[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

A Synthesis of Tryptophan and Tryptophan Analogs[1]

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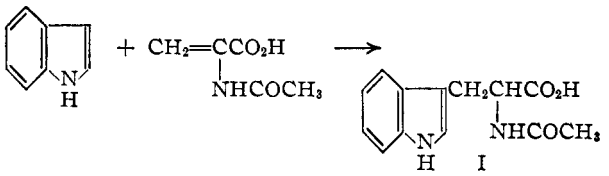
Acetyltryptophan has been prepared from α-acetamidoacrylic acid and indole in the presence of acetic acid and acetic anhydride. Variations in the method of conducting the reaction were studied, and under certain conditions a 57.7% yield of acetyltryptophan was obtained. Acetyl-2-methyltryptophan and acetyl-7-methyltryptophan have been prepared from α-acetamidoacrylic acid and the corresponding indoles by the same method. Indole-2-carboxylic acid and ethyl indole-2-carboxylate did not react with α-acetamidoacrylic acid under conditions similar to those used in the preparation of acetyltryptophan. The possible involvement of an azlactone, an oxazoline, or diacetylserine as an intermediate in the formation of acetyltryptophan has been investigated and found unlikely.

Although a number of syntheses of tryptophan have been described recently, a short synthesis of this compound in good yield remains desirable. It appeared that if indole could be added to α-acetamidoacrylic acid in such a way as to yield acetyltryptophan (I), this addition reaction might provide the basis for the desired simple method of preparing tryptophan.

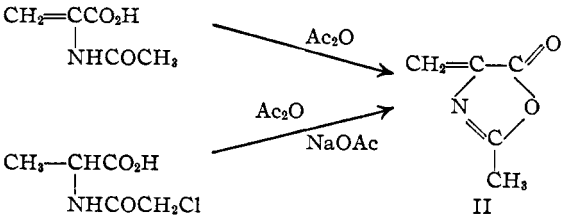


Among a number of known processes similar to the proposed reaction are the additions of indole and its derivatives to acrylonitrile. In basic solution N-cyanoethylindoles are the chief products, although in some cases 1,3-dicyanoethylindoles are obtained as by-products.[3] In acidic solution, and in the presence of catalysts such as copper borate, β-3-indolylpropionitriles are obtained.[4] Acrylic acid derivatives also react with indoles to yield 3-substituted indoles under acidic conditions.[5]

A number of unsuccessful experiments in which indole was treated with α-acetamidoacrylic acid, in various solvents and in the presence of various acidic, basic, and copper compounds, were carried out. In a reaction medium consisting of acetic acid and acetic anhydride the desired product, acetyltryptophan, was obtained. When equimolar proportions of indole and α-acetamidoacrylic acid were used it was possible, under certain conditions, to obtain a 49% yield of acetyltryptophan. When a large excess (3 moles) of α-acetamidoacrylic acid was used a 57.7% yield was obtained.

No acetyltryptophan could be isolated from reaction mixtures in which the only solvent used was acetic anhydride. Acetic acid alone, in quantities comparable to those used in reactions employing both acetic acid and acetic anhydride, did not dissolve the α-acetamidoacrylic acid and no reaction occurred. When indole and α-acetamidoacrylic acid were heated together in dioxane solution containing acetic acid it was possible to recover 79% of the α-acetamidoacrylic acid unchanged, but no acetyltryptophan could be isolated. The addition of dehydrating agents, other than acetic anhydride, to such reaction mixtures was likewise unproductive.

Since acetyltryptophan was obtained *only in the presence of acetic anhydride*, it appeared possible that an azlactone (II), arising from the action of acetic anhydride on α-acetamidoacrylic acid, might be involved as an intermediate in its formation. A compound analogous to α-acetamidoacrylic acid, α-phenylacetamidoacrylic acid, has been converted to an azlactone in low yield by the action of acetic anhydride.[6] Although 2-methyl-4-formal-5-oxazolone (II) has not been isolated, it has been postulated by Bergmann and Delis[7] as an intermediate



in the conversion of serine to pyruvic acid which occurs when the former is warmed with acetic anhydride and then boiled with dilute phosphoric acid. Its homolog, 2-ethyl-4-formal-5-oxazolone, has been prepared in low yield by the action of sodium acetate and benzoic anhydride on α-bromopropionylalanine.[8] This compound, which was rather unstable, was hydrolyzed readily to pyruvic acid. Although this product was isolated in low yield by distillation, its formation in at least 50% yield by the same general procedure was detected by the preparation of the phenylhydrazone of the pyruvic acid formed on hydrolysis of the undistilled reaction product. A number of other unsaturated azlactones have been prepared from α-haloacylamino acids by treatment with acetic anhydride and base.[6,9] For the present work it seemed adequate to prepare 2-methyl-4-formal-5-oxazolone by a reaction of this type, and treat it, without isolation, with indole. If the azlactone II is indeed an inter-

(1) Abstracted from the Thesis submitted by J. A. MacDonald to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, October, 1954.

(2) Dow Chemical Company Fellow, 1953–1954.

(3) N. Roh and W. Wolff, German Patent 641,597 (*Frdl.*, **23**, 152 (1936)).

(4) W. Reppe and H. Ufer, German Patent 698,273 (*Frdl.*, **25**, 173 (1938)).

(5) After the submission of this manuscript, W. E. Noland and P. J. Hartmann [THIS JOURNAL, **76**, 3227 (1954)] described the addition of indole to nitroethylene.

(6) J. A. King and F. H. McMillan, THIS JOURNAL, **72**, 833 (1950).

(7) M. Bergmann and D. Delis, *Ann.*, **458**, 76 (1927).

(8) M. Bergmann and F. Stern, *ibid.*, **448**, 20 (1926).

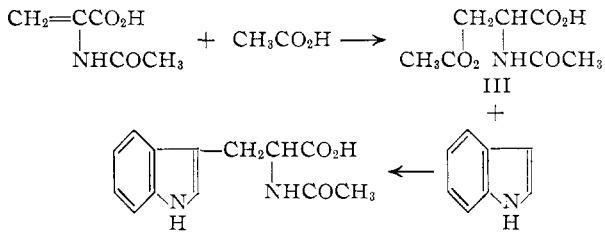
(9) J. C. Sheehan and W. E. Duggins, THIS JOURNAL, **72**, 2475 (1950).

mediate in the formation of acetyltryptophan from indole and α-acetamidoacrylic acid, such treatment would be expected to yield acetyltryptophan.

For the preparation of II, α-chloroacetylalanine was used. That this compound is converted to the azlactone by sodium acetate and acetic acid was shown by treating a portion of it with these reagents, hydrolyzing the reaction product, adding phenylhydrazine, and isolating the phenylhydrazone of pyruvic acid in 53% yield. That sodium acetate, necessarily present in the reaction mixture containing 2-methyl-4-formal-5-oxazolone, does not interfere with the formation of acetyltryptophan from indole and α-acetamidoacrylic acid was shown by carrying out this reaction in the presence of the quantities of sodium acetate and acetic anhydride used in the preparation of the azlactone. Sufficient acetic acid was added to give a ratio of acetic acid to acetic anhydride equal to that employed in previous successful preparations of acetyltryptophan. A 33% yield of acetyltryptophan was obtained.

No acetyltryptophan was isolated from the products of any of the reactions in which indole was treated with solutions of 2-methyl-4-formal-5-oxazolone. It appears, therefore, that this compound is not an intermediate in the formation of acetyltryptophan from indole and α-acetamidoacrylic acid.

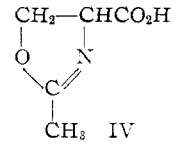
Since acetic acid is known to add to olefins and to acrolein[10] it appeared possible that the product of its addition to α-acetamidoacrylic acid might be an intermediate in the formation of acetyltryptophan. If such a compound were an intermediate, indole



would have to replace its acetoxyl group in order to yield acetyltryptophan. That indole might be capable of such a replacement is indicated by its reaction with propiolactone to yield 3-indolylpropionic acid.[11]

The addition product III, diacetylserine, has not been described, but the analogous compound diacetylcysteine is prepared very readily. If III were involved in the formation of acetyltryptophan, diacetylcysteine would be expected to yield acetyltryptophan by replacement of the thioacetoxyl group. Diacetylcysteine therefore was prepared and treated with indole in the presence of acetic acid and acetic anhydride. No acetyltryptophan was isolated from the reaction product. In view of this result it seems unlikely that the analogous compound, diacetylserine, is involved in the formation of acetyltryptophan.

Another possible intermediate in the formation of acetyltryptophan is 2-methyl-4-carboxyoxazoline (IV). A convenient synthesis of 2-phenyl-4-carboxyoxazoline, differing from IV only in the substituent at the 2-position, has been described.[12] This oxazoline reacts with hydrochloric acid to give 1-benzoylamino-2-chloropropionic acid[12] and with thiobenzoic acid to give dibenzoylcysteine.[13] It appeared possible that indole also might attack at the 4-position and yield benzoyltryptophan. 2-Phenyl-4-carboxyoxazoline was prepared and treated with indole in the presence of acetic acid and acetic anhydride. No benzoyltryptophan was obtained from this treatment, and it would therefore appear unlikely that an oxazoline intermediate is involved in the formation of acetyltryptophan from indole and α-acetamidoacrylic acid.



The possibility of preparing nuclear-substituted acetyltryptophans by the reactions of substituted indoles with α-acetamidoacrylic acid was investigated. 2-Methyl- and 7-methylindole were found to yield the corresponding acetyltryptophans, but neither ethyl indole-2-carboxylate nor indole-2-carboxylic acid added to α-acetamidoacrylic acid.

Experimental[14]

Preparation of Acetyltryptophan.—A mixture consisting of 1.17 g. (0.01 mole) of indole, 1.30 g. (0.01 mole) of α-acetamidoacrylic acid,[15] 6 ml. of acetic acid, and 2 ml. of acetic anhydride was heated in an oil-bath maintained at 95–96° for 20 minutes. The reaction mixture, which was stirred throughout the heating period, turned red soon after heating was begun. The reaction product was cooled in ice, diluted with 25 ml. of ether, and made basic with 30% sodium hydroxide. The ether layer, after several extractions with dilute sodium hydroxide, was washed with water, dried, and concentrated to yield an oily residue weighing 0.31 g. and possessing an indole odor. A few grains of sodium hydrosulfite were added to the collected sodium hydroxide solutions, which were then cooled in ice, neutralized with concentrated hydrochloric acid, and concentrated at the water-pump to about 50 ml. The residue was acidified to congo red, and allowed to stand in the refrigerator overnight. The solid was collected, washed with water, and recrystallized from ethanol–water. A yellow solid, 1.22 g. (49%), m.p. 204–205° after softening at 195°, was obtained. In some experiments the maximum yield of acetyltryptophan was obtained only after concentrating the mother liquors to yield one or two additional crops of solid.

The best yields of acetyltryptophan were obtained when the reaction mixture was heated for only five minutes after solution was complete. The use of a catalytic amount of copper acetate did not increase the yield. When a threefold excess (3.90 g., 0.03 mole) of α-acetamidoacrylic acid was used and a reaction time of 45 minutes was employed, a 57.7% yield of acetyltryptophan was obtained. In this case complete solution of the α-acetamidoacrylic acid did not occur. A sample of acetyltryptophan prepared in this way and recrystallized from ethanol–water had m.p. 206–207°, alone or mixed with authentic acetyltryptophan.[16] Hydrolysis with 10% sodium hydroxide gave tryptophan, m.p. 273–276° dec. The infrared spectra of this compound and of authentic tryptophan in Nujol mulls were essentially identical.

(10) S. A. Ballard and B. P. Geyer, U. S. Patent 2,459,677 (*C. A.*, **43**, 3026 (1949)).

(11) J. Harley-Mason, *Chemistry and Industry*, 886 (1951).

(12) E. M. Fry, *J. Org. Chem.*, **14**, 887 (1949).

(13) E. M. Fry, *ibid.*, **15**, 438 (1950).

(14) All melting points are corrected. Microanalyses were performed by Mr. J. Nemeth, Mrs. E. Fett and Mrs. L. Chang. The infrared spectra were obtained by Miss Helen P. Miklas.

(15) M. Bergmann and K. Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).

(16) C. P. Berg, W. C. Rose and C. S. Marvel, *J. Biol. Chem.*, **85**, 207 (1929).

Treatment of Indole with α-Acetamidoacrylic Acid and Hydrolysis of the Resulting Reaction Product.—A mixture of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.17 g. (0.01 mole) of indole, 4 ml. of acetic acid and 1 ml. of acetic anhydride was heated on the steam-bath, with stirring, for 15 minutes. The product was cooled, made basic with an excess of 7 ml. of 30% sodium hydroxide and refluxed for 20 hours. The product was extracted twice with ether, heated with charcoal, filtered, cooled, brought to a *p*H of 6.5 by the addition of acetic acid, and cooled in the refrigerator overnight. A precipitate, chiefly inorganic, was collected. The tryptophan was extracted from this material with hot ethanol and hot water, and recovered from the combined extracts by concentration. The product was recrystallized from ethanol–water, using a few grains of sodium hydrosulfite to reduce the color. The resulting product weighed 0.40 g. (19.6%) and melted at 260–264°.

Treatment of Indole with 2-Methyl-4-formal-5-oxazolone.—To 1.66 g. (0.01 mole) of chloroacetylalanine[17] was added 7 ml. of acetic anhydride and 1.60 g. of fused sodium acetate. The mixture was allowed to stand at room temperature for 10 minutes, and then 1.17 g. (0.01 mole) of indole was added. The solution was stirred at room temperature for 5 minutes and then heated, with stirring, at 80–85°, for 20 minutes. No acetyltryptophan was obtained when this product was worked up by the method employed in the preparation of acetyltryptophan from indole and α-acetamidoacrylic acid. The alkali-insoluble fraction yielded 0.81 g. of solid melting at 46–48° and possessing an indole odor.

Similar results were obtained in other experiments in which the order of addition of the reagents was varied.

Preparation of Acetyltryptophan in the Presence of Sodium Acetate.—A mixture consisting of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.17 g. (0.01 mole) of indole, 1.60 g. of sodium acetate, 21 ml. of acetic acid and 7 ml. of acetic anhydride was heated, with stirring, at 80–85° for 20 minutes. The product was concentrated at about 2 mm. pressure to about one-half of its original volume. This residue, worked up by the general method employed in the other preparations of acetyltryptophan, yielded 0.82 g. (33.3%) of product melting at 203–205°.

Treatment of Indole with Diacetylcysteine.—A mixture of 2.05 g. (0.01 mole) of diacetylcysteine,[18] 1.17 g. (0.01 mole) of indole, 6 ml. of acetic acid and 2 ml. of acetic anhydride was heated, with stirring, at 95–96° for 20 minutes. Solution occurred soon after heating was begun. The deep red reaction product was worked up by the procedure used in the reaction of indole with α-acetamidoacrylic acid. No acetyltryptophan was obtained. The alkali-insoluble portion of the reaction mixture yielded 1.06 g. of brown viscous oil.

Treatment of 2-Phenyl-4-carboxyoxazoline with Indole.—A mixture of 0.50 g. (0.00262 mole) of 2-phenyl-4-carboxyoxazoline,[12] 0.307 g. (0.0262 mole) of indole, 1.6 ml. of acetic acid and 0.5 ml. of acetic anhydride was heated, with stirring, at 95–96° for 5 minutes. Solution occurred almost immediately. The product was worked up by the method used in the reaction of indole with α-acetamidoacrylic acid. No benzoyltryptophan was obtained. A similar result was obtained when the 2-phenyl-4-carboxyoxazoline was added to the other reagents.

(17) E. Fischer, *Ber.*, **37**, 2486 (1904).

(18) M. W. Farlow, *J. Biol. Chem.*, **176**, 71 (1948).

Preparation of Acetyl-2-methyltryptophan.—A mixture of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.31 g. (0.01 mole) of 2-methylindole, 6 ml. of acetic acid and 2 ml. of acetic anhydride was heated, with stirring, at 95–96° for 20 minutes. Solution was complete after 15 minutes. The dark red reaction product was worked up by the method used in the preparation of acetyltryptophan. It yielded 0.50 g. (19.2%) of white solid melting at 207.5–209°. A portion of this material, on further recrystallization from ethanol–water, yielded a sample melting at 208–209°.

Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.77. Found: C, 64.42; H, 6.16; N, 10.85.

Acetyl-2-methyltryptophan was prepared by Rydon[19] as an intermediate in the synthesis of 2-methyltryptophan, but its melting point was not recorded. A portion of the above product was hydrolyzed by refluxing it for 30 hours with a solution of barium hydroxide. The resulting 2-methyltryptophan, after recrystallization from ethanol–water, melted at 235–236° (lit.[19] 234–235°). It darkened several degrees below its melting point.

Preparation of Acetyl-7-methyltryptophan.—A mixture consisting of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.31 g. (0.01 mole) of 7-methylindole, 6 ml. of acetic acid and 2 ml. of acetic anhydride was heated, with stirring, at 95–96° for 25 minutes. Solution was complete after 20 minutes. The reaction product, worked up by the method used in the preparation of acetyltryptophan, yielded 0.95 g. (36.5%) of orange solid which melted at 221–222° after previous softening. A portion of this product was recrystallized twice from ethanol–water, Darco being used in the first recrystallization. The recrystallized material, light yellow in color, melted at 223–224.5°; acetyl-7-methyltryptophan has been reported to melt at 217–218°.[19]

Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.77. Found: C, 64.71; H, 6.35; N, 10.70.

Treatment of Ethyl Indole-2-carboxylate with α-Acetamidoacrylic Acid.—A mixture of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.89 g. (0.01 mole) of ethyl indole-2-carboxylate, 6 ml. of acetic acid and 2 ml. of acetic anhydride was heated, with stirring, at 110° for 20 minutes. Solution was complete after 15 minutes. The reaction product was worked up by the method used in the synthesis of acetyltryptophan. No solid organic material was obtained from the alkali-soluble portion of the reaction product. The alkali-insoluble portion yielded 1.60 g. of yellow solid melting at 122–123° alone, and at 123–124.5° when mixed with authentic ethyl indole-2-carboxylate. A similar experiment, employing a reaction period of 40 minutes, was carried out at 95–96°, but no addition product could be isolated.

Treatment of Indole-2-carboxylic Acid with α-Acetamidoacrylic Acid.—A mixture consisting of 1.30 g. (0.01 mole) of α-acetamidoacrylic acid, 1.61 g. (0.01 mole) of indole-2-carboxylic acid, 4 ml. of acetic acid and 1 ml. of acetic anhydride was heated, with stirring, at 110° for 20 minutes. The cooled reaction mixture, on treatment with 30 ml. of water, yielded a precipitate which was collected. This precipitate weighed 1.20 g. and melted at 202.5–206°. When mixed with authentic indole-2-carboxylic acid it melted at 205–206°.

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(19) H. N. Rydon, *J. Chem. Soc.*, 705 (1948).