tion from benzene; 5.5 g. (93.7% of the theoretical yield) of the desired product was obtained as needles which sintered at 96° and melted at 102–103°.

Anal. Calcd. for $C_{13}H_{16}O_7$: C, 54.91; H, 5.67. Found: C, 54.88; H, 5.84.

Acid Chloride of 3,4-Dicarboxy-2-furanpentanol-acetate (XIII).—Five grams of the above compound (XII) was heated for one hour to 90° with 10 cc. of thionyl chloride. The excess of thionyl chloride was evaporated and the residue was distilled *in vacuo;* 5.2 g. (92% of the theoretical yield) of the desired acid chloride was obtained as a colorless oil which boiled at 160–165° at 0.03 mm.

A small amount of the acid chloride was hydrolyzed with an excess of 5 *N* potassium hydroxide and the resulting acid was isolated and recrystallized from ethyl acetate. Needles which melted at 124–126° were obtained which did not depress the melting point when mixed with an authentic sample of 3,4-dicarboxy-2-furanpentanol (VI).

Dianilide of 3,4-Dicarboxy-2-furanpentanol (XIV).—A solution of 900 mg. of the above acid chloride (XIII) was added to an ether solution of 2 cc. of aniline. The mixture was kept at room temperature for thirty minutes and was then washed with 2 *N* hydrochloric acid, 10% sodium bicarbonate and water, was dried over sodium sulfate and the ether removed on the steam-bath. The crystalline residue was refluxed for one hour with 10 cc. of methanol and 2 cc. of 5 *N* potassium hydroxide. The methanol was removed *in vacuo* and the residue was acidified with concentrated hydrochloric acid, and extracted with a large amount of ethyl acetate. The ethyl acetate solution was washed with water, dried over sodium sulfate, filtered, and concentrated on the steam-bath. The resulting crystals of the anilide were purified by recrystallization from methanol; 1.2 g. of silky needles melting at 99–101° was obtained.

Anal. Calcd. for $C_{23}H_{24}O_4N_2$: C, 70.39; H, 6.16; N, 7.13. Found: C, 70.17; H, 6.35; N, 6.84.

The author wishes to express his thanks to Mrs. Florence Baker for technical assistance.

Summary

1. The preparation of 2-furanpentanol and of 3,4-dicarboxy-2-furanpentanol has been described.

2. 3,4-Dicarboxy-2-furanpentanol was characterized by a number of derivatives.

3. 3,4-Dicarbethoxy-2-furanpentanol was selectively hydrolyzed and the structure of a 3-carbethoxy-4-carboxy-2-furanpentanol was suggested for the reaction product.

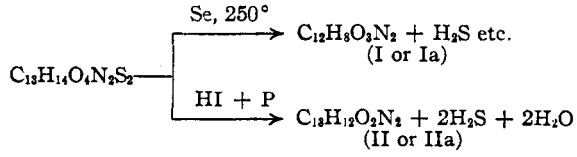
PITTSBURGH, PENNSYLVANIA
RECEIVED NOVEMBER 3, 1944

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

Gliotoxin. V. The Structure of Certain Indole Derivatives Related to Gliotoxin[1,2]

BY JOHN R. JOHNSON, RICHARD B. HASBROUCK,[3] JAMES D. DUTCHER[4] AND WILLIAM F. BRUCE

The antibiotic substance gliotoxin is produced by at least two microörganisms other than *Gliocladium fimbriatum*—it has been isolated from cultures of *Aspergillus fumigatus*[5] and of a species of *Penicillium* not yet definitely identified.[6] Investigations of the chemical constitution of the crystalline antibiotic principle have led to the isolation of two crystalline primary degradation products which are free of sulfur and have been identified definitely as derivatives of indole-2-carboxylic acid. Gliotoxin on heating with selenium[1] loses one carbon atom and gives a yellow crystalline compound, m. p. 253–255°, having the formula $C_{12}H_8O_3N_2$. The latter on hydrolysis gives oxalic acid and a colorless compound, $C_{10}H_{10}ON_2$, which has been shown to be the N-methylamide of indole-2-carboxylic acid. A milder degradation is accomplished by reduction with hydriodic acid and red phosphorus in glacial acetic acid,[7] which leads to a colorless crystalline compound, m. p. 122°, having the formula $C_{13}H_{12}O_2N_2$ and containing all of the carbon and nitrogen atoms of the gliotoxin molecule. This product on mild hydrolysis gives a 13-carbon monobasic acid of the formula $C_{13}H_{14}O_3N_2$, which on further hydrolytic cleavage breaks down into indole-2-carboxylic acid and *dl*-α-N-methylalanine. Although gliotoxin is optically active, $[\alpha]^{26}D$ −255°, both of the primary degradation products are optically inactive.



The selenium degradation product has been synthesized previously from the methylamide of indole-2-carboxylic acid and ethoxalyl chloride, and the hydriodic acid reduction product has been prepared from indole-2-carbonyl chloride and an ester of *dl*-α-N-methylalanine. Although these syntheses represent an important advance toward elucidation of the constitution of the primary degradation products, they do not suffice to establish the structures unambiguously. Both syntheses involve the ring closure of a substituted amide of indole-2-carboxylic acid, and it is known

(1) This is a continuation of a series previously entitled "Gliotoxin, the Antibiotic Principle of *Gliocladium fimbriatum*," the last instalment of which appeared in THIS JOURNAL, **66**, 619 (1944).

(2) Presented before the New York Section of the American Chemical Society, June 9, 1944.

(3) Abbott Laboratories Fellow in Chemistry, 1940–1941; Allied Chemical and Dye Corporation Fellow, 1941–1942.

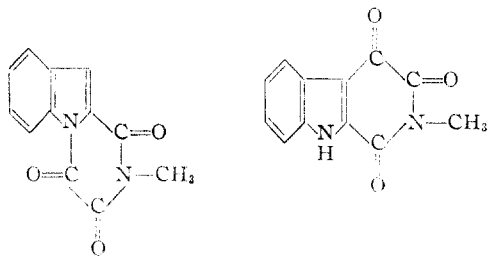
(4) Du Pont post-doctorate Fellow; present address, Squibb Institute for Medical Research, New Brunswick, N. J.

(5) Menzel, Wintersteiner and Hoogerheide, *J. Biol. Chem.*, **152**, 419 (1944); Glister and Williams, *Nature*, **153**, 651 (1944); also private communication from Dr. Neville F. Stanley, The Prince Henry Hospital, Sydney, Australia.

(6) Professor Harold Raistrick (London), private communication to J. R. J., October 1943; *cf.* Johnson, McCrone and Bruce, THIS JOURNAL, **66**, 501 (1944).

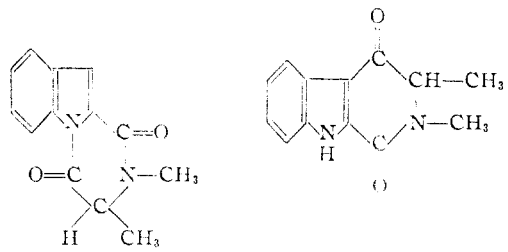
(7) Dutcher, Johnson and Bruce, *ibid.*, **66**, 617 (1944).

that such cyclizations can occur either to the nitrogen atom in the 1-position of the indole nucleus or to the carbon atom in the 3-(β) position.[8] Closure to the nitrogen atom leads to a derivative of α-pyrazindole (pyrazino[1.2-a]indole; Ring Index No. 1630)[9] whereas closure to the carbon atom gives a derivative of 2,9-pyridindole (9-pyrid[3.4-b]indole; β-carboline; Ring Index No. 1646), as shown in the alternative formulas I and Ia, respectively, for the selenium degradation product and in formulas II and IIa for the hydriodic acid reduction product.



I Ia

Alternatives for selenium degradation product

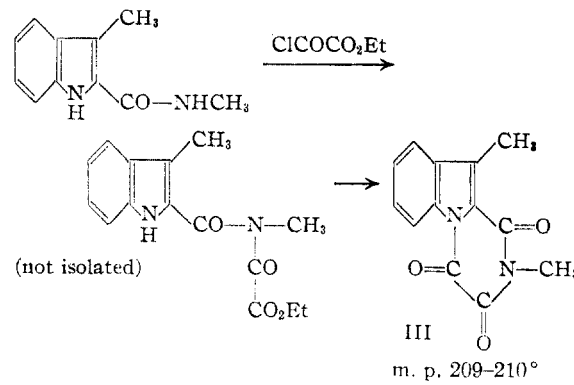


II IIa

Alternatives for hydriodic reduction product

In view of the important bearing of the structure of these products on the constitution of gliotoxin itself, it became imperative to determine with certainty which type of ring closure had occurred. After consideration of various physical and chemical methods, it was decided to attack the problem by preparing a number of model compounds known definitely to be cyclized in the 1- or 3-position and comparing their ultraviolet absorption spectra with the spectra of the compounds from gliotoxin.

To obtain compounds of known structure syntheses were carried out with derivatives of indole-2-carboxylic acid having either the 1- or the 3-position blocked by a methyl group. 3-Methylindole-2-carboxylic acid, prepared from the phenylhydrazone of α-ketobutyric acid,[10] was converted to the N-methylamide and condensed with ethoxalyl chloride to give a pyrazino[1.2-a]indole derivative of formula III.

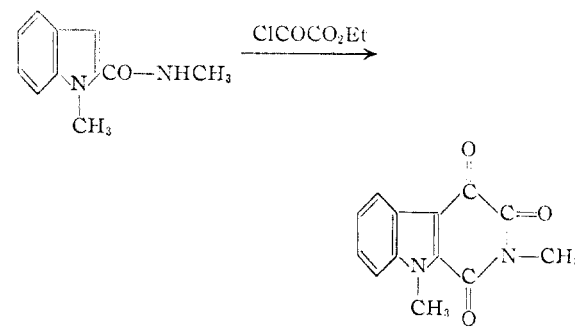


(not isolated)

III

m. p. 209–210°

In a similar way 1-methylindole-2-carboxylic acid, prepared from the α-methyl-α-phenylhydrazone of pyruvic acid,[11] was converted into the isomeric pyridindole derivative of formula IV. Yields of nearly 90% of the theoretical were obtained in both cyclizations and the products were readily obtained in a pure state.



IV

M. p. 269–270°

The ultraviolet absorption spectra[12] of the selenium degradation product and of compounds III and IV (Fig. 1) show that the selenium degradation product and compound III have absorption minima at approximately 290 mμ and maxima at 265 and 340–345 mμ. Compounds IV and Ia (see below), in which the cyclization occurs to the 3-position of the indole nucleus, show displacement of the minima and of the second maxima about 20 mμ toward the longer wave lengths. These considerations indicate that the selenium degradation product of gliotoxin is represented by the pyrazino[1.2-a]indole structure of formula I.

Confirmation of the conclusion drawn from the comparison of absorption spectra was achieved by synthesis of the isomeric pyridindole of formula Ia. Kermack, Perkin and Robinson[8] have shown that the condensation product obtained by interaction of the acid chloride of indole-2-carboxylic acid and N-methylaminoacetaldehyde dimethyl acetal undergoes ring closure at the 3-position to give the pyridindolone derivative V. It seemed

(8) Kermack, Perkin and Robinson, *J. Chem. Soc.*, **121**, 1872 (1923).

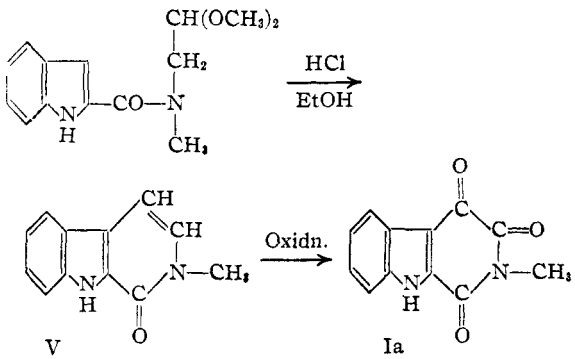
(9) Patterson and Capell, "The Ring Index. A List of Ring Systems used in Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1943.

(10) Wislicenus and Arnold, *Ann.* **246**, 315, 344 (1888).

(11) Fischer and Hess, *Ber.*, **17**, 559 (1884).

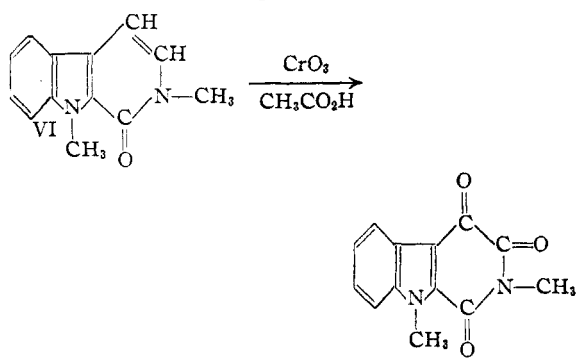
(12) We are indebted for the absorption spectra to Dr. Nettie Cox of the Squibb Institute for Medical Research, who carried out the spectroscopic work in collaboration with Dr. Dutcher.

possible that the latter could be oxidized under appropriate conditions to compound Ia.



V Ia

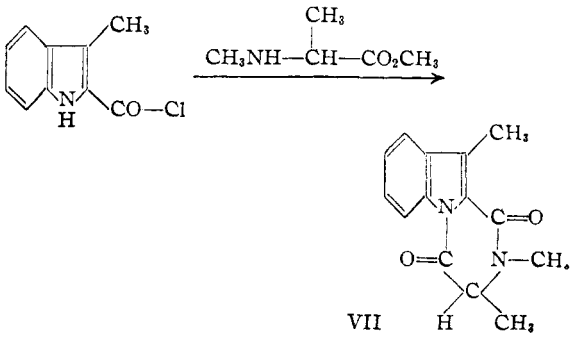
As such oxidations have not been reported for pyridindoles it was deemed advisable to test first the oxidation of the N-methyl derivative, VI, to compound IV. The pyridindolone VI was prepared by ring closure of the product obtained by treating N-methylaminoacetaldehyde dimethyl acetal with the acid chloride of 1-methylindole-2-carboxylic acid. Oxidation of VI with chromic acid in glacial acetic acid produced the desired result, for the yellow crystalline oxidation product melted at 268.5–269° and showed no depression when mixed with compound IV.



VI IV, M. p. 268.5–269°

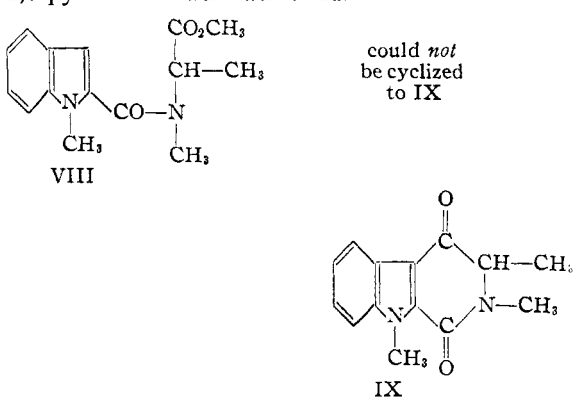
Oxidation of compound V with chromic acid or with acid potassium permanganate gave yellow needles, m. p. above 290°, which should correspond to structure Ia. Since the selenium degradation product melts at 253–255° it is obvious that the two are different. Likewise, the absorption spectrum of the oxidation product Ia differs from the spectra of the selenium degradation product and compound III but resembles that of compound IV (*cf.* Fig. 1).

Although it appeared quite probable that the same ring structure would be common to both of the primary degradation products, the preparation of model compounds related to the hydriodic acid reduction product was undertaken to confirm this inference. The acid chloride of 3-methylindole-2-carboxylic acid reacted smoothly with the methyl ester of *dl*-α-N-methylalanine and ring closure occurred spontaneously in the reaction mixture, giving directly the α-pyrazindole derivative of formula VII, m. p. 117.5–118.5°.



VII

Reaction of the acid chloride of 1-methylindole-2-carboxylic acid with *dl*-α-N-methylalanine methyl ester under similar conditions gave an oily liquid, presumably the methyl ester of N-(1-methylindole)-2-carbonyl-N-methylalanine(VIII). Neither the ester nor the corresponding free acid could be induced to undergo ring closure to the 2,9-pyridindole derivative IX.



VIII could *not* be cyclized to IX

IX

Failure to effect this ring closure is not in itself a valid argument against the structure IIa, since the factors influencing the cyclization of indole-2-carboxylic acid derivatives are not well understood and it has been noted that small differences in constitution can alter completely the course of reaction.[8] A few exploratory experiments were carried out on the cyclization of derivatives of indole-2,3-dicarboxylic acid but the preliminary results indicated that this method would not afford a convenient means of access to reference compounds of known constitution.

Comparison of the ultraviolet absorption curve (Fig. 2) for the hydriodic acid reduction product of gliotoxin with that of the α-pyrazindole VII reveals a very close similarity of the absorption maxima and minima for the two compounds. Although the corresponding 2,9-pyridindoles (IIa or IX) were not available for comparison, the striking resemblance to compound VII affords strong evidence that the hydriodic acid reduction product has the α-pyrazindole structure shown in formula II. This conclusion has been confirmed by examination of the infrared absorption spec-

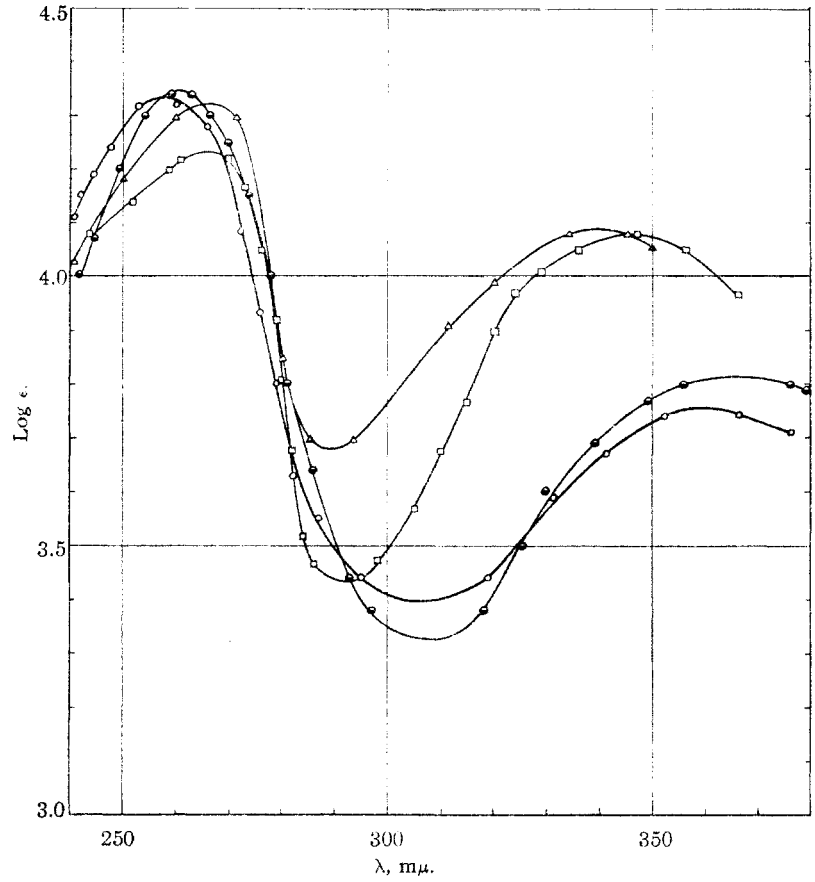
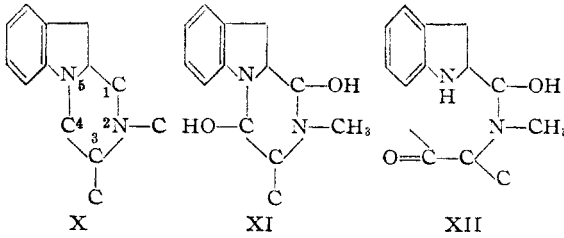


Fig. 1.—Ultraviolet absorption curves: △, selenium degradation product of gliotoxin; □, α-pyrazindole derivative III; ◒, pyridindole derivative IV; ○, pyridindole derivative Ia.

trum,[13] which shows the absence of a free —NH— group that would be present in the alternative 2,9-pyridinole structure IIa.

The circumstance that the α-pyrazindole nucleus is common to both of the primary degradation products has led us to assume as a working hypothesis, that the complete carbon and nitrogen skeleton of the gliotoxin molecule can be represented by formula X. As gliotoxin yields one



X XI XII

mole of methylamine on hydrolysis with alkalies,[14] it appears probable that a methyl group is present as such in the 2-position of the fused pyrazine ring. Acylation reactions indicate that gliotoxin has two reactive hydrogens,[14] suggesting that two of the four oxygen atoms of gliotoxin may be present as hydroxyl groups, tentatively assigned to positions 1 and 4 as shown in XI. In the absence of direct evidence that the α-pyrazindole ring is present in gliotoxin, the observed acylation reactions would be compatible with an alternative structural type (XII) containing a free —NH— group in the indole nucleus and an hydroxyl in the side chain. Negative color tests for a free indole —NH— group and other chemical observations lead us to prefer the structure XI.

Further studies of the chemical behavior of gliotoxin and its degradation products, and evidence bearing on the disposition of the oxygen and sulfur atoms, will be presented in subsequent reports.

Experimental

Indole-2-carboxylic Acid.—In view of the extensive use of this acid in our synthetic work, several methods of preparation were investigated. The most convenient proved to be the condensation of *o*-nitrobenzaldehyde with aceturic acid to give *o*-nitro-α-acetaminocinnamic azlactone, hydrolysis of the azlactone to *o*-nitrophenylpyruvic acid, and reduction of the latter with ferrous sulfate and ammonia.

An intimate mixture of 6 g. (0.04 mole) of *o*-nitrobenzaldehyde, 5.5 g. (0.047 mole) of aceturic acid, 2.6 g. (0.032 mole) of fused sodium acetate, and 16.2 g. of acetic anhydride was heated for 2.5 hours on a steam-bath in an open Erlenmeyer flask. After cooling to room temperature and standing for several hours the solid crystalline product was broken up, washed with three 20-cc. portions of water, and dried in vacuum over calcium chloride and solid sodium hydroxide. The dark yellow azlactone weighed 6 g. (65% yield) and melted at 110–112°.[15] Recrystallization from petroleum ether (b. p. 90–100°) gave the pure *o*-nitro-α-acetaminocinnamic azlactone; orange-yellow crystals, m. p. 114–115°.

Eight grams of the crude azlactone was refluxed for two and a half hours with 200 cc. of *N* hydrochloric acid in an apparatus with ground glass joints. The solution was treated with a little activated carbon and filtered while hot. The filtrate deposited an oil which crystallized on standing and scratching (or seeding). After cooling for ten to twelve hours at 0° to complete the crystallization, the crude *o*-nitrophenylpyruvic acid was filtered and washed with 5 cc. of water. After drying in vacuum the product weighed 4.3 g. and melted at 117–120°. Concentration of the mother liquor to 50 cc. in vacuum gave an

(13) We are indebted to Dr. J. R. Downing of the du Pont Experimental Station for investigating the infrared absorption spectra of gliotoxin and its degradation products. This work has been interrupted but it is hoped that it can be resumed later.

(14) Bruce, Dutcher, Johnson and Miller, THIS JOURNAL, **66**, 614 (1944).

(15) All melting points are uncorrected.

additional 1.7 g. of material melting at 119–120°, making a total of 6 g. (83% yield). The crude product is sufficiently pure for synthetic purposes; it may be purified by crystallization from water with small loss. Satisfactory results were not obtained in attempting to hydrolyze the azlactone obtained from *o*-nitrobenzaldehyde and hippuric acid.

o-Nitrophenylpyruvic acid was converted to indole-2-carboxylic acid in 63–65% yields by reduction with ferrous sulfate and ammonia, following essentially the procedure of Kermack, Perkin and Robinson.[16] The crude reaction product, m. p. 201–202°, was sufficiently pure for most purposes. Indole-2-carboxylic acid of higher purity was obtained by alkaline hydrolysis of the ethyl ester, obtained by reduction of ethyl *o*-nitrophenylpyruvate (see below). The yield in the saponification was 85–88%, and the acid formed lustrous, silvery flakes melting at 203–204°.

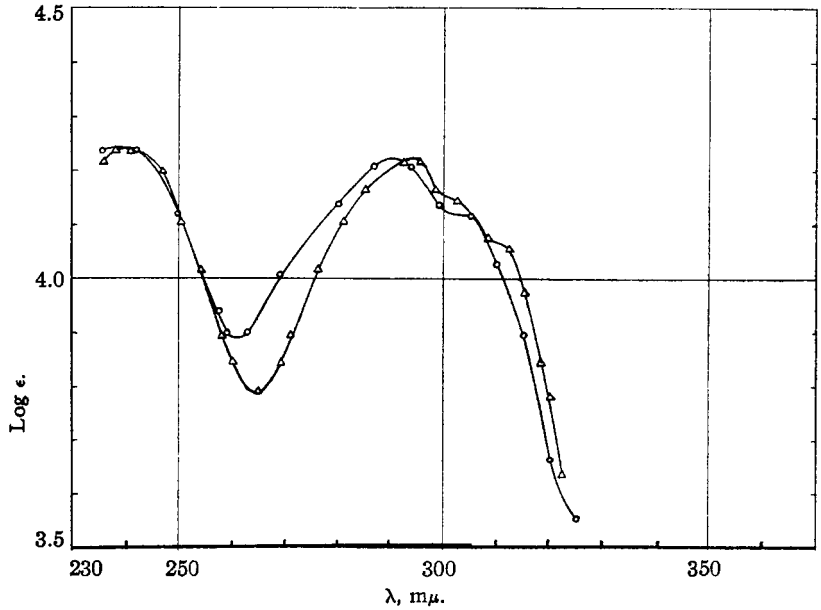


Fig. 2.—Ultraviolet absorption curves: ○, hydriodic acid reduction product of gliotoxin; △, α-pyrazindole derivative VII.

Ethyl Indole-2-carboxylate.—Ethyl *o*-nitrophenylpyruvate was prepared by condensation of ethyl oxalate with *o*-nitrotoluene by means of potassium ethoxide (60% yield).[17] The product was reduced directly with zinc and acetic acid, using a little cobalt nitrate as catalyst,[18] to give the ethyl ester of indole-2-carboxylic acid (65–68% yield). This ester formed light yellow elongated prisms, m. p. 123–125°.

N-Methylamide of Indole-2-carboxylic Acid.—A solution of 2.5 g. of the ethyl ester of indole-2-carboxylic acid in 75 cc. of absolute alcohol was saturated with dry, gaseous methylamine at 20°. After standing for four days the solution was evaporated under reduced pressure and the residual solid was treated with 125 cc. of boiling benzene. On cooling and standing the N-methylamide crystallized in colorless needles, m. p. 219–220°. The yield was 1.8 g. (77% of the theoretical). This amide is identical with the neutral compound $C_{10}H_{10}ON_2$ obtained from the selenium degradation product, and its properties have been described in an earlier paper. Preparation of the amide by reaction of methylamine with the acid chloride of indole-2-carboxylic acid in benzene solution is less satisfactory than the method given above, and the yield is only 30–35%.

Indole-2-carbonyl Chloride.—The preparation of this acid chloride by treatment of the acid with acetyl chloride and phosphorus pentachloride has been described.[16] This method was employed in a few experiments but the use of thionyl chloride proved more convenient and appeared to give a purer product.

To a cool solution of 0.75 g. (4.65 millimoles) of indole-2-carboxylic acid in 25 cc. of anhydrous ether was added 1.15 g. (9.6 millimoles) of thionyl chloride which had been purified by distillation from quinoline and from linseed oil. After standing at 25° for forty minutes the ether was distilled off under reduced pressure at a temperature not exceeding 35°. The residual acid chloride was treated with 15 cc. of anhydrous ether, which was distilled off as before. This process was then repeated once more to remove the last traces of thionyl chloride and hydrogen chloride. The residual acid chloride was dissolved in 20 cc. of anhydrous ether and used immediately. The yield of the acid chloride is 80–90%, based on the yields of reaction products obtained in subsequent transformations.

The Selenium Degradation Product (I): 2-Methyl-1,2,3,4-tetrahydropyrazino[1.2-a]indole-1,2,3-trione.[19]—In a flask fitted with ground joints a suspension of 0.52 g. (0.003 mole) of the N-methylamide of indole-2-carboxylic acid in 25 cc. of pure benzene was treated with 1.64 g. (0.012 mole) of freshly distilled ethoxalyl chloride. A gas inlet tube of 2 mm. bore was inserted through the condenser tube to conduct a slow stream of nitrogen over the surface of the liquid to remove the hydrogen chloride formed in the reaction. After refluxing for five hours no more hydrogen chloride was escaping from the system. The mixture was cooled to 20° and after standing for an hour the crystalline product was collected and washed with two 1-cc. portions of benzene. The product weighed 0.42 g. and formed yellow prisms, m. p. 254–255°. Concentration of the mother liquor to a volume of 5 cc. gave an additional quantity of 0.14 g. of quite pure crystals melting at 255.5–256.5°. The total yield amounted to 82% of the theoretical. This product showed no m. p. depression when mixed with the selenium degradation product from gliotoxin (m. p. 253–255°).[1]

The Hydriodic Acid Reduction Product (II): 2,3-Dimethyl-1,2,3,4-tetrahydropyrazino[1.2-a]indole-1,4-dione.[7]—An ether solution of the methyl ester of *dl*-α-N-methylalanine prepared by esterification of 1.5 g. (15 millimoles) of the amino acid was added to an ether solution of indole-2-carbonyl chloride prepared from 0.75 g. (4.65 millimoles) of indole-2-carboxylic acid by the thionyl chloride method. After standing at 20° for four hours the clear ethereal solution was decanted from a small amount of oil which had separated and was evaporated to dryness on a steam-bath. The residue was recrystallized from 6 cc. of methanol, from which on cooling there was obtained 275 mg. of stout yellow prisms, m. p. 121–123°. This material was shown by mixed m. p. to be identical with

(16) Kermack, Perkin and Robinson, *J. Chem. Soc.*, **119**, 1626 (1921).

(17) Wislicenus and Thoma, *Ann.*, **436**, 45 (1924).

(18) Maurer and Moser, *Z. physiol. Chem.*, **161**, 135 (1926).

(19) The names and numbering used for the α-pyrazindole and 2,9-pyridinole systems are based on the Ring Index (No. 1630 and 1646, respectively). In an earlier paper[1] the selenium product was inadvertently named as a 1,3,5-triketo derivative instead of being designated properly as a 1,3,5-trione. A similar fault in nomenclature was made also in naming the hydriodic acid reduction product as a 1,4-diketo derivative instead of a 1,4-dione.[7] On the basis of the nomenclature used by the British investigators (ref. 16, pp. 1627 and 1642) the selenium product would be named 4-methyl-2,3,5-triketo-2,3,4,5-tetrahydroindolediazine-1,4.

the hydriodic acid reduction product of gliotoxin, m. p. 122°.

Concentration of the mother liquor gave an additional 250 mg. of crystals melting at 125–139°, which consisted largely of the same product. Further concentration of the final mother liquor gave 130 mg. of colorless crystals melting at 146–148°, which were not identified.

Methyl Ester of N-Methyl-N-(indole-2-carbonyl)-alanine.—An ether solution of the methyl ester of N-methylalanine prepared from 6.7 g. (65 millimoles) of the amino acid was added to a chloroform solution of indole-2-carbonyl chloride prepared from 4 g. (25 millimoles) of indole-2-carboxylic acid (by the phosphorus pentachloride method). The mixture was allowed to stand for twenty minutes and then shaken with 5 g. of anhydrous sodium carbonate to remove hydrogen chloride. After filtering, the solution was concentrated under reduced pressure at 55°. The residual reddish-brown sirup was dissolved in 20 cc. of warm methanol and cooled at −10° overnight. The solution deposited 2.6 g. of crystals, m. p. 140–142°, and a further quantity of 0.9 g. was obtained by concentration of the mother liquor (total 3.5 g., 58% yield). Recrystallization from methanol gave colorless needles, m. p. 140–142°. This product is the methyl ester of the 13-carbon atom acid, $C_{13}H_{14}O_3N_2$, obtained by alkaline hydrolysis of the hydriodic acid reduction product of gliotoxin.

A sample of 260 mg. of the methyl ester was hydrolyzed by allowing it to stand at 20° with approximately 2% methanolic potassium hydroxide. The consumption of alkali was equivalent to 10.04 cc. of 0.1 *N* KOH, giving a saponification equivalent of 259 (calcd. for $C_{14}H_{16}O_3N_2$, 260). Acidification of the alkaline solution gave N-methyl-N-(indole-2-carbonyl)-alanine, m. p. 186–187°, identical with the 13-carbon acid derived from the hydriodic acid reduction product.

Syntheses of the type described above indicate that indole-2-carbonyl chloride reacts quite rapidly with N-methylalanine ester to produce the open-chain indolyl derivative, which can be isolated readily if the hydrogen chloride formed in the reaction mixture is removed by neutralization. In the presence of hydrogen chloride the open-chain indolyl derivative cyclizes to the α-pyrazindole II at a slower rate than the initial condensation. The same reaction carried out under similar conditions but in different solvents may give either the open-chain amide or the cyclization product, owing to differences in the retention of hydrogen chloride in the reaction medium and in the rate of ring closure in different media.

Oxidation of 2-Methyl-1,2-dihydro-9-pyrid[3.4-b]-indole-1-one (V) to 2-Methyl-1,2,3,4-tetrahydro-9-pyrid-[3.4-b]indole-1,3,4-trione (Ia)—(a) **With Chromium Trioxide.**—The starting material was prepared by ring closure of the condensation product obtained from indole-2-carbonyl chloride and N-methylaminoacetaldehyde dimethyl acetal, following the general procedure described below for compound VI. The pyridindolone V crystallized from benzene in tiny, colorless prisms, m. p. 269–270°; previously reported,[8] 263°.

To a cold, stirred solution of 132 mg. (0.66 millimole) of 2-methyl-1,2-dihydro-9-pyrid[3.4-b]indole-1-one in 10 cc. of glacial acetic acid (distilled over chromium trioxide) and 1 cc. of water, a solution of 135 mg. (1.35 millimoles, =3 atoms of oxygen) of chromium trioxide in 10 cc. of 50% acetic acid was added from a microburet over a period of about two hours. After standing overnight the reaction mixture was concentrated in vacuum to dryness and dissolved in a few cc. of ethanol. The solution was transferred to a vacuum sublimation apparatus, concentrated to dryness, and sublimed. A yield of about 10 mg. of long, yellow needles was obtained at 248–250° (11 mm.). The product melted above 290° in a sealed capillary and is obviously different from the selenium degradation product of gliotoxin, which melts at 253–255°.

Anal. Calcd. for $C_{12}H_8O_3N_2$: C, 63.20; H, 3.51; N, 12.30. Found: C, 63.25; H, 3.91; N, 12.25.

(b) **With Permanganate.**—The successful use of potassium permanganate in acetic acid for the oxidation of unsaturated steroids[20] prompted us to try this reagent in the pyridindole series. The results were as satisfactory as the chromic oxidation.

To a solution of 132 mg. (0.00066 mole) of 2-methyl-1,2 dihydro-9-pyrid[3.4-b]indole-1-one in 20 cc. of glacial acetic acid, 8.5 cc. of 0.5 *N* potassium permanganate solution (a slight excess) was added at room temperature over a period of forty-five minutes. After standing overnight the solution was diluted with water to about 250 cc. and seeded with a little of the product obtained in the preceding section. After further standing the green precipitate was collected and washed with a little water. From the mother liquor, after neutralizing most of the acetic acid, a second portion of crude product was secured; total yield, 41 mg. The material was sublimed twice at 235–250° (11 mm.) and recrystallized by dissolving in a mixture of methanol and benzene and then distilling off the methanol. A yield of 11.2 mg. of fine yellow needles was obtained. A microscopic mixed fusion of this material with the chromic oxidation product showed the two to be identical.

Model Compounds of the 2,9-Pyrindole Series

1-Methylindole-2-carboxylic Acid.—The α-methyl-α-phenylhydrazone of pyruvic acid, m. p. 79–80°, prepared from 20.6 g. (0.17 mole) of the hydrazine and 16 g. (0.18 mole) of pyruvic acid, was heated for thirty minutes on a steam-bath with 320 cc. of 12% hydrochloric acid according to the general procedure of Fischer and Hess.[11] The crude product weighed 13.9 g. (47% yield, based on the hydrazine) and melted at 200–205°. Recrystallization from 95% ethanol raised the m. p. to 208–209°, and a further recrystallization gave practically colorless needles melting at 209–210°.

The iodo derivative is a convenient compound for characterization of this acid. A warm solution of 200 mg. of 1-methylindole-2-carboxylic acid in 10 cc. of 10% aqueous sodium hydroxide was treated with 15 cc. of 0.1 *N* iodine–potassium iodide solution. After a few minutes the solution was acidified to congo red with concd. hydrochloric acid. Two recrystallizations of the crude product from dilute methanol, with addition of decolorizing carbon, gave nearly colorless, tiny prisms of 3-iodo-1-methylindole-2-carboxylic acid, m. p. 177–178° (dec.).

Methyl 1-Methylindole-2-carboxylate.—A solution of 3 g. (0.017 mole) of crude 1-methylindole-2-carboxylic acid in 90 cc. of commercial absolute methanol was saturated with dry hydrogen chloride and allowed to stand at room temperature for fifteen to eighteen hours. The solution was evaporated to dryness in vacuum (100 mm.), the residue taken up in 60 cc. of methanol, and the solution again evaporated to dryness. Crystallization from methanol gave light pink plates, m. p. 97–98°; yield, 2.35 g. Concentration of the mother liquor to about 6 cc. yielded 80 mg. of crystals melting at 93–95°; total yield of ester, 2.43 g. (75%). Recrystallization of a sample from methanol gave colorless plates, m. p. 97.5–98.5°; saponification equivalent, observed, 191; calcd. 189.

Ethyl 1-Methylindole-2-carboxylate.—The ethyl ester was prepared in a similar manner from the acid and ethanol. The crude product (50% yield, m. p. 62–63°) on recrystallization from ethanol furnished the pure ester in the form of colorless plates, m. p. 63–64°; saponification equivalent, observed, 199; calcd. 203.

N-Methylamide of 1-Methylindole-2-carboxylic Acid.—A solution of 2.94 g. (15.5 millimoles) of the methyl ester of 1-methylindole-2-carboxylic acid in 120 cc. of commercial absolute methanol was saturated with dry methylamine gas and allowed to stand at room temperature for forty to forty-five hours. The reaction mixture was concentrated in vacuum to a volume of about 10 cc., filtered hot and then diluted to about 20 cc. with water and cooled. A good-sized crop of crystals was obtained. Further dilution of the mother liquor yielded an additional amount of product. The total yield of amide was 2.65 g., or 90% of the theoretical. The crude material was nearly colorless and melted at 109–111°. Two recrystallizations from

(20) Ehrenstein and Decker *J. Org. Chem.*, **5**, 551 (1940).

dilute methanol yielded colorless prisms of m. p. 112–112.5°. The amide may also be recrystallized as rosets of colorless needles from mixtures of methanol and petroleum ether or benzene and petroleum ether.

Anal. Calcd. for $C_{11}H_{12}ON_2$: C, 70.21; H, 6.38. Found: C, 70.30, 70.03; H, 6.64, 6.52.

1-Methylindole-2-carbonyl Chloride.—To a solution of 2 g. (0.0114 mole) of 1-methylindole-2-carboxylic acid in 135 cc. of anhydrous ether was added 2.6 g. (0.0125 mole) of phosphorus pentachloride. The mixture was shaken frequently until all the phosphorus pentachloride dissolved (about twenty minutes). The solution was allowed to stand for four hours, after which it was concentrated to dryness in vacuum at a temperature of not over 45°. The residue was dissolved in about 20 cc. of anhydrous ether and concentrated to dryness in vacuum. This concentration was repeated twice more with ether and finally with one 10-cc. portion of dry chloroform in order to remove all phosphorus compounds and hydrogen chloride. The final residue of acid chloride was dissolved in anhydrous ether or chloroform, depending upon the reaction in which it was to be used. The solution was quickly filtered and was used immediately.

Reaction of the N-Methylamide of 1-Methylindole-2-carboxylic Acid with Ethoxalyl Chloride: 2,9-Dimethyl-1,2,3,4-tetrahydro-9-pyrid[3.4-b]indole-1,3,4-trione (IV).—To a solution of 1 g. (5.32 millimoles) of 1-methylindole-2-carboxylic N-methylamide in 45 cc. of anhydrous, thiophene-free benzene was added 2.9 g. (21.3 millimoles) of ethoxalyl chloride. The color of the solution darkened and a noticeable amount of heat was generated. The solution was heated under reflux for seventeen hours in the presence of nitrogen. A considerable quantity of product had crystallized out in the hot solution as yellow prisms. The reaction mixture was cooled to room temperature and the product collected and washed with a little cold benzene. The first crop of crystals melted at 269–270° and weighed 1.10 g. The second crop, obtained by concentrating the mother liquor to 4 cc., weighed 56 mg., and melted at 268–269°. The total yield was 1.156 g., or 90%. Recrystallization of the product from benzene gave fine, yellow prisms of m. p. 269–270°.

Anal. Calcd. for $C_{13}H_{10}O_3N_2$: C, 64.50; H, 4.13. Found: C, 64.57, 63.40; H, 4.18, 4.17.

The same compound was obtained by chromic acid oxidation of the unsaturated pyridindole VI, as described in the following section.

Reaction of 1-Methylindole-2-carbonyl Chloride with N-Methylaminoacetal: 2,9-Dimethyl-1,2-dihydro-9-pyrid[3.4-b]indole-1-one (VI).—To an ice-cold solution of the acid chloride prepared from 2 g. (1.14 millimoles) of 1-methylindole-2-carboxylic acid, in 11 cc. of dry chloroform, a solution of 3.6 g. (3 millimoles) of the dimethyl acetal of N-methylaminoacetaldehyde in 11 cc. of chloroform was added dropwise with stirring over a period of 30 minutes. After the addition was completed the reaction mixture was kept at 0–5° for an hour. The solution was evaporated nearly to dryness under reduced pressure and the residue dissolved in benzene. The solution was evaporated again to remove the last of the chloroform. This residue was treated with 40 cc. of benzene and 10 cc. of water, and the benzene layer was separated and evaporated under reduced pressure. The residual red oil crystallized slowly but no attempt was made to obtain the pure intermediate, N-methyl-N-(2',2'-dimethoxyethyl)-1-methylindole-2-carbonamide.

The crude amide was treated with 45 cc. of saturated ethanolic hydrogen chloride and warmed to 40–45° for ten minutes. After standing for an hour at room temperature the solution was diluted with water to 150 cc. and made slightly alkaline with concd. ammonia (color change from red to yellow). The crystalline pyridindole was collected and washed with 10 cc. of water. The yield of tan-colored product, m. p. 153.5–155° was 1.95 g. (81% of the theoretical). Microscopical observations indicated that this material was about 95% pure. A portion of the product was purified by treatment with 20% hydrochloric acid, collecting the precipitate of the hydrochloride, dissolving in water and reprecipitating the pyridindolone. The pure material (VI) formed minute plates, m. p. 158°.

Oxidation of this condensation product to the corresponding pyridindole-1,3,4-trione (IV) was carried out with chromic acid, as described above for the oxidation of V to Ia. A solution of 425 mg. (2 millimoles) of the pyridindolone VI in 33 cc. of 90% acetic acid was oxidized with 400 mg. (4 millimoles) of chromium trioxide in 30 cc. of 50% acetic acid. The crude oxidation product on sublimation at 275° (9 mm.) gave 10 mg. of yellow crystals, which on recrystallization from benzene formed bright yellow needles, m. p. 268.5–269°. Mixed m. p. determination and microscopical examination showed the oxidation product to be identical with the compound obtained by condensation of the methylamide of 1-methylindole-2-carboxylic acid and ethoxalyl chloride.

Reaction of 1-Methylindole-2-carbonyl Chloride with N-Methylalanine Ester: N-Methyl-N-(1-methylindole-2-carbonyl)-alanine.—To an ether solution of 1-methylindole-2-carboxylic acid chloride prepared from 1.4 g. (8 millimoles) of the acid was added an ether solution containing approximately 0.0117 mole of the methyl ester of *dl*-N-methylalanine. The mixture immediately became cloudy, and on standing at room temperature overnight deposited a small amount of a red oil. The ether solution was decanted from the insoluble oil, washed with three 20-cc. portions of water and dried over anhydrous sodium sulfate. Concentration of the ether solution gave a red oily liquid that would not crystallize on scratching and cooling. The oil was dissolved in 20 cc. of dry benzene, 2 drops of glacial acetic acid was added, and the mixture refluxed for two hours. Concentration of this solution gave a small amount of oil which could not be induced to crystallize and is evidently the methyl ester (VIII) of the open chain amide, N-methyl-N-(1-methyl-indole-2-carbonyl)-alanine. The ester was saponified with methanolic potassium hydroxide by standing for seven hours at room temperature. Acidification and crystallization from methanol, followed by petroleum ether, gave almost colorless crystals of N-methyl-N-(1-methylindole-2-carbonyl)-alanine, m. p. 147–148.5°.

A sample of the free acid (0.55 g.) suspended in 25 cc. of benzene containing 1 cc. of acetic anhydride was refluxed for two hours. The solution yielded an oil which could not be induced to crystallize. The small quantities of material available did not permit further attempts at this time to effect cyclization to the pyridindole derivative (IX).

Model Compounds of the α-Pyrazindole Series

3-Methylindole-2-carboxylic Acid.—This acid was not prepared directly but was obtained by saponification of the ethyl ester, which was synthesized by an extended series of reactions. Five grams (2.5 millimoles) of ethyl 3-methylindole-2-carboxylate was refluxed for 1.5 hours with a solution of 2.4 g. (3.65 millimoles) of potassium hydroxide in 10 cc. of water and 65 cc. of methanol. After distilling off all of the methanol, 40 cc. of water was added and the solution was acidified to congo red with 4 *N* sulfuric acid. The white precipitate of 3-methylindole-2-carboxylic acid was collected, washed well with water and dried. The yield was 4.0 g. (94%) and the product melted at 164–166°.

Ethyl 3-Methylindole-2-carboxylate.[10]—Ethyl methyloxalacetate prepared from ethyl oxalate and ethyl propionate was hydrolyzed with 10% sulfuric acid to give α-ketobutyric acid, b. p. 67.5–69° (11 mm.) (54% yield), and the latter was treated with phenylhydrazine to form the phenylhydrazone (93% yield). Eight grams of the crude phenylhydrazone, m. p. 135–141°, on refluxing with 8 cc. of concd. sulfuric acid and 72 cc. of absolute ethanol gave 6.1 g. of colorless crystals of ethyl 3-methylindole-2-carboxylate (72% yield). The purified ester melted at 135–136°.

N-Methylamide of 3-Methylindole-2-carboxylic Acid.—A solution of 2.34 g. of ethyl 3-methylindole-2-carboxylate in 100 cc. of absolute methanol was saturated with dry gaseous methylamine at 20°, sealed in a 400-cc. bomb-

tube and heated at 100° for six hours. After cooling to 20° and standing overnight the reaction mixture was evaporated to dryness under reduced pressure. The residual white solid was boiled for a few minutes with 20 cc. of benzene (to dissolve unchanged ester), and the mixture was allowed to cool. The colorless needles of the N-methylamide weighed 1.58 g. (73% yield) and melted at 197–197.5°. One recrystallization of this material from benzene gave long colorless needles, m. p. 198.5–199.5°. The N-methylamide is readily soluble in methanol, moderately soluble in hot benzene and very sparingly soluble in cold benzene.

Anal. Calcd. for $C_{11}H_{12}ON_2$: C, 70.21; H, 6.38. Found: C, 68.63, 68.74; H, 6.57, 6.46.

A similar reaction was carried out without the use of a bomb tube, by sealing the reaction mixture in a flask and allowing it to stand at room temperature for forty-eight hours. The isolation of the product was carried out as before but only 42% conversion to the amide was realized.

Reaction of the N-Methylamide of 3-Methylindole-2-carboxylic Acid with Ethoxalyl Chloride: 2,10-Dimethyl-1,2,3,4-tetrahydropyrazino[1.2-a]indole-1,3,4-trione (III).—A mixture of 0.84 g. (4.5 millimoles) of the N-methylamide, 40 cc. of dry benzene and 2.46 g. (18 millimoles) of ethoxalyl chloride was refluxed for 12 hours in a slow stream of nitrogen. The hot solution was filtered from a small amount of sediment, concentrated to a volume of 15 cc. and allowed to cool gradually to 20°. The crop of small, elongated yellow prisms which separated was collected and washed with 2 cc. of cold benzene. The product weighed 0.78 g. and melted at 208–209°. Concentration of the mother liquor to 4 cc. gave a further quantity of material, m. p. 207–208°. The total yield was 0.965 g. (90% of the theoretical). Recrystallization from benzene raised the melting point to 209–210°.

Anal. Calcd. for $C_{13}H_{10}O_3N_2$: C, 64.50; H, 4.13. Found: C, 64.66; H, 4.38.

Reaction of 3-Methylindole-2-carbonyl Chloride with N-Methylalanine Ester: 2,3,10-Trimethyl-1,2,3,4-tetrahydropyrazino[1.2-a]indole-1,4-dione (VII).—An ether solution of the acid chloride was prepared from 1 g. (5.7 millimoles) of 3-methylindole-2-carboxylic acid by means of phosphorus pentachloride, according to the procedure given above for 1-methylindole-2-carbonyl chloride. This solution was treated with an ether solution of the methyl ester of *dl*-α-N-methylalanine prepared by esterification of 2.08 g. (18 millimoles) of the amino acid. The mixture turned cloudy immediately, and on standing deposited a small amount of an oil (presumably N-methylalanine ester hydrochloride). The clear supernatant solution was decanted, washed with three 15-cc. portions of water, and dried over magnesium sulfate. After filtering and evaporating the solution to dryness on a steam-bath, there was obtained 0.82 g. (62% yield) of the yellow crystalline condensation product. Recrystallization from 50–50 benzene–petroleum ether (b. p. 60–70°) followed by crystallization from 50% aqueous methanol gave pale yellow crystals melting at 117.5–118.5°. This substance crystallizes quite slowly, and cooling at −10° for several hours is necessary to insure complete crystallization.

Anal. Calcd. for $C_{14}H_{14}O_2N_2$: C, 69.43; H, 5.78. Found: C, 69.60, 69.50; H, 5.50, 5.36.

Summary

The synthesis of a series of model compounds of the α-pyrazindole and 2,9-pyridindole series has been described. Comparisons of absorption spectra and evidence from the synthetic investigations show that the selenium degradation product and the hydriodic acid reduction product from gliotoxin are α-pyrazindole derivatives. These observations suggest strongly that the carbon and nitrogen skeleton of gliotoxin itself is that of 2,3-dimethyl-1,2,3,4-tetrahydropyrazino-[1.2-a]indole.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Reactions Involving Ester-Exchange

BY WILLIAM S. JOHNSON AND ARTHUR GOLDMAN

In a previous communication[1] a report was given on the preparation, structures and configurations of all of the four possible isomeric half-esters (I, II, IV and V) of γ-methyl-γ-(2-naphthyl)-itaconic acid. It was demonstrated that ethyl 3-carboxy-4-(2-naphthyl)-*cis*-3-pentenoate (I) was cyclized by the action of acetic acid, acetic anhydride and sodium acetate to ethyl 3-methyl-6,7-benz-1-indone-2-acetate (III). Under the same conditions the isomeric 3-carbethoxy-4-(2-naphthyl)-*cis*-3-pentenoic acid (II) failed to cyclize into the aromatic nucleus. Parallel behavior was exhibited by the half-esters in the *trans* series. Thus 3-carbethoxy-4-(2-naphthyl)-*trans*-3-pentenoic acid (IV), but not the isomeric ethyl 3-carboxy-4-(2-naphthyl)-*trans*-3-pentenoate (V), cyclized to give ethyl 4-acetoxy-1-methylphenanthrene-2-carboxylate (VI).

During the course of this work it was found that if a catalytic amount of *zinc chloride*[2] was used in place of the sodium acetate in the cyclization reaction, the ring closure I → III proceeded more rapidly and quantitatively (*cf.* the 18% yield previously reported[1]). More striking, however, was the discovery that under these conditions the *half-ester II also was cyclized to the indoneacetic ester III.* This reaction obviously involved an ester-exchange; and, moreover, it likewise proceeded rapidly and in practically quantitative yield, which is all the more surprising considering the fact that acetic acid—a potential participant in the ester-exchange reaction—was used as a diluent. Since the results of the previously reported cyclization experiments held a prominent part in the argument of the proof of structures of the half-esters, it now appeared advisable, in view of the anomalous cyclization behavior with zinc chloride, to obtain further evidence for the structures. That the half-ester believed to be II, actually has the carbethoxyl group on the double bonded carbon was shown by ozonolysis. To avoid cleavage of the ester, the reaction was con-

(1) Johnson and Goldman, THIS JOURNAL, **66**, 1030 (1944).

(2) *Cf.* Fieser and Hershberg, *ibid.*, **59**, 1028 (1937).