was added dropwise, with stirring, over a period of thirty minutes. After stirring for an hour, the mixture was refluxed for twenty-four hours.

To the cold reaction mixture was added 100 cc. of 10% sodium hydroxide solution and the mixture was steam distilled to remove the solvent and facilitate solution of any unalkylated starting material. The insoluble organic product was taken up in benzene, and the filtered solution was distilled to remove the solvent. The excess of diethylaminoethyl bromide was removed by distillation under reduced pressure (b. p. 60° (4 mm.)) and the residual liquid dissolved in 150 cc. of dry benzene. Under anhydrous conditions, dry hydrogen chloride was passed over the solution for several hours. The benzene was removed by decantation and the sticky hydrochloride of the base was dissolved in hot butanol. An equal volume of dry acetone and several volumes of dry ether were added, and the solution was chilled thoroughly. The purified hydrochloride was collected by centrifuging, and subjected to several reprecipitations in a similar way. Eventually there was obtained 1 g. of fine white crystals of the hydrochloride of the base; m. p. 173–174°.

Anal. Calcd. for $C_{18}H_{23}N_3O \cdot HCl$: N, 12.59. Found: N, 12.39, 12.31.

Several grams of slightly less pure material were recovered from the filtrates. The free base was converted to the sulfate, phosphate and *p*-toluenesulfonate, but these salts were hygroscopic and difficult to purify.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra were obtained with a Beckmann spectrophotometer and ethanol was used as solvent. Curves are shown in detail for three pairs of isomers (Figs. 1–3). Tabulations of the characteristic maxima and minima for the pyrazinoindolones and pyridindolones are given in Tables I and II.

Summary

A number of pyrazino[1.2-a]indole-1(2)-ones and pyrid[3.4-b]indole-1(2)-ones have been prepared by ring closure of the 2-indolecarbonyl derivatives of α-aminoacetals. It has been found that these two different structural types can be distinguished from one another by means of their ultraviolet absorption spectra.

Pyrazinoindole-1(2)-ones and pyridindole-1(2)-ones having a hydrogen in the 2-position undergo N-alkylation at that position by the conventional methods of alkylation. This procedure permits the preparation of 2-alkylpyrazinoindole-1(2)-ones that are not accessible by direct cyclization.

2,3-Dimethylpyrazinoindole-1(2)-one and the isomeric 2,3-dimethylpyridindole-1(2)-one, which are important reference structures for gliotoxin degradation products, have been synthesized.

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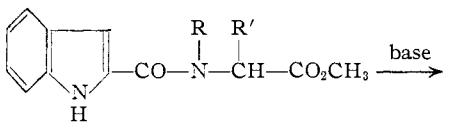
[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

Pyrazinoindole-1,4-diones. Ring Closure of 2-Indolecarbonyl Derivatives of α-Aminoesters

BY JOHN R. JOHNSON, JOHN H. ANDREEN[1] AND ANN D. HOLLEY[1]

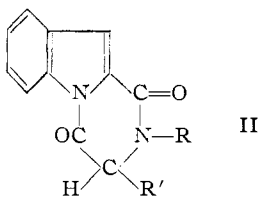
One of the important degradation products of the antibiotic substance gliotoxin is a crystalline compound of the formula $C_{13}H_{12}N_2O_2$, which is obtained readily by reduction of gliotoxin with hydriodic acid and red phosphorus.[2] A synthesis of this degradation product was effected by reaction of 2-indolecarbonyl chloride with the methyl or ethyl ester of *dl*-N-methylalanine to form the 2-indolecarbonyl derivative of the aminoester (I), which underwent ring closure spontaneously in the reaction mixture.[2,3] Evidence from ultraviolet absorption studies indicated that the cyclization product is 2,3-dimethylpyrazino[1.2-a]indole-1,4(2,3)dione (II, R=R′=CH_3) rather than the isomeric pyridindole-1,4-(2,3)-dione (III, R=R′=CH_3).

In the earlier report[2] the spontaneous cyclization of the 2-indolecarbonyl derivative of the aminoester was attributed to the presence of free hydrogen chloride formed in the first step of the reaction, as it is known that this reagent effects ring closure of analogous derivatives of α-amino-



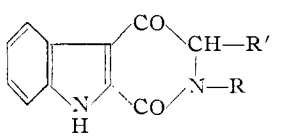
I

2-Indolecarboxamido ester



II

Pyrazinoindole-1,4(2,3)-dione
(observed ring closure)



III

Pyridindole-1,4(2,3)-dione
(alternative ring closure)

(1) The Wm. S. Merrell Company Fellow in Chemistry.

(2) Dutcher, Johnson and Bruce, THIS JOURNAL, **66**, 617 (1944).

(3) Johnson, Hasbrouck, Dutcher and Bruce, *ibid.*, **67**, 423 (1945).

acetals.[4,5] The present study has shown however that the acylaminoesters, unlike the corresponding acetals, fail to undergo ring closure under the influence of hydrogen chloride or other acidic reagents but do so only in the presence of bases, such as ammonia, amines and sodium methoxide. It is now evident that the spontaneous ring closures observed in the earlier experiments were due to the presence of free aminoester and not free hydrogen chloride. This view is confirmed by the fact that 2,3-dimethylpyrazinoindole-1,4(2,3)-dione is formed in almost quantitative yields when the corresponding acylaminoester (I, $R=R'=CH_3$) is warmed in methanol with a small quantity of the methyl ester of *dl*-N-methylalanine.

The unusual ease of ring closure of the 2-indolecarbonyl derivative of N-methylalanine esters under the influence of basic catalysts led us to investigate the cyclization of 2-indolecarbonyl derivatives of other α-aminoesters under similar conditions. This study disclosed that the structural factors involved in ring closure of the acylaminoesters are quite different from those concerned with cyclization of the corresponding acylaminoacetals.

The 2-indolecarbonyl derivative of N-methylglycine ethyl ester (I, $R=CH_3$, $R'=H$) was found to undergo cyclization readily with the formation of a crystalline compound which was shown to be 2-methylpyrazino[1.2-a]indole-1,4-(2,3)-dione (II, $R=CH_3$, $R'=H$), by comparison of its ultraviolet absorption spectrum with that of a reference compound of known structure synthesized from 3-methylindole-2-carbonyl chloride and N-methylalanine ester.[3]

Negative results were obtained with the 2-indolecarboxamides from esters of glycine (I, $R=R'=H$) and alanine (I, $R=H$, $R'=CH_3$). These amides, which differ from the preceding ones in having a hydrogen atom on the amide nitrogen, failed to undergo cyclization on warming in methanolic solution with amines or with sodium methoxide, and were recovered unchanged. They are also much less readily saponified by cold methanolic potassium hydroxide than the corresponding N-methyl compounds.

Particular attention was directed to cyclization of the amide from 1-methylindole-2-carbonyl chloride and N-methylalanine methyl ester, as this compound could undergo only the alternative type of ring closure (*cf.* formula III) to form 2,3,9-trimethylpyridindole-1,4(2,3)-dione. Attempts to effect the desired ring closure were completely unsuccessful.

Although the present study has been limited to only six examples of 2-indolecarboxamidoesters, the results indicate: (a) that ring closure occurs readily if an alkyl group is present on the amide nitrogen but does not take place if a hydrogen atom is present in this position; (b) that the N-alkylated amides yield only the corresponding pyrazinoindole-1,4(2,3)-diones, in marked contrast to the corresponding N-alkylated 2-indolecarboxamidoacetals, which give only the pyridindolone type of ring closure.

We wish to thank The Wm. S. Merrell Company for a generous research grant in support of this work.

Experimental

Esters of N-(2-Indolecarbonyl)-N-methylalanine (I, $R=R'=CH_3$).—A solution of approximately 38 millimoles of freshly prepared *dl*-N-methylalanine ethyl ester in 50 cc. of dry ether was added to an ethereal solution (25 cc.) of 2-indolecarbonyl chloride[3] prepared from 3 g. (19 millimoles) of the acid. The mixture became slightly warm and developed a turbidity immediately. The solution was decanted from the oily hydrochloride of the aminoester and allowed to stand at 20° for an hour. The amide separated in a mass of needles; the product was collected on a filter and washed with 10% sodium carbonate solution and with water. The first crop of crystals weighed 3.7 g. (72% yield); a small additional quantity was recovered by concentration of the filtrate. Recrystallizations of the first crop from 95% ethanol (3 cc./g.) gave an 85% recovery of the **ethyl ester** of N-(2-indolecarbonyl)-N-methylalanine; colorless needles, m. p. 125–127°. The corresponding **methyl ester** was obtained in a similar way from the methyl ester of *dl*-N-methylalanine; it formed colorless needles from methanol, m. p. 140–142°.

More than twenty unsuccessful tests were carried out in an effort to effect cyclization of the ethyl or methyl ester by means of acidic reagents, which included: hydrogen chloride (1–15%) at temperatures from 20–80° in methanol, ethanol, benzene and ether; hydriodic acid in ethanol, ether and glacial acetic acid; sulfuric acid in ethanol and in ether; boron trifluoride etherate in ether. Further, the ethyl ester was recovered unchanged after heating alone at 145° for one hour or after refluxing in neutral ethanol for twenty-four hours.

2,3-Dimethylpyrazino[1.2-a]indole-1,4(2,3)-dione.—The experiments described in the preceding paragraph rendered untenable our earlier view that cyclization of 2-indolecarboxamidoesters occurred by acid catalysis.[3] Scrutiny of the experiments in which spontaneous ring closure had occurred indicated that an excess of N-methylalanine ester was present in these reaction mixtures, as we had assumed a conservative yield of 65% in the esterification of N-methylalanine. Experiments then disclosed that the free aminoesters are very effective catalysts for the cyclization, and that other amines and basic reagents such as sodium methoxide in absolute methanol also effect ring closure.

A slurry of 1 g. of the ethyl ester in 20 cc. of methanol at 20° was treated with 1 cc. of 25% aqueous dimethylamine. The crystals of the indolecarboxamidoester dissolved immediately and the solution was set aside for several hours. Upon concentration of the solution and cooling to −10°, there was obtained 0.35 g. of crystals as the pyrazinoindoledione, m. p. 120–122°. A further quantity was isolated from the mother liquors. In a similar experiment using 1 cc. of 28% aqueous ammonia of the basic catalyst, 0.5 g. of the cyclized product was isolated. Superior yields and quality of cyclized product were obtained when the aminoester itself was used as the basic catalyst, as described below.

A solution of 1 g. of the ethyl ester (3.6 millimoles) in 25 cc. of warm methanol was treated with 3 cc. of an ethereal solution of N-methylalanine methyl ester containing approximately 0.6 millimole of aminoester. The reaction mixture was allowed to stand at 20° for twelve hours and then concentrated to one-half its volume on a steam-bath. The cooled solution deposited 0.73 g.

(4) Kermack, Perkin and Robinson, *J. Chem. Soc.*, **119**, 1502 (1921); **121**, 1872 (1922).

(5) Johnson, Larsen, Holley and Gerzon, THIS JOURNAL, **69**, 2364 (1947).

(88% yield) of white crystals, m. p. 122.5–123.5°. A small second crop was isolated from the mother liquor, indicating that the cyclization was practically quantitative. Recrystallizations from methanol raised the melting point to 123.5–124°. The product was identical with the previous preparations of this compound.

The ultraviolet absorption spectrum of the cyclized product (in ethanol) is very similar to the spectra of the parent 2-indolecarboxamidoesters and of the esters and methylamide of 2-indolecarboxylic acid, which have a maximum in the region of 290 μ (log ϵ *ca.* 4.2). The pyrazinoindole-1,4(2,3)-diones differ slightly from the uncyclized esters in showing a definite inflection in the region of wave length 300–305 mμ which appears to be characteristic.

Ethyl Ester of N-(2-Indolecarbonyl)-N-methylglycine (I, R = CH_3, R′ = H).—N-Methylaminoacetonitrile was prepared according to the method of Staudt[6] and converted to the hydrochloride of N-methylglycine ethyl ester by the procedure of Baumann.[7] An ethereal solution of 37 millimoles of freshly prepared N-methylglycine ethyl ester was added to an ethereal solution of 2-indolecarbonyl chloride prepared from 3 g. (18.6 millimoles) of the acid. The amide precipitated at once; after one-half hour the product was collected and washed thoroughly with water; weight 3.5 g. (78% yield), m. p. 140–145°. On recrystallization from methanol the amidoester formed fine, colorless needles, m. p. 146–146.5°.

When a suspension of 0.25 g. of the amidoester in 1.25 cc. of methanol was treated with 1.25 cc. of *N* methanolic potassium hydroxide solution, a brilliant yellowish-green color developed immediately. The solid ester dissolved within ten minutes and soon a colorless precipitate formed; after fifteen minutes the solid and the solution had become colorless. After four hours of standing at 20° the solution was acidified to congo red with strong hydrochloric acid and diluted with an equal volume of water. The solid acid was collected, washed thoroughly with water, and finally with small amounts of ethanol and ether (yield, 80%). Recrystallization from 95% ethanol gave colorless crystals of N-(indole-2-carbonyl)-sarcosine, m. p. 223° (Dennis–Shelton bar); neutralization equivalent, calcd. 232, found 232.

2-Methylpyrazino[1.2-a]indole-1,4(2,3)-dione.—A slurry of 350 mg. (1.43 millimoles) of the ethyl ester of N-(2-indolecarbonyl)-N-methylglycine in 3.5 cc. of methanol was treated with approximately 0.1 millimole of N-methylglycine ethyl ester in 3 cc. of ether. After standing at 20° for twenty-four hours the supernatant liquid was decanted and evaporated under reduced pressure; the residual solid weighed 14 mg. The remaining undissolved solid was treated with 1 cc. of methanol and an ethereal solution of about 0.04 millimole of N-methylglycine ester and allowed to stand for twenty-four hours. The colorless solid was collected and washed with alcohol, followed by ether; weight, 237 mg. (84% yield), m. p. 203–205°. After crystallization from ethanol the material melted at 204.5–205.5°. The ultraviolet absorption of this compound is different from the parent ester and very similar to that of the hydriodic acid degradation product of gliotoxin.

Anal. Calcd. for $C_{12}H_{10}N_2O_2$: N, 13.08. Found: N, 13.24, 13.28.

In a subsequent experiment the cyclized product was obtained in 65% yield after the mixture had been allowed to stand for only one hour. Lower yields were obtained when ammonia was used as the basic catalyst under similar conditions.

(6) Staudt, *Z. physiol. Chem.*, **146**, 286 (1925).

(7) Baumann, *J. Biol. Chem.*, **21**, 563 (1915).

Ethyl Ester of N-(2-Indolecarbonyl)-glycine (I, R = R′ = H).—An ethereal solution of 2-indolecarbonyl chloride prepared from 3 g. (18.6 millimoles) of the acid was treated with an ethereal solution of 38 millimoles of freshly prepared glycine ethyl ester. The product precipitated immediately, and after several hours it was collected, washed with 10% sodium carbonate solution, and finally with water; weight, 4.1 g. (90% yield). The purified material formed colorless crystals; m. p. 225–227°. Attempts to effect ring closure of this compound by means of glycine ethyl ester in methanol, or sodium methoxide in absolute methanol, were unsuccessful.

Anal. Calcd. for $C_{13}H_{14}N_2O_3$: N, 11.38. Found: N, 11.26, 11.41.

Unlike the corresponding N-methyl derivative, the rate of saponification of this ester at 20° is exceedingly slow; after four hours of contact with methanolic potash a large portion of the ester remained intact. Saponification was effected by heating the amidoester with methanolic potassium hydroxide on a steam-bath for five to ten minutes. After acidification with strong hydrochloric acid the methanol was removed under reduced pressure. The amidoacid was taken up in sodium carbonate solution and the free acid regenerated by acidification (yield, 55%). Recrystallization from 95% ethanol furnished pale yellow crystals of N-(indole-2-carbonyl)-glycine, m. p. 224° (Dennis–Shelton bar); neutralization equivalent, calcd. 218, found 214, 214.

Methyl Ester of N-(2-Indolecarbonyl)-alanine (I, R = H, R′ = CH_3).—This compound was prepared from alanine methyl ester and 2-indolecarbonyl chloride by the same procedure used with glycine ethyl ester. The yield was 88% of the theoretical; the product formed colorless crystals, m. p. 205–207°. All attempts to effect ring closure of this ester by means of basic catalysts were unsuccessful.

Anal. Calcd. for $C_{13}H_{14}N_2O_3$: N, 11.38. Found: N, 11.34, 11.44.

The amido ester was saponified by warming with methanolic potassium hydroxide solution as described for the corresponding glycine ester. The free amidoacid (yield, 63%) was recrystallized from 95% ethanol. The purified N-(indole-2-carbonyl)-alanine formed colorless crystals, m. p. 219° (Dennis–Shelton bar); neutralization equivalent, calcd. 232, found 235, 234.

Methyl Ester of N-Methyl-N-(1-methylindole-2-carbonyl)-alanine.—The reaction of *dl*-N-methylalanine with 1-methylindole-2-carbonyl chloride, as described in a previous paper,[3] furnished the acylamino ester as an oily liquid which could not be obtained in a crystalline state. Heating the product in methanol solution with N-methylalanine ester, and other basic catalysts, failed to bring about cyclization.

Summary

It has been shown that the cyclization of 2-indolecarbonyl derivatives of α-amino esters is catalyzed by bases and not, as hitherto supposed, by acids.

Ring closure did not occur with amides having a hydrogen atom present on the amide nitrogen, but took place readily with the corresponding N-methyl compounds. The successful cyclization reactions gave invariably the corresponding pyrazinoindole-1,4(2,3)-diones.

ITHACA, N. Y. RECEIVED MAY 7, 1947