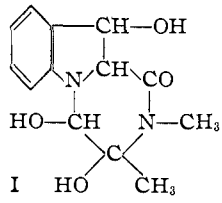
[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

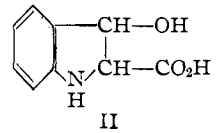
Gliotoxin. VIII. Derivatives of 3-Hydroxyindoline-2-carboxylic Acid[1]

BY JOHN R. JOHNSON AND JOHN H. ANDREEN[2]

Studies of gliotoxin and the sulfur-free reduction product, desthiogliotoxin, have led to the view that the latter may be formulated as a pyrazinoindolone derivative (such as I)[3] in which one of the structural units is related to 3-hydroxyindoline-2-carboxylic acid (II). The other structural unit of gliotoxin is evidently related to N-methylcysteine, and this portion of the antibiotic molecule bears some resemblance to the penaldic acid fragment of the penicillins.



I

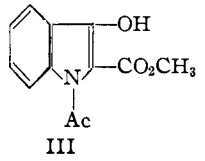


II

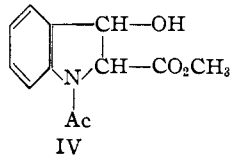
The formation of indole-2-carboxylic acid and its derivatives in the previously described degradation reactions of gliotoxin can be attributed to loss of the elements of water from the 3-hydroxyindoline system under the influence of acidic or basic reagents. The synthesis of model compounds related to 3-hydroxyindoline-2-carboxylic acid was undertaken for the purpose of comparing their properties with those of gliotoxin and desthiogliotoxin.

The parent compound 3-hydroxyindoline (3-indolinol) and several of its simple derivatives have been obtained by hydrogenation of indoxyl and N-acetylated indoxyl derivatives over nickel-copper catalysts at high pressures.[4] After several unsuccessful attempts to effect hydrogenation of the methyl ester of indoxylic acid under similar conditions, and also with other catalysts, it was found that the desired hydrogenation could be accomplished by using the corresponding N-acetyl derivative (III).

The methyl ester of N-acetylindoxylic acid (III) was prepared from the N-acetyl derivative of dimethylphenylglycine-*o*-carboxylate by an intramolecular ester condensation.[5] Hydrogenation of III over nickel or copper chromite catalysts[6] at 1800–2000 lb. pressure gave 10–30% yields of the dihydro compound, methyl 1-acetyl-3-hydroxyindoline-2-carboxylate (IV), which formed colorless crystals melting at 166–167°. More satisfactory results (70–80% yields) were obtained with Adams platinum catalyst at room temperature and low pressures.



III

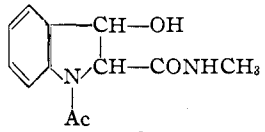


IV

It was observed that the model hydroxyindolinecarboxylic ester (IV) underwent the anticipated transformation to indole derivatives by the action of acids or alkalies. Aqueous 50% sulfuric acid, at room temperature, brought about elimination of the elements of water and concurrent hydrolysis of the N-acyl group to produce methyl indole-2-carboxylate. The same reaction occurred, but not so rapidly, with aqueous methanolic solutions of sulfuric or hydrochloric acid.

When dissolved in 10% methanolic potassium hydroxide solution and allowed to stand at room temperature for several hours, the ester (IV) was merely saponified and crystals of the potassium salt of 1-acetyl-3-hydroxyindoline-2-carboxylic acid separated. Careful acidification of the potassium salt gave the free acid, which regenerated the original ester (IV) when treated with diazomethane. On heating with aqueous alkalies, and subsequent acidification, the ester is converted to indole-2-carboxylic acid.

Acetylation of the hydroxyindoline ester (III) by acetyl chloride and pyridine, according to Shriner's quantitative procedure,[7] showed the presence of one reactive hydrogen and furnished the crystalline O-acetyl derivative, methyl 1-acetyl-3-acetoxyindoline-2-carboxylate. Benzoylation in pyridine solution gave the O-benzoyl derivative, methyl 1-acetyl-3-benzoxyindoline-2-carboxylate.



V

By the action of anhydrous methylamine at low temperature, the ester (IV) was converted to the corresponding N-methylamide (V), a model compound which is closely related to the hydroxyindoline structural unit postulated for gliotoxin. This N-methylamide, like gliotoxin, evolved methylamine when refluxed with aqueous alkali and the hydrolysate gave indole-2-carboxylic acid

(1) Preceding paper, THIS JOURNAL, **69**, 2364 (1947).

(2) The Wm. S. Merrell Company Postdoctorate Fellow, 1945–1946; present address, E. I. du Pont de Nemours and Co., Inc., Rayon Department, Buffalo, N. Y.

(3) Dutcher, Johnson and Bruce, THIS JOURNAL, **67**, 1740 (1945). Further studies of the reactions of desthiogliotoxin will be described in a forthcoming paper.

(4) German Patents 515,544, 516,675, 516,676 and 518,515 (1928); *Frdl.*, **17**, 638, 642, 649 (1930).

(5) German Patents 117,059 and 126,962 (1900); *Frdl.*, **6**, 542, 556 (1901).

(6) Adkins and Coonradt, THIS JOURNAL, **63**, 1563 (1941).

(7) Shriner, "Quantitative Analysis of Organic Compounds," Edward Brothers, Inc., Ann Arbor, Michigan, 1938.

upon acidification. Methanolic potassium hydroxide solution at room temperature converted the compound V into the methylamide of indole-2-carboxylic acid. This behavior also parallels that of gliotoxin and desthiogliotoxin, which are converted by mild action of methanolic alkalies into derivatives of the methylamide of indole-2-carboxylic acid. The model amide (V) was transformed also into the methylamide of indole-2-carboxylic acid by the action of 50% aqueous sulfuric acid at room temperature.

The observed behavior of the model compounds IV and V appears to be compatible with the view that a 3-hydroxyindoline unit is present in gliotoxin and desthiogliotoxin.

Experimental

Dimethyl N-Acetylphenylglycine-*o*-carboxylate.[5]—A solution of 500 g. of methyl anthranilate and 180 g. of methyl chloroacetate in 600 ml. of methanol was refluxed for six days on a steam-bath. After distilling off the methanol, the reaction mixture was shaken with chloroform and water, and the chloroform layer washed with sodium carbonate solution and water. The chloroform was distilled off from a steam-bath, and unreacted methyl chloroacetate and methyl anthranilate were removed by distillation at low pressure (up to 105° at 2 mm.). The residual liquid, while still warm, was poured into 600 ml. of 95% ethanol. After standing for several hours at 20°, the crystals of dimethyl phenylglycine-*o*-carboxylate were collected and washed with cold ethanol. The product formed colorless needles, m. p. 92–96°; the yield was 265 g. (72%).

Fifty grams of the crude ester from the preceding preparation was mixed thoroughly, by swirling, with 35 g. of acetyl chloride. After the initial vigorous evolution of hydrogen chloride had subsided, the mixture was heated for an hour on a steam-bath. The liquid was cooled to room temperature and poured into 100 ml. of water. The product was taken up in chloroform and the solvent distilled off (finally under reduced pressure). The residue was dissolved in 30 ml. of methanol, and sufficient water (about 100 ml.) was added to produce a permanent turbidity. Addition of seed crystals and shaking caused the product to separate in crystalline form. After standing overnight at −10°, the crystals were collected with suction, washed with water, and dried thoroughly. The product weighed 50 g. (84% yield); m. p. 77–80°.

Methyl Ester of N-Acetylindoxylic Acid (III).[5]—A cold solution of sodium methoxide prepared from 4.5 g. of sodium and 50 ml. of methanol was added, with thorough mixing, to a solution of 50 g. of dimethyl N-acetylphenylglycine-*o*-carboxylate in 200 ml. of absolute methanol. The mixture was allowed to stand at room temperature for twenty minutes and then acidified with 65 ml. of 20% aqueous acetic acid. The resulting solution was stirred mechanically during the addition of 275 ml. of water, which gave a permanently turbid mixture. Over a period of four hours, with continued stirring, the crude product separated in the form of grayish green crystals (weight, 27 g.). After crystallization from 125 ml. of methanol, with addition of decolorizing carbon, the purified material formed colorless crystals, m. p. 124–126°, weighing 22.5 g. (50% yield).

Methyl Ester of 1-Acetyl-3-hydroxyindoline-2-carboxylic Acid (IV).—Hydrogenation of the methyl ester of N-acetylindoxylic acid was carried out at first over nickel and copper catalysts at 1800–2000 lb. pressure and at room temperature, but the yields of the dihydro compound were only 10–30%, and the purification of the reduction product was difficult. The following procedure proved to be much more satisfactory.

A solution of 5 g. of the methyl ester of N-acetylindoxylic acid in 150 ml. of methanol and 0.1 g. of Adams platinum oxide was shaken at room temperature for two and one-half hours with hydrogen at an initial pressure of 60 lb./sq. in. After removal of the catalyst, the solution was concentrated to a volume of 50 ml. and allowed to stand overnight at −10°. The initial crop of colorless needles of the dihydro compound (IV) weighed 4 g. (80% yield); m. p. 165–167°. Additional material was obtained by concentration of the mother liquor. After recrystallization from methanol the purified product melted at 166–167°.

Anal. Calcd. for $C_{12}H_{13}O_4N$: N, 5.95. Found (micro-Dumas): N, 5.96.

The ultraviolet absorption spectrum of this compound (IV) showed maxima at 250 mμ (log ε, 4.2) and 280 mμ (log ε, 3.45) and an inflexion in the 285–290 mμ region; minima were observed at 225 mμ (log ε, 3.6) and between 270–275 mμ (log ε, 3.3).

Reaction with Acids.—A small sample (0.5 g.) of methyl 1-acetyl-3-hydroxyindoline-2-carboxylate was added to 5 ml. of 50% aqueous sulfuric acid at room temperature. At first a clear solution was formed but after stirring for one minute a marked turbidity developed. After standing for two hours at 25° the mixture was diluted with water and the crystalline solid was collected. After two crystallizations from aqueous methanol the product melted at 148–151°, and was identified as methyl indole-2-carboxylate by comparison with an authentic specimen and by saponification to indole-2-carboxylic acid.

The same product was obtained also by allowing the hydroxyindoline ester to stand for several days with aqueous-methanolic sulfuric acid or hydrochloric acid.

Reaction with Alkali.—A solution of 250 mg. of methyl 1-acetyl-3-hydroxyindoline-2-carboxylate in 10 ml. of 10% methanolic potassium hydroxide solution was allowed to stand at room temperature for four hours. The needle-like crystals of the potassium salt which separated were collected and washed with methanol; a second crop was obtained by cooling the combined mother liquor and washings. The crystals dissolved readily in water and left a white ash on ignition.

When an aqueous solution of the salt was acidified carefully with hydrochloric acid, colorless crystals of an organic acid separated. The acid was purified by dissolving it in a small amount of hot methanol, cooling the solution and adding benzene to the point where crystallization occurred. The purified acid decomposed at 163–167° (in a capillary tube) with evolution of gas. On the Dennis-Shelton bar it decomposed instantaneously at *ca.* 190°.

A 500-mg. sample of the free acid was dissolved in 30 ml. of pure acetone and the solution treated at 0° with 5 ml. of a cold ethereal solution of diazomethane. The solution was filtered and allowed to stand at 25° for two hours. The solution was concentrated to a volume of 2–3 ml. and allowed to stand at −5°. The colorless crystals which separated weighed 450 mg. and melted at 164–166°. Under the microscope the crystals were identical with those of the original methyl 1-acetyl-3-hydroxyindoline-2-carboxylate and showed no melting-point depression when mixed with the latter.

Acetylation.—Two quantitative acetylations with acetyl chloride and pyridine according to the procedure of Shriner,[7] using 1-g. samples of the ester, showed consumption of 0.97 and 0.98 mole of acetyl chloride per mole. The O-acetyl derivative was recovered from the neutralized acetylation mixtures and recrystallized twice from methanol; colorless crystals, m. p. 135.5–137°.

Benzoylation.—A solution of 1 g. of the hydroxyindoline ester in 10 ml. of pyridine was cooled in an ice-bath and treated with 1 g. of benzoyl chloride. After standing for two days at 25° the mixture was cooled in an ice-bath and treated with an equal volume of water. The crystals of the O-benzoyl derivative which separated were recrystallized from methanol; white needles, m. p. 140–141°.

Methylamide of 1-Acetyl-3-hydroxyindoline-2-carboxylic Acid (V).—A cold solution of 250 mg. of methyl 1-acetyl-3-hydroxyindoline-2-carboxylate (IV) in 20 ml. of anhydrous methylamine was sealed tightly and allowed

to stand at room temperature for two days. Evaporation of the methylamine left a white solid; weight, 185 mg. (73% yield). After crystallization from acetonitrile the methylamide formed colorless crystals which melted with decomposition at 235–240° (in a capillary tube); on the Dennis–Shelton bar the substance decomposed instantly at 258°.

Anal. Calcd. for $C_{12}H_{14}O_3N_2$: N, 11.2. Found (micro-Dumas): N, 11.24.

Reaction with Alkali.—(a) A mixture of 433 mg. of the methylamide and 30 ml. of 20% aqueous potassium hydroxide solution was refluxed for one and one-half hours. The methylamine formed was swept out of the system by a current of nitrogen and absorbed in 20 ml. of 0.1 *N* hydrochloric acid. The alkaline hydrolysate was cooled and acidified by the addition of 9 ml. of concentrated hydrochloric acid. The precipitated acid was collected and washed with water. Without further purification the product melted at 203–204.5°, and was identified as indole-2-carboxylic acid by comparison with an authentic sample. The accessory product of hydrolysis, methylamine, was identified as the hydrochloride, m. p. 222–225°.

(b) A 200-mg. sample of the methylamide (V) was dissolved by gentle warming in 15 ml. of *N*/3 methanolic potassium hydroxide solution. After four hours of standing at 25°, carbon dioxide was bubbled into the solution to convert excess alkali to the carbonate. The reaction mixture was evaporated to dryness under diminished pressure and the solid residue was taken up in water. All but a small portion of the solids dissolved in water; the identity of the insoluble material, m. p. 165–175°, was not established. The filtered aqueous solution was acidified with hydrochloric acid and cooled at −5° for several hours. The colorless crystalline solid which separated was collected and identified as the methylamide of indole-2-carboxylic acid, m. p. 219–220°. The yield was 65% of the theoretical.

Reaction with Acid.—A 50-mg. sample of the methylamide (V) was dissolved in 0.5 ml. of 50% aqueous sulfuric acid. After standing for one-half hour at room temperature colorless crystals began to separate. After two hours of standing 1 ml. of water was added and the crystals were collected and washed with water; weight, 16 mg. Recrystallization from 95% ethanol gave colorless crystals, m. p. 220–221°, which were identified as the methylamide of indole-2-carboxylic acid by comparison with an authentic sample.

Summary

The methyl ester and methylamide of 1-acetyl-3-hydroxyindoline-2-carboxylic acid have been synthesized as model compounds of the hydroxyindoline system that has been postulated to occur in gliotoxin and desthiogliotoxin. The anticipated transformation of the model compounds into derivatives of indole-2-carboxylic acid by the action of acids and of alkalies was confirmed experimentally. The behavior of the model compounds is therefore compatible with the view that the various derivatives of indole-2-carboxylic acid formed from gliotoxin and desthiogliotoxin arise from the presence of a 3-hydroxyindoline grouping.

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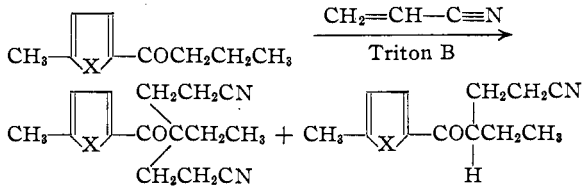
The Cyanoethylation of Certain Alkylated Heterocyclic Ketones[1]

BY NANCY A. ACARA AND ROBERT LEVINE

The cyanoethylation of a number of different types of active hydrogen compounds has been studied by Bruson and co-workers.[2] Among the compounds which were cyanoethylated are alkyl, alkylaryl and alkylheterocyclic ketones. In this laboratory, we have recently extended this work to the cyanoethylation of a number of β-keto-esters and β-diketones.[3]

The present study on the cyanoethylation of several alkylated ketones in the thiophene and furan series was undertaken in order to determine what effect an alkyl group present in the heterocyclic ring would have on the nature of the condensation products formed. Thus, 5-methyl-2-(acetyl, propionyl and *n*-butyryl)-furan, 5-methyl-2-(acetyl, propionyl and *n*-butyryl)-thiophene and 2,5-dimethyl-3-(acetyl, propionyl and *n*-butyryl)-furan were treated with acrylonitrile in the presence of the basic condensing agent, "Triton B."

The yields and physical constants of the condensation products are found in Table I. It may be seen that 5-methyl-2-acetyl-(furan and thiophene) have been tricyanoethylated and the corresponding ethyl ketones dicyanoethylated in good yields. However, the homologous *n*-propyl ketones gave a mixture of mono- and dicyanoethylated derivatives as indicated by the following equation in which X represents an oxygen or sulfur atom.



In order to determine whether a heterocyclic *n*-propyl ketone of the furan or thiophene series which does not contain an alkyl group in the ring would also give rise to a mixture of condensation products, 2-*n*-butyrylthiophene was cyanoethylated and a mixture of the mono (36%) and the dicyanoethylated (48.5%) ketones was isolated. This result is unexpected because the cyanoethylation of 2-*n*-butyrylfuran has apparently

(1) This paper is based on a thesis submitted by Nancy A. Acara in partial fulfillment of the requirements for the degree of Master of Science at the University of Pittsburgh.

(2) See Bruson, "Organic Reactions," Vol. 5, Roger Adams, Editor-in-Chief, John Wiley and Sons, Inc., New York, N. Y., 1949, Chapter 2.

(3) Zellars and Levine, *J. Org. Chem.*, **13**, 911 (1948).