Carbo-*p*-nitrobenzyloxyglycine.—Glycine (3.78 g., 0.05 mole) was dissolved in 15.6 ml. of 4 *N* sodium hydroxide (0.0625 equivalent) and the solution was cooled in an ice-bath. *p*-Nitrobenzyl chloroformate (13.5 g., 0.0625 mole) was dissolved in dioxane, the solution was made up to 32 ml. volume and cooled in an ice-bath. The dioxane solution, along with 15.6 ml. of cold 4 *N* sodium hydroxide was added to the glycine solution in five approximately equal portions, with at least five minutes being allowed between additions. The reaction mixture was shaken on a mechanical shaker in an apparatus designed to permit simultaneous cooling in an ice-bath. After the final addition the mixture was shaken for an additional hour to ensure complete hydrolysis of the excess *p*-nitrobenzyl chloroformate. A crystalline by-product which had formed during the reaction was removed by filtration; weight 1.8 g., m.p. 150–168°. After this product had been recrystallized from amyl acetate, it possessed a melting point of 171–172°. When this product was mixed with authentic di-(*p*-nitrobenzyl) carbonate, no depression of the melting point was noted.

The filtrate was then acidified with concentrated hydrochloric acid. The carbo-*p*-nitrobenzyloxy derivative, which separated as an oil, was extracted into ethyl acetate (three 35-ml. portions). The ethyl acetate phase was washed several times with *N* hydrochloric acid and then the product was extracted into *N* sodium bicarbonate. The alkaline solution was washed several times with fresh ethyl acetate and then treated with decolorizing carbon. After the solution had been cooled in an ice-bath, it was acidified with concentrated hydrochloric acid. The product, which separated as a crystaline solid, was collected, washed with water, and allowed to dry in air; weight 9.93 g. (78%), m.p. 120–123°. The compound was recrystallized from water, a satisfactory recrystallization being obtained when the concentration was no greater than about 1.8 g. per 100 ml. of water and the rate of cooling was slow. The melting point of the purified product was 122.5–124°. A sample was dried at 100° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{10}H_{10}O_6N_2$: C, 47.25; H, 3.97; neut. equiv., 254.2. Found: C, 47.45; H, 3.92; neut. equiv., 260.

N-Carbo-*p*-nitrobenzyloxyhydroxy-L-proline.—The product separated as an oil which crystallized after being allowed to stand overnight in the cold; yield 10.81 g. (66% based on the monohydrate).[10] After the derivative had been recrystallized from amyl acetate, the material melted at 136.5–139°, $[\alpha]^{26}D$ −41.6° (*c* 1, *N* sodium hydroxide). A sample was dried at 100° over phosphorus pentoxide *in vacuo* for analysis.

Anal. Calcd. for $C_{13}H_{14}O_7N_2$: C, 50.32; H, 4.55; neut. equiv., 310.3. Found: C, 50.18; H, 4.48; neut. equiv., 309.

Carbo-*p*-nitrobenzyloxy-L-proline Monohydrate.—The product which separated as an oil was induced to crystallize by cooling the mixture and scratching the walls of the flask. When the material was dried over phosphorus pen-

(6) G. Wolf and A. M. Seligman, THIS JOURNAL, **73**, 2080 (1951).

(7) All melting points were taken on the hot-stage. The analyses reported here were performed by the Microchemical Laboratory, Department of Chemistry, University of California, Berkeley. The water analyses reported were by the method of Karl Fischer as modified by E. Almy, W. Griffin and C. Wilcox, *Anal. Chem.*, **12**, 392 (1940).

(8) According to E. Eigenberger, see A. Weissberger and E. Proskauer, "Organic Solvents," Oxford University Press, New York, N. Y., 1935, p. 139.

(9) "Organic Syntheses," Coll. Vol. II, ed. by A. H. Blatt. John Wiley and Sons, Inc., New York, N. Y., 1934, p. 4.

(10) When the derivative was heated on a hot-stage, it lost water of hydration over a range of 66–88° and then recrystallized into the anhydrous product which then melted at 133–138°. The presence of water of hydration was indicated by the fact that when a sample of the crude material was dried at 100° *in vacuo* over phosphorus pentoxide to constant weight it decreased 5.67% in weight and melted at 133–138°. Calculated weight loss for one mole of water of hydration is 5.49%.

toxide *in vacuo*, the crystals were converted to a glass, which indicated the loss of water of hydration; weight of the glass, 11.53 g. (78%). The glass was dissolved in sodium bicarbonate solution and the product reprecipitated by acidification. The air-dried product melted at 50–56°. It was recrystallized from 50% acetic acid to give purified material which exhibited a melting range from about 44 to 58° (the range depended upon the rate of heating), $[\alpha]^{25}D$ −38.9° (*c* 1, *N* sodium hydroxide). The sample was air-dried for analysis.

Anal. Calcd. for $C_{13}H_{14}O_6N_2 \cdot H_2O$: C, 50.00; H, 5.17; H_2O, 5.76; neut. equiv., 312.3. Found: C, 50.20; H, 5.12; H_2O, 5.78; neut. equiv., 325.

Carbo-*p*-nitrobenzyloxy-L-leucine Monohydrate.—The final product separated as an oil which readily crystallized upon cooling the mixture and scratching the walls of the vessel. The air-dried crystalline product weighed 12.94 g. (79%, based on the monohydrate), m.p. 56–58.5°. It was also a hydrate and turned into a glass when dried over phosphorus pentoxide *in vacuo*. After the product had been recrystallized from *n*-butyl ether, the purified material melted at 60–61°, $[\alpha]^{27}D$ −15.8° (*c* 1, *N* sodium hydroxide). The sample was dried in air for analysis.

Anal. Calcd. for $C_{14}H_{18}O_6N_2 \cdot H_2O$: C, 51.21; H, 6.14; H_2O, 5.49; neut. equiv., 328.3. Found: C, 51.26; H, 6.16; H_2O, 5.45; neut. equiv., 332.

Carbo-*p*-nitrobenzyloxy-DL-isoleucine.—The yield of the final product, which was easily induced to crystallize, was 13.8 g., or 90% of theory, m.p. 102–115°. After the material was recrystallized from *n*-butyl ether, it melted at 114.5–116.5°. A sample was dried at 56° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{14}H_{18}O_6N_2$: C, 54.19; H, 5.85; neut. equiv., 310.3. Found: C, 54.46; H, 5.65; neut. equiv., 309.

Carbo-*p*-nitrobenzyloxy-L-isoleucine.—A sample of pure L-isoleucine[11] (200 mg.) was used for this preparation. The alkali and the dioxane solution of *p*-nitrobenzyl chloroformate were added in two portions to the alkaline solution of L-isoleucine, which was cooled in an ice-bath and stirred with a mechanical stirrer; yield 346 mg. (73%), m.p. 77.5–80°. Three recrystallizations of the derivative from *n*-butyl ether produced no change in the melting point, $[\alpha]^{23}D$ −12.6° (*c* 1, *N* sodium hydroxide). A sample was dried at 56° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{14}H_{18}O_6N_2$: C, 54.19; H, 5.85; neut. equiv., 310.3. Found: C, 54.25; H, 5.89; neut. equiv., 311.

Reaction of *p*-Nitrobenzyl Chloroformate in 4 *N* Sodium Hydroxide.—*p*-Nitrobenzyl chloroformate (2.16 g., 0.01 mole) was dissolved in dioxane, the solution was made up to 5 ml. volume and cooled in an ice-bath. In place of the usual aqueous solution of the sodium salt of an amino acid, 2.5 ml. of water was placed in the reaction tube and the mixture was cooled in an ice-bath. To the reaction tube were added the solution of *p*-nitrobenzyl chloroformate and 2.5 ml. of ice-cold 4 *N* sodium hydroxide in five portions. At least five minutes was allowed between additions. The reaction mixture was shaken on a mechanical shaker and simultaneously cooled in an ice-bath. A thick yellowish precipitate formed during the course of the reaction.

After completion of the reaction the mixture was filtered. A crystalline product remained on the filter, while an oil, which consisted of unreacted *p*-nitrobenzyl chloroformate, passed through with the filtrate. The crystalline product was washed twice with 50% dioxane, twice with water, and allowed to dry in air; weight 0.93 g., m.p. 110–150°. After it was recrystallized from amyl acetate, the product melted at 171.5–172°. The sample was dried at 100° over phosphorus pentoxide *in vacuo* for 6 hours for analysis.

Anal. Calcd. for $C_{15}H_{12}O_7N_2$: C, 54.23; H, 3.64; N, 8.43. Found: C, 54.28; H, 3.31; N, 8.38.

No depression of the melting point was noted when a sample was mixed with authentic di-(*p*-nitrobenzyl) carbonate (m.p. 171.5–172°).

Carbo-*p*-nitrobenzyloxyglycyl Chloride.—Finely powdered carbo-*p*-nitrobenzyloxyglycine (5.67 g., 0.022 mole) was suspended in 125 ml. of dry ether and the suspension was cooled in an ice–salt-bath. Phosphorus pentachloride (5.0 g.) was added and the reaction was allowed to proceed with cooling and swirling for one hour. The mixture was then brought quickly to room temperature which caused most of the solids to pass into solution. The mixture was rapidly filtered through a sintered glass filter into a round bottom flask. When the ether was removed under vacuum, the product crystallized. The crystals were washed three times with petroleum ether which had been dried over phosphorus pentoxide and then were redissolved in 150 ml. of dry ether and kept in an ice–salt-bath until used.

The acid chloride was characterized by conversion to the amide. The acid chloride (200 mg.) was allowed to react with 1 ml. of concentrated ammonium hydroxide solution. A crystalline product separated which amounted to 90 mg., m.p. 195.5–198°. After the compound had been recrystallized three times from ethanol–water, the amide melted at 198.5–199°. It was dried at 100° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{10}H_{11}O_6N_3$: C, 47.39; H, 4.38; N, 16.60. Found: C, 47.71; H, 4.40; N, 16.26.

Carbo-*p*-nitrobenzyloxyglycyl-L-leucine Methyl Ester.—The freshly prepared ethereal solution of carbo-*p*-nitrobenzyloxyglycyl chloride (*ca.* 0.022 mole) described above was allowed to warm to room temperature in order to effect complete solution and was added to an ethereal solution of 7.25 g. (0.05 mole) of L-leucine methyl ester prepared from L-leucine methyl ester hydrochloride[12] according to the method described by Fischer and Fourneau.[13] A precipitate of L-leucine methyl ester hydrochloride began to form immediately. The mixture was allowed to stand at room temperature for two hours and the precipitate of L-leucine methyl ester hydrochloride was removed by filtration. The ethereal solution of carbo-*p*-nitrobenzyloxyglycyl-L-leucine methyl ester was successively washed with *N* hydrochloric acid (twice), water, *N* potassium bicarbonate (twice) and water (twice). After the solution had been dried over sodium sulfate, the ether was removed. The product separated as a mixture of crystals and oil. The mixture was dissolved in 25 ml. of diisobutyl ketone–*n*-butyl ether (1:1) and induced to crystallize. The crystals were collected and washed with diisobutyl ketone–*n*-butyl ether (1:1), then petroleum ether and allowed to dry over concentrated sulfuric acid; weight 5.16 g., m.p. 56–61°. A second crop (0.51 g., m.p. 54–60°) was collected by addition of petroleum ether to the mother liquor; total yield 5.67 g. or 68% based on the amount of carbo-*p*-nitrobenzyloxyglycine. After the ester had been recrystallized from diisobutyl ketone–*n*-butyl ether, it melted at 59–62°, $[\alpha]^{26}D$ −26.5 ± 0.7° (*c* 1, ethanol). A sample was dried at 35° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{17}H_{23}O_7N_3$: C, 53.54; H, 6.08. Found: C, 53.68; H, 6.20.

Carbo-*p*-nitrobenzyloxyglycyl-L-leucine.—Carbo-*p*-nitrobenzyloxyglycyl-L-leucine methyl ester (3.81 g., 0.01 mole) was dissolved in 75 ml. of methanol and 11 ml. of 1.00 *N* sodium hydroxide was added. After the solution had stood for two hours at room temperature, the mixture was acidified with concentrated hydrochloric acid and the methanol was removed by vacuum distillation. The oil which remained was dissolved in 25 ml. of ethyl acetate and the resulting solution was washed with *N* hydrochloric acid and water. The product was then extracted into *N* potassium bicarbonate. The alkaline solution was washed with ethyl acetate, cooled in an ice-bath, and acidified with concentrated hydrochloric acid. The oil which separated was induced to crystallize by cooling the mixture and scratching the walls of the vessel. The crystalline product was washed with water and dried over phosphorus pentoxide; weight 3.06 g. (83%), m.p. 115.5–120.5°.

After the peptide derivative had been recrystallized from chloroform or chloroform–*n*-butyl ether (1:1) it melted at 118–120.5°, $[\alpha]^{27}D$ −15.6° (*c* 1, *N* sodium hydroxide). A sample was dried at 78° over phosphorus pentoxide *in vacuo* for analysis.

Anal. Calcd. for $C_{16}H_{21}O_7N_3$: C, 52.31; H, 5.76; neut. equiv., 367.4. Found: C, 52.27; H, 5.69; neut. equiv., 368.

(11) We are indebted to J. P. Greenstein for this sample of pure L-isoleucine, $[\alpha]D$ +41.2°.

(12) E. Abderhalden and H. Spinner, *Z. physiol. Chem.*, **107**, 1 (1919).

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

Glycyl-L-leucine.—Carbo-*p*-nitrobenzyloxyglycyl-L-leucine (2.20 g., 0.006 mole) was dissolved in 30 ml. of methanol and 0.7 ml. of glacial acetic acid was added. The solution was placed in a 125-ml. erlenmeyer flask containing 220 mg. of palladous oxide[14] and equipped with a magnetic stirrer. Hydrogen was bubbled through the solution with stirring, the course of the reduction being followed by trapping the carbon dioxide evolved in barium hydroxide solution. One hour was required for complete reduction. The product, which had crystallized, was brought back into solution by the addition of water. The catalyst was removed by filtration and the methanol and water were removed by vacuum distillation. The by-product of the reduction, presumably *p*-toluidinium acetate, was removed by drying the residue to constant weight over phosphorus pentoxide and sodium hydroxide pellets under high vacuum; yield 1.10 g. (97%), m.p. 231–232° with some decomposition.

For analysis the peptide was recrystallized from water–ethanol and dried at 100° over phosphorus pentoxide *in vacuo*. The pure peptide melted at 234–237° with decomposition (cap.), $[\alpha]^{25}D$ −36.0° (*c* 1, water).[15]

Anal. Calcd. for $C_8H_{16}O_3N_2$: C, 51.04; H, 8.57; N, 14.89. Found: C, 51.46; H, 8.63; N, 14.82.

Carbo-*p*-nitrobenzyloxy-L-leucyl Chloride.—This compound was prepared in the same manner as that described for the preparation of carbo-*p*-nitrobenzyloxyglycyl chloride. The carbo-*p*-nitrobenzyloxy-L-leucine monohydrate (5.62 g., 0.0171 mole) was first dehydrated *in vacuo* over phosphorus pentoxide to remove water of hydration. The acid chloride was characterized by conversion to the amide, which was prepared in the manner described for carbo-*p*-nitrobenzyloxyglycinamide. The amide was recrystallized from 50% ethanol. When the amide was heated on the hot-stage, it melted at 160–161.5°, then this melt crystallized and the new crystals melted at 164.5–165.5°. It was dried at 100° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{14}H_{19}O_5N_3$: C, 54.36; H, 6.19. Found: C, 54.45; H, 6.09.

Carbo-*p*-nitrobenzyloxy-L-leucyl-L-leucine Methyl Ester.—This compound was prepared in the same manner as that described for the preparation of carbo-*p*-nitrobenzyloxyglycyl-L-leucine methyl ester. The product, which was easily induced to crystallize, amounted to 5.6 g. (78% yield based on the amount of carbo-*p*-nitrobenzyloxy-L-leucine used for preparation of the acid chloride) and melted at 79.5–81°. After the ester had been recrystallized from *n*-butyl ether, it melted at 79.5–81.5°, $[\alpha]^{24}D$ −24.1° (*c* 1, ethanol). It was dried at 56° *in vacuo* over phosphorus pentoxide for analysis.

Anal. Calcd. for $C_{21}H_{31}O_7N_3$: C, 57.65; H, 7.14. Found: C, 57.68; H, 7.05.

Carbo-*p*-nitrobenzyloxy-L-leucyl-L-leucine.—Saponification of the ester (5.03 g., 0.0115 mole) was carried out in the same manner as the saponification of the corresponding ester of the glycyl-L-leucine derivative. The rate of hydrolysis of the former, however, was significantly slower than that of the latter, since 1.3 g. or about 25% of the ester (m.p. 79–80.5°) was recovered from the organic phase after the peptide derivative had been extracted with bicarbonate solution. The peptide derivative, which separated as an oil when its alkaline solution was acidified, was crystallized from *n*-butyl ether. Based upon results of an analysis and the weight loss upon drying, the product, when crystallized from *n*-butyl ether, contained a mole of *n*-butyl ether for each four moles of the derivative. The melting point was 65–90° and the yield was 3.2 g. or 84%, taking into account the recovered unsaponified ester and the solvent of crystallization. When the derivative was dried at 56° *in vacuo* to constant weight, it melted at 89.5–91°, $[\alpha]^{24}D$ −33.3° (*c* 1, *N* sodium hydroxide).

Anal. Calcd. for $C_{26}H_{39}O_7N_3$: C, 56.72; H, 6.90; neut. equiv., 423.5. Found: C, 56.63; H, 6.94; neut. equiv., 425.

L-Leucyl-L-leucine Hydrate.—The peptide derivative was hydrogenated in the usual manner and freed of *p*-toluidine in the same way as was glycyl-L-leucine. The yield was quantitative. For analysis the peptide was recrystallized from 95% ethanol from which it separated with a variable amount of water of hydration. This same phenomenon was noted by Fischer[16] in his original preparation of L-leucyl-L-leucine. Since Fischer reported that the dipeptide was converted in part to the anhydride when it was dried over phosphoric anhydride at 100°, the peptide was dried in air for analyses. On the hot-stage the crystals were observed to undergo a change in crystalline form at about 155°. The new crystals sublimed from between the cover slips without melting. When the compound was placed in a sealed capillary tube and heated in an oil-bath, it melted at 252–254° (uncor.) with decomposition, $[\alpha]^{22}D$ −13.7° (*c* 0.95, *N* sodium hydroxide).[17]

Anal. Calcd. for $C_{12}H_{24}O_3N_2 \cdot {}^3/_4H_2O$: C, 55.89; H, 9.97; H_2O, 5.2. Found: C, 55.60; H, 10.13; H_2O, 4.8.

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(14) R. L. Shriner and R. Adams, THIS JOURNAL, **46**, 1683 (1924).

(15) E. Fischer and J. Steingroever [*Ann.*, **365**, 167 (1909)] reported that glycyl-L-lencine decomposes at about 242° (cor.) with yellowing at 234°; $[\alpha]^{20}D$ −35.1° (*c* 4.2, water).

(16) E. Fischer, *Ber.*, **39**, 2893 (1906).

(17) Calculated rotation for the anhydrous material. Fischer (ref. 16) reported a melting point of 270° (cor.) for L-leucyl-L-leucine hydrate and a calculated specific rotation for the anhydrous compound of $[\alpha]^{20}D$ −13.4° (*c* 8.1, *N* sodium hydroxide).