[CONTRIBUTION FROM THE LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

The Synthesis of Tryptophan from Gramine

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Recently Snyder and Smith[1] reported the details of an excellent synthesis of tryptophan in 45% over-all yield. In the same issue of THIS JOURNAL a report from this Laboratory[2] presented in outline an almost identical procedure for preparing this compound. It is believed that our method differs sufficiently in details from that of Snyder to warrant publication at this time.

Snyder and co-workers condensed gramine methiodide (I)[3] with ethyl sodioacetamidomalonate in dry dioxane to obtain ethyl α-acetamido-α-carbethoxy - β - (3 - indolyl) - propionate (II) in yields ranging from 63–70%. The quaternary salt (I) was obtained by a two-step process from indole in 80% of the theoretical quantity.

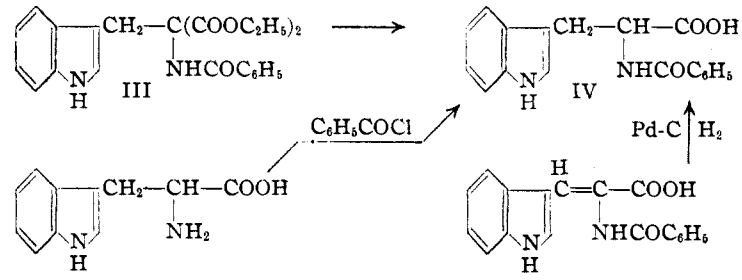
It has been found that it is unnecessary and indeed undesirable to use I in the condensation. When ethyl iodide is added slowly to a warm solution of gramine and ethyl sodioacetamidomalonate in absolute ethanol the desired product, II, was isolated in a yield of 73%. Since it is possible to recover 16% of the gramine in a state of purity suitable for re-use, the yield based on the consumed Mannich base is actually 86%. Furthermore, the reaction in dry ethyl alcohol is over in about seven to eight hours whereas the dioxane procedure requires about thirty-six hours for completion. Saponification of II to the free dicarboxylic acid was quantitative. Simultaneous decarboxylation and deacetylation was carried out in boiling 2 *N* sulfuric acid. The yield of *dl*-tryptophan, m. p. 279–280°, was 61%, which is not as good (81%) as was realized by the alkaline degradation of the ester, II, as reported by Snyder.

It was possible to substitute the less expensive methyl sulfate for ethyl iodide in the above condensation. The yield of II in this case was 72% with a 10% recovery of gramine.

A practically complete conversion of gramine to ethyl α-acetamido-α-carbethoxy-β-(3-indolyl)-propionate was realized by proceeding according to a suggestion of Mr. B. F. Tullar of these Laboratories. Since dimethylethylamine, a product of the reaction, forms quaternary salts with the alkylating agents, the gramine is incompletely converted to the methosulfate or ethiodide. When *two* moles of methyl sulfate are used, the demands of both the volatile and Mannich base are satisfied and the latter then reacts completely. When this technique was followed the reaction was over in four hours at room temperature and the yield of II increased to 95%.

The attempt also was made to carry out the synthesis of the amino acid with ethyl benzamidomalonate[4] but the yields in some of the steps (especially the hydrolysis of IV) were so low as to render the procedure impractical.

Ethyl α-carbethoxy-α-benzamido-β-(3-indolyl)-propionate (III) was prepared in 50% yield from gramine (23% recovered), ethyl iodide, and ethyl benzamidomalonate. Alkaline saponification followed by decarboxylation in 2 *N* sulfuric acid furnished benzoyl-*dl*-tryptophan IV in 88% of the theoretical yield. This compound was previously mentioned by Ellinger and Flamand[5] but no properties were given. Accordingly the compound was prepared by benzoylation of a known specimen of *dl*-tryptophan and by reduction of α-benzamido-β-(3-indolyl)-acrylic acid. All three specimens proved to be identical in both physical and chemical properties.



Experimental[6]

Ethyl benzamidomalonate was prepared by the method of Redemann and Dunn.[4] On large scale runs the distillation of the intermediate ethyl isonitrosomalonate was omitted. Ethyl acetamidomalonate was prepared by a modification of the method described by Snyder.[1] The indole used in this work was the technical variety that melted between 48–52° and ranged from tan to light brown in color. Gramine was obtained by the procedure of Kuhn and Stein.[7] The yield was 90–93% of material which melted at 128–130°. This was suitable for use in the condensation experiments. A purer product (m. p. 134°) could be obtained by crystallization from acetone but the losses were excessive.

Ethyl α-Carbethoxy-α-acetamido-β-(3-indolyl)-propionate (II).—To a solution of 2.88 g. (0.125 m.) of sodium in 250 cc. of 100% ethanol[8] there was added 27.2 g. (0.125 mole) of ethyl acetamidomalonate followed by 21.8 g. (0.125 mole) of gramine. The clear yellow solution was warmed on the steam-bath with stirring as a solution of

(1) Snyder and Smith, THIS JOURNAL, **66**, 350 (1944).
(2) Albertson, Archer and Suter, *ibid.*, **66**, 500 (1944).
(3) Snyder, Smith and Stewart, *ibid.*, **66**, 200 (1944).
(4) Redemann and Dunn, *J. Biol. Chem.*, **130**, 345 (1939).
(5) Ellinger and Flamand, *Ber.*, **40**, 3029 (1907); *Z. physiol. Chem.*, **55**, 8 (1908).
(6) The microanalyses were done by Esther Bass, Alice Rainey and Patricia Curran of this Laboratory.
(7) Kuhn and Stein, *Ber.*, **70B**, 567 (1937).
(8) Smith, *J. Chem. Soc.*, 1288 (1927).

19.5 g. (0.125 mole) of ethyl iodide in 20 cc. of 100% ethanol was added dropwise over a period of one-half hour. Refluxing was continued for six hours, during which time dimethylethylamine was evolved and some diethyldimethylammonium iodide precipitated.

Most of the alcohol (about 225 cc.) was removed and the residue taken up in a mixture of 75 cc. of water and 100 cc. of chloroform. The aqueous layer was extracted with three 50-ml. portions of chloroform and then discarded. The combined organic layers were washed with 10% hydrochloric acid, saturated sodium bicarbonate solution and finally with water.

The acid extract was made basic and cooled. It yielded 3.5 g. of gramine, m. p. 128–129° This represents 16% of the base used originally.

The crystalline residue obtained from the evaporated chloroform extract weighed 35.8 g. Crystallization from aqueous alcohol gave 31.4 g. of a solid, m. p. 152.5–155°. It was completely saponifiable. The yield was 73% of the theoretical. On the basis of gramine consumed the yield is 86%. Further crystallization raised the melting point to 157°.

Anal. Calcd. for $C_{18}H_{22}N_2O_5$: C, 62.40; H, 6.39; N, 8.09. Found: C, 62.78; H, 6.58; N, 8.27.

When an equivalent quantity of methyl sulfate was substituted for the ethyl iodide in the above experiment, the yield of II was 72%. The gramine recovery amounted to 10%.

When 25.2 g. (0.20 mole) of methyl sulfate was stirred into an alcoholic solution of 17.4 g. (0.12 mole) of gramine, 2.76 g. (0.12 mole) of sodium and 26.0 g. (0.12 mole) of ethyl acetamidomalonate, an exothermic reaction occurred which necessitated occasional cooling of the flask. The solution was allowed to stand at room temperature protected from moisture for four hours before being poured into water. After the suspension was chilled in ice, the solid was collected and dried. The compound melted at 153° and weighed 32.8 g. (95%).

When 99.5% alcohol was used as the reaction medium a 59% yield of condensaton product was obtained, but the recovery of gramine was 28%. On the basis of consumed base the yield is 82%.

In one experiment piperidylmethylindole[7] was substituted for gramine. The reaction gave the normal products but in lower yield.

Ethyl α-Carbethoxy-α-benzamido-β-(3-indolyl)-propionate (III).—When ethyl benzamidomalonate was substituted for the acetyl homolog, the desired ester (III), m. p. 138.5–140°, was isolated in 50% of the theoretical yield. It was completely saponifiable. The acid extracts contained 23% of the original gramine. The pure compound melted at 142°.

Anal. Calcd. for $C_{23}H_{24}N_2O_6$: N, 6.86. Found: N, 7.20.

Benzoyl-*dl*-tryptophan (IV).—To a solution of 10 g. of sodium hydroxide in 60% ethanol there was added 12.2 g. of the ester III. After refluxing for ninety minutes, the mixture was concentrated to 70 cc. to remove most of the alcohol. About 3 volumes of water was added and the whole cooled in ice. Twenty-five cubic centimeters of concentrated hydrochloric acid was added slowly. The acid which precipitated was collected on a filter and air-dried. The yield was quantitative. The compound decomposed rapidly at 90–100° with a vigorous evolution of carbon dioxide.

A solution of 3.52 g. of this acid in 18 cc. of 2 *N* sulfuric acid was refluxed for three hours. At the end of this time the mixture was cooled and filtered to give 2.70 g. of benzoyl-*dl*-tryptophan, (88%). On recrystallization from aqueous methanol with the aid of Norite it melted at 192–193°.

dl-Tryptophan was benzoylated in 77% yield according to the procedure given by Berg, *et al.*, for the *l*-isomer.[9] Crystallization from aqueous alcohol gave a substance of m. p. 191–192° identical with the product obtained by the decarboxylation of the above dibasic acid. Ellinger and Flamand[5] mention the compound but report no properties. Benzoyl-*dl*-tryptophan was also prepared by catalytic reduction of α-benzamido-β-(3-indolyl)-acrylic acid. This compound was prepared from indole-β-aldehyde and hippuric acid by the method of Ellinger and Flamand.[5]

Reduction was effected by dissolving 200 mg. of the acrylic acid, m. p. 236°, in 75 cc. of ethanol and adding an equal weight of palladium on charcoal (5% Pd). A pressure of 40 lb. at 50° was used. After one and one-half hours of shaking the catalyst was removed by filtration and the alcohol was distilled off under vacuum. The residual sticky gray residue was dissolved in a small amount of ethanol, filtered, and the product precipitated by the addition of water. The white crystalline product melted at 188–191°. When a sample was mixed with benzoyl-*dl*-tryptophan prepared by the other methods, the melting point was not depressed.

Anal. Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.2; H, 5.24; N, 9.09. Found: C, 70.02; H, 5.24; N, 8.79.

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Summary

1. An improved procedure for preparing ethyl α - acetamido - α - carbethoxy - β - (3 - indolyl) - propionate, an intermediate in the synthesis of tryptophan, is described.

2. A new method of synthesizing benzoyl-*dl*-tryptophan is described.

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(9) Berg, Rose and Marvel, *J. Biol. Chem.*, **85**, 209 (1929–1930).