the reaction increases the yields. While a low reaction temperature is important during the first twenty-four hours, a further extension of the reaction at a low temperature does not increase the yields appreciably as indicated in Table II.

Omission of anhydrous sodium sulfate resulted in lower yields. A 2:1 ratio of concd. sulfuric acid to dipotassium nitroacetate is needed to get optimum yields as indicated by the data in Table III.

Experimental

Preparation of the Dipotassium Salt of Nitroacetic Acid.—In a three-liter flask provided with stirrer, condenser and dropping funnel was placed 1500 ml. (20.2 moles) of freshly prepared 50% potassium hydroxide. When the temperature had risen to 60° on dissolving of the potassium hydroxide, 300 g. (4.91 moles) of nitromethane was added dropwise with vigorous stirring. During the one and one-half hours required for the addition of the nitromethane, the temperature rose to 102°. Ammonia was liberated during the latter half of the addition and the reflux condenser was removed after the entire amount of nitromethane had been added. The reddish brown mixture was then heated until the first crystals appeared. After cooling, the potassium salt which had separated out was filtered and washed with methanol. This first crop weighed 210 g. representing a yield of 44.5%.

Anal. Calcd. for $C_2HNO_4K_2$: K, 43.03. Found: K, 42.61.

The mother liquor was evaporated further and after cooling a second crop of crystals was obtained which weighed 60 g., representing a yield of 12.7%.

Anal. Found: K, 42.43.

Still further evaporation of the mother liquor gave a third crop of crystals (70 g.) which was found by analysis to be 43% contaminated with potassium carbonate which formed in the reaction.

Anal. Found: K, 48.90.

Preparation of Methyl Nitroacetate.—In a one-liter flask fitted with stirrer, thermometer and dropping funnel were placed 90.5 g. (0.5 mole) of dipotassium nitroacetate, 500 ml. (12.38 moles) of methanol and 15 g. (0.1 mole) of anhydrous sodium sulfate. The flask was placed in a Dewar and cooled down to −50 to −60° by a trichloroethylene-dry ice mixture. One hundred grams (1 mole) of concentrated sulfuric acid (d. 1.84) was added dropwise to the mixture during a period of one and one-half hours. The reaction mixture was kept in the Dewar for twenty-four hours with stirring (temp. −50 to −60°) and then for one hundred and forty-four hours at 23–25° with occasional shaking. The precipitated potassium sulfate was then filtered off and the excess methanol was evaporated under vacuum. The oily residue was diluted with ether and neutralized with a 5% solution of sodium carbonate. The ether layer, which contained the ester, was dried and the ether was evaporated. Vacuum distillation of the residue at 15 mm. yielded 36 g. (60%) of the ester, boiling at 93–94°; $n^{20}D$ 1.4245; neut. equiv. calcd. for $C_3H_5O_4N$, 119, found 121.

Preparation of Ethyl Nitroacetate. The procedure was the same as for the synthesis of methyl nitroacetate. Forty grams (60%) of ethyl nitroacetate was obtained, boiling at 105–107° at 25 mm., d^{20}_4 1.11950, $n^{20}D$ 1.4252.

Acknowledgments.—The authors wish to express their appreciation to Mr. L. Friedman for his technical assistance and express their thanks to the General Tire and Rubber Company, Akron, Ohio, for financial assistance in this work.

Summary

An improved synthesis of esters of nitroacetic acid has been presented. The methyl and ethyl esters of nitroacetic acid have been prepared in two steps from nitromethane in 60% yield.

LAFAYETTE, INDIANA RECEIVED APRIL 7, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

The Chemistry of Nitroacetic Acid and its Esters. II. The Synthesis of Ethyl α-Nitro-β-(3-indole)-propionate from Gramine and Ethyl Nitromalonate[1]

BY D. I. WEISBLAT AND D. A. LYTTLE

We have recently reported a synthesis of *dl*-tryptophan (V) in which the key intermediate, ethyl α-nitro-β-(3-indole)-propionate (IV), was prepared from gramine and ethyl nitroacetate.[2] A new synthesis of this intermediate, starting with ethyl nitromalonate (I), is described in this communication.

The alkylation of ethyl nitromalonate (I) by gramine (II) under reaction conditions similar to those reported for the alkylation of ethyl nitroacetate[2] gives ethyl α-nitro-α-carbethoxy-β-(3-indole)-propionate (III) in excellent yield. The structure of ethyl nitromalonate, in contrast to that of ethyl nitroacetate, excludes dialkylation[2,3] as a possible side reaction and this, together with the activating effect of the second carbethoxy group, probably accounts for the higher yields and purer product obtained in the present alkylation.

Since powdered sodium hydroxide is an effective catalyst for the dialkylation of ethyl nitroacetate by gramine,[2] it was expected that it would greatly accelerate the present alkylation. Contrary to our expectations, however, it was found that not only sodium hydroxide but basic substances in general cause extensive decomposition. For this reason the rapid removal of dimethylamine, which is formed in the reaction, is essential for optimum yield and purity of product.

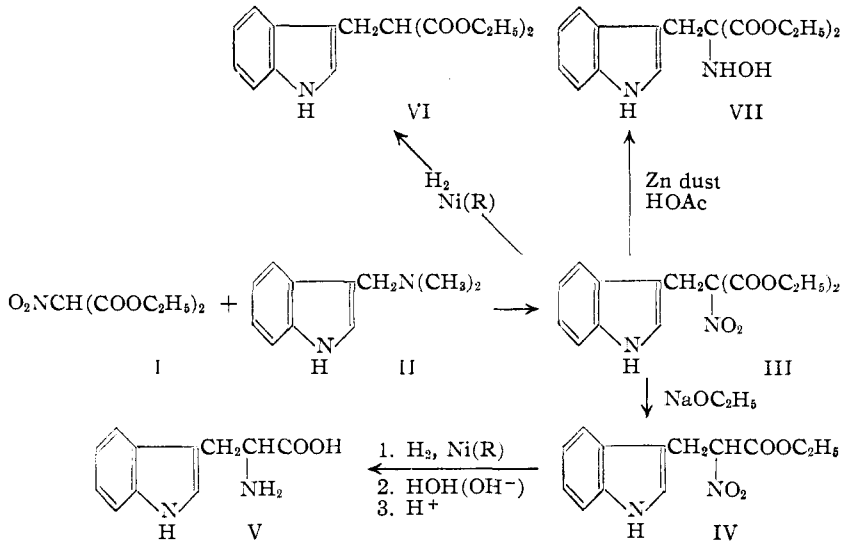
The conversion of III to IV in a yield of 91.6% was readily accomplished by treating a solution of III in ether with one equivalent of sodium in alcohol.[4]

(1) This paper was presented before the Organic Division at the 112th Meeting of the American Chemical Society, New York City, September, 1947.

(2) Lyttle and Weisblat, THIS JOURNAL, **69**, 2118 (1947).

(3) Snyder and Katz, *ibid.*, **69**, 3140 (1947).

(4) Ulpiani, *Gazz. chim. ital.*, **34**, 174 (1904).



Improvements in the reduction of IV and the hydrolysis of ethyl *dl*-tryptophan to *dl*-tryptophan (V) have increased the over-all yield on these steps to 87 from 50% as reported previously.[2]

Attempts to obtain ethyl α-amino-α-carbethoxy-β-(3-indole)-propionate by catalytic reduction of III with Raney nickel and hydrogen at 2500 p. s. i. and 100° were unsuccessful. The hydrogen uptake was almost 4 moles per mole of III, indicating that hydrogenolysis had occurred. The only product isolated was the known ethyl α-carbethoxy-β-(3-indole)-propionate (VI).[5]

Chemical reduction of III with zinc dust and acetic acid gave a product representing only partial reduction of the nitro group, ethyl α-hydroxylamino-β-(3-indole)-propionate (VII), in a yield of 56%.

When the nitration of Arndt and Rose,[6] which consists of treating methyl malonate with fuming nitric acid, was applied to the preparation of ethyl nitromalonate, yields varying from 45 to 76% were obtained. An investigation of this reaction showed that a higher temperature is necessary for optimum yields. When the nitration is allowed to proceed for several hours at 15–20°, consistent yields of around 92% can be obtained.

We have found, however, that ethyl nitromalonate prepared by these nitration procedures invariably is contaminated with oxides of nitrogen which initiate autocatalytic decomposition of the nitro ester. These oxides, which cannot be removed by repeated washing and/or distillation, are completely removed by treatment of the nitro ester with an amide such as urea or acetamide. Ethyl nitromalonate so treated is stable over long periods of time.

Experimental

Ethyl Nitromalonate (I).—Ethyl malonate (80.0 g., 0.5 mole) was placed in a 500-cc. three-necked flask fitted with a dropping funnel, a stirrer, a thermometer and an outlet protected by a drying tube. The flask was cooled by tap water at 12°, and 184 cc. of fuming nitric acid (d. 1.5) was added at a rate sufficient to maintain the temperature between 15 and 20°. The addition required one hour, after which the mixture was allowed to stir for three and one-half hours at 15°. The solution was poured onto 1 l. of ice and water and the ester extracted with 200- and 100-cc. portions of toluene. The combined toluene extracts were washed twice with water, and then with 200-cc. portions of 5% aqueous urea solution until a starch-potassium iodide test for oxides of nitrogen in the wash was negative. The toluene solution was extracted with 10% sodium carbonate solution in portions until acidification of a test portion of extract showed that it contained no nitro ester. The sodium carbonate extracts were combined and washed once with 200 cc. of toluene. The aqueous solution was then carefully acidified to congo red paper with concentrated hydrochloric acid, with cooling by the occasional addition of ice. The ester was collected by extracting with 500-, 200- and 100-cc. portions of toluene. The toluene solution was washed twice with 200-cc. portions of water and then with 5% aqueous urea solution, checking again with starch-potassium iodide test paper for the complete absence of oxides of nitrogen. Drying of the toluene solution was done over magnesium sulfate. The yield of ester was determined by weighing the toluene solution, taking an aliquot, adding an equal volume of alcohol, and titrating the nitro ester with 1 *N* sodium hydroxide to a phenolphthalein end-point. The assay showed that the yield was 94.1 g. or 91.7%. If the analytically pure ester is desired, it may be obtained by concentrating and distilling; b. p. 81–83° at 0.3 mm.; $n^{21}D$ 1.4274.

Ethyl α-Nitro-α-carbethoxy-β-(3-indole)-propionate (III).—Distilled ethyl nitromalonate,[7] 43.3 g. (0.25 mole), 250 cc. of toluene dried by distillation, and gramine,[8] 51.3 g. (0.25 mole) were placed in a 500-cc., three-necked flask fitted with stirrer, nitrogen inlet, thermometer in the mixture, and an efficient reflux condenser. With a vigorous stream of nitrogen[9] passing through the well-stirred mixture, it was heated rapidly to vigorous reflux. Dimethylamine evolution began at about 90 to 95° and was very rapid at the boiling point. Refluxing, nitrogen flow and stirring were continued until evolution of dimethylamine ceased, usually after three hours. The solution was cooled and extracted twice with 50-cc. portions of 10% hydrochloric acid, washed with 50 cc. of water, then extracted with two 50-cc. portions of 5% sodium hydroxide and washed twice with water. The toluene solution was dried over magnesium sulfate and concentrated at reduced pressure. The last traces of solvent were removed by heating at 80° and 0.5 mm. with stirring. There remained 80.5 g. (96.5%) of III as a light red, thick sirup.

Anal. Calcd. for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.29, 57.19; H, 5.14, 5.14; N, 8.45, 8.23.

Ethyl α-Nitro-β-(3-indole)-propionate (IV).—Ethyl α-nitro-α-carbethoxy-β-(3-indole)-propionate, 80.5 g. (0.24 mole), in 500 cc. of anhydrous ether was cooled in an ice-

(5) Snyder, Smith and Stewart, THIS JOURNAL, **66**, 203 (1944).

(6) Arndt and Rose, *J. Chem. Soc.*, 1 (1935).

(7) Equally good results are obtained, however, when a stoichiometric amount of undistilled ester in toluene solution is used.

(8) Kuhn and Stein, *Ber.*, **70**, 567 (1937).

(9) This is necessary to insure rapid and complete removal of dimethylamine. If this is not done, both yield and quality of product are poor.

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.

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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).